Article

Sestrin mediates detection of and adaptation to low-leucine diets in *Drosophila*

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Mechanistic target of rapamycin complex 1 (mTORC1) regulates cell growth and metabolism in response to multiple nutrients, including the essential amino acid leucine[1]. Recent work in cultured mammalian cells established the Sestrins as leucine-binding proteins that inhibit mTORC1 signalling during leucine deprivation[2,3], but their role in the organismal response to dietary leucine remains elusive. Here we find that *Sestrin*-null flies (*Sesn*$^{-/-}$) fail to inhibit mTORC1 or activate autophagy after acute leucine starvation and have impaired development and a shortened lifespan on a low-leucine diet. Knock-in flies expressing a leucine-binding-deficient Sestrin mutant (*Sesn*^{L431E}) have reduced, leucine-insensitive mTORC1 activity. Notably, we find that flies can discriminate between food with or without leucine, and preferentially feed and lay progeny on leucine-containing food. This preference depends on Sestrin and its capacity to bind leucine. Leucine regulates mTORC1 activity in glial cells, and knockdown of *Sesn* in these cells reduces the ability of flies to detect leucine-free food. Thus, nutrient sensing by mTORC1 is necessary for flies not only to adapt to, but also to detect, a diet deficient in an essential nutrient.

The protein kinase mTORC1 regulates growth and metabolism in response to diverse signals, including growth factors and nutrients such as amino acids[1]. Amino acids activate mTORC1 by promoting its translocation to the lysosomal surface, where its essential activator Rheb resides[4–6].The heterodimeric Rag GTPases, which are under the control of several multi-component protein complexes, including GATOR1 and GATOR2 (ref. [7]), regulate the lysosomal localization of mTORC1 (refs. [4,5]). GATOR1 is a GTPase-activating protein for RagA and RagB and is necessary for amino acid deprivation to inhibit mTORC1 signalling[8,9]. By contrast, GATOR2 is required for amino acids to activate mTORC1 and directly interacts with several of the amino acid sensors so far discovered, indicating that it acts as a nutrient-sensing hub despite its still unknown biochemical function[7].

Among the proteogenic amino acids, leucine is the best-established activator of mTORC1 (refs. [10–13]). Work in cultured mammalian cells has shown that leucine controls mTORC1 by regulating the interaction of GATOR2 with the Sestrin family of proteins[3,14,15], which are repressors of mTORC1 signalling[16,17]. Human Sestrin1 and Sestrin2 bind leucine at affinities consistent with the leucine concentration needed to activate mTORC1 and are required for leucine deprivation to inhibit mTORC1 signalling[3]. Moreover, a Sestrin2 mutant that does not bind leucine fails to dissociate from GATOR2 in the presence of leucine, and in cells expressing this mutant, mTORC1 activity remains low even when the cells are cultured in leucine-replete conditions[2,3]. Despite the evidence that Sestrin is a leucine sensor for the mTORC1 pathway in cultured mammalian cells, the roles of Sestrin-mediated leucine sensing in the physiology of an intact organism remain largely unexplored.

Although much of the work on leucine sensing has been in mammalian systems, Sestrin and the core nutrient-sensing machinery, including the Rag GTPases, GATOR1 and GATOR2, are conserved in most invertebrates, including the fly *Drosophila melanogaster*[18]. Unlike in mammals, flies express only one gene for Sestrin (*Sesn*)[16], greatly facilitating the in vivo study of leucine sensing by mTORC1. Here we show that Sestrin and its leucine-binding pocket are required for leucine to regulate mTORC1 activity in fly tissues in vivo and for flies to detect and adapt to leucine-deficient diets.

Fly mTORC1 senses leucine in vivo through Sestrin

In an equilibrium binding assay, *Drosophila* Sestrin bound leucine with a dissociation constant (K_d) of about 100 μM (Fig. 1a), an affinity several fold lower than those of human Sestrin1 and Sestrin-2 (K_d values of about 15–20 μM)[3]. This reduced affinity is probably the result of a difference between the leucine-binding pockets of human and fly Sestrin. Structural studies show that in human Sestrin2 a tryptophan (W444) forms the floor of the pocket, but in the fly protein, the analogous residue is a leucine (L431), a smaller residue that when introduced into human Sestrin2 (W444L) is sufficient to reduce its leucine-binding capacity by several fold[2]. The low leucine affinity of fly Sestrin is consistent with the observation that fly haemolymph has substantially higher amino acid concentrations than human plasma[18,19], a difference probably reflected

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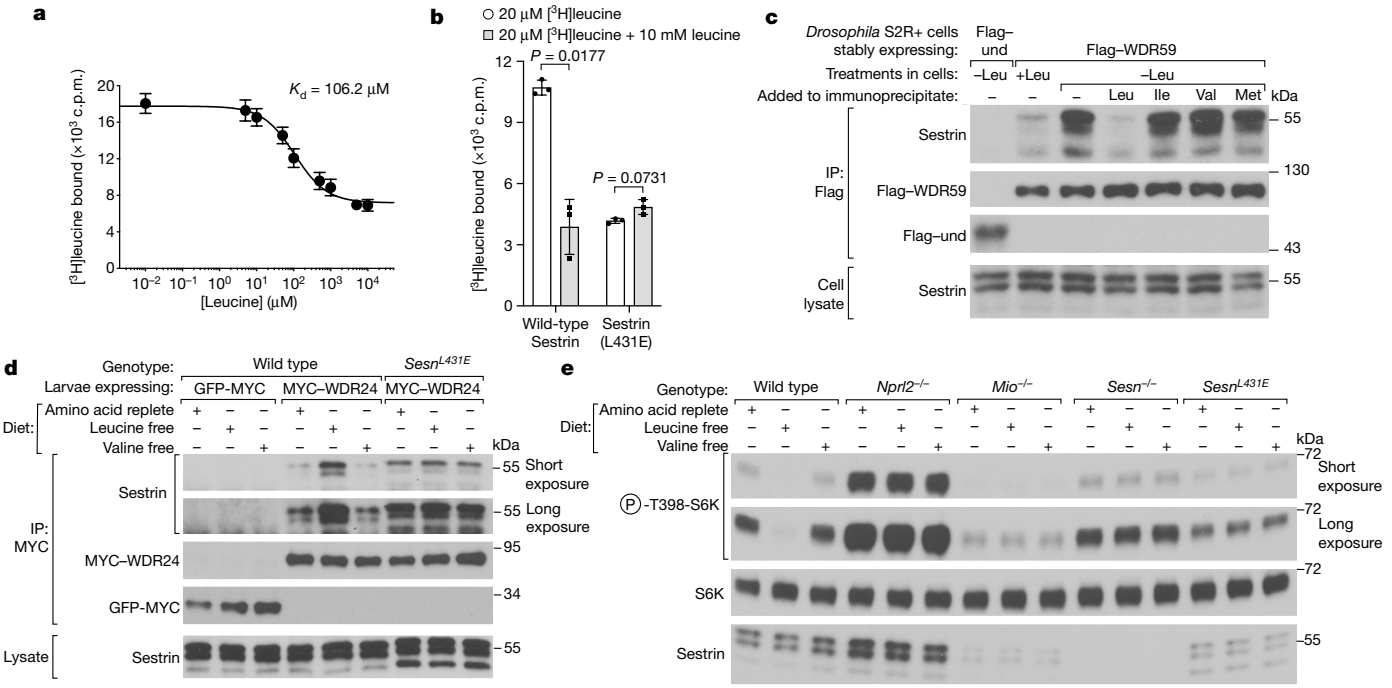

Fig. 1 | *Drosophila* Sestrin binds GATOR2 and regulates mTORC1 in vivo in response to dietary leucine. a, Data from an equilibrium binding assay showing that purified Flag–Sestrin bound leucine, $K_d \approx 100$ μM. The values are the mean ± s.d. of three technical replicates from a representative experiment. **b**, The L431E alteration blocks leucine binding by *Drosophila* Sestrin. HA-tagged wild-type Sestrin and Sestrin(L431E) were prepared from HEK293T cells expressing the appropriate cDNAs. The binding assays were performed as in **a**. The values are the mean ± s.d. of three technical replicates from a representative experiment. The *P* values were determined using an unpaired *t*-test with Welch correction, and the Holm-Šídák multiple comparison method. **c**, Leucine starvation enhances the Sestrin–GATOR2 interaction. Flag–immunoprecipitates (IPs) were prepared from S2R+ cells stably expressing Flag-tagged und (negative control) or WDR59 (a GATOR2 component) and starved or not of leucine. Immunoprecipitates and lysates were analysed by immunoblotting for the indicated proteins. Addition to the immunoprecipitates of 1 mM leucine, but not other amino acids, disrupted the Sestrin–GATOR2 interaction. **d**, Dietary leucine regulates in vivo the interaction of Sestrin with GATOR2 depending on the leucine-binding site of Sestrin. Immunoprecipitates were prepared from lysates of fat bodies from wild-type (*OreR*) or $Sesn^{L431E}$ larvae expressing the MYC-tagged control protein GFP or the MYC-tagged GATOR2 component WDR24 in the fat body (*lpp-gal4*). Animals were fed the indicated diets for 4.5 h before sample collection. Amino acid replete: chemically defined diet containing all amino acids; leucine free or valine free: chemically defined diet lacking leucine or valine, respectively. **e**, Sestrin binding to leucine regulates mTORC1 signalling in vivo. Shown are immunoblots of Sestrin, S6K and phospho-S6K in fat bodies prepared as in **d** from larvae with the indicated genotypes. *Nprl2* and *Mio* encode core components of the GATOR1 and GATOR2 complexes, respectively. The dietary composition and feeding period were as in **d**. The data are representative of three (**a**,**b**) or two (**c**–**e**) independent experiments with similar results.

intracellularly. Like the analogous mutant of human Sestrin2 (W444E), fly Sestrin(L431E) does not bind leucine (Fig. 1b).

To examine whether leucine regulates the interaction of fly Sestrin with GATOR2, we stably expressed in *Drosophila* S2R+ cells a Flag-tagged control protein (und, the *Drosophila* orthologue of mammalian metap2, methionyl aminopeptidase) or WDR59, one of the five core components of the GATOR2 complex. Sestrin co-immunoprecipitated with GATOR2, but not und, and removal of leucine from the cell medium strongly enhanced the interaction. The addition of leucine, but not isoleucine, valine or methionine, to the immunoprecipitates was sufficient to release Sestrin from GATOR2 (Fig. 1c). Thus, like the human protein, fly Sestrin binds to GATOR2 in a fashion that is specifically disrupted by leucine.

To extend our work in vivo, we generated flies that ectopically express MYC-tagged WDR24, another core component of GATOR2 (*lpp>myc–WDR24* flies), in the fat body, and are either wild type at the *Sesn* locus or have a knock-in mutation causing the L431E substitution that renders Sestrin unable to bind leucine ($Sesn^{L431E}$). For a period of 4.5 h, we fed third instar larvae a chemically defined diet (see Methods and Extended Data Tables 1–4 for details) containing all proteogenic amino acids (amino acid replete) or the same diet lacking just leucine (leucine free) or valine (valine free). Regardless of genotype, larvae eating the leucine- or valine-free diets had reduced levels of leucine or valine, respectively (Extended Data Fig. 1a,b). In lysates prepared from isolated fat bodies, endogenous Sestrin co-immunoprecipitated with GATOR2, but not a control protein (GFP-MYC), and deprivation of leucine, but not valine, strongly boosted the interaction. In contrast, Sestrin(L431E) bound equally well to GATOR2 under all dietary conditions, consistent with the mutant being leucine insensitive (Fig. 1d). In cultured cells and in fat bodies, we observed that Sestrin has multiple isoforms (Fig. 1c,d), probably the result of differential splicing[16].

In wild-type larvae, feeding of the diet free in leucine, but not valine, inhibited mTORC1 in the fat body, as assessed by the phosphorylation of S6K, a canonical mTORC1 substrate. The loss of Sestrin ($Sesn^{-/-}$) did not impact mTORC1 activity in larvae eating the amino-acid-replete diet, but completely prevented the inhibition of mTORC1 normally caused by leucine deprivation (Fig. 1e). Sestrin was also required for the leucine-free diet to activate autophagy, a process suppressed by mTORC1, as monitored by the formation of mCherry–Atg8a-positive puncta (Extended Data Fig. 1c). In $Sesn^{L431E}$ larvae, mTORC1 activity was low relative to that in wild-type larvae and also unaffected by leucine deprivation, indicating that the leucine-binding mutant of Sestrin acts as a non-repressible inhibitor of mTORC1 (Fig. 1e). Notably, mTORC1 signalling was inhibited in $Sesn^{-/-}$ larvae deprived of all food to a similar extent as in wild-type larvae (Extended Data Fig. 1d), which is consistent with work in cultured mammalian cells showing that Sestrin has a specific role in transmitting leucine availability to mTORC1 (refs. [3,14]). Last, in larvae lacking a component of GATOR1 ($Nprl2^{-/-}$) or GATOR2 ($Mio^{-/-}$),

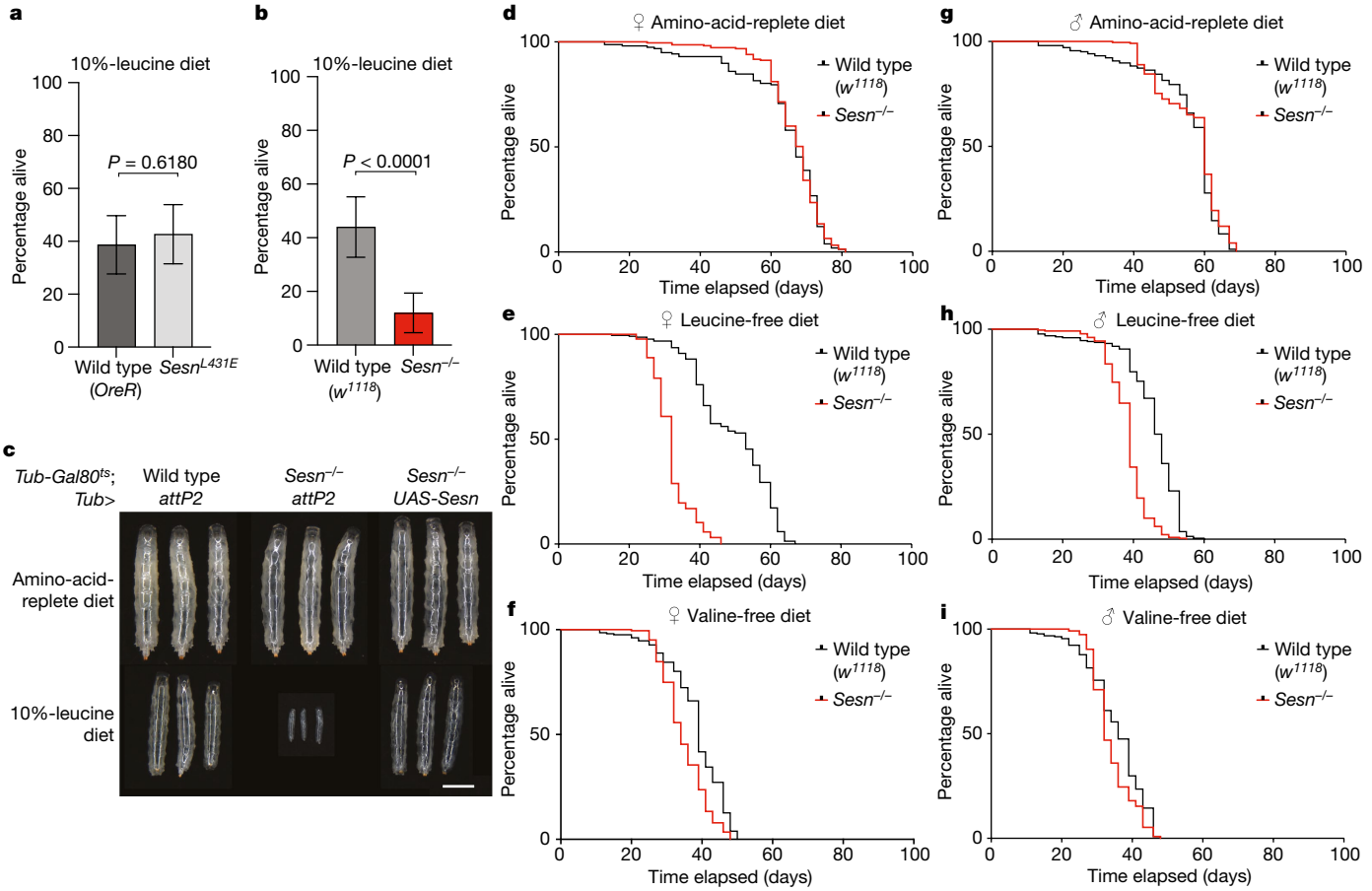

Fig. 2 | *Drosophila* require Sestrin to adapt to a low-leucine diet. a,**b**, Loss of Sestrin reduces survival during development after leucine starvation. The bar charts show survival (%) of larvae raised for 10 days on a chemically defined diet containing 10% of the leucine in the control diet. The *P* values were determined using a two-proportion *z*-test (two-sided). The bars show the percentage of surviving larvae in each genotype and the error bars represent the 95% Wald confidence interval. **c**, Sestrin is required for larval growth on a low-leucine diet. Shown are age-synchronized animals of the indicated genotypes raised for 9 days on either an amino-acid-replete diet or a reduced (10%)-leucine diet. Scale bar, 1 mm. **d**–**i**, Loss of Sestrin reduces survival of adult flies after leucine starvation. $Sesn^{-/-}$ animals show reduced lifespan when fed a diet lacking leucine (0% leucine). Shown are survival curves of age-synchronized adult male (♂) and female (♀) animals of the indicated genotypes fed the indicated diets. In **c**, wild type (w^{1118}) $n = 157$; $Sesn^{-/-}$ $n = 217$; in **d**, wild type (w^{1118}) $n = 221$; $Sesn^{-/-}$ $n = 225$; in **e**, wild type (w^{1118}) $n = 206$; $Sesn^{-/-}$ $n = 203$; in **f**, wild type (w^{1118}) $n = 205$; $Sesn^{-/-}$ $n = 226$; in **g**, wild type (w^{1118}) $n = 222$; $Sesn^{-/-}$ $n = 230$; in **h**, wild type (w^{1118}) $n = 221$; $Sesn^{-/-}$ $n = 228$. See statistics in Supplementary Data 1 and Methods. The data in **a**–**i** are representative of three independent experiments with similar results.

the absence of dietary leucine did not impact mTORC1 activity and it remained as hyperactive or suppressed, respectively, as when the larvae were fed the amino-acid-replete diet (Fig. 1e). Consistent with mTORC1 promoting *Sesn* transcription as part of a feedback loop[16,20], $Nprl2^{-/-}$ and $Mio^{-/-}$ flies had increased and decreased Sestrin levels, respectively (Fig. 1e). Collectively, these results show that dietary leucine modulates mTORC1 in vivo and that this regulation requires Sestrin and its leucine-binding pocket as well as the GATOR1 and GATOR2 complexes.

Sestrin mediates adaption to low-leucine diets

We reasoned that Sestrin-mediated suppression of mTORC1 helps animals adapt to and thus survive a diet low in leucine. We first tried to test this idea by feeding larvae food lacking leucine, but all larvae, independently of genotype, died within 2–3 days of starting the diet, consistent with leucine being an essential amino acid required for larval growth. When given food containing one-tenth of the normal leucine content, about 40% of wild-type larvae survived over a period of 16 days (Fig. 2a,b). In contrast, only about 10% of $Sesn^{-/-}$ larvae did so (Fig. 2b). Moreover, the surviving larvae grew to a much smaller size than their wild-type counterparts (Fig. 2c), a defect rescued by the expression of wild-type Sestrin from the ubiquitous *Tubulin*-Gal4, *Tubulin*-Gal80^{ts} promoter (Fig. 2c). When fed the standard laboratory diet, $Sesn^{-/-}$ and wild-type larvae developed indistinguishably (Extended Data Fig. 2a).

Consistent with previous work showing that adult flies can live for weeks on a diet lacking any amino acid source[21], our observations showed that wild-type flies also survived for many weeks on a leucine-free diet (Fig. 2e,h, Extended Data Fig. 2c,f and Supplementary Data 1). As with larvae, adult flies also required Sestrin to adapt to leucine scarcity, as $Sesn^{-/-}$ male and female animals had greatly shortened lifespans on the leucine-free, but not amino-acid-replete, diet (Fig. 2d,e,g,h and Supplementary Data 1). On the other hand, $Sesn^{-/-}$ flies had slightly shorter lifespans than wild-type counterparts only when eating the valine-free food (Fig. 2f,i and Supplementary Data 1), a diet on which the activity of processes controlled by mTORC1, such as protein synthesis and autophagy, would be expected to impact survival. When the $Sesn^{L431E}$ flies were fed the same chemically defined diets, they survived similarly to the wild-type flies (Extended Data Fig. 2b–g and Supplementary Data 1). Consistent with the chronic suppression of mTORC1 signalling, $Sesn^{L431E}$ larvae reared on the standard laboratory diet developed more slowly than wild-type ones (Extended Data Fig. 2h).

We monitored mTORC1 activity in whole-fly lysates of female and male adult flies that had been fasted overnight and then refed for 90 min with the chemically defined diets used above. The loss of Sestrin prevented the inhibition of mTORC1 caused by the leucine-free diet in male and female flies (Extended Data Fig. 3a,b).

We further focused on oogenesis, a physiological trait that is known to be regulated by diet[22]. Moreover, diet is known to regulate ovarian function through the GATOR1–GATOR2 complexes[21,23–25], and *Mio*, the gene for one of the components of GATOR2, was so named because mutations in it result in a missing oocyte phenotype[26]. We found that mTORC1 activity was strongly increased in the ovaries of $Sesn^{-/-}$ flies eating the standard laboratory diet, and as in larval fat bodies (Fig. 1e), it was suppressed in the ovaries of $Sesn^{L431E}$ flies (Extended Data Fig. 3c).

When fed the amino-acid-replete or valine-free diet, $Sesn^{-/-}$ and wild-type flies had ovaries of similar sizes, but the loss of Sestrin greatly reduced ovarian size in flies under conditions of acute leucine deprivation (Extended Data Fig. 3d,e), again pointing to a specific role for Sestrin in adapting to leucine scarcity. The ovaries of the $Sesn^{L431E}$ flies were equally small on all of the diets (Extended Data Fig. 3d,e), consistent with a role for mTORC1 in the control of gonad development. $Sesn^{L431E}$ flies also had reduced fecundity as they laid fewer eggs than wild-type flies (Extended Data Fig. 3f). Eggs from wild-type, $Sesn^{L431E}$ and $Sesn^{-/-}$ flies had comparable hatching rates, suggesting that Sestrin does not impact fertility (Extended Data Fig. 3g). Collectively, these data reveal that in larvae and adult flies Sestrin promotes survival on a low-leucine diet and has a particularly important role in controlling ovarian size and function.

Sestrin regulates feeding behaviour

Having established that Sestrin is important for flies to adapt to and survive on diets low in leucine, we examined whether flies also require Sestrin to detect and thus avoid food that is poor in leucine. To do so, we developed an assay to test whether adult flies prefer eating leucine-rich over leucine-poor food. The experimental set-up consisted of 15 female and 5 male flies in a bottle containing 2 apple pieces, the first painted with a solution of one or more amino acids and the second with an appropriate control (Fig. 3a). Each also contained a trace amount of a unique DNA oligonucleotide, which served as a barcode for measuring the food consumption, an approach previously described[27] and that we validated (Extended Data Fig. 4a–c and Methods). We chose apple as the base food because it is carbohydrate rich and protein poor[28], allowing us to set up food choices that have different amino acid compositions but the same content of sugars. Apples are reported to contain very little leucine and valine[29–31].

We found that wild-type female flies prefer to eat apples coated with leucine rather than water. This preference emerges after the flies have been eating the food for about 6 h and increases to 5–6-fold by 24 h, the time point we used in subsequent experiments (Fig. 3b). The preference for leucine is concentration dependent (Extended Data Fig. 4d) and not every amino acid elicits a preference, as flies do not distinguish between apples coated with valine or water (Extended Data Fig. 4e). Given a choice between equal amounts of leucine and valine, flies still prefer leucine, suggesting that the leucine preference is not simply the result of a nitrogen imbalance (Extended Data Fig. 4e). Moreover, the leucine preference requires differential mTORC1 activity, as when flies were fed the mTORC1 inhibitor rapamycin, they no longer showed a preference (Fig. 3c). Rapamycin treatment also lowered the total amount of food consumed by the flies (Extended Data Fig. 4f), consistent with previous reports[32,33].

Remarkably, neither $Sesn^{-/-}$ nor $Sesn^{L431E}$ female flies—both of which have leucine-insensitive mTORC1 signalling—had a preference for leucine as they ate similar amounts of leucine-rich and leucine-poor foods (Fig. 3d,e and Extended Data Fig. 4g). However, the two *Sesn* mutants probably differ in the total amount of food each ate. The amount of food (leucine-rich or leucine-poor) that $Sesn^{-/-}$ female flies ate was similar to the amount of leucine-rich food consumed by wild-type (w^{1118}) flies (Extended Data Fig. 4h). The opposite was true for $Sesn^{L431E}$ female flies. These flies ate an amount of food (leucine-rich or leucine-poor) similar to the amount of leucine-poor food consumed by the wild-type (*OreR*) flies (Extended Data Fig. 4i). That $Sesn^{L431E}$ files, which have low mTORC1 signalling, eat less food than wild-type controls is consistent with rapamycin causing a reduction in food consumption (Extended Data Fig. 4f). Whole-body re-expression in the $Sesn^{-/-}$ female flies of Sestrin driven by *Tub*>Gal4 partially restored the leucine preference of the animals (Extended Data Fig. 4j).

We also examined whether flies can distinguish between foods with a more subtle difference in amino acid composition: an apple coated with the 20 proteogenic amino acids versus just 19 of them (that is, lacking only leucine). Indeed, this was the case and this preference was also absent in the $Sesn^{-/-}$ and $Sesn^{L431E}$ flies (Fig. 3f). Valine again served as a control: when removed from the 20-amino-acid cocktail, neither wild-type nor *Sesn* mutant flies showed preference for the valine-containing food (Extended Data Fig. 4k).

To obtain temporal control of Sestrin suppression, we generated a conditional knockdown system using a short hairpin RNA (shRNA) targeting *Sesn*. Ubiquitous expression of the shRNA reduced Sestrin protein levels (Fig. 3g), and as expected, the preference of the flies for the leucine-containing food (Fig. 3h). Using a temperature-sensitive shRNA driver, we suppressed Sestrin specifically during adulthood (Fig. 3i,j). This too reduced their leucine preference (Fig. 3k), indicating that the acute loss of Sestrin in adult flies is sufficient to impact the leucine preference. Notably, the temperature shift to 29 °C increased Sestrin levels (Fig. 3j), consistent with previous work showing that multiple stresses induce its transcription[17,34]. Thus, female flies can readily detect food lacking leucine even if it contains sugars and other amino acids. This ability requires Sestrin and its capacity to bind leucine.

To further analyse the physiological relevance of leucine sensing through the Sestrin–mTORC1 axis, we tested the impact of both leucine and Sestrin on the choice between low- and high-protein diets: apple coated with a low or high amount of yeast extract, which is a complex type of food and the major protein source for laboratory-raised flies. Wild-type flies had a strong preference for the apple with a higher protein content. The addition of leucine to the protein-poor food reduced the preference of wild-type female flies for the protein-rich food, but only minimally impacted the preference of the $Sesn^{L431E}$ mutants (Extended Data Fig. 5a). $Sesn^{-/-}$ mutants showed a similar trend (Extended Data Fig. 5b), but it was not statistically significant. Together, these data suggest that flies use leucine sensing through the Sestrin–mTORC1 axis as a proxy for the food protein content.

Sestrin regulates egg-laying behaviour

We found that female flies prefer to lay eggs on the leucine-coated apples. To explore this further, we put 15 female and 5 male flies in the assay bottle and 24 h later counted the number of eggs on each piece of apple (Extended Data Fig. 6a). In an initial test, we found that flies laid many more eggs on an apple piece painted with a yeast suspension instead of water, consistent with yeast being a food rich in nutrients and the olfactory cues that attract flies[35–38] (Extended Data Fig. 6b).

Wild-type flies that had been deprived of protein overnight deposited 5–6-fold more eggs on an apple piece coated with the 20 proteogenic amino acids instead of water (Extended Data Fig. 6c,d,f). Flies had a similar, albeit smaller (threefold), preference for leucine-coated apples, and this preference was more profound when the flies had been starved for protein. Importantly, flies did not distinguish between apple pieces painted with the same substance (Extended Data Fig. 6d,f).

We found that $Sesn^{L431E}$ mutant flies lacked a strong preference for laying eggs on the apple coated with leucine and had a reduced preference for the apple with the 20 amino acids (Extended Data Fig. 6e),

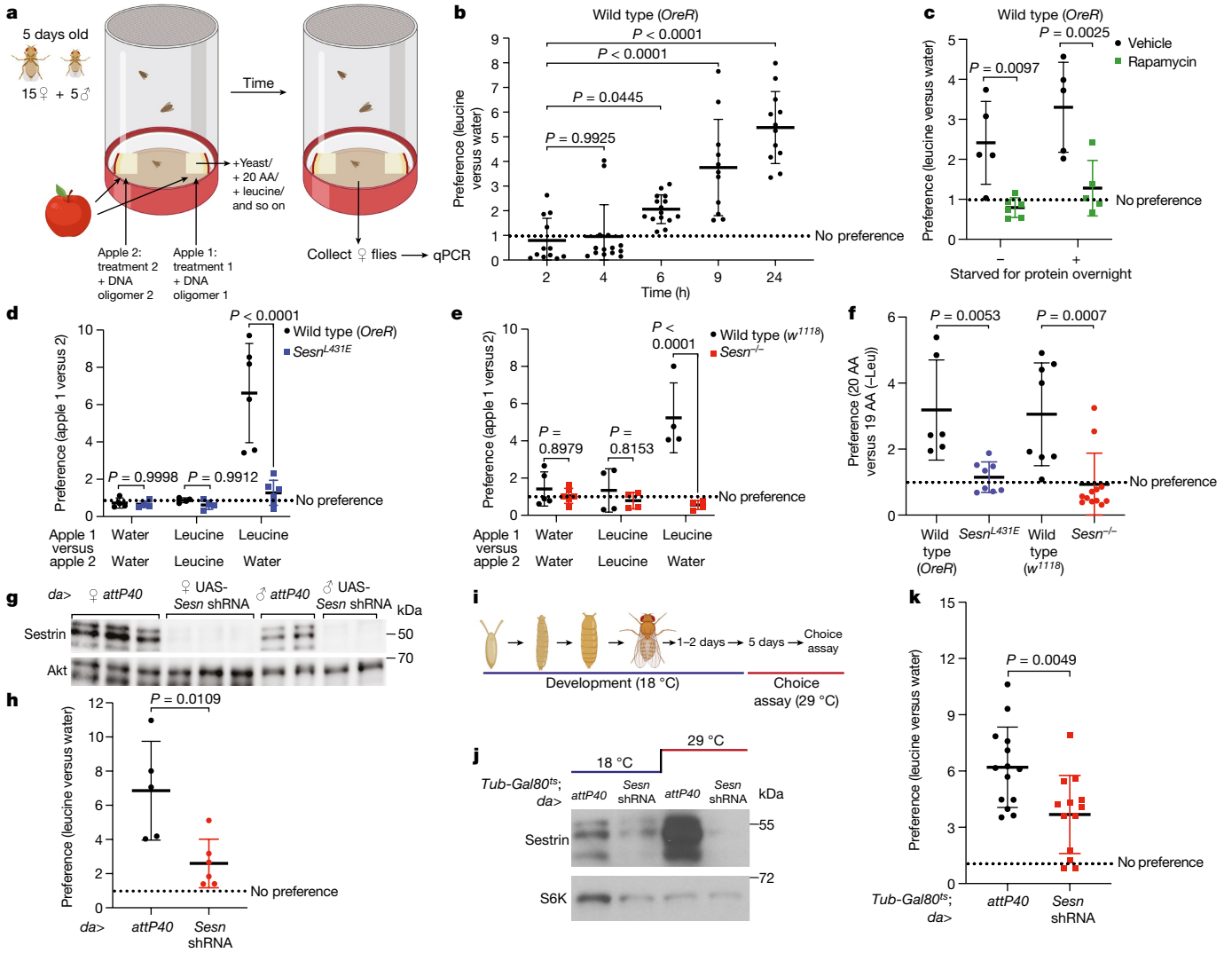

Fig. 3 | Flies prefer to eat leucine-containing food in a fashion that depends on the capacity of Sestrin to bind leucine. a, A schematic of the two-choice food preference assay (see Methods for details). AA, amino acids. **b**, Wild-type female animals develop a preference for leucine over the course of several hours. The data show the fold difference in relative food intake for the leucine-coated compared to water-coated apples. $n \geq 11$ per time point. **c**, Rapamycin prevents flies from developing a preference for the leucine-coated apple. $n \geq 5$ per condition. **d**–**f**, $Sesn^{L431E}$ and $Sesn^{-/-}$ animals fail to develop a preference for the leucine-containing apple. In **d**,**e**, $n \geq 4$ per condition; in **f**, $n \geq 6$ per condition. **g**, Immunoblotting for Sestrin following knockdown of *Sesn* in adult flies. Akt serves as a loading control. **h**, Ubiquitous knockdown of *Sesn* reduces the preference of adult female flies for leucine. The data show the fold difference in food intake for the leucine-coated apple relative to the water-coated apple. $n \geq 5$ per condition. **i**, The approach used to achieve temporal control of *Sesn* knockdown in **j**,**k**. **j**, Sesn immunoblot showing $Gal80^{ts}$-mediated depletion of Sestrin in adult, but not developing, animals. Extracts were prepared from flies raised at the indicated temperatures. S6K serves as a loading control. Note that heat shock induces Sestrin protein levels in control flies. **k**, Knockdown of *Sesn* during adulthood is sufficient to decrease the preference of female flies for leucine-containing apples. $n \geq 13$ per condition. **a**,**i**, Created with BioRender.com. In **b**–**f**,**h**,**k** the values are mean ± s.d. of biological replicates from a representative experiment. Each experiment was repeated three (**d**–**k**) or two (**b**,**c**) times with similar results. Statistical analyses were carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test (**b**), two-way ANOVA followed by Šídák's multiple comparisons test (**c**–**e**), one-way ANOVA followed by Šídák's multiple comparisons test (**f**) and two-tailed unpaired *t*-test (**h**,**k**).

although the total number of eggs $Sesn^{L431E}$ mutant flies laid was about 25% reduced compared to that for the wild-type flies (Extended Data Fig. 3f). This altered egg-laying behaviour was also observed in the $Sesn^{-/-}$ flies, which laid a similar number of eggs to the wild-type animals (Extended Data Fig. 6g). Furthermore, the wild-type flies mildly preferred to deposit eggs on an apple piece painted with the 20 proteogenic amino acids instead of 19 (that is, lacking leucine), a much more complex choice, and this ability was reduced in the $Sesn^{L431E}$ flies (Extended Data Fig. 6h). When facing the same complex choice, $Sesn^{-/-}$ flies did not show a statistically significant different behaviour compared to the wild-type flies (Extended Data Fig. 6h), which might reflect the subtleness and noise of this complex choice set-up. Consistent with the leucine preference we observed in the food choice assay, we found that female flies also laid fewer eggs on food lacking leucine, and this capacity requires the intact leucine-binding pocket of Sestrin. This finding might reflect an active choice for egg deposition or the amount of time that flies spend on each apple owing to their preference for eating leucine-containing food.

Glial Sestrin regulates leucine preference

To determine in which tissue(s) Sestrin is required for flies to distinguish between food with or without leucine, we suppressed Sestrin with the *Sesn* shRNA under the control of a variety of cell-type-specific

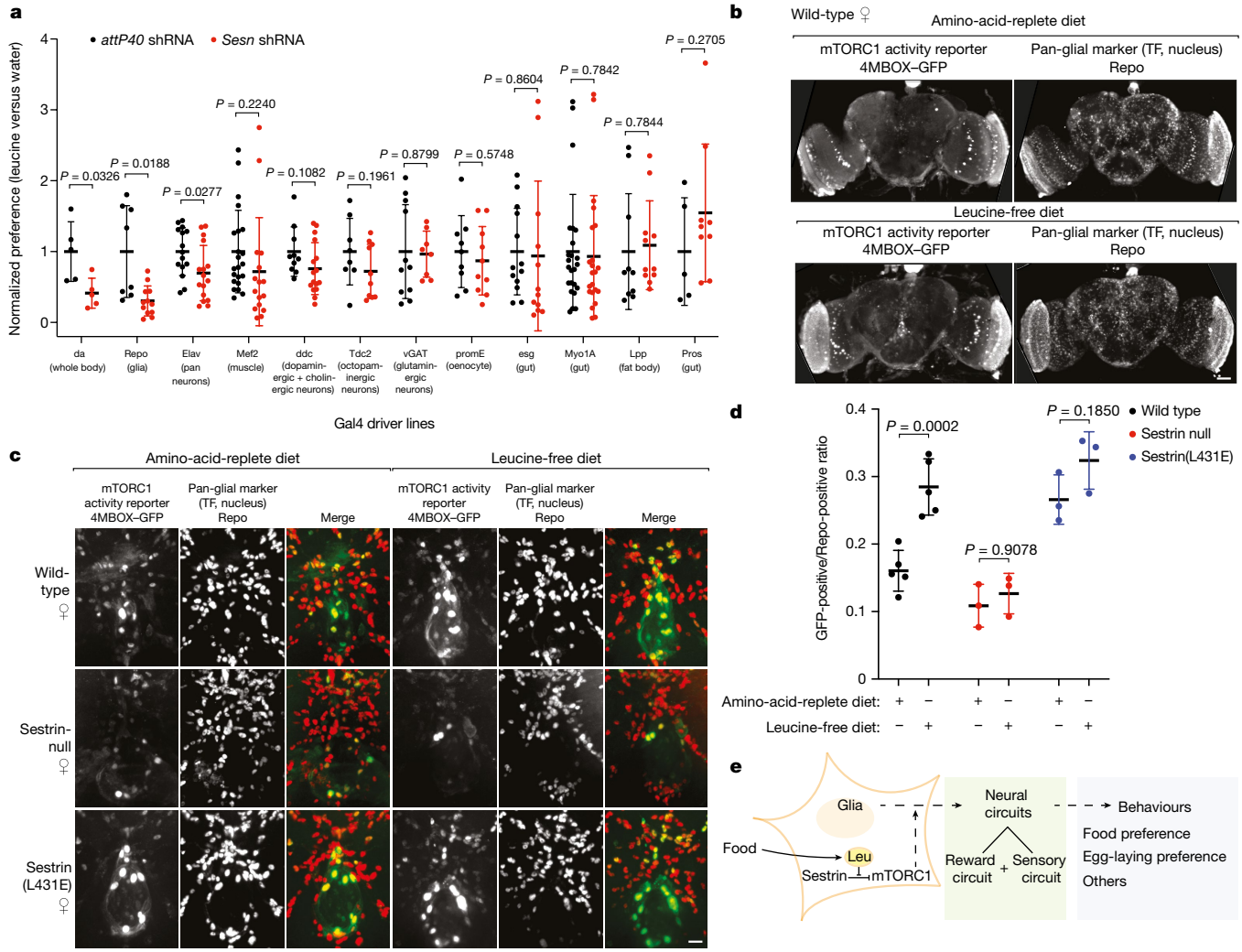

Fig. 4 | Sestrin-regulated mTORC1 signalling in glial cells controls the preference of flies for leucine-containing food. a, A genetic screen identifies a role for Sesn in glial cells in mediating the leucine preference. *Sesn* RNA-mediated interference was performed in various tissues with the indicated Gal4 lines. Knockdown of *Sesn* in glial cells (*Repo*-Gal4) and ubiquitously (*da*-Gal4), but not in other tissues, reduces the preference for the leucine-coated versus water-coated apple. For each Gal4 line, the data are normalized to the leucine preference of control flies. See non-normalized data in Extended Data Fig. 7a. $n \geq 5$ per condition. **b**,**c**, Confocal projection of brains of adult female flies of the indicated genotypes expressing 4MBOX–GFP, a reporter for the MITF transcription factor (TF) that is negatively regulated by mTORC1. Animals were fed the indicated diets for 1 day and brains were stained for GFP and Repo. The images in **b**,**c** were taken with 10× and 40× objectives, respectively. Scale bars, 50 µm (**b**) and 10 µm (**c**). **d**, In wild-type flies, but not $Sesn^{L431E}$ or $Sesn^{-/-}$ flies, leucine starvation increases the number of GFP-positive peri-oesophageal glial cells. Each point represents the ratio of the number of GFP- to Repo-positive cells in the oesophageal area of one fly brain. $n \geq 3$ per condition. **e**, Proposed role of the Sestrin–mTORC1 pathway in regulating the preference of flies for leucine-containing food. In **a**,**d**, the values are mean ± s.d. of biological replicates from a representative experiment. The data are representative of three independent experiments with similar results. Statistical analysis was performed using two-tailed unpaired *t*-test (**a**), and two-way ANOVA followed by Šídák's multiple comparisons test (**d**).

Gal4 drivers. Notably, *Sesn* knockdown specifically in glial cells (*repo*-Gal4) was sufficient to reduce the preference of flies for the leucine-containing food to a similar extent as when it was expressed ubiquitously (*da*-Gal4; Fig. 4a). In contrast, *Sesn* knockdown in many other tissues, including the fat body and muscle, did not impact the leucine preference. It is important to note that the intrinsic capacity of each Gal4 driver line to distinguish between food with or without leucine varied considerably (Extended Data Fig. 7a), probably owing to their different genetic backgrounds. Thus, although we are confident that the preference of flies for leucine-containing food requires Sestrin in glial cells, we are cautious in ruling out contributions from other tissues, particularly those examined with driver lines with intrinsically lower leucine preferences, such as the pan (*elav*-Gal4) and dopaminergic and cholinergic (*ddc*-Gal4) neuronal lines (Extended Data Fig. 7a).

Consistent with an important role for glial Sestrin in regulating the leucine preference, expression of wild-type Sestrin just in glial cells in Sestrin-null flies partially rescued the defect in detecting leucine-poor food (Extended Data Fig. 7b). In wild-type flies, expression in the glial cells of either wild-type Sestrin or Sestrin(L431E) decreased the leucine preference, consistent with the inhibition of mTORC1 caused by Sestrin overexpression (Extended Data Fig. 7b). Indeed, overexpression under the control of *repo*-Gal4 of TSC1 and TSC2—well-established inhibitors of mTORC1 signalling—was also sufficient to decrease the leucine preference (Extended Data Fig. 7c).

Analyses of a single-cell RNA-sequencing dataset indicated that Sestrin is expressed in most glial subtypes[39] (Extended Data Fig. 7d). Expression of the *Sesn* shRNA under the control of Gal4 driver lines that target subtypes of glial cells revealed that none caused as strong a suppression of the leucine preference as with the pan-glial driver

repo-Gal4 (Extended Data Fig. 7e), although *Wrapper*-Gal4-driven *Sesn* knockdown led to a partial reduction of the leucine preference. Thus, multiple glial subtypes probably participate in mediating the leucine preference.

Given the importance of glial Sestrin in mediating the leucine preference, we examined mTORC1 signalling in glial cells in the brains of adult female flies. To do so, we used a line expressing a GFP-based reporter for the MITF transcription factor[40], which is the *Drosophila* orthologue of mammalian TFEB (ref. [41]). mTORC1 suppresses MITF so that after mTORC1 inhibition, MITF activity increases[41] and drives GFP expression. In wild-type flies, starvation for total protein activated, as indicated by elevated GFP expression, MITF in Repo-positive glial cells, particularly in those surrounding the oesophagus (Extended Data Fig. 7f). Remarkably, starvation for just leucine also increased the number of peri-oesophageal GFP-positive glial cells (Fig. 4b–d and Extended Data Fig. 8a,b). In contrast, in $Sesn^{-/-}$ flies, leucine starvation did not increase the number of peri-oesophageal GFP-positive glial cells, which were few in number irrespective of the diet (Fig. 4c,d and Extended Data Fig. 8a,b). In $Sesn^{L431E}$ flies, there were many peri-oesophageal GFP-positive glial cells, and, like in $Sesn^{-/-}$ flies, leucine starvation did not increase their numbers (Fig. 4c,d and Extended Data Fig. 8a,b). Notably, quantification of GFP-positive cells in the mushroom body and optic lobe areas showed that, unlike in peri-oesophageal glial cells, the mTORC1 activity in these cells did not significantly respond to acute dietary treatments (Extended Data Fig. 8b–e). Thus, dietary leucine regulates mTORC1 signalling in a subset of glial cells in a fashion that depends on Sestrin and its capacity to bind leucine, and this regulation correlates with the ability of flies to distinguish between food that is rich or poor in leucine.

Discussion

We show that *D. melanogaster* requires Sestrin to regulate mTORC1 signalling in response to dietary leucine, survive a leucine-poor diet, and control leucine-sensitive physiological measures such as food choice and ovarian size. Flies with a point mutation that eliminates the leucine-binding capacity of Sestrin(L431E) have suppressed, leucine-insensitive mTORC1 signalling. Moreover, whereas wild-type flies can live on leucine-free diets for weeks, flies lacking Sestrin die much faster. In all, our results establish Sestrin as a physiologically relevant leucine sensor in vivo. Recently, Lu et al. reported complementary findings of an amino acid-sensing role of Sestrin upstream of mTORC1 in the control of *Drosophila* development, fecundity and longevity[42].

We find that Sestrin and its leucine-binding pocket are required for the preference of adult female flies for consuming, as well as laying eggs on, leucine-rich instead of leucine-poor food even when it contains sugars and other amino acids. To our knowledge, the ability of flies to choose food that is rich in leucine over food that lacks leucine but still retains a complex set of other nutrients has not been previously documented, although such behaviour has been reported in mice[43]. When given a starker choice than we provided—a pure sugar, such as sucrose or glucose, versus an individual amino acid—flies prefer to eat a variety of essential amino acids in sex- and developmental stage-dependent fashions[44–46].

There has been a long-standing interest in understanding the mechanisms that enable animals, including flies and rodents[43,47], to prefer diets rich in protein. A variety of mechanisms in flies have been implicated, including amino acid transporters[44], taste receptors[45,48,49], GCN2 (ref. [50]), serotonin[51] and dopamine signalling[50,52], sex peptide receptor[53], microbiome[54], and mTOR and S6K (refs. [51,53]). How these mechanisms coordinate together to impact organismal protein detection in the diet remains unclear.

Our work raises several questions for future study. One such question concerns whether there is crosstalk between the food preference behaviour controlled by glial cells and acute changes in ovarian size caused by nutritional stress. Another question is whether female flies actively choose to lay more eggs on the leucine-containing food because it has the nutrients needed for larval growth, or whether the apparent preference simply reflects the amount of time they spend on it owing to their dietary preference. As it takes flies many hours to distinguish between leucine-containing and leucine-free food (Fig. 3b), it seems unlikely that the alterations in Sestrin eliminate the preference for leucine by substantially interfering with the capacity of flies to taste leucine. Rather, we favour the idea that leucine, through Sestrin–mTORC1, turns on a neuronal reward circuit that drives food consumption (see potential model in Fig. 4e). Previous work has identified a set of dopaminergic neurons that controls protein hunger[52], and it will be interesting to examine whether Sestrin-mediated leucine-sensitive mTORC1 signalling can impact these cells. In this regard, it is intriguing that the preference for leucine requires the expression of Sestrin in glia as there is increasing evidence that glial cells can be key intermediates between an environmental signal and its modulation of a neuronal circuit[55–57]. Last, it will be interesting to investigate why mTORC1 activity in a set of peri-oesophageal glial cells is particularly sensitive to Sestrin-dependent regulation by dietary leucine.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-022-04960-2.

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Methods

Materials

Reagents were obtained from the following sources: HRP-labelled anti-rabbit secondary antibody and the antibodies against *Drosophila* phospho-70 S6 kinase (Thr398) (number 9209), phospho-ERK (number 9101), ERK (number 4695), Akt (number 9272), MYC (number 2278) and the Flag (number 2368) epitope from Cell Signaling Technology; anti-GFP antibody from Aves Labs (GFP-1020); 8D12 anti-Repo antibody from Developmental Studies Hybridoma Bank; Alexa 488-, 568- and 647-conjugated secondary antibodies and Complete Protease Cocktail from Roche; Schneider's medium and inactivated fetal bovine serum from Invitrogen; amino-acid-free Schneider's medium from US Biologicals; [^{3}H]leucine from American Radiolabeled Chemicals; leucine from Sigma (L8912); rapamycin from LC Laboratories (number R-5000); and Reliance One-Step Multiplex RT-qPCR Supermix from Bio-Rad. Fresh apples (Gala) were from Star Market. The dS6K antibody was a gift from Mary Stewart (North Dakota State University) and the *Drosophila* Sestrin antibody was a gift from Jun Hee Lee (University of Michigan).

Methods

Tissue culture. Drosophila S2R+ cells were cultured in Schneider's medium with 10% inactivated fetal bovine serum at 25 °C and 5% CO_2. The S2R+ cell line was obtained from the Drosophila RNAi Screening Center/Transgenic RNAi Project Functional Genomics Resources and Drosophila Research & Screening Center-Biomedical Technology Research Resource at Harvard Medical School. It has been molecularly validated by DNA and RNA sequencing (see Table 2 of a recent authentication[58]).

Suspension FreeStyle 293F cells were obtained from Thermo Fisher and cultured in FreeStyle 293 expression medium (Thermo Fisher (12338018)), supplemented with 100 IU ml^{-1} penicillin and 100 μg ml^{-1} streptomycin, at a shaking speed of 125 r.p.m. at 37 °C and 8% CO_2, 80% humidity. No mycoplasma contamination was detected using PCR.

Lysis of cells, tissues and flies, and immunoprecipitations. Cells were rinsed with cold phosphate-buffered saline (PBS) and lysed in lysis buffer (1% Triton, 10 mM β-glycerol phosphate, 10 mM pyrophosphate, 40 mM HEPES pH 7.4, 2.5 mM $MgCl_2$ and 1 tablet of EDTA-free protease inhibitor (Roche) (per 25 ml buffer)). Cell lysates were cleared by centrifugation in a microcentrifuge (15,000 r.p.m. for 10 min at 4 °C). Cell lysate samples were prepared by the addition of 5× sample buffer (0.242 M Tris, 10% SDS, 25% glycerol, 0.5 M dithiothreitol and bromophenol blue), resolved by 8–12% SDS–polyacrylamide gel electrophoresis (PAGE), and analysed by immunoblotting.

Dissected tissues and whole flies were crushed physically using a bead beater in 1% Triton lysis buffer (same as above). The resulting lysates were cleared by centrifugation in a microcentrifuge (15,000 r.p.m. for 10 min at 4 °C) and analysed as above. For anti-Flag immunoprecipitations, the anti-Flag M2 affinity gel (Sigma number A2220) was washed with lysis buffer three times and then resuspended to a ratio of 50:50 affinity gel to lysis buffer. A 25 μl volume of a well-mixed slurry was added to cleared lysates and incubated at 4 °C in a shaker for 90–120 min. For anti-MYC immunoprecipitations, magnetic anti-MYC beads (Pierce) were washed three times with lysis buffer. A 30 μl volume of resuspended beads in lysis buffer was added to cleared lysates and incubated at 4 °C in a shaker for 90–120 min. Immunoprecipitates were washed three times; once with lysis buffer and twice with lysis buffer with 500 mM NaCl. Immunoprecipitated proteins were denatured by addition of 50 μl of SDS-containing sample buffer (0.121 M Tris, 5% SDS, 12.5% glycerol, 0.25 M dithiothreitol and bromophenol blue) and heated in boiling water for 5 min. Denatured samples were resolved by 8–12% SDS–PAGE, and analysed by immunoblotting.

Leucine-binding assay and K_d calculation. For radiolabelled leucine-binding assays using Flag-tagged *Drosophila* Sestrin, suspension HEK293F cells were seeded at 2.5 million cells ml^{-1}, and transfected with the pRK5-Flag-Sestrin cDNA using polyethylenimine. At 72 h after transfection, cells were rinsed once in cold PBS and lysed in 1% Triton lysis buffer (1% Triton, 40 mM Hepes pH 7.4, 2.5 mM $MgCl_2$ and 1 tablet of EDTA-free protease inhibitor (Roche) per 25 ml buffer). Following an anti-Flag immunoprecipitation, the beads were washed four times with lysis buffer containing 500 mM NaCl and then incubated for 1 h on ice in cytosolic buffer (0.1% Triton, 40 mM HEPES pH 7.4, 10 mM NaCl, 150 mM KCl, 2.5 mM $MgCl_2$) with the indicated amount of [^{3}H] leucine and unlabelled leucine. After 1 h, the beads were aspirated dry and rapidly washed four times with binding wash buffer (0.1% Triton, 40 mM HEPES pH 7.4, 300 mM NaCl, 2.5 mM $MgCl_2$). The beads were aspirated dry again and resuspended in 80 μl of cytosolic buffer. Each sample was mixed well, and then 15 μl aliquots were separately quantified using a TriCarb scintillation counter (Perkin Elmer). This process was repeated in pairs for each sample, to ensure similar incubation and wash times for all samples analysed across different experiments.

The affinity for leucine of *Drosophila* Flag–Sestrin was determined by first normalizing the bound [^{3}H]-labelled leucine concentrations across three separate binding assays performed with varying amounts of cold leucine. These values were plotted and fitted to a hyperbolic equation (Cheng–Prusoff equation) to estimate the half-maximum inhibitory concentration (IC_{50}) value. The K_d value was derived from the IC_{50} value using the equation:

$$K_d \text{ or } K_i = IC_{50}/(1 + ([^3\text{H}]\text{leucine})/K_d).$$

In vitro GATOR2–Sestrin dissociation assay. *Drosophila* S2 cells stably expressing Flag-tagged *Drosophila* WDR59 were leucine-starved for 1 h or kept in full medium were lysed and subjected to anti-Flag immunoprecipitations as described above. The GATOR2–Sestrin complexes immobilized on the Flag beads were washed twice in lysis buffer with 250 mM NaCl, and then incubated for 25 min in 0.3 ml of cytosolic buffer (0.1% Triton, 40 mM HEPES pH 7.4, 10 mM NaCl, 150 mM KCl, 2.5 mM $MgCl_2$) with the indicated concentrations of leucine or other amino acids in the cold. The beads were then washed three times in the cytosolic buffer. The Flag-tagged WDR59 and the amount of Sestrin that remained bound to the beads were assayed by SDS–PAGE and immunoblotting.

Liquid chromatography–mass spectrometry-based metabolomics and quantification of metabolite abundances. Liquid chromatography–mass spectrometry (LC–MS)-based metabolomics was performed and data were analysed as previously described[59,60] using 500 nM isotope-labelled internal standards. Briefly, an 80% methanol extraction buffer with 500 nM isotope-labelled internal standards was used for whole-fly metabolite extraction. Samples were dried by vacuum centrifugation, and stored at −80 °C until analysed. On the day of analysis, samples were resuspended in 100 μl of LC–MS-grade water, and insoluble material was cleared by centrifugation at 15,000 r.p.m. The supernatant was then analysed as previously described by LC–MS (refs. [59,60]).

Fly stocks and maintenance. All flies were reared at 25 °C and 60% humidity with a 12 h on/off light cycle on standard laboratory food (12.7 g l^{-1} deactivated yeast, 7.3 g l^{-1} soy flour, 53.5 g l^{-1} cornmeal, 0.4% agar, 4.2 g l^{-1} malt, 5.6% corn syrup, 0.3% propionic acid, 1% Tegosept in ethanol). The following stocks were used: *nprl^{l}* (ref. [21]), *Mio^{1}* (ref. [26]), *Sesn^{8A11}* (ref. [16]), *Lpp-gal4* (gift from S. Eaton and P. Léopold); *promE-Gal4* (ref. [61]), *yw,hs-Flp; mCherry–Atg8a; Act>CD2>GAL4, UAS–nlsGFP/TM6B* (gift from Eric Baehrecke), *hs-Flp; act>CD2>Gal4, UAS-nlsGFP* (ref. [62]), and *w; UAS-sfGFP^{MODC}-3xMyc* (ref. [63]). *Elav-Gal4* (number 458), *Repo-Gal4* (number 7415), *Mef2-Gal4* (number 27390), *ddc-gal4* (number 7010), *Tdc2-Gal4* (number 9313), *vGAT-Gal4* (number 58980), *attP40*

(number 36304), *attP2* (number 36303) and *Sesn* RNAi (number 64027) were obtained from the Bloomington Drosophila Stock Center. *da*-Gal4, esg-*Gal4*, *Myo1A-Gal4*, *Pros-Gal4* were constructed (stocks from the laboratory of N.P.).

UAS-Sesn, *UAS-Sesn*^{L431E}, *UAS-Myc-WDR24* were constructed using the Gateway system. cDNAs were cloned into entry plasmids used for the LR clonase reaction (Invitrogen, 11791-020), with the destination vector pWALIUM10-roe (ref. [64]) or equivalent (Frederik Wirtz-Peitz, unpublished data). The plasmids were then microinjected into embryos for ϕ31-mediated recombination at *attP2* or *attP40* landing sites, as per standard procedures to create transgenic flies. *attP40*, *attP2*, *w*^{1118} and *OreR* were used as controls.

For Fig. 2c, the larvae of genotype *w*; *Sesn*$^{-/-}$; *tubulin-Gal4*, *tubulin-Gal80*^{ts}/*UAS-Sesn* were raised at a mildly permissive temperature (25 °C) to express relatively physiological levels of *UAS-Sesn* in Sestrin-null larvae.

Sesn^{L431E} knock-in flies were generated with CRISPR–Cas9 technology to achieve dinucleotide replacement at the endogenous locus. A single-stranded oligonucleotide donor was used, containing the codon change (CTG>GAG) flanked by 20-base-pair homology arms (sequence: 5′-ACCAAGGACTACGATAGTGTGGAGGTCGAGCTGCAGGACAGTGA-3′). A single single-guide RNA with a cutting site abutting the nucleotide replacement locus (sequence forward/reverse: 5′-GTCGCAAGGACTAC GATAGTGTGC-3′/5′-AAACGCACACTATCGTAGTCCTTG-3′) was cloned into the pCFD3 expression vector as in a previous report[65]. pCFD3-sgRNA and single-stranded oligonucleotide donor were injected into nos-Cas9 embryos, and emerging adults were crossed to Sco/Cyo. Progenies were screened by sequencing heterozygous animals (5–10 animals per founder cross) (PCR/sequencing primers: forward primer, 5′-CGACGACTACGACTATGGCGAA-3′; reverse primer, 5′-GCATGTGTGGGTATGTGTGTGGT-3′). Individual stocks were established, and backcrossed nine times onto a control *OreR* background (using the same PCR and sequencing primers as above for genotyping).

Synthetic fly food formulation and preparation. *Drosophila* diet formulations were derived from previous recipes[66,67] with the following modifications: the type of agar (Micropropagation Agar-Type II; Caisson Laboratories number A037); the final percentage of Agar (1%); the amount of sucrose (25 g per litre of food); and the amino acids that were added to stock solutions before or after autoclaving[68] whose order is described below. The amino acid composition of the diet including the concentrations of leucine, isoleucine and valine were based on the exome-matched (that is, the concentrations used for a given amino acid correspond with the prevalence of exons for that amino acid in the *Drosophila* genome) and *Drosophila* diet formulation developed in a previous study[67] that was found to be optimal for growth and fecundity without compromising lifespan. The rationale for which amino acids were part of the autoclaving process was based on solubility considerations[68].

The complete procedure, formula and stock solutions for food production are as follows: prepare mixture 1 (Extended Data Tables 1, 3 and 4); stir using stir bar; autoclave mixture 1 for 15 min; prepare mixture 2 (Extended Data Tables 2–4) and set aside; remove mixture 1 from the autoclave, combine it with mixture 2 and stir, making sure to mix well; quickly pipette the food into *Drosophila* vials (5–10 ml food per vial); allow the food to solidify/cool for roughly an hour, and then cover the vials (either with cotton plugs or with plastic wrap) and store food at 4 °C. The food is good for about 3 weeks at 4 °C (it will shrink and pull away from the sides of the vials owing to evaporation). (Note, after autoclaving, mixture 1 containing agar can start solidifying (both before and after the two mixtures are combined, but combining the two mixtures will cause food to cool down and solidify fast). Quickly combine and pour the food while the autoclaved mixture is still hot to avoid this. Adding water to the autoclave tray and keeping mixture 1 in this hot water until ready to combine and pour helps prevent premature solidification.)

The catalogue numbers for the reagents not listed in Extended Data Tables 1–4 are as follows: sucrose (Sigma, S7903), agar (Caisson, A037), propionic acid (Sigma, P5561). Stocks can be stored at 4 °C for several months unless otherwise specified.

Generation of clones expressing the *Sesn* shRNA. Clones were generated by crossing yw,hs-flp; mCherry–Atg8a; Act>CD2>GAL4, UAS–nlsGFP/TM6B with the indicated UAS lines. Progeny of the relevant genotype was reared at 25 °C and spontaneous clones were generated in the fat body owing to the leakiness of the heat-shock flipase (hs-flp).

Food preference assay. Determination of relative food consumption from two different food sources using unique DNA oligomers was performed as previously reported[27]. The sequences were as follows: DNA oligomer 1, 5′-ACCTACACGCTGCGCAACCGAGTCAT GCCAATATAAGCAGATTAGCATTACTTTGAGCAACGTATCGGCGATCAG TTCGCCAGCAGTTGTAATGAGCCCC-3′ ; forward quantitative PCR (qPCR) primer 1, 5′-GCAACCGAGTCATGCCAATA-3′; reverse qPCR primer 1, 5′-TTACAACTGCTGGCGAACTG-3′; DNA oligomer 2, 5′-GGGCA GCAGGATAACTCGAATGTCTTAGTGCTAGAGGCTTGGGGCGTGTAAGT GTATCGAAGAAGTTCGTGTTAAACGCTTTGGAATGACTGTAATGTAG-3′; forward qPCR primer 2, 5′-CAGCAGGATAACTCGAATGTCTTA-3′; reverse qPCR primer 2, 5′-CAGTCATTCCAAAGCGTTTAACA-3′; genomic *Cyp1* forward qPCR primer, 5′-ACCAACCACAACGGCACTG-3′; genomic *Cyp1* reverse qPCR primer, 5′-TGCTTCAGCTCGAAGT TCTCATC-3′.The DNA oligomers and their corresponding qPCR primers were purchased from Integrated DNA Technologies with 4 nmol per tube and diluted in nuclease-free water to final stocks with a DNA concentration of 3.5 μg μl^{-1}.

For the assay, the surface of fresh Gala apples was sprayed and cleaned using 70% ethanol. Fresh Gala apple pieces (about 1 g) containing both a piece of peel and pulp were cut on a clean field using a knife (both the knife and the field were precleaned by 70% ethanol). Two apple pieces with similar shape and weight were placed in the opposite corners of a 6 oz (177 ml; 57 length × 57 width × 103 height (in mm)) clean *Drosophila* bottle. Solutions of 100 μl in volume that contained one DNA oligomer (final concentration 3.5 ng μl^{-1}) and substances (that is, sterile water, amino acid solutions and so on) were placed evenly on top of the apple pieces and allowed to soak in for 1.5–2 h. Age-synchronized adult flies (15 female and 5 male animals) were flipped into these assay bottles and allowed to feed ad libitum on the apples for the indicated times in the time course experiments (Fig. 3b and Extended Data Fig. 4g) and for 24 h in the other food preference experiments.

CO_2-anaesthetized flies were collected using a tweezer. From each bottle, two tubes of female flies were collected with five flies per tube. Five flies were homogenized for each qPCR sample. Homogenization was performed using a beads beater in the cold after adding 250 μl of squishing buffer (10 mM Tris-HCl pH 8.2, 1 mM EDTA, 1 mM NaCl) and 0.5 μl of 20 mg ml^{-1} proteinase K (Thermo Fisher number AM2546). The whole-fly lysates were digested at 37 °C for 30–40 min after homogenization followed by proteinase K inactivation at 95 °C for 5 min. The samples were centrifuged for 10 min at 15,000 r.p.m. at room temperature and 2 μl of the supernatant was loaded in each qPCR reaction in a 96-well qPCR plate. We used the SYBR green qPCR master mix from Bio-Rad and a CFX96 Touch Real-Time PCR Detection System with a melting temperature of 60 °C and 40 cycles per run.

Genomic *Cyp1* qPCR Ct values were used to control for extraction efficiency. For every batch of samples, an average of *Cyp1* qPCR Ct values was taken and all samples beyond ±0.5 Ct away from the average were discarded. Standard curves for DNA oligomers 1 and 2 were generated, and the amount of DNA oligomer from each tube of flies was calculated by fitting their Ct values to the standard curves. The preference index was generated by dividing the calculated amount of DNA oligomer 1 by that of DNA oligomer 2.

To remove external oligomer that may stick to the outside of the flies, we used a four-step protocol described previously[27]: a 10-min wash with 10% Contrex AP Powdered labware detergent (catalogue number 5204, Decon Laboratories); a 5-min wash in double-distilled H_2O; a 2-min wash in 30% bleach; and a 5-min wash in double-distilled H_2O. All washes were performed in a 1,500 µl microfuge tube with continuous rocking at room temperature.

For Fig. 3c and Extended Data Fig. 4f, we fed the flies with food containing either 25 µM rapamycin or 25 µM ethanol for 2 days before either protein starvation overnight or not (including 25 µM Rapamycin or 25 µM ethanol). Then for the final choice assay, 25 µM of rapamycin or 25 µM ethanol was added to both apple pieces in the container.

Immunofluorescence assays. Fat bodies from aged larvae (96 h after egg laying) were dissected in PBS at room temperature, fixed for 25–30 min in 4% formaldehyde, washed twice for 10 min in PBS 0.3% Triton (PBST), blocked for 30 min (PBST, 5% BSA, 2% FBS, 0.02% NaN_3), incubated with primary antibodies in the blocking buffer overnight, and washed four times for 15 min. Secondary antibodies diluted 1:500 in PBST were added for 1 h and tissues were washed four times before mounting in Vectashield (Vector Laboratories) containing 4′,6-diamidino-2-phenylindole (DAPI). Brains from 5–10-day-old adult female flies were dissected and processed as in a previous study[69].

Images for Fig. 2c and Extended Data Fig. 3d were acquired on a Zeiss Axio Zoom v16. Images for Fig. 4b,c and Extended Data Figs. 1c, 7f and 8b,c were acquired on a Zeiss AxioVert200M microscope with a 63× or 40× oil-immersion objective or a 10× objective and a Yokogawa CSU-22 spinning-disc confocal head with a Borealis modification (Spectral Applied Research/Andor) and a Hamamatsu ORCA-ER CCD camera. The MetaMorph software package (Molecular Devices) was used to control the hardware and image acquisition. The excitation lasers used to capture the images were 405 nm, 488 nm and 561 nm. Images for Extended Data Fig. 6b,c were acquired on an iPhone XR camera through a binocular microscope.

Egg-laying preference assay. The set-up for the egg-laying preference assay was identical to that for the food preference assay. Instead of collecting female flies for qPCR analyses, the two apple pieces were removed from the bottle and examined under a binocular microscope. The number of eggs on each apple piece was determined.

Ovary size quantification. Ovaries were dissected in PBS and bright-field images were acquired using a Zeiss Axio Zoom v16 scope. The size of the ovaries was quantified using the average area of individual ovaries on ImageJ.

Developmental timing. Three-day-old crosses were used for 3–4-h periods of egg collection on standard laboratory food. Newly hatched L1 larvae were collected 24 h later for synchronized growth using the indicated diets at a density of 30 animals per vial. The time to develop was monitored by counting the number of animals that underwent pupariation, every 2 h in fed conditions, or once/twice a day in starved conditions. The time at which half the animals had undergone pupariation is reported. For larva developmental timing experiments, 10%-leucine chemically defined diet was used because complete leucine starvation quickly caused lethality before any size comparison across genotypes could be efficiently and meaningfully performed.

Lifespan experiments. To generate age-synchronized adult flies, larvae were raised on laboratory food at low density, transferred to fresh food after emerging as adults and allowed to mate for 48 h. Animals were anaesthetized with low levels of CO_2 and sorted at a density of 25 flies per vial. Each condition examined used 8–10 vials of flies. Flies were transferred to fresh vials three times per week at which point deaths were also scored. For adult flies, leucine-free diet or valine-free diet was used.

Statistical analyses. For non-survival experiments, two-tailed unpaired *t*-tests, multiple *t*-tests, one-way or two-way ANOVA analyses followed my post hoc tests were used for comparison between two groups in GraphPad Prism (GraphPad Software v9). All comparisons were two-sided unless specified otherwise. All analysed *P* values are indicated for each comparison made within all figure panels. *P* values of less than 0.05 were considered to indicate statistical significance.

For survival comparisons in Fig. 2a,b, two-proportion *z*-tests were performed. Pupariation percentage (Extended Data Fig. 2a,h) data were compared using permutation tests, in which the test statistic was the difference in mean pupariation times of the two genotypes. The distribution of the test statistic under the null hypothesis was estimated by simulating 100 million rearrangements of the data. Permutation tests were performed in R (script available in Supplementary Data 2). Results for all statistical analyses were summarized in source data files corresponding to each figure.

Analysis of survival data. All data were complete and uncensored. Kaplan–Meier estimates of the survival function were plotted and used to compute median survival times. Log-rank tests were used to compare survival distributions, and univariate Cox proportional hazard analysis (with ties handled by Efron approximation) was used to compute hazard ratios between Sestrin-mutant versus wild-type flies within individual dietary conditions. To examine the interaction between genotype and diet (specifically using the alternative hypothesis that the lifespan defect of Sestrin-mutant versus wild-type flies is exacerbated on a leucine-free compared to a valine-free diet), one-tailed Wald tests were conducted on the interaction coefficients generated by two-factor Cox proportional hazard models with interaction terms (with ties handled by Efron approximation). All statistical analyses on survival data were performed in R (script available in Supplementary Data 3).

Reporting summary

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

Data availability

The data that support the findings of this study are available from the corresponding authors and the Whitehead Institute (sabadmin@wi.mit.edu) upon reasonable request. Source data are provided with this paper.

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Author contributions X.G. and D.M.S. developed the research plan and together with P.J. and N.P. interpreted experimental results. X.G. and P.J. designed and performed all experiments. R.B. helped with ordering and maintaining fly stocks. M.L.V. and P.V.L. helped with experimental design and data analysis. N.K. helped with statistical analysis. M.A.R., A.E.A. and J.W.L. provided the recipe for the chemically defined food and prepared the first batches of it. X.G. and D.M.S. wrote the manuscript and P.J. and N.P. helped edit it.

Competing interests D.M.S. is a shareholder of Navitor Pharmaceuticals, which is targeting for therapeutic benefit the amino-acid-sensing pathway upstream of mTORC1. J.W.L. advises Raphael Pharmaceuticals, Nanocare Technologies, Petri Biologics, and Restoration Foodworks. M.A.R. is currently employed by Amgen, which has interests in neurodegenerative diseases. These relationships have no overlap with this study. The other authors declare no competing interests.

Additional information

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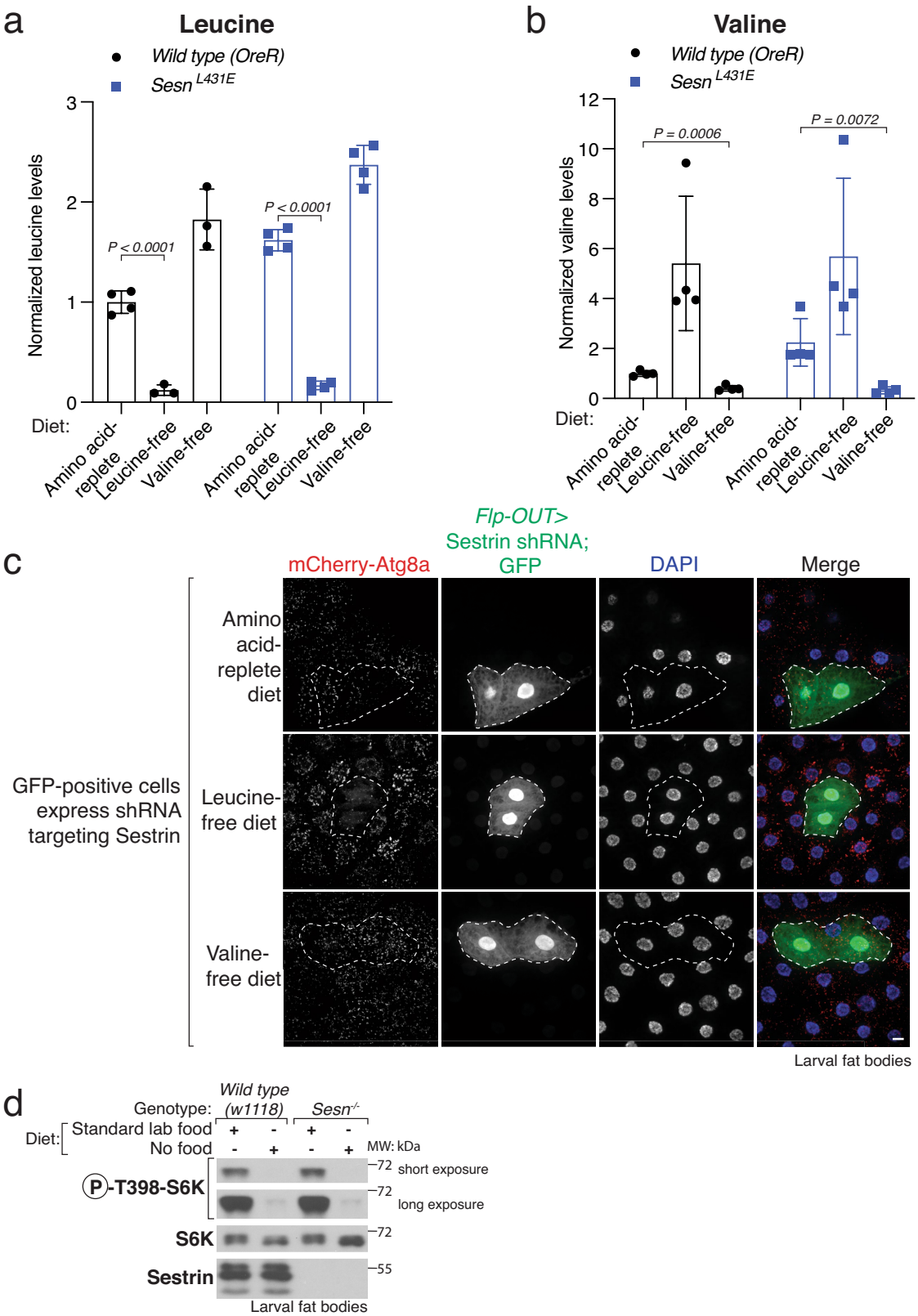
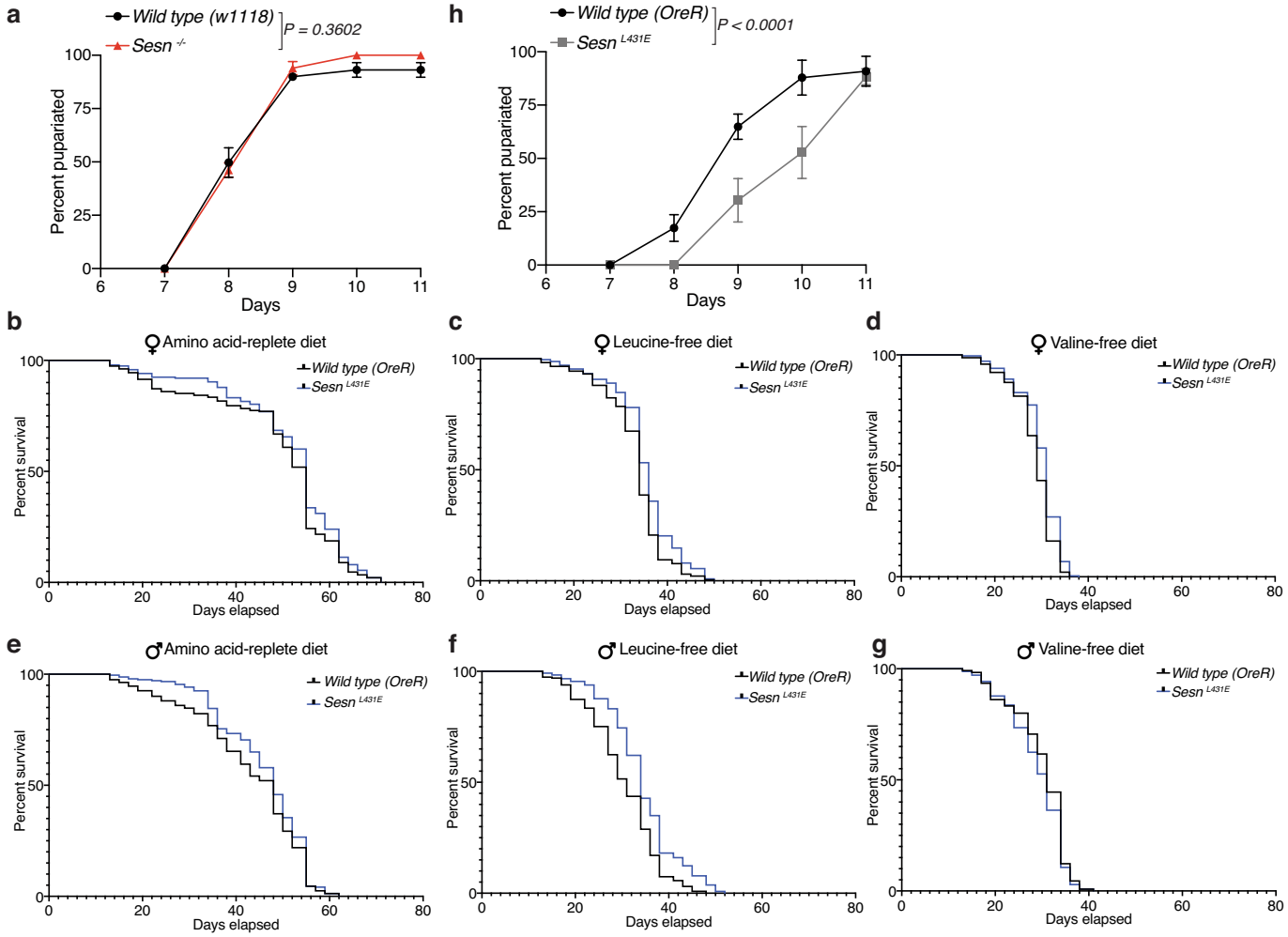
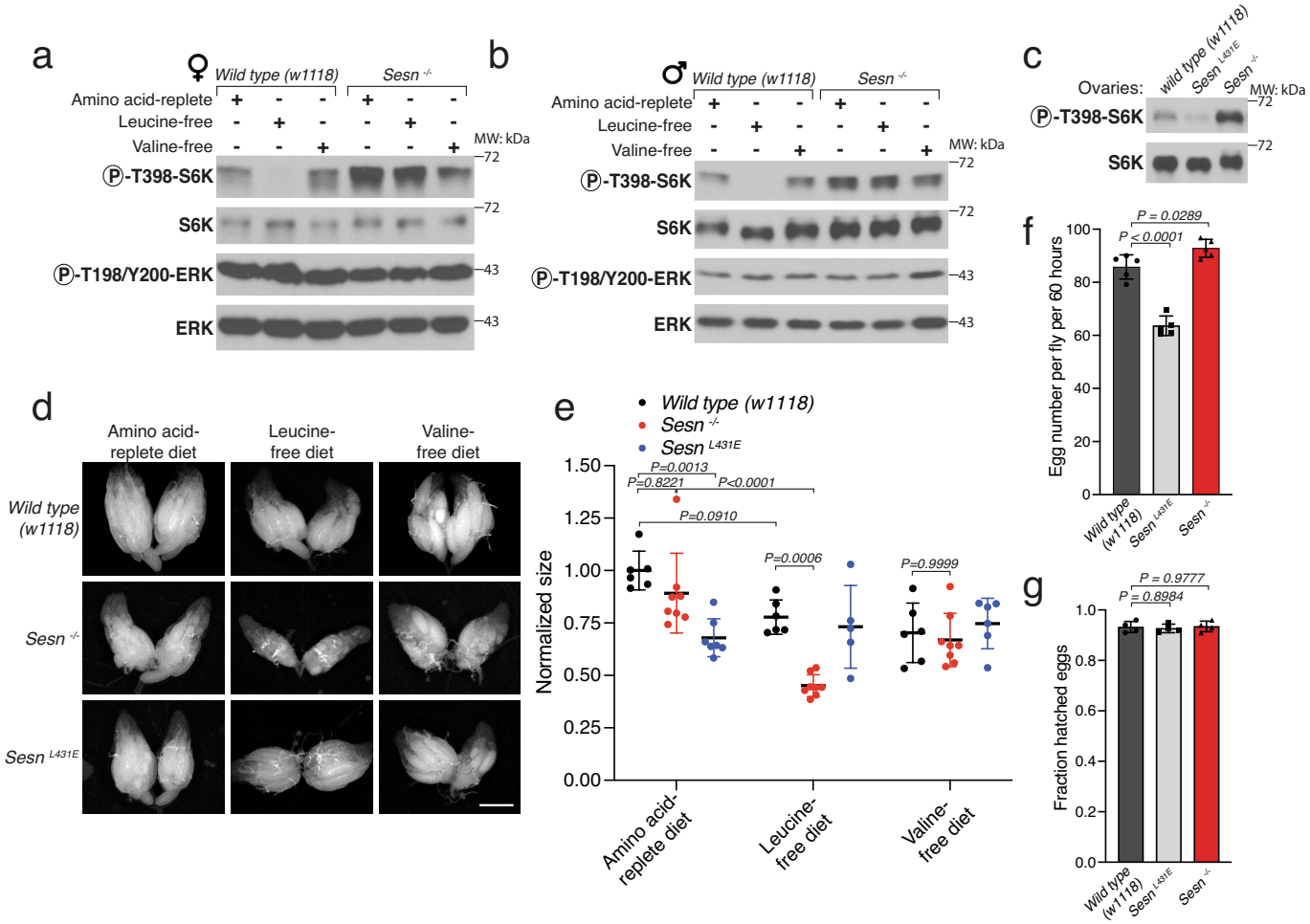


Extended Data Fig. 1 | See next page for caption.

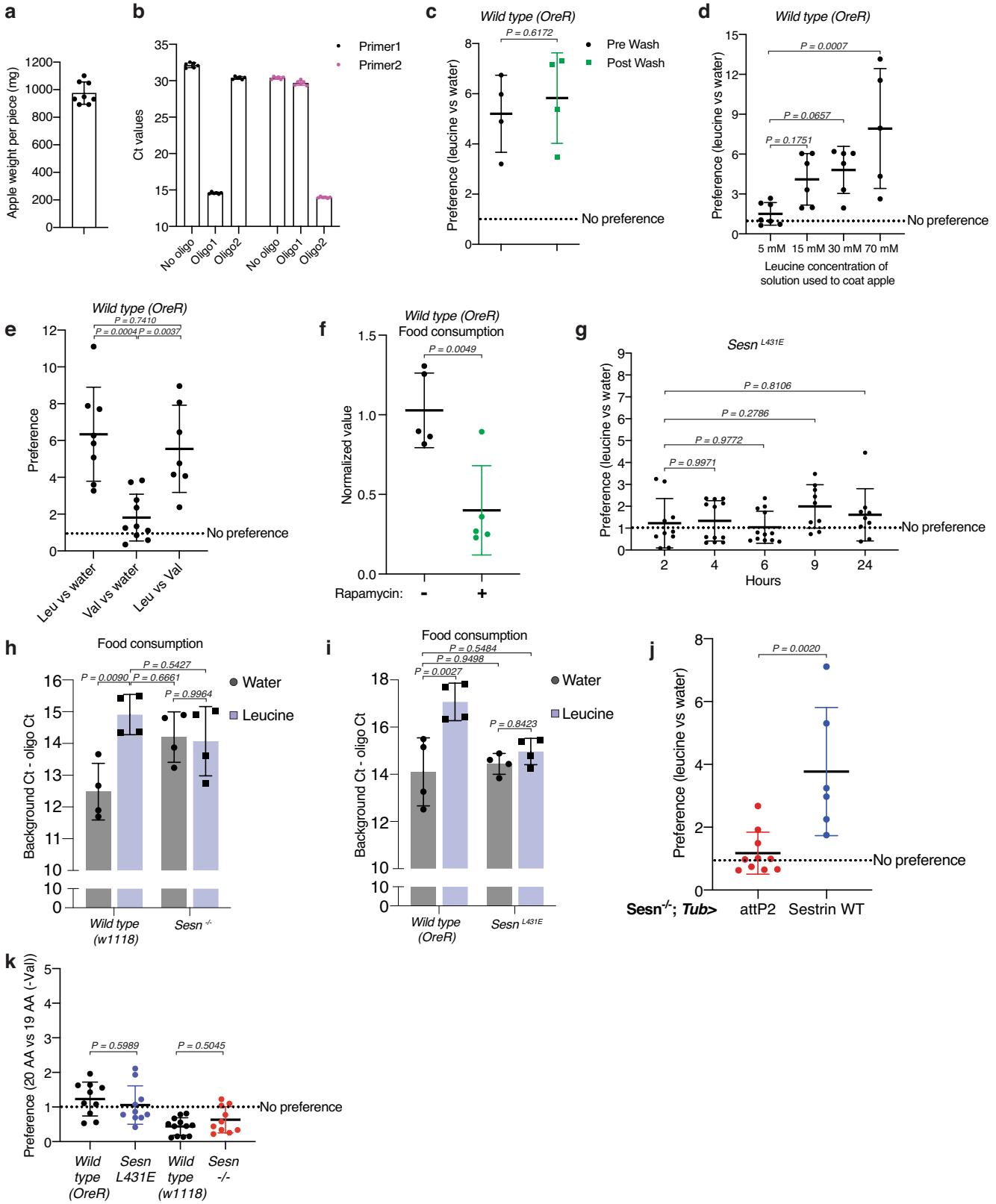
Extended Data Fig. 1 | Validation of chemically-defined diets and loss of Sestrin phenotypes in larval fat bodies. a, b, *Drosophila* larvae eating chemically-defined diets lacking individual amino acids have reduced levels of the missing amino acid. Relative levels of leucine (a) and valine (b) measured by LC-MS/MS in whole larval extracts of *Wild-type (OreR)* or $Sesn^{1431E}$ larvae fed the indicated diet for 4.5 h. Values are mean ± SD of biological replicates from a representative experiment. n = 4 independent biological samples. Two samples from *wild type (OreR)* leucine-free and valine-free, respectively, failed to yield decent peaks for leucine levels, thus discarded. Multiple unpaired t tests, Holm-Šídák multiple comparison method. c, Sesn knockdown prevents autophagy induction upon leucine deprivation. Fat body cells in mid-third instar larvae expressing mCherry-Atg8a were fed the indicated diets for 4.5 h. The *Sesn* RNAi was expressed in clones of cells (GFP, outlined) with a FLP-out system[70]. Scale bar, 10 µm. d, Loss of Sestrin does not affect the inhibition of mTORC1 caused by the deprivation of all food. Immunoblot analyses of phospho-S6K and S6K in adult female flies in the fed state or starved of all food for 1 day.



Extended Data Fig. 2 | The *Sesn*^{L431E} mutation does not affect adult fly lifespan on the chemically defined diets but does mildly delay larvae development, while loss of Sestrin does not affect larvae development. a, Loss of Sestrin does not the affect development of larva feeding on a complete diet. Time to pupariation for w^{1118} *and Sesn*$^{-/-}$ larvae fed the standard yeast-based diet. b-g, Survival curves for animals of the indicated sex and genotypes fed the indicated chemically-defined diets. (a) nWT(*OreR*)=235; n(*Sesn*^{L431E})=238; (b) nWT(*OreR*)=233; n(*Sesn*^{L431E})=237; (c) nWT(*OreR*)=242; n(*Sesn*^{L431E})=248; (d) nWT(*OreR*)=242; n(*Sesn*^{L431E})=240; (e) nWT(*OreR*)=229; n(*Sesn*^{L431E})=243; (f) nWT(*OreR*)=245; n(*Sesn*^{L431E})=245. See statistics in Supplementary Data 1 and methods. h. *Sesn*^{L431E} larvae raised on a standard yeast-based diet are developmentally delayed. Data are representative of three independent experiments with similar results. Statistical analysis was performed using a permutation test on the difference of the mean pupariation times of the two genotypes (a, h).

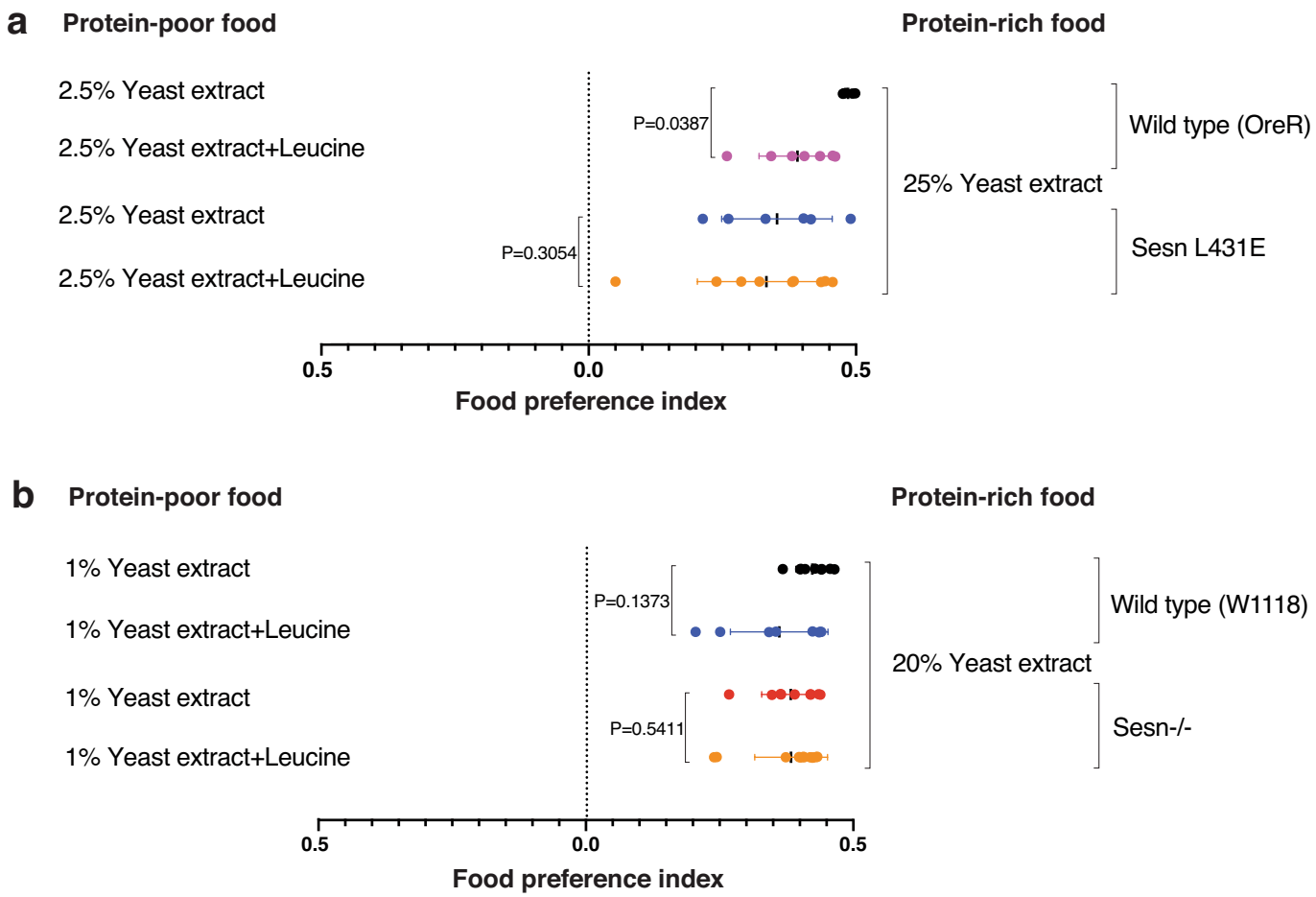


Extended Data Fig. 3 | Sestrin-mediated mTORC1 signaling in ovaries. a, b, Sestrin mediates leucine-sensing by mTORC1 in adult animals. Immunoblot analyses of whole adult animals of the indicated sex and genotype following overnight starvation and 1.5 h of refeeding with the indicated diets. c, In flies feeding a standard diet and lacking Sestrin or expressing the leucine-binding deficient Sestrin mutant (L431E), mTORC1 activity is increased or decreased, respectively. Lysates were prepared from isolated ovaries from animals of the indicated genotypes and fed a standard yeast-based diet. d, e, Loss of Sestrin accelerates the reduction in ovary size caused by leucine starvation. (d) Ovarian size in females of the indicated genotypes fed the indicated diets for 24 h. Results are quantified in (e). Scale bar, 500 µm. f, g, *Sesn*^{L431E} flies have reduced fecundity but not fertility. (f) Number of eggs laid over a period of 60 h by females of the indicated genotypes maintained on the standard yeast-based diet. (g) Hatching rate of eggs laid in the same conditions as in (f). (e, f, g) Values are mean ± SD of biological replicates from a representative experiment. (e) n = 6 (*Wild type* (*w*^{1118})), 8 (*Sesn*$^{-/-}$), 7 (*Sesn*^{L431E} amino acid-replete diet), 5 (*Sesn*^{L431E} leucine-free diet), and 6 (*Sesn*^{L431E} valine-free diet). (f) n = 5. (g) n = 4. Data are representative of three independent experiments with similar results. Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparisons test (e), and one-way ANOVA (f, g) followed by Dunnett's multiple comparisons test.

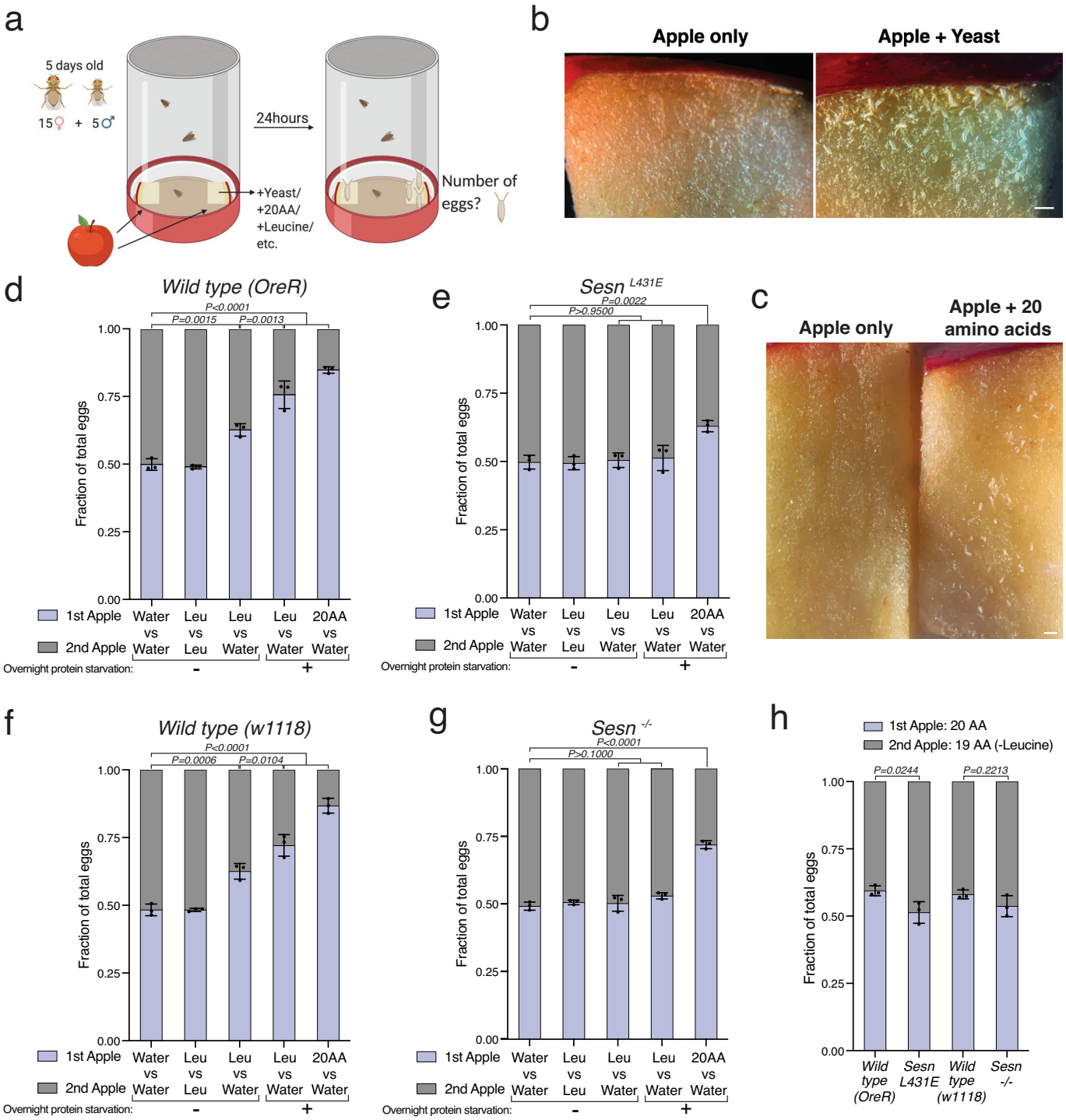


Extended Data Fig. 4 | See next page for caption.

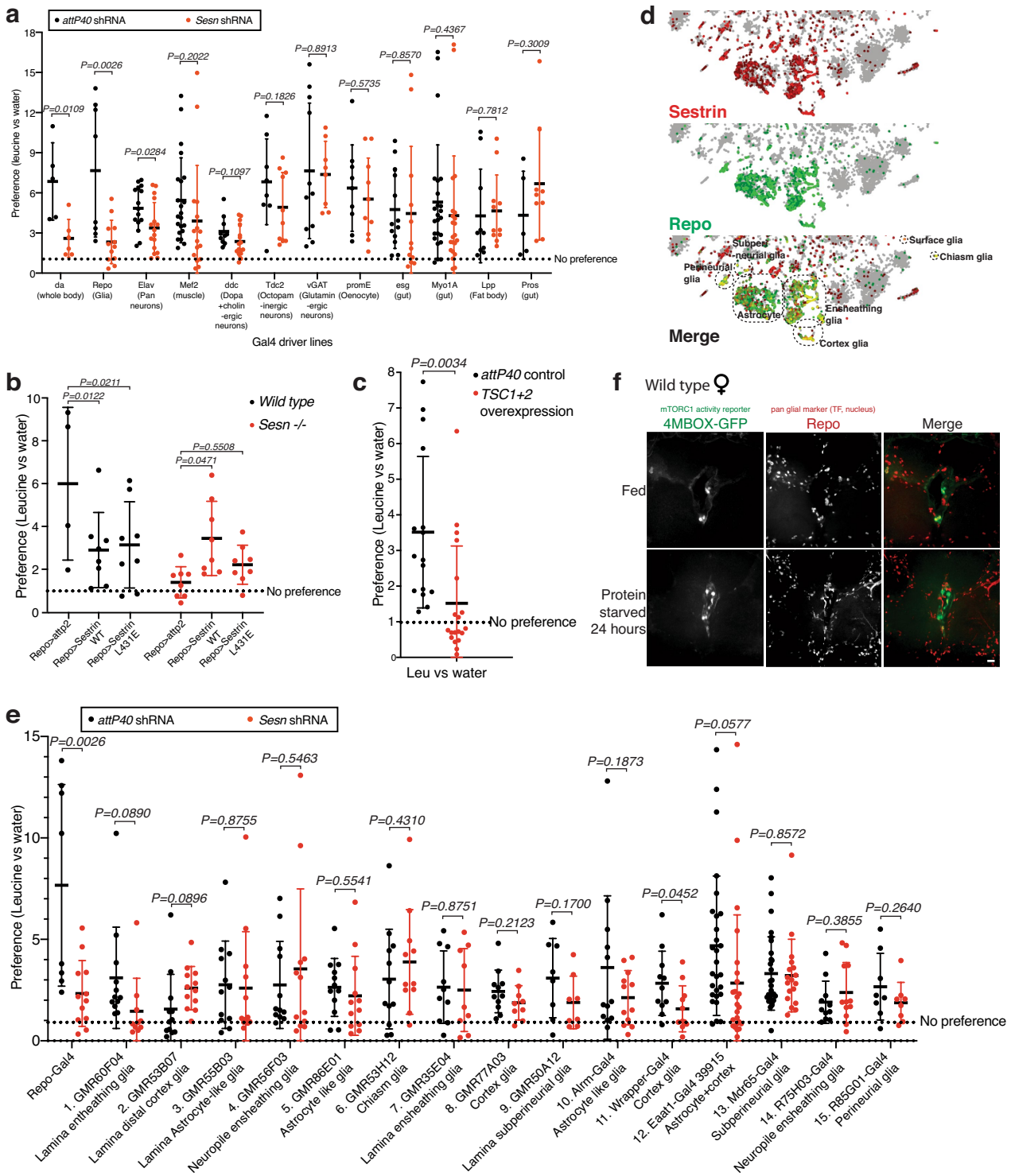
Extended Data Fig. 4 | Sestrin mediates the preference for leucine-containing food and influences total food intake. a-c, Characterization of the methods used in the food two-choice assay. (a) Measurement of the weight of the apple pieces used in the assay. n = 8. (b) Background qPCR signal determination for each oligonucleotide barcode used in assay. n = 6 for each condition. (c) The qPCR signals used to determine the leucine preference of the wild-type flies come primarily from internal DNA oligonucleotides instead of external ones that might contaminate the outside of the body of female flies. qPCR for oligonucleotide barcodes in a leucine versus water choice assay before and after washing animals as previously described[27]. n = 4 for both pre and post wash conditions. d, Preference of the flies for apple pieces painted with the indicated leucine concentrations. Animals were given a choice between leucine- or water-coated apples. Indicated leucine concentrations (5 mM, 15 mM, 30 mM, and 70 mM) were the solution concentrations used to coat apples. The final concentration on the food should be ~10 times more diluted. n (5 mM) = 7, n (15 mM and 30 mM) = 6, n (70 mM) = 5. e, Adult female flies do not have a preference for valine- versus water-painted apple pieces. Wild-type (*OreR*) animals were given indicated food choices and the preference fold-difference was shown. n (leucine vs water) = 8, n (valine vs water) = 10, n (leucine vs valine) = 7. f, Rapamycin treatment reduces fly food consumption. Vehicle or Rapamycin pre-treated animals were given a choice between leucine- or water-coated apples. For the Rapamycin group during the choice assay, animals were fed on apples painted with Rapamycin in addition to either leucine or water. Data show the normalized values of food consumption. n = 5 for both conditions. g, $Sesn^{L431E}$ animals do not have a preference for valine- over water-painted apples. Animals were given a choice between valine- or water-coated apples and food preference was measured at the indicated time points. Data show the fold-difference in relative food intake for the valine-coated apple compared to the water-coated apple. n = 10 (2 hrs), 12 (4 hrs), 12 (6 hrs), 9 (9 hrs), and 9 (24 hrs). h,i, $Sesn^{L431E}$ animals have decreased food intake regardless of the leucine content of the food (h), and $Sesn^{-/-}$ animals have increased food intake regardless of the leucine content of the food (i). n = 4 for all conditions. j, Whole-body re-expression of wild-type Sestrin driven by *Tub*>Gal4 is sufficient to partially restore the preference for leucine-containing food of $Sesn^{-/-}$ adult female flies. Animals with indicated genotypes were given the choice between leucine- or water- coated apples. Data show the preference of fold-difference. n (attP2) = 10, n (Sestrin WT) = 6. k, Adult female flies do not develop a preference for valine-containing apple regardless of their genotype. Animals with indicated genotypes were given the choice between leucine- or water- coated apples. Data show the preference of fold-difference. n (*Wild type OreR*, $Sesn^{L431E}$, $Sesn^{-/-}$) = 10, n (*Wild type* w^{1118}) = 12. Values are mean ± SD of biological replicates from a representative experiment. Data are representative of three independent experiments with similar results. Statistical analysis was performed using two-tailed unpaired t test (c, f, j), one-way ANOVA followed by Dunnett's multiple comparisons test (d, g), one-way ANOVA followed by Tukey's multiple comparisons test (e), two-way ANOVA followed by Tukey's multiple comparisons test (h, i), and one-way ANOVA followed by Šídák's multiple comparisons test (k).



Extended Data Fig. 5 | Leucine-sensing via the Sestrin-mTORC1 axis contributes to the detection of the protein content of food. a, *Wild-type (OreR)* flies prefer food containing a high amount of yeast extract and this preference is reduced by the addition of leucine to food containing a low amount of yeast extract. $Sesn^{L431E}$ flies have a reduced preference for the food containing a high amount of the yeast extract and the addition of leucine has minimal impact on the preference. How the food preference index was calculated is described in the methods. n (*Wild type OreR*, no leucine)=5, n (*Wild type OreR*, with leucine)=7, n ($Sesn^{L431E}$, no leucine)=6, n ($Sesn^{L431E}$, with leucine)= 9. b, As in (a) a choice experiment for *wild type* w^{1118} and $Sesn^{-/-}$ flies. n (*Wild type* w^{1118}, no leucine)=9, n (*Wild type* w^{1118}, no leucine)=8, n ($Sesn^{-/-}$, no leucine)=9, n ($Sesn^{-/-}$, with leucine)=12. Values are mean ± SD of biological replicates from a representative experiment. Data are representative of three independent experiments with similar results. Statistical analysis was performed using two-tailed unpaired t test, Holm-Šídák method.

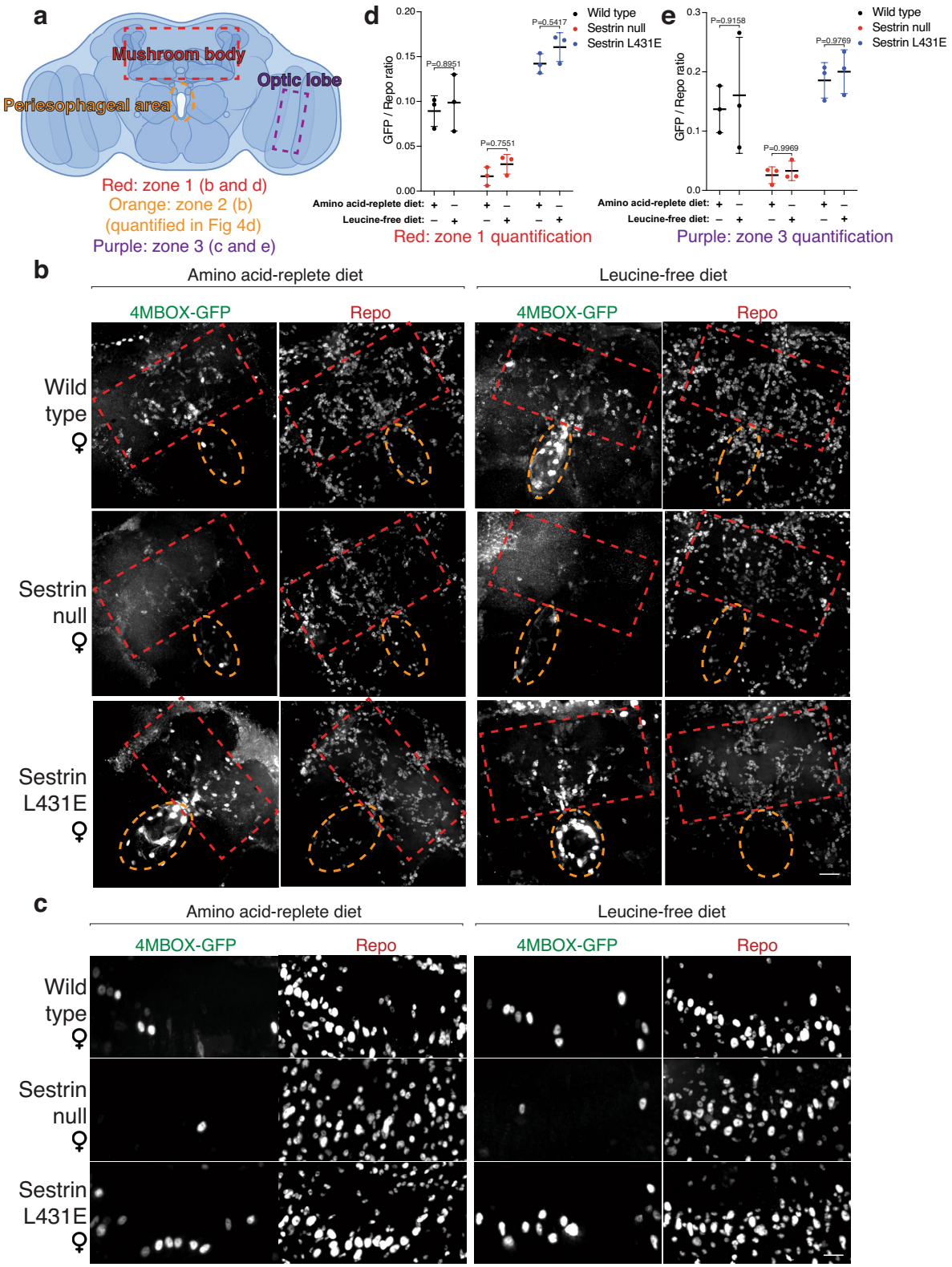


Extended Data Fig. 6 | Flies prefer to lay eggs on leucine-containing food in a fashion that requires the leucine-binding capacity of Sestrin. a, Schematic of the setup used in the egg-laying preference assay. Two identical apple pieces were painted with solutions containing different substances and placed on opposite sides of a container. Animals were allowed to feed *ad libitum* over the course of the assay and the number of eggs deposited on each apple was counted after 24 h. b, c, Wild-type flies prefer to lay eggs on yeast- or amino acid-painted apples over water-painted apples. Scale bars, 1 mm. d-h, $Sesn^{L431E}$ and $Sesn^{-/-}$ animals do not prefer to lay eggs on the leucine-containing apple. (a) created with BioRender.com. Values are mean ± SD of three biological replicates from a representative experiment. Data are representative of two independent experiments with similar results. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test (d-g), and Šídák's multiple comparisons test (h).



Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | Sestrin-regulated mTORC1 signaling in glial cells controls the preference of flies for leucine-containing food. a, Same data as in Figure 4a except that the values were not normalized to the values from the flies expressing the control shRNA from each of the indicated drivers. n = 5 (*da, pros attP40 shRNA; da Sesn shRNA*), 8 (*repo, tdc2 attP40 shRNA; vGAT Sesn shRNA*), 12 (*repo, esg Sesn shRNA*), 15 (*Elav attP40 shRNA*), 16 (*Elav, Mef2, ddc Sesn shRNA*), 22 (*Mef2 attP40 shRNA; Myo1A Sesn shRNA*), 10 (*ddc, Lpp attP40 shRNA; tdc2, promE Sesn shRNA*), 11 (*vGAT attP40 shRNA; Lpp Sesn shRNA*), 9 (*promE attP40 shRNA; pros Sesn shRNA*), 13 (*esg attP40 shRNA*), 24 (*Myo1A attP40 shRNA*). Each point represents the ratio of the amount of two oligonucleotide barcodes per 5 flies. b, Expression of wild-type Sestrin under *repo*-Gal4 driver in $Sesn^{-/-}$ flies is sufficient to partially rescue the leucine preference phenotype. n (*repo*-attP40 in *wild type* w^{1118}) = 4, n (other conditions) = 8. c, Overexpression of TSC1+TSC2 in glial cells using *repo*-Gal4; *Tub*-Gal80ts reduces the preference of flies for leucine-containing food. n (attP40) = 16, n (TSC1+2) = 19. d, The *Sesn* mRNA (red) is expressed in all classified subtypes of glial cells as indicated by co-expression of a pan glial marker, *Repo* (green). The single cell RNA sequencing dataset is from a previous study[39]. e, The knockdown of Sestrin using a pan glial cell driver (*repo*-Gal4) reduces the leucine preference of flies much more significantly than a knockdown using drivers for glial subtypes. The knockdown of Sestrin in cortex glial cells using the *wrapper*-Gal4 driver line significantly decreased the leucine preference of flies. n = 8 (*repo, 9.GMR50A12, 15.R85G01-Gal4 attP40 shRNA; 9.GMR50A12, 15.R85G01-Gal4 Sesn shRNA*), 12 (*1.GMR60F04, 2.GMR53B07, 3.GMR55B03, 4.GMR56F03, 5.GMR86E01, 6.GMR53H12, 10.Alrm-Gal4 attP40 shRNA; repo, 2.GMR53B07, 3.GMR55B03, 4.GMR56F03, 5.GMR86E01, 10.Alrm-Gal4, 14.R75H03-Gal4 Sesn shRNA*), 10 (*7.GMR35E04 attP40 shRNA, 1.GMR60F04 Sesn shRNA*), 11 (*8. GMR77A03, 11.Wrapper-Gal4, 14.R75H03-Gal4 attP40 shRNA; 6.GMR53H12, 11.Wrapper-Gal4 Sesn shRNA)*, 28 (*12.Eaat1-Gal4 39915, 13.Mdr65-Gal4 attP40 shRNA), 9 (7.GMR35E04, 8.GMR77A03 Sesn shRNA)*, 24 *(12.Eaat1-Gal4 39915 Sesn shRNA), 18 (13.Mdr65-Gal4 Sesn shRNA).* f, Confocal projection of wild-type female brains expressing 4MBOX-GFP fed the standard yeast-based food or starved of protein for 24 h. Scale bar, 10 µm. Values are mean ± SD of biological replicates from a representative experiment. Data are representative of two independent experiments with similar results. Statistical analysis was performed using two-tailed unpaired t test (a, c, e), and two-way ANOVA followed by Dunnett's multiple comparisons test (b).



Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Dietary leucine regulates mTORC1 signaling in glial cells in the peri-esophageal area in a fashion that depends on Sestrin and its capacity to bind leucine. a, Schematic of the areas imaged and quantified for the ratio of GFP-positive cells to Repo-positive cells. The red rectangle represents zone 1, the orange rectangle represents zone 2, and the purple rectangle represents zone 3. b, Representative confocal images of zone 1 and zone 2 brain areas from wild-type, *Sesn*$^{-/-}$, *and Sesn*^{L431E} female flies fed with an amino acid-replete or leucine-free diet. Scale bar, 25 µm. Note: images are reprocessed during revision from the same batch of samples as Figure 4c for the purpose of showing all zones 1, 2, and 3 clearly. The exact fly brains in the representative images and stacks might vary from Figure 4c, despite they are all from the same batch of samples. c, Representative confocal images of zone 3 brain areas of wild-type, *Sesn*$^{-/-}$, *and Sesn*^{L431E} female flies fed an amino acid-replete or leucine-free diet. Scale bar, 10 µm. Note: images are from the same brains shown in (b). (a) created with BioRender.com. d, e, Quantification of the GFP-positive to Repo-positive ratio in zone 1 (d) and zone 3 (e). n = 3 individual brains with indicated dietary treatment and genotype for each condition. Values are mean ± SD of biological replicates from a representative experiment. Data are representative of three independent experiments with similar results. Statistical analysis was performed using two-way ANOVA followed by Šídák's multiple comparisons test.

Extended Data Table 1 | Chemically defined food "Part 1" mixture

Part 1 (AUTOCLAVE 15 minutes)		
Category	Ingredient	Amount of stock per liter
Gelling Agent	Agar-Type II	10 g
Sugar	Sucrose	25 g
Metal Ions	CaCl2*6h2o	1 mL
	CuSO4*5h2o	1 mL
	FeSO4*7h2o	1 mL
	MgSO4 (anhydrous)	1 mL
	MnCl2*4h2o	1 mL
	ZnSO4*7h2o	1 mL
Cholesterol	Cholesterol	15 mL
Amino Acids	Tyrosine	0.93g
	Histidine	50 mL
	Isoleucine	50 mL
	Methionine	50 mL
	Phenylalanine	50 mL
	Threonine	50 mL
	Valine	50 mL
Water	Water (milliQ)	158 mL

Extended Data Table 2 | Chemically defined food "Part 2" mixture

Part 2		
Category	Ingredient	Amount of stock per liter
Base	Buffer	100 ml
Amino Acids	Arginine	10 mL
	Cysteine	10 mL
	Glutamate	10 mL
	Glycine	10 mL
	Lysine	10 mL
	Proline	10 mL
	Serine	10 mL
	Alanine	50 mL
	Asparagine	50 mL
	Aspartate	50 mL
	Glutamine	50 mL
	Leucine	50 mL
	Tryptophan	50 mL
Vitamin Solution	see Part 1	21 mL
Folic Acid	Folic Acid	1 mL
Other Nutrients Solution	see Part 1	8 mL
Preservatives	Propionic acid	6 mL
	methyl 4-hydroxybenzoate	15 mL

Extended Data Table 3 | Amino acid stock solutions

Amino Acids	Catalog Number	g/50mL	Suspend in:
L-Alanine	Sigma, A7469	1.1	H2O
L-Asparagine	Amresco, 94341	1.03	H2O
L-Aspartic Acid	Alfa Aesar, A13520	1.17	0.5N NaOH
L-Glutamine	Amresco, 0374	1.12	H2O
L-Histidine	Amresco, 1B1164	0.65	H2O
L-Isoleucine	Amresco, E803	1.12	H2O
L-Leucine	Sigma, L8912	2.03	0.2N HCl
L-Methionine	Amresco, E801	0.6	H2O
L-Phenylalanine	Sigma, P5482	1.01	H2O
L-Threonine	Sigma, T8441	1.11	H2O
L-Tryptophan	Amresco, E800	0.32	H2O
L-Valine	Amresco, 1B1102	1.2	H2O
L-Arginine HCl	Amresco, 0877	8.16	H2O
L-Cysteine	Sigma, 30089	1.71	1N HCl
L-Glutamic acid	Alfa Aesar, A12919	7.59	H2O
L-Glycine	Alfa Aesar, A13816	3.84	H2O
L-Lysine HCl	Amresco, 0437	6.83	H2O
L-Proline	Sigma, P5607	4.89	H2O
L-Serine	Sigma, S4311	6.89	H2O
L-Tyrosine	Sigma, T8566	**add Tyr powder	N/A

Extended Data Table 4 | Other stock solutions

Vitamin solution	**Catalog Number**	**g/50mL**	**Suspend in:**
Biotin	Sigma, B4501	0.001	H2O
Ca pantothenate	Sigma, 21210	0.039	H2O
Nicotinic acid	Sigma, N4126	0.03	H2O
Pyridoxine HCl	Sigma, P9755	0.006	H2O
Riboflavin	Sigma, R4500	0.003	H2O
Thiamine (aneurin)	Sigma, T4625	0.005	H2O
Folic acid solution	**Catalog Number**	**g/50mL**	**Suspend in:**
Folic acid	Sigma F8758	0.025	0.004N NaOH
Other Nutrients solution	**Catalog Number**	**g/50mL**	**Suspend in:**
Choline chloride	MP Biomedicals, 194639	0.3125	H2O
Inosine	Sigma, I4125	0.4065	H2O
Myo-Inositol	Sigma, I7508	0.0315	H2O
Uridine	Sigma, U3003	0.375	H2O
Methyl 4-hydroxybenzoate solution	**Catalog Number**	**g/50mL**	**Suspend in:**
Methyl 4-hydroxybenzoate	Sigma, H3647	5	95% EtOH
Buffer	**Catalog Number**	**50mL stock**	
Glacial Acetic Acid	Millipore, AX0074	1.5 mL	
KH2PO4	JT Baker, 3246	1.5 g	
NaHCO3	Sigma, S8875	0.5 g	
Water		Up to 50mL	
Metal Ions	**Catalog Number**	**g/50mL**	**Suspend in:**
CaCl2*6h2o	Sigma, 21108	12.5	H2O
CuSO4*5h2o	Sigma, C7631	0.125	H2O
FeSO4*7h2o	Sigma, F7002	1.25	H2O (store - 20C)
MgSO4 (anhydrous)	Sigma, M7506	12.5	H2O
MnCl2*4h2o	Sigma, M3634	0.05	H2O
ZnSO4*7h2o	Sigma, Z0251	1.25	H2O
Cholesterol solution	**Catalog Number**	**g/50mL**	**Suspend in:**
Cholesterol	Sigma, C8253	1	EtOH

nature research

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Last updated by author(s): 07/04/2022

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	MetaMorph 7 (for all confocal images), iPhone XR (for eggs on apple pictures), CFX Maestro Software for Bio-Rad CFX Real-Time PCR Systems, Xcalibur 4.0
Data analysis	GraphPad Prism v8.0, XCalibur v4.0, (Fiji Is Just) ImageJ v2.1.0/1.53c, Microsoft Excel 16.40

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Extended Data Fig. 10d: the single cell RNAseq dataset analyzed is Aerts_Fly_AdultBrain_Filtered_57k, which is available here: scope.aerslab.org. All codes required to run the CPH and permutation statistical analyses are provided as source data.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We chose a minimal sample size of n=3 for quantitative measurements based on the standards of the field. For food preference experiments performed on adult flies, we anticipated higher variance due to the nature of animal behavior. Therefore, we chose to include more samples per condition. Same reason goes for the ovarian size measurement as well as the lifespan measurement.
Data exclusions	No data were excluded other than the lifespan measurement and DNA oligonucleotide-based food preference assay. For the lifespan measurement, if we observe more than 5 flies were stuck in the food (20% of total flies in this tube), this tube of flies would be discarded due to the impossibility to interpret the survival curve. For the food preference assay, we stated clearly in the Methods that to control for the extraction efficiency, if the qPCR Ct number for a sample's genomic locus control is more than 0.5 away from the average (which means the DNA extraction efficiency is not passing the quality control for this particular sample), this sample would be discarded.
Replication	Key findings were reproducible in this work and we have encountered no problems in reproducibility. All experiments were repeated at least twice independently.
Randomization	For lifespan measurement, different tubes of flies were placed on tube racks randomly to rule out the potential effects from the location/air flow/light. For food preference assay, we processed all flies from the same batch at the same time but randomized orders of assay bottle set-up and sample collection for different genotypes as well as the location of assay bottles in bottle racks to rule out effects of location/air flow/light. For the metabolomics experiment, we randomized the sample order in LC/MS run to prevent the potential systematic bias. For the rest of the experiments, samples were handled at the same time, internally controlled and processed independently. Thus randomization was not necessary.
Blinding	Blinding was performed wherever possible during sample preparation and analysis. We especially blinded the qPCR sample run and analysis for food preference experiments due to the big sample size. The quantification of ovarian size and fly brain imaging was also performed in a blinded manner.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	HRP-labeled anti-rabbit secondary antibody and the antibodies against Drosophila Phospho-70 S6 Kinase (Thr398) (#9209), Akt (#9272), p44/42 MAPK (Erk1/2) (#4695), Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (#9101). myc (#2278), and the FLAG (#2368) epitope from Cell Signaling Technology (CST); Anti-Green Fluorescent protein (GFP) antibody from Aves Labs (GFP-1020); 8D12 Anti-Repo antibody from Developmental Studies Hybridoma Bank (DSHB); Alexa 488, 568, and 647-conjugated secondary antibodies are from Invitrogen. The dS6K antibody was a gift from Mary Stewart (North Dakota State University) and the Drosophila Sestrin antibody one from Jun Hee Lee (University of Michigan).
Validation	All used commercially available antibodies have validations from the corresponding company's website. The dS6K antibody has been validated in numerous previously published studies (for example: Bar-Peled et al. Science. 2013). And Drosophila Sestrin antibody was validated in J.H.Lee et al. Science. 2010.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	S2R+ cells were originally from Dr. Schneider and have been passaging in Perrimon lab and DRSC at Harvard Medical School for decades. HEK-293F cells were from ThermoFisher (R79007).
Authentication	S2R+ cells were authenticated via DNA and RNA sequencing. And the authentication work has been cited in the methods section.
Mycoplasma contamination	HEK293F cells were tested negative for mycoplasma by PCR.
Commonly misidentified lines (See ICLAC register)	None.