Lysozyme lipid signalling from the periphery to neurons regulates longevity

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Lysozymes are key cellular organelles that metabolize extra- and intracellular substrates. Alterations in lysosomal metabolism are implicated in ageing-associated metabolic and neurodegenerative diseases. However, how lysosomal metabolism actively coordinates the metabolic and nervous systems to regulate ageing remains unclear. Here we report a fat-to-neuron lipid signalling pathway induced by lysosomal metabolism and its longevity-promoting role in Caenorhabditis elegans. We discovered that induced lysosomal lipolysis in peripheral fat storage tissue upregulates the neuropeptide signalling pathway in the nervous system to promote longevity. This cell-non-autonomous regulation is mediated by a specific polyunsaturated fatty acid, dihomo-γ-linolenic acid, and acts through NHR-49 nuclear receptor and NLP-11 neuropeptide in neurons to extend lifespan. These results reveal lysosomes as a signalling hub to coordinate metabolism and ageing, and lysosomal signalling mediated inter-tissue communication in promoting longevity.

Aging is a process of progressive decline occurring at all levels. Mechanisms that govern the crosstalk across different organelles and among different tissues contribute to longevity regulation1–3. In particular, lipids are crucial signals in mediating organelle crosstalk and tissue interactions4–6, and dietary supplementation of specific lipids influences lifespan7. Lysosomes actively participate in lipid metabolism, and lipid breakdown by lysosomal acid lipases releases free fatty acids (FFA) from triacylglycerols (TAGs) and cholesteryl esters (CEs)8. Lysosomes also serve as a signalling hub inside the cell. In Caenorhabditis elegans, LIPL-4 is a lysosomal acid lipase specifically expressed in the intestine, the peripheral fat storage tissue. It is upregulated upon fasting8,9 and in the long-lived mutant that reduces insulin/IGF-1 signalling (IIS)10,11. Lysosomes actively participate in lipid metabolism, and lipid breakdown by lysosomal acid lipases releases free fatty acids (FFA) from triacylglycerols (TAGs) and cholesteryl esters (CEs). Lysosomes also serve as a signalling hub inside the cell. In Caenorhabditis elegans, LIPL-4 is a lysosomal acid lipase specifically expressed in the intestine, the peripheral fat storage tissue. It is upregulated upon fasting8,9 and in the long-lived mutant that reduces insulin/IGF-1 signalling (IIS)10,11. Lysosomes actively participate in lipid metabolism, and lipid breakdown by lysosomal acid lipases releases free fatty acids (FFA) from triacylglycerols (TAGs) and cholesteryl esters (CEs). Lysosomes also serve as a signalling hub inside the cell. In Caenorhabditis elegans, LIPL-4 is a lysosomal acid lipase specifically expressed in the intestine, the peripheral fat storage tissue. It is upregulated upon fasting8,9 and in the long-lived mutant that reduces insulin/IGF-1 signalling (IIS)10,11. Lysosomes actively participate in lipid metabolism, and lipid breakdown by lysosomal acid lipases releases free fatty acids (FFA) from triacylglycerols (TAGs) and cholesteryl esters (CEs).

Results

Peripheral lysosomal lipolysis turns on neuronal signalling.

We first systematically profiled transcriptional changes associated with LIPL-4-induced lysosomal lipolysis using RNA sequencing (RNA-seq) analysis of lipl-4 transgenic worms (lipl-4 Tg), which constitutively express this lipase in the intestine and have extended lifespan (Fig. 1a and Supplementary Table 4). A series of genes are differentially expressed in lipl-4 Tg compared with wild-type (WT) worms (fold change >1.5, P < 0.05; Supplementary Table 1, and Fig. 1a). DAVID functional annotation of the genes upregulated in lipl-4 Tg revealed the enrichment of distinct biological processes (Fig. 1b). Besides ‘immune response’ and ‘defence response’ Gene Ontology categories that are commonly associated with longevity, we discovered the enrichment of ‘neuropeptide signalling pathway’, which consists of genes encoding neuropeptides and their processing enzymes. We used quantitative PCR with reverse transcription (qRT–PCR) to confirm the induction of neuropeptide genes and pro-longevity effect. These studies reveal that lysosome-derived signals are crucial not only for organellar crosstalk in the cell12, but also for tissue coordination in the organism, making them exciting targets for optimal pro-longevity intervention at the systemic level.

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Fig. 1 | Peripheral lysosomal lipolysis upregulates neuropeptide signalling. a, Transgenic strains constitutively expressing lipl-4 in the intestine (lipl-4 Tg) show lifespan extension. b, Gene Ontology of upregulated genes in lipl-4 Tg compared with WT worms. c, Genes encoding neuropeptide-processing enzymes egl-3, egl-21, sbt-1, pgal-1 and pghm-1 are transcriptionally upregulated by lipl-4 Tg. d, Schematic diagram of the neuropeptide processing and maturation pathway. e, List of significantly upregulated neuropeptide genes (P < 0.05) by lipl-4 Tg in RNA-seq transcriptome profiling. f,egl-21 mRNA is detected by smFISH (Red Quasar 670), while nuclei are stained by DAPI. g, egl-21 is detected in neurons (boxed region) of WT but not egl-21(lf) worms. The intestine region marked by dashed lines shows no egl-21. h, GFP driven by the egl-21 endogenous promoter is expressed in neurons but not the intestine (marked by dashed lines). Scale bar, 10 μm.
carboxypeptidase E, peptidylglycine α-amidating monoxygenase and neuroendocrine chaperone 7B2, respectively (Fig. 1d). Among them, egl-21, pgal-1 and pghm-1 are specifically expressed in neurons\textsuperscript{9,14}. Their inductions suggest a cell-non-autonomous regulation of neuronal genes by peripheral lysosomal lipolysis. There are also neuropeptides transcriptionally upregulated in \textit{lipl-4} \textit{Tg}, including 3 insulin-like peptides (ILPs), 12 FMRFamide-related peptides (FLPs) and 19 neuropeptide-like proteins (NLPs) (Fig. 1e and Supplementary Table 1).

Next, we examined the role of the neuropeptide signalling pathway in longevity regulation using the loss-of-function mutant of egl-21, \textit{egl-21}(\textit{lf}). The EGL-21 enzyme is required for neuropeptide processing by removing basic residues from the C-terminus of cleaved peptides\textsuperscript{18,19}, and the \textit{egll-21} gene is exclusively expressed in neurons as visualized by both single-molecule fluorescence in situ hybridization (smFISH) and its green fluorescent protein (GFP) transgenic reporter (Fig. 1f,g). We found that \textit{lipl-4} \textit{Tg} cannot prolong the lifespan of \textit{egl-21}(\textit{lf}) (Extended Data Fig. 1b and Supplementary Table 4), suggesting that the induction of neuropeptide signalling contributes to \textit{lipl-4}-induced longevity. Previous genomic RNA interference (RNAi) screens found that inactivation of egl-3, encoding the convertase upstream of EGL-21, extends lifespan, which is suppressed by inactivation of the \textit{daf-16}/FOXO transcription factor\textsuperscript{9,12,13}. Similarly, we found that \textit{egl-21}(\textit{lf}) has extended lifespan and this lifespan extension requires \textit{daf-16} (Extended Data Fig. 1c and Supplementary Table 5). Given that ILPs regulate lifespan\textsuperscript{11,12} and DAF-16/FOXO is the key mediator of longevity caused by IIS reduction\textsuperscript{9}, the requirement of DAF-16 suggests that the longevity effect conferred by \textit{egl-21}(\textit{lf}) is possibly due to reduced agonist IIL maturation and IIS. In contrast, the lifespan extension in \textit{lipl-4} \textit{Tg} is not suppressed by \textit{daf-16} RNAi (Extended Data Fig. 1d and Supplementary Table 5), indicating a negligible role of ILPs for \textit{lipl-4}-induced longevity. These results suggest that, in order to test whether the reduction of NLPs or FLPs in \textit{egl-21}(\textit{lf}) affects \textit{lipl-4}-induced longevity, we should inactivate \textit{daf-16} to eliminate the contribution from IIL reduction. We found that, with \textit{daf-16} RNAi, \textit{egl-21}(\textit{lf}) fully abrogates the lifespan extension conferred by \textit{lipl-4} \textit{Tg} (Fig. 1b and Supplementary Table 5). Together, these results suggest that neuropeptide processing is required for intestinal lysosomal lipolysis to promote longevity, which is probably associated with NLPs and/or FLPs but not ILPs.

**Neuronal NLP-11 neuropeptide promotes longevity**

To identify specifically involved neuropeptides, we performed an RNAi-based screen to search for neuropeptide-encoding genes whose inactivation suppresses the lifespan extension in \textit{lipl-4} \textit{Tg} (Supplementary Table 3). We discovered that RNAi inactivation of \textit{nlp-11} in a neuronal RNAi-sensitive background suppresses \textit{lipl-4} \textit{Tg} longevity without affecting WT lifespan (Fig. 2a and Supplementary Tables 3 and 5). We further generated a clustered regularly interspaced short palindromic repeats (CRISPR) deletion mutant for \textit{nlp-11}, \textit{nlp-11}(\textit{lf}) (Extended Data Fig. 2a) and crossed it with \textit{lipl-4} \textit{Tg}. We found that \textit{nlp-11}(\textit{lf}) reduces the lifespan extension in \textit{lipl-4} \textit{Tg} but not WT lifespan (Fig. 2b and Supplementary Table 4). \textit{nlp-11} is transcriptionally upregulated in \textit{lipl-4} \textit{Tg} (Extended Data Fig. 2b), and overexpression of \textit{nlp-11} driven by its endogenous promoter sufficiently prolongs lifespan (Fig. 2c, Extended Data Fig. 2c and Supplementary Table 4). \textit{nlp-11} expresses in both neurons and the intestine (Extended Data Fig. 2d). To examine where \textit{nlp-11} functions to regulate longevity, we knocked down \textit{nlp-11} selectively in the intestine and found that this intestine-only inactivation does not affect the lifespan extension in \textit{lipl-4} \textit{Tg} (Fig. 2d and Supplementary Table 5). We also overexpressed \textit{nlp-11} in either neurons or the intestine using tissue-specific promoters and found that only neuron-specific overexpression of \textit{nlp-11} is sufficient to prolong lifespan (Fig. 2e,f, Extended Data Fig. 2e,f and Supplementary Table 4). Together, these results demonstrate that neuronal \textit{nlp-11} is specifically responsible for the longevity effect conferred by intestinal lysosomal lipolysis.

**Lysosome-derived PUFAs regulate neuropeptide and longevity**

Lysosomal acid lipase catalyses FFA release from TAGs and/or CE\textsuperscript{4}. Through lipidomic profiling of FFAs, we found that the levels of polyunsaturated fatty acids (PUFAs) are increased in \textit{lipl-4} \textit{Tg} (Fig. 3a). To test whether these PUFAs are derived from lysosomal lipolysis, we purified lysosomes and profiled different classes of lipids. We found that, compared with WT, the level of TAGs is reduced by approximately threefold in lysosomes purified from \textit{lipl-4} \textit{Tg} (Extended Data Fig. 3a). Moreover, 186 out of 305 detected TAG species (61%) are decreased in \textit{lipl-4} \textit{Tg} lysosomes, with 63% of them containing PUFAs (Fig. 3b). These results suggest that the induction of PUFAs is probably due to increased lysosomal lipolysis of TAGs.

To test the hypothesis that these PUFAs serve as cell-non-autonomous signals to regulate neuropeptides, we utilized loss-of-function mutants of \textit{fat-1} and \textit{fat-3} that encode \(\alpha\)-3 fatty acid desaturases and \(\Delta6\)-desaturase, respectively required for PUFA biosynthesis\textsuperscript{20} (Fig. 3c). With these desaturase mutants, the upregulation of neuropeptide genes (Fig. 3d) and the lifespan extension are suppressed in \textit{lipl-4} \textit{Tg} (Extended Data Fig. 3b,c and Supplementary Table 4). FAT-1 and FAT-3 function in the intestine and neurons to catalyse PUFA biosynthesis locally\textsuperscript{8,15}. We selectively reduced intestinal PUFAs by knocking down \textit{fat-1} and \textit{fat-3} only in the intestine and found that intestine-only inactivation of either \textit{fat-1} or \textit{fat-3} fully abrogates the lifespan extension in \textit{lipl-4} \textit{Tg} (Fig. 3e,f and Supplementary Table 5). Together, these results suggest that PUFAs derived from intestinal lysosomal lipolysis mediate both neuropeptide induction and longevity.

**Peripheral lipid chaperone LBP-3 promotes longevity**

FFAs have low aqueous solubility and must be bound to proteins in order to diffuse through the lipophobic environment. A family of proteins termed fatty acid binding proteins (FABPs) function as lipid chaperones, which reversibly bind FFAs and their derivatives to mediate their trafficking and signalling effects\textsuperscript{21,22}. We tested whether specific FABPs facilitate the action of intestinal PUFAs on neurons, and focused on three FABPs, LBP-1, LBP-2 and LBP-3, that carry putative secretory signals. We found that RNAi inactivation of \textit{lbp-2} or \textit{lbp-3} but not \textit{lbp-1} specifically suppresses the induction of neuropeptide genes caused by \textit{lipl-4} \textit{Tg} (Extended Data Fig. 4a,b). To confirm the RNAi knockdown results, we generated CRISPR deletion mutants of \textit{lbp-2} and \textit{lbp-3} (Extended Data Fig. 4c) and crossed them with \textit{lipl-4} \textit{Tg}. We found that only \textit{lbp-3} but not \textit{lbp-2} deletion suppresses the induction of neuropeptide genes (Fig. 4a). Deletion of \textit{lbp-3} also suppresses \textit{lipl-4} \textit{Tg} longevity without affecting WT lifespan (Fig. 4b and Supplementary Table 4).

We also found that transgenic strains that constitutively express \textit{lbp-3} (\textit{lbp-3} \textit{Tg}) live longer than WT worms (Fig. 4c, Extended Data Fig. 4d and Supplementary Table 4) and show upregulation of \textit{egl-3}, \textit{egl-21} and \textit{nlp-11} (Fig. 4d). We further profiled transcriptome changes in \textit{lbp-3} \textit{Tg} using RNA-seq (Supplementary Table 2, and PCA analysis in Extended Data Fig. 4e). Among 39 neuropeptide genes upregulated in \textit{lipl-4} \textit{Tg}, all 5 neuropeptide-processing genes and 16 neuropeptide genes were upregulated in \textit{lbp-3} \textit{Tg} (Fig. 4e and Supplementary Table 2). Similar to \textit{lipl-4} \textit{Tg}, the lifespan extension conferred by \textit{lbp-3} \textit{Tg} is not suppressed by \textit{daf-16} RNAi (Extended Data Fig. 4f and Supplementary Table 5), but it is suppressed by \textit{egl-21}(\textit{lf}) in the \textit{daf-16} RNAI background (Fig. 4f and Supplementary Table 5). Moreover, \textit{nlp-11} inactivation partially suppresses the lifespan extension in \textit{lbp-3} \textit{Tg} (Fig. 4g and Supplementary Table 4). These results support that specific neuropeptides act downstream of
LBP-3 signalling to regulate longevity. Next, we found that transgenic strains that selectively overexpress lbp-3 in the intestine exhibit the upregulation of neuropeptide genes (Fig. 4b) and lifespan extension (Fig. 4i, Extended Data Fig. 4g and Supplementary Table 4). Together, these results support that the specific lipid chaperone LBP-3 mediates fat-to-neuron communication to regulate neuropeptides and longevity.

LBP-3 relies on secretion for its regulation. To further understand the function of LBP-3 in this endocrine regulation, we examined whether LBP-3 can be secreted from the intestine. In C. elegans, coelomocytes are scavenger cells that take up secreted materials from the body cavity and serve as a monitor of secreted proteins25. We discovered that this secretion of LBP-3–RFP is elevated in lbp-3 RNAi to reduce PUFA biosynthesis in peripheral tissues and found reduction of LBP-3–RFP secretion in lbp-3 RNAi (Fig. 6a,b). Thus, LBP-3 protein requires secretion from the intestine to systemically regulate neuropeptides and longevity, which is triggered by LIPL-4-induced lysosomal lipolysis.

DGLA regulates LBP-3 secretion, neuropeptides and longevity. To examine how specific PUFAs and LBP-3 coordinate with each other, we first examined the effect of PUFAs on LBP-3 secretion. We generated transgenic strains that overexpress LBP-3 without its secretory signal only in the intestine and found no neuropeptide gene induction (Fig. 5d) or lifespan extension (Fig. 5e, Extended Data Fig. 5b) in these strains. RFP fusion of this non-secretable LBP-3 was not detected in coelomocytes (Fig. 5f). Thus, LBP-3 protein requires secretion from the intestine to systematically regulate neuropeptides and longevity, which is triggered by LIPL-4-induced lysosomal lipolysis.
LBP-3, amphipathic 1-anilinonaphthalene-8-sulfonic acid (1-ANS) shows enhanced fluorescence that is quenched once outcompeted by FFAs. We found that DGLA (Kₐ = 10.96 μM), AA (Kₐ = 2.9 μM) and EPA (Kₐ = 4.76 μM) but not ETA bind to LBP-3 (Fig. 6c).

Next, we supplemented DGLA, AA or EPA to worms and measured neuropeptide gene expression. We found that, in fat-3(lf), DGLA supplementation is able to restore the upregulation of neuropeptide genes caused by lipl-4 Tg (Fig. 6d). Neither AA nor EPA supplementation shows such an ability (Fig. 6d).

DGLA binding specificity of LBP-3 mediates its effects. To further confirm that the effect of DGLA on neurons is dependent on LBP-3, we supplemented DGLA to lipl-4 Tg with both fat-3(lf) and lipb-3(lf) mutants. We found that, in the absence of LBP-3, DGLA supplementation fails to restore the neuropeptide gene induction (Fig. 7a).

Thus, DGLA requires LBP-3 to regulate neuropeptides. Next, we supplemented DGLA, AA or EPA to worms and measured neuropeptide gene expression. We found that, in fat-3(lf), DGLA supplementation is able to restore the upregulation of neuropeptide genes caused by lipl-4 Tg (Fig. 6d). Neither AA nor EPA supplementation shows such an ability (Fig. 6d). To examine tissue specificity of this restoration, we conducted qRT–PCR analysis using dissected intestine and found that DGLA supplementation causes no intestinal induction of egl-3 or egl-21 (Extended Data Fig. 6a). We also imaged egl-21 messenger RNA transcripts using smFISH and found an increase in neurons by DGLA supplementation (Extended Data Fig. 6b). Moreover, DGLA supplementation sufficiently restores the increased LBP-3–RFP secretion (Fig. 6e,f) and lifespan extension in lipl-4 Tg with fat-3 inactivation (Fig. 6g and Supplementary Table 5). Together, these results suggest that the induction of DGLA by lysosomal lipolysis promotes secretion of the LBP-3 lipid chaperone from the intestine, and LBP-3–DGLA signals to neurons to regulate neuropeptides and longevity.

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Supplementary Table 5 for full lifespan data. Source numerical data are available in source data.
Peripheral lipid chaperone LBP-3 regulates neuropeptide and longevity. a, The loss-of-function mutation of the lipid chaperone lbp-3(lf) but not lbp-2(lf) suppresses the transcriptional up-regulation of egl-3 and egl-21 by lipl-4 Tg. b, lbp-3(lf) suppresses lipl-4 Tg longevity. c, Constitutive expression of lbp-3 driven by its own endogenous promoter (lbp-3 Tg) prolongs lifespan. d, The transcriptional levels of egl-3, egl-21 and nlp-11 are induced in the lbp-3 Tg worms. e, Out of 39 upregulated neuropeptide genes by lipl-4 Tg, 22 are also significantly induced by lbp-3 Tg (P < 0.05). f, The loss-of-function mutation of egl-21(lf) suppresses lbp-3 Tg longevity. daf-16 RNAi knockdown is used to eliminate the influence from ILP reduction in egl-21(lf). g, The loss-of-function mutation of nlp-11(lf) suppresses lbp-3 Tg longevity. h, Intestine-specific lipl-4 overexpression upregulates the transcriptional levels of egl-3, egl-21 and nlp-11. i, Overexpression of lbp-3 selectively in the intestine extends lifespan. In a, error bars represent mean ± s.e.m., n = 3 (lbp-2(lf), lipl-4 Tg and lipl-4 Tg;lbp-3(lf)) and n = 6 (WT, lbp-3(lf) and lipl-4 Tg;lbp-3(lf)) biologically independent samples, ***P = 0.0001 by two-way ANOVA with Holm-Sidak correction, ~2,000 worms per replicate. In b, c, f and i, n = 3 biologically independent samples, ***P < 0.001 by log-rank test, 60–120 worms per replicate. Lifespan, error bars represent mean ± s.e.m., and ***P < 0.001 by two-way ANOVA with Holm-Sidak correction, ~2,000 worms per replicate. Source numerical data are available in source data.

tested whether the lipid binding specificity of LBP-3 is responsible for its regulatory effects. Despite the close homology between LBP-2 and LBP-3, LBP-2 is not required for the upregulation of neuropeptide genes by lipl-4 Tg (Fig. 4a), and its overexpression does not induce neuropeptide genes (Fig. 7b). We then analysed their lipid binding preferences through profiling C. elegans liposome that binds to either LBP-2 or LBP-3. We found that LBP-2 and LBP-3 exhibit distinct lipid binding preferences (Fig. 7c and Extended Data Fig. 7a). In particular, DGLA shows 25% occupancy among LBP-3-bound PUFA, but only 1% occupancy among LBP-2-bound PUFA (Fig. 7c). Interestingly, for EPA, the highest-abundant PUFAs in C. elegans liposome (Fig. 7d), both LBP-2 and LBP-3 show low percentage of occupancy (6% and 3%, respectively) (Fig. 7c). These results suggest that LBP-2 and LBP-3 have different binding specificity towards PUFAs.

Next, we compared the predicted structures of LBP-2 and LBP-3 using AlphaFold2 and found changes in two cap-like α-helices that are responsible for lipid binding (Fig. 7e). A sequence alignment between LBP-2 and LBP-3 reveals that ten amino acids are different in these regions (Fig. 7f). We thus designed a chimeric protein by replacing the two cap-like α-helices from N38 to K60 with those present in LBP-2 (Fig. 7f and Extended Data Fig. 7b). We generated
transgenic lines expressing this chimeric protein selectively in the intestine and confirmed that the chimeric protein expresses normally (Extended Data Fig. 7c). We found that overexpression of the chimeric protein, like LBP-2, does not induce neuropeptide gene expression (Fig. 7g and Extended Data Fig. 7d). No lifespan extension was detected in these transgenic strains either (Fig. 7h, Extended Data Fig. 7e and Supplementary Table 4). These results suggest that the lipid binding specificity of LBP-3 towards DGLA is necessary for its regulation of neuropeptides and longevity.

Neuronal transduction of peripheral lipid signals. To examine whether secreted LBP-3 is taken up by neurons, we generated a transgenic strain that specifically expresses GFP nanobody (GBP) in neurons together with polycistronic mKate, and then crossed it with a transgenic strain expressing GFP-fused LBP-3 only in the intestine. In this line, if secreted LBP-3–GFP proteins from the intestine are taken up by neurons, neuronal GBP will capture them, making GFP visible in neurons (Fig. 8a). In supporting LBP-3–GFP uptake by neurons, we detected GFP signals in mKate-positive neurons (Fig. 8b). As controls, we did not detect neuronal GFP signals in either the GBP or the LBP-3–GFP transgenic strain alone (Extended Data Fig. 8a,b). Moreover, we generated a transgenic strain that expresses GBP fused with the extracellular domain of SAX-7 (GBP-SAX-7) in neurons, and then crossed it with the intestine-specific LBP-3–GFP transgenic strain. In this line, LBP-3–GFP signals were also detected in neurons (Extended Data Fig. 8c), supporting a close proximity between secreted LBP-3 and the neuronal surface. Together, these results reveal LBP-3 as an endocrine lipid chaperone, which is transported from the intestine to neurons to mediate lpl-4-induced longevity.

Previously, we discovered LBP-8 as a cell-autonomous mediator of lpl-4-induced longevity. To investigate the interaction between LBP-3 and LBP-8, we examined the transcriptional levels of the neuropeptide genes in the long-lived lbp-8 transgenic strain (lbp-8 Tg) and found only negligible changes (<35%) (Fig. 8c). Both lbp-3 and lbp-8 are partially required for the lifespan extension caused by lpl-4 Tg (Fig. 4b), and lbp-3 Tg and lbp-8 Tg have an additive effect in prolonging lifespan (Fig. 8d). In the intestine, LBP-8 facilitates the lysosome-to-nucleus retrograde transport of lipid signals that activate nuclear receptors NHR-49 and NHR-80 to promote longevity12.

See Supplementary Table 4 for full lifespan data. Source numerical data are available in source data.
When examining the involvement of NHR-49 and/or NHR-80, we found that the loss-of-function mutation of nhr-49 fully suppresses the upregulation of neuropeptide genes in lipl-4 Tg and lip-3 Tg (Fig. 8e,f), but the nhr-80 mutation has a negligible effect (<18% reduction; Fig. 8e). Importantly, in the nhr-49 mutant background, neuron-specific restoration of nhr-49 fully rescues the upregulation of neuropeptide genes (Fig. 8f) and the lifespan extension (Fig. 8g and Supplementary Table 4) conferred by lip-3 Tg. Furthermore, we confirmed the neuronal expression of nhr-49 using a transgenic strain expressing NHR-49-mKate2 fusion driven by its endogenous promoter (Fig. 8h). After crossing this line with the GFP reporter line of nlp-11, we found many overlaps between neurons with nlp-11 expression and NHR-49 localization (Fig. 8h). On the basis of CenGenApp analysis, there are 57 overlapping neurons (Extended Data Fig. 8d and Supplementary Table 6). Thus, lysosomal lipid signals from the periphery act through neuronal NHR-49 to regulate neuropeptides and longevity (Fig. 8i).

Discussion

This study supports an emerging paradigm that lysosomes are the critical signalling hub for longevity regulation. We have identified two lipid chaperones mediating the signalling role of lysosomes, LBP-8 for lysosome-to-nucleus retrograde signalling and LBP-3 for fat-to-neuron endocrine signalling, which act in parallel to regulate longevity. Overexpression of non-secretable LBP-3 causes decreased transcription of neuropeptide genes, which is probably
Fig. 7 | DGLA binding specificity of LBP-3 mediates its effects. a, DGLA supplementation fails to restore the induction of egl-3 and egl-21 by lipl-4 Tg in the fat-3(lf) and lbp-3(lf) double-mutant background. DMSO serves as the vehicle control. b, Constitutive expression of lbp-2 (lbp-2 Tg) does not affect the transcription of egl-3 or egl-21. c, After incubation with C. elegans liposome, fatty acids bound to LBP-2 or LBP-3 proteins were analysed with mass spectrometry. DGLA shows a high percentage of occupancy in LBP-3 but not LBP-2. d, In the C. elegans liposome, the percentage of EPA is more than ten times higher than that of DGLA or AA. e, LBP-2 (blue) and LBP-3 (grey) superposition structures predicted using AlphaFold2. f, An LBP-2 and LBP-3 protein alignment generated using t-coffee. Secondary structures are displayed above the alignment. The LBP-2 sequence utilized for the replacement in the LBP-3 chimeric protein is highlighted in purple. g, h, Intestine-specific overexpression of chimeric lbp-3(chim) does not affect the transcription of egl-3 and egl-21 (g) nor prolongs lifespan (h). In a and b, error bars represent mean ± s.e.m., n = 3 biologically independent samples; NS, P > 0.05 by two-way ANOVA with Holm–Sidak correction, ~2,000 worms per replicate. In c and d, n = 3 biologically independent samples. In g, error bars represent mean ± s.e.m., n = 4 biologically independent samples; NS, P > 0.05 by two-way ANOVA with Holm–Sidak correction, ~2,000 worms per replicate. In h, n = 3 biologically independent samples; NS, P > 0.05 by log-rank test, 98–120 worms per replicate. See Supplementary Table 4 for full lifespan data. Source numerical data are available in source data.
due to reduced DGLA secretion. Thus, secreted LBP-3 may regulate the basal expression of neuropeptides even without the induction of lysosomal lipolysis. Mammalian FABP4 secreted from adipocytes has been implicated in the hormonal control of metabolism, and FABP5 at the blood–brain barrier contributes to the brain uptake of docosahexaenoic acid, a PUFA essential for cognitive function. Therefore, FABP secretion may function as an evolutionarily conserved mechanism to facilitate lipid transportation from peripheral metabolic tissues to the central nervous system.

Nuclear receptors are the best-known mediators of lipid signals in transcriptional responses, and several C. elegans nuclear receptors have been implicated in regulating longevity, including NHR-49, NHR-80, NHR-62 and DAF-12 (refs. 1,16–18). In particular, PPARα has high binding affinity for FAs and plays a crucial role in metabolic tissues to regulate lipid catabolism. PPARα also expresses at a high level in the nervous system; however, its neuronal function and regulation remain poorly understood. Our studies reveal that, in the nervous system, NHR-49 regulates the longevity effect conferred by neuronal AMPK activation. Thus, lipids may be crucial endocrine signals that couple peripheral metabolic status.

Fig. 8 | Neuronal transduction of peripheral lipid signals to regulate longevity. a, Schematic design using neuron-expressing GBP to detect the uptake of intestine-secreted LBP-3 into neurons. b, In the transgenic line expressing GBP in the intestine and GBP polycistronic mKate in neurons, GFP signals from secreted LBP-3 are detected in neurons (mKate positive). Scale bars, 30 μm and 10 μm in the inset. c, Constitutive expression of lbp-8 (lbp-8 Tg) does not affect the transcription of egl-3, while the induction of either egl-21 or nlp-11 is negligible (<35%). d, lbp-3 Tg and lbp-8 Tg have an additive effect on lifespan extension. e, The loss-of-function mutation of the nuclear receptor nhr-49(lf) fully suppresses the induction of the neuropeptide genes by lpl-4 Tg, while the loss-of-function of nhr-80(lf) only decreases the induction by less than 17%. f, nhr-49(lf) fully suppresses the induction of the neuropeptide genes by lbp-3 Tg, which is rescued by neuronal restoration of nhr-49. g, nhr-49(lf) fully suppresses the lifespan extension in the lbp-3 Tg worms, which is rescued by neuronal restoration of nhr-49. h, In the transgenic line expressing nlp-11 polycistronic GBP and mKate-fused NHR-49 driven by their endogenous promoters, GFP and mKate signals overlap in many neurons. Scale bar, 10 μm. i, Cartoon illustrating the overall model. In e, error bars represent mean ± s.e.m., n = 4 biologically independent samples, *P = 0.034, **P = 0.002 by two-way ANOVA with Holm–Sidak correction, ~2,000 worms per replicate. In d and g, n = 3 biologically independent samples, ***P < 0.001 by log-rank test, 80–101 worms per replicate. See Supplementary Table 4 for full lifespan data. In e, error bars represent mean ± s.e.m., n = 4 biologically independent samples, ***P = 0.0004 and ****P < 0.0001 by two-way ANOVA with Holm–Sidak correction, ~2,000 worms per replicate. In f, error bars represent mean ± s.e.m., n = 4 biologically independent samples, *P = 0.019, **P = 0.009 and ***P = 0.0006 for WT versus lbp-3 Tg, P = 0.0003 for lbp-3 Tg versus lbp-3 Tg:nhr-49(lf) and P = 0.0003 for lbp-3 Tg:nhr-49(lf) versus lbp-3 Tg:nhr-49(lf);neu-nhr-49, ****P < 0.0001 by two-way ANOVA with Holm–Sidak correction, ~2,000 worms per replicate. Source numerical data are available in source data.
with neuronal transcription via PPARγ. We observed the internalization of secreted LBP-3 into neurons, and FABPs are known to cooperate with PPARs in regulating transcriptional responses. We thus hypothesize that LBP-3–DGLA could directly act in concert with neuronal NHR-49 to regulate neuropeptide genes. However, we could not rule out the possibility that, within neurons, secondary lipid signals are derived from internalized DGLA and activate NHR-49 in the nucleus.

Our work highlights the crucial role of PUFAs in regulating lupil-4–induced longevity. The induction of PUFAs has also been linked with dietary-restriction-mediated longevity. Previous studies have reported that monounsaturated fatty acid (MUFA) supplementation is sufficient to extend C. elegans lifespan. One MUFA species, palmitoleic acid, is increased in lupil-4 Tg. However, through profiling the liposome bound to LBP-3, we did not detect an enrichment of palmitoleic acid. Although we could not rule out the possibility that the induction of palmitoleic acid contributes to lupil-4–induced longevity, it might not be involved in the LBP-3–mediated endocrine signalling mechanism.

Online content
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Methods

C. elegans strain maintenance. C. elegans strains obtained from Caenorhabditis Genome Center or generated in this study are listed in Supplementary Table 7. Strains were maintained on standard nematode growth medium (NGM) agar plates seeded with corresponding bacteria at 20 °C. The full list of C. elegans strains is provided in Supplementary Table 7.

Molecular cloning and generating transgenics. Tissue-specific lbp-3 or nlp-11 expression vectors were generated using Multisite Gateway System (Invitrogen) as previously described.

For generating nlp-11 and lbp-3 transgenic lines driven by their endogenous promoters, the whole genomic region including the promoter, 5′, untranslated region (UTR), coding sequence and 3′ UTR were first PCR-amplified and then fused together with sl2-GFP::znac-5 3′ UTR via fusion PCR. For nlp-11 and lbp-3, 2.2 kbp and 1.1 kbp of the upstream promoter region was used, respectively. The Gibson Assembly Method (NEB) was used to generate the following vectors. To amplify egl-21 and nhr-49, 612 bp and 2.4 kbp of the upstream promoter region was used, respectively. lbp-3ns fused to both RFP and sl2::gfp, egl-21 vectors. To amplify and nlp-11, 612 bp and 2.4 kbp of the upstream promoter region was used, respectively. lbp-3 fused to GFP, SAX-7 fused to GBP and GBP alone were amplified using tissue-specific promoter vectors. The chimeric lbp-3 sequence was ordered using IDT, while the 3xHA sequence was PCR-amplified and ligated into the tissue-specific promoter vectors.

Transgenic strains were generated and integrated as previously described and backcrossed to N2 at least five times.

Generating deletion mutants using CRISPR. All gene-specific mutations were generated using saturated single guide RNA (sgRNA) targeting throughout the nlp-11 loci. sgRNAs were identified using the http://crispr.mit.edu/ website. Possible sgRNAs were then screened for predicted efficacy using http://crispr.wustl.edu/. For nlp-11 deletion, we followed the protocol suggested by Dickinson et al. and Ward et al., while for lbp-3 deletion, we identified candidates using the protocol suggested by Paix et al. Genotyping PCR was performed using nlp-11 and lbp-3 spanning primers listed in Supplementary Table 8. Candidate worms with notable band shifting were saved and back-crossed at least four times with N2.

Lifespan assays. Lifespan assays were performed as previously described. For integrated transgenic strains and newly isolated CRISPR deletion mutants, the strains were backcrossed at least five times before lifespan analysis (Supplementary Tables 4 and 5).

For lifespan assays involving strains containing mutation of egl-21 or fat-3, 5′-fluoroerythroidine (FUDR) at a final concentration of 100 μM was added at L4 stage to prevent ageing irrelevant lethality due to internal eggs hatching. All the other lifespan assays did not use FUDR.

qRT–PCR. Total RNA was isolated as previously described. Synthesis of complementary DNA was performed using the amfiRvert Platinum cDNA Synthesis Master Mix (GenDEPOT). Quantitative PCR was performed using Kapa SYBR Ready PCR kit (Kapa Biosystems) in a Realplex PCR machine (Eppendorf), and values were normalized to rpl-32 as an internal control. All data shown represent three to four biologically independent samples. Primers used in this study are listed in Supplementary Table 8.

Intestines were dissected from day 1 adult lbp-3Tg with fat-3 RNAI supplemented by vehicle or DGLA for 12 h, and spun down at 20,000g for 2 min at 4 °C. Serum content was removed, and 10 μl of worm lysis buffer (containing 1:100 diluted DNase; both from Ambion Power SYBR Green Cells-to-Ct kit) was added. Lysis reaction was incubated at room temperature for 5 min, stopped by adding 2 μl of Stop Solution and incubated at room temperature for 2 min following the manufacturer protocol. The synthesized cDNA was used undiluted for qRT–PCR.

Fluorescent microscopy. Day 1 adult worms were mounted on 2% agarose pads containing 0.5% Na2, as anaesthetic on glass microscope slides. Fluorescent images were taken using confocal FV3000 (Olympus). Polygon selection tool was used to select coelomocytes’ area to be quantified, and average pixel intensity was averaged to obtain mean and standard deviation. In each imaging session, around 40,000 age-synchronized worms were acquired in full-scan and data-dependent MS2 mode.

For MS2 scanning, 20 dependent scans were acquired in each cycle. The MS2 resolution was 30k; HCD was used to fragment precursor ions with stepped collision energy 25, 30, 35; AGC target was 50,000. High-throughput analysis of lipidomic data was performed using Lipidsearch software (Thermo Fisher Scientific). Lipid quantification use precursor ion area and lipid were identified by matching product ion spectrum to lipid search library. Both precursor and product ions tolerance were set at 5 ppm. M-score threshold was set 2.0. Both positive and negative data were aligned on the basis of retention time tolerance 0.1 min and mean value. The final data were filtered on the basis of preferred ion adduct for each lipid class.

Lipid feeding. Age-synchronized worms were grown on NGM plates seeded with OP50 bacteria to day 1 adulthood. AA, DGLA and EPA (Nu-Check Prep) were dissolved in DMSO and diluted into OP50 bacterial food to a final concentration of 1 mM. Then, 300 μl of each mixture was added to standard 6-cm NGM plates that were dried in a laminar flow hood under dark conditions. Worms were collected after 12 h of lipid feeding under dark conditions followed by RNA extraction and qRT–PCR.

Protein expression and purification. WT C. elegans LBP-2 (resides 19–161) and LBP-3 (resides 16–165) were subcloned into pMCsG7-His vector. LBP-2 and LBP-3 in the pMCsG7 vector was transformed into Escherichia coli strain BL21 (DE3) cells. Cultures (1 litre in LB) were grown to an OD600 of 0.6 and induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside at 30 °C for 4 h, then harvested by centrifugation. For affinity purification and mass spectrometry studies with LBP-2, cells were lysed through sonication in a buffer containing 20 mM Tris–HCl pH 7.4, 500 mM NaCl, 25 mM imidazole, 5% glycerol, 5 mM 2-mercaptoethanol and 8 M urea. Unfolded LBP-2 was purified by nickel affinity chromatography in buffers containing 8 M urea. LBP-2 was refolded through stepwise dialysis over multiple days to remove urea. Refolded LBP-2 was further purified through size exclusion chromatography using a HiLoad 16/60 Superdex 75 column into a buffer containing 20 mM HEPES pH 7.5, 150 mM NaCl and 5% glycerol, 0.5 mM tris(2-carboxyethyl)phosphine. For affinity purification, ligand binding assays and mass spectrometry with LBP-3, cells were lysed through sonication in a buffer containing 20 mM Tris–HCl pH 7.4, 500 mM NaCl, 25 mM imidazole, 5% glycerol, 5 mM 2-mercaptoethanol, lysozyme, Dnase A and 100 μM phenylmethylsulfonyl fluoride. LBP-3 was purified by nickel affinity chromatography and followed by size exclusion chromatography using a HiLoad 16/60 Superdex 75 column.

Competitive fluorescence-based binding assay. Quantification of ligand binding was conducted via competition of the probe 1,8-ANS as previously described. LBP-2 and LBP-3 binding with C. elegans liposome. For each sample, 100,000 age-synchronized worms were grown on NGM plates seeded with OP50 bacteria and collected as young adults. The worms were washed three times in M9 buffer, one time in 1x PBS, pelleted in a minimal volume of 1.2 ml and resuspended in liquid nitrogen. Lipids were extracted from C. elegans lysates using the Bligh and Dyer method.
Fatty acids were extracted from LBP-2 and LBP-3 after binding with C. elegans lipid extracts. Fatty acid derivatives were generated as previously described. Briefly, dried lipid extracts were incubated with 200 µl of oxylal chloride (2 M in dichloromethane) for 5 min, then dried down under nitrogen gas.

Mass spectrometry for LBP-2: 5 µl of the LBP-2-derived fatty acid sample resuspended in methanol was injected onto a ThermoScientific Accucore C18 (4.6 × 100 mm, 2.6 µm) column using the Exxon/LC AD UPLC system at a 0.8 ml min⁻¹ flow rate, and a gradient solvent system containing 10 mM ammonium acetate, pH 7 in H₂O (solvent A) and 10 mM ammonium acetate, pH 7 in 100% acetonitrile (solvent B). Samples were chromatographically resolved using a stepwise gradient starting at 40% solvent B for 4 min, 100% solvent B for 5 min and then 65% solvent B for 5 min. Derivatized fatty acids were detected using a triple quadrupole mass spectrometer in positive ion mode. The following multiple reaction-monitoring transitions were used to detect the most abundant derivatized fatty acids. Derivatized fatty acids were quantified in MultiQuant 3.0.2 software (AB Sciex) using a calibration curve with the following fatty acids: myristic acid, palmitic acid, oleic acid, linoleic acid, stearic acid, AA and docosahexaenoic acid.

Western blot. At least 300 worms per genotype were grown on seeded NGM plates before being collected and snap-frozen on dry ice. The samples were lysed in worm lysis buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1 mM EDTA and 0.1% NP-40) containing a protease and phosphatase inhibitor cocktail (cOmplete Protease Inhibitor Cocktail, cat. no. 1169749801; PhosSTOP, cat. no. 4906845001; both from Sigma) and homogenized with motorized pestle pestle. The lysates were then centrifuged, and the supernatants were used for protein quantification using WT-1 and LBP-3/WT-3 lysates. The anti-β-actin antibody is from Santa Cruz (sc-7337, 1:2,000). Protein detection was performed using chemiluminescent substrate (ECL kit (Illumina) following the manufacturer's instructions. Lanes were pooled together and sequenced using Illumina NextSeq 500 system. RNA-Seq reads were aligned to the C. elegans reference genome using hisat2 with the default setting. HTSeq 0. 9. 17c for 5 min to count the reads mapped to each gene. DESeq2 was used to normalize the raw counts and identify differentially expressed genes (fold change ≥ 1.5; false discovery rate < 0.05).

Protein sequence alignment. The alignment was generated by toffee and illustrated by ESMrpt.

AlphaFold2 structures of the C. elegans LBP-2 and LBP-3. The C. elegans LBP-2 (F40F4.2) and LBP-3 protein (F40F4.4) sequences were downloaded from wormbase. The LBP-2 and LBP-3 structures were predicted using AlphaFold2 through the jupyter notebook for ColabFold (https://doi.org/10.1101/2021.08.15.456425 and https://doi.org/10.1038/s41586-021-03819-2). The ColabFold was used with the default parameters, and sequence alignments were generated by MMseq2 (https://doi.org/10.1101/2021.08.15.456425 and https://doi.org/10.1038/nbt.3988). The structure images were generated by UCSF Chimera.

Statistics and reproducibility. For all figure legends, asterisks indicate statistical significance as follows: NS, not significant (P > 0.05), *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001. Data were obtained by performing independently at least three biological replicates, unless specified in the figure legends. No statistical methods were used to predetermine the sample size. Non-parametric tests were used from the analyses. Two-tailed Student’s t-test or one-way or two-way analysis of variance (ANOVA) with Holm– Sidak corrections was used as indicated in the corresponding figure legends. n indicates the number of biological replicates. For survival analysis, statistical analyses were performed with SPSS software (IBM) using Kaplan–Meier survival analysis and log-rank test. Details on samples size, number of biological replicates and statistics for each experiment are provided in Supplementary Tables 4 and 5. For FIA profiling, statistical analysis was performed using two-way ANOVA test with Holm– Sidak correction comparing lipo-4 Tg (n = 9 biological replicates) vs. WT (n = 6 biological replicates), while for lipid profiling using lysosomal isolation, t-test was used to compare lipo-4 Tg (n = 4 biological replicates) vs. WT (n = 3 biological replicates). For RNA-seq, two-sided Wilcoxon rank test in R package DESeq2 was used. For qRT–PCR, t-test or one-way or two-way ANOVA with Holm– Sidak correction was used as indicated in the corresponding figure legends. Figures and graphs were constructed using GraphPad Prism 7 (GraphPad Software) and Illustrator (CC 2019; Adobe). The researchers involved in the study were not blinded during experiments or outcome assessment.

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

Data availability. Deep-sequencing (RNA-seq) data that support the findings of this study have been deposited into the NCBI Sequence Read Archive, and the accession codes for each biological sample are SAMN25414088, SAMN25414093, SAMN25414090, SAMN25414091, SAMN25414092, SAMN25414093, SAMN25414094, SAMN25414095, SAMN25414096, SAMN25414097 and SAMN25414098. Source data are provided with this paper. The lipidomics data are deposited into the metabolights database at the following link: https://www.ebi.ac.uk/metiobits/met/METBLS5654. The wormbase https://wormbase.org is used for searches related to C. elegans. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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Author contributions

M.S., A.F. and M.C.W. conceived the project and designed the experiments. M.S., A.F., L.Y., F.J., A.C., M.C.T., I.A.N., P.H. and Y. Yu performed experiments. Q. Zhang conducted lip-4 Tg RNA-seq, and J.D.D. conducted lbp-3 Tg RNA-seq. Q. Zhao conducted the structural simulation and alignment, and Y. Ye conducted the bioinformatic transcriptome analysis. M.S. and M.C.W. wrote the manuscript. M.S., F.J., Q. Zhao, M.C.T., A.C., W.B.M., E.A.O., L.H., J.W. and M.C.W. edited the manuscript.

Competing interests

J.W. is a cofounder of Chemical Biology Probes LLC and Coactigon Inc. The focuses of these companies are unrelated to this study. The other authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Peripheral lysosomal lipolysis up-regulates neuropeptide signaling (related to Fig. 1).  

(a) Principal Component Analysis (PCA) of lipl-4 Tg and WT worms falling in two distinct clusters.  
(b) The loss-of-function mutant of egl-21(lf) shows lifespan extension, and lipl-4 Tg cannot further enhance this lifespan extension as it does in the WT condition.  
(c) In the background of daf-16 RNAi inactivation, the longevity effect caused by egl-21(lf) is suppressed. daf-16 RNAi is thus used to eliminate the contribution from ILP reduction in the egl-21(lf) mutant.  
(d) Inactivation of daf-16 does not affect the longevity effect of lipl-4 Tg.  

(a) PCA analysis using two-sided statistical test, (b–d) n=3 biologically independent samples, n.s. p>0.05 and ***p<0.001 by long-rank test, 60-120 worms per replicate. See Supplementary Tables 4-5 for full lifespan data.
Extended Data Fig. 2 | NLP-11 neuropeptide acts in neurons to promote longevity (related to Fig. 2). **a** A schematic representation of the nlp-11(rax51) loss-of-function mutation. White boxes represent the nlp-11(rax51) deletion, black lines represent introns. The mutant lacks the three exons and the transcriptional start site. **b** nlp-11 is transcriptionally up-regulated by lipl-4 Tg. **c** Constitutive expression of nlp-11 driven by its endogenous promoter extends lifespan. **d** The transgenic strains expressing nlp-11 under its endogenous promoter (nlp-11p::nlp-11::sl2-GFP) reveals the expression of nlp-11 in intestinal and neuronal cells. Scale bar 100 µm. **e, f** Neuron-specific overexpression of nlp-11 prolongs lifespan (**e**), but intestine-specific overexpression has no such effect (**f**). (b) Error bars represent mean ± s.e.m., n=3 biologically independent samples, ***p<0.0001 by two-tailed Student’s t-test, ~2000 worms per replicate. (c, e, f) n=3 biologically independent samples, n.s. p>0.05 and *** p<0.001 by long-rank test, 60-120 worms per replicate. See Supplementary Table 4 for full lifespan data. Source numerical data are available in source data.
Extended Data Fig. 3 | Lysosome-derived PUFAs in the periphery regulate neuropeptide and longevity (related to Fig. 3). a) The level of TAG is decreased in the purified lysosomes from lipl-4 Tg compared to those from WT worms. b) The loss-of-function mutant fat-1(lf) suppresses lipl-4 Tg longevity. c) In the loss-of-function mutant of fat-3(lf), the lifespan-extending effect of lipl-4 Tg is shortened. (a) Error bars represent mean ± s.e.m., n=3 (WT) and n=4 (lipl-4 Tg) biologically independent samples, **p=0.0025 by two-tailed Student’s t-test, ~ 200,000 worms per replicate. (b, c) n=3 biologically independent samples, n.s. p>0.05 and *** p<0.001 by log-rank test, 60-120 worms per replicate. See Supplementary Table 4 for full lifespan data. Source numerical data are available in source data.
Extended Data Fig. 4 | Peripheral lipid chaperone LBP-3 regulates neuropeptide and longevity (related to Fig. 4). 

a, b) The induction of egl-3 (a) and egl-21 (b) in lipl-4 Tg is suppressed by RNAi inactivation of either lbp-2 or lbp-3, but not lbp-1. 
c) Schematic representation of lbp-2 and lbp-3 loss-of-function mutants. White boxes represent the lbp-2(rax63) and lbp-3(rax60) deletions, black boxes represent insertions, while black lines represent introns. The mutants lack the entire first exon and transcriptional start site. 
d) The transgenic strain that carries constitutive expression of lbp-3 (lbp-3 Tg) driven by its own endogenous promoter prolongs lifespan. 
e) PCA analysis of lbp-3 Tg and WT worms falling in two distinct clusters. 
f) Inactivation of daf-16 does not affect the longevity effect of lbp-3 Tg. daf-16 RNAi knockdown used to eliminate the influence from ILP reduction in egl-21(lf). 
g) Overexpression of lbp-3 selectively in the intestine extends lifespan. (a, b) Error bars represent mean ± s.e.m., n=4 biologically independent samples, n.s. p>0.05, *p=0.0104, **p=0.0018, ***p=0.0001 and ****p<0.0001 by two-way ANOVA with Holm-Sidak correction, ~2000 worms per replicate. (e) PCA analysis using two-sided statistical test. (d, f, g) n=3 biologically independent samples, n.s. p>0.05 and *** p<0.001 by long -rank test, 60-120 worms per replicate. See Supplementary Tables 4-5 for full lifespan data. Source numerical data are available in source data.
Extended Data Fig. 5 | LBP-3 secreted from the periphery regulates neuropeptide and longevity (related to Fig. 5). a) LBP-3 and the lysosomal membrane protein LMP-1 are visualized by their GFP and RFP fusions, respectively. LBP-3::GFP colocalizes with LMP-1::RFP and Lysotracker Red staining at lysosomes, and is also detected in the cytosol. Scale bar 10µm. b) Intestine-specific overexpression of lbp-3 lacking its secretory signal (lbp-3ns) fails to extend lifespan. n=3 biologically independent samples, n.s. p>0.05 by long -rank test, 72-100 worms per replicate. See Supplementary Table 4 for full lifespan data.
Extended Data Fig. 6 | DGLA regulates LBP-3 secretion, neuropeptides and longevity (related to Fig. 6). a) Intestine was dissected from *lipl-4 Tg* with *fat-3 RNAi* knockdown and used for qPCR analysis. The expression of *egl-21* is not detectable in the dissected intestine, while the expression of *egl-3* is weakly detected. The transcriptional level of either *egl-3* or *egl-21* is not affected by DGLA supplementation. DMSO serves as the vehicle control. 

b) *egl-21* transcripts are measured in *lipl-4 Tg* with *fat-3 RNAi* using smFISH (red Quasar 670). Upon DGLA supplementation, the *egl-21* transcript level is increased compared to the vehicle control. Scale bar 10µm. Representative images from three biological repeats. (a) Error bars represent mean ± s.e.m., n=3 biologically independent samples, n.s. p>0.05 by two-way ANOVA with Holm-Sidak correction, ~20 dissected intestines per replicate. (b) n=3 biologically independent samples, ****p<0.0001 by two-tailed Student’s t-test, 12 worms per replicate. Source numerical data are available in source data.
Extended Data Fig. 7 | DGLA binding specificity of LBP-3 mediates its effects (related to Fig. 7).  

**a)** After incubation with the *C. elegans* liposome, fatty acids bound to LBP-2 or LBP-3 proteins were analyzed with mass spectrometry. In addition to PUFAs shown in Fig. 5, SFAs and MUFAs also bind to LBP-2 and LBP-3 with different preferences.  

**b)** The LBP-3 chimeric protein structure predicted using AlphaFold2. The two helix regions from LBP-2 are highlighted in purple.  

**c)** Western-blots of WT and transgenic worms overexpressing LBP-3 chimeric proteins fused with 3XHA-tag. LBP-3::3xHA fusion proteins are detected in both chimeric lines. β-actin is used as a control.  

**d, e)** Intestine-specific overexpression of the chimeric *lbp-3(chim)* does not affect the transcription of *egl-3* or *egl-21* (d) and fails to extend lifespan (e). (d) Error bars represent mean ± s.e.m., n=3 biologically independent samples, n.s. p>0.05 by two-way ANOVA with Holm-Sidak correction, ~300 worms per replicate. (e) n=3 biologically independent samples, n.s. p>0.05 by long-rank test, 72-100 worms per replicate. See Supplementary Table 4 for full lifespan data. Source numerical data and unprocessed blots are available in source data.
Extended Data Fig. 8 | Neuronal transduction of peripheral lipid signals to regulate longevity (related to Fig. 8). a) In the transgenic line expressing GBP polycistronic mKate in neurons alone, no GFP signals are detected in neurons. Scale bar 30µm and 10µm in the inset. b) In the transgenic line expressing secretable LBP-3::GFP selectively in the intestine alone, GFP signals are only strongly detected in the intestine and pharynx. Scale bar 30µm and 10µm in the inset. c) In the transgenic line expressing GBP tagged with the extracellular domain of SAX-7 (GBP::SAX-7) and polycistronic mKate in neurons and secretable LBP-3::GFP in the intestine, strong GFP signals are detected in neurons. Scale bar 30µm and 10µm in the inset. d) Heatmap showing averaged nlp-11 and nhr-49 gene expression per neuronal cell type (threshold equal to two). The color of circles represents relative gene expression, while the size of circles indicates the percentage of cells expressing the gene within each neuron cluster. The heatmap is generated using the online webtool CenGeneApp. Neuronal cell types shared between nhr-49 and nlp-11 are marked by black arrows.
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  Give P values as exact values whenever suitable.
- [ ] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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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: Fluorescent images were taken using confocal FV3000 (Olympus). Western-blot images were acquired using Image Quant LAS 500. Free fatty acids were identified using lipidssearch 4.2.27 software (Thermo Fisher Scientific).

Data analysis: The commercial softwares used for data collection are specified in the methods section.

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All data generated or analyzed during this study are included in this manuscript and the Extended Data Materials. The RNA-seq data has been deposited into the NCBI Sequence Read Archive (SRA) and the accession codes for each biological sample are SAMN25414087, SAMN25414088, SAMN25414089, SAMN25414090, SAMN25414091, SAMN25414092, SAMN25414093, SAMN25414094, SAMN25414095, SAMN25414096, SAMN25414097, and the BioProject accession code is PRJNA801907 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA801907).
Field-specific reporting

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Life sciences study design

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

Sample size
No sample-size calculation was performed. Sample size was used based on previous literature and recognized in the worm field. For each experiment, n values are provided in the figure legends and in the section of Statistics and Reproducibility. The amount of animals used for each experiment is reported in the Method session.

Data exclusions
No data were excluded from the analysis in the study

Replication
All experimental replications were repeated at least three independent times and the detailed information is specified in Figure Legends and Statistics And Reproducibility

Randomization
Worms with the same genotype were randomly chosen and grouped into experiments.

Blinding
Investigators were not blinded, as for each experiment and analysis worms from different genotypes were compared to wild-type/control

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
Anti-HA, Rabbit, Cell Signaling, #C29F4, https://www.cellsignal.com/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724 Anti-beta-actin, Mouse, Santa Cruz, sc-47778, https://www.scbt.com/p/beta-actin-antibody-c4

Validation
Validation statement from the company: HA-Tag (C29F4) Rabbit mAb detects exogenously expressed proteins containing the HA epitope tag in all the species specified including C. elegans. In our study, wild type worms that do not express HA-tagged proteins were used as negative controls for the Anti-HA antibody (Cell Signaling, #C29F4). Validation of Anti-beta-actin, Mouse, Santa Cruz, sc-47778 was previously described (PMID:27534274, PMID:32966783, PMID:30599151).

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals
Caenorhabditis elegans used in this study:
N2 Bristol Strain
raxis[ges-1::pil-4::sl2-GFP; myo-2p::mCherry]
egp-21[n476]
raxis[ges-1::pil-4::sl2-GFP; myo-2p::mCherry],egp-21(n476)
fat-3[wa22]
raxis[ges-1::pil-4::sl2-GFP; myo-2p::mCherry],fat-3[wa22]
fat-1[wa9]
Wild animals

No wild animals were used in this study

Field-collected samples

No field-collected samples were used in this work

Ethics oversight

No ethical approval or guidance required

Note that full information on the approval of the study protocol must also be provided in the manuscript.