MALT-1 mediates IL-17 neural signaling to regulate C. elegans behavior, immunity and longevity

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Besides pro-inflammatory roles, the ancient cytokine interleukin-17 (IL-17) modulates neural circuit function. We investigate IL-17 signaling in neurons, and the extent it can alter organismal phenotypes. We combine immunoprecipitation and mass spectrometry to biochemically characterize endogenous signaling complexes that function downstream of IL-17 receptors in C. elegans neurons. We identify the paracaspase MALT-1 as a critical output of the pathway. MALT1 mediates signaling from many immune receptors in mammals, but was not previously implicated in IL-17 signaling or nervous system function. C. elegans MALT-1 forms a complex with homologs of Act1 and IRAK and appears to function both as a scaffold and a protease. MALT-1 is expressed broadly in the C. elegans nervous system, and neuronal IL-17–MALT-1 signaling regulates multiple phenotypes, including escape behavior, associative learning, immunity and longevity. Our data suggest MALT1 has an ancient role modulating neural circuit function downstream of IL-17 to remodel physiology and behavior.
Immune signaling pathways can regulate the development and function of the nervous system in both health and disease. Many of these effects are mediated by cytokines, small, secreted proteins that can participate in neuroimmune and interneuronal communication. For example, low levels of IL-1β and TNFα regulate synaptic and homeostatic plasticity in healthy animals; pathological levels of proinflammatory cytokines during inflammation can disrupt fetal brain development, alter adult behavior, and drive hyperalgesia and neuroinflammatory diseases. Progression of neurodegenerative diseases, including Alzheimer’s, Parkinson’s and Amyotrophic lateral sclerosis (ALS), has also been associated with chronic inflammation.

Recent work shows that the interleukin 17 (IL-17) proinflammatory cytokine can modify neural circuit activity. In a rodent model of infection during pregnancy, IL-17 secretion during maternal immune activation drives autism-related behaviors in the pups. This phenotype is associated with hyperactivity of a specific cortical sub-region that expresses IL-17 receptors (IL-17R) in mice. IL-17 can also lower the activation threshold of nociceptive neurons, and contributes to mechanical hyperalgesia. In C. elegans, IL-17Rs are expressed throughout the nervous system, and ILC-17.1 (interleukin cytokine 17 related 1), a homolog of mammalian IL-17s, has been shown to act on the RMG hub interneurons, increasing their response to presynaptic input from oxygen (O2) sensors. The increased circuit gain conferred by ILC-17.1 enables C. elegans to persistently escape 21% O2, an aversive cue associated with surface exposure. Specific sensory responses and behaviors are thus modulated by IL-17 across distantly-related species, suggesting IL-17 has broad and conserved roles in regulating neuronal properties.

While IL-17’s action on the nervous system is now established, its molecular effectors there are poorly understood. Moreover, the extent to which IL-17 signaling contributes to brain function and physiology is unclear, even in the well-defined C. elegans nervous system.

Here, we report that IL-17 signaling in the C. elegans nervous system is mediated by the paracaspase MALT-1. MALT-1 is an ancient protein studied extensively, and almost exclusively, in the mammalian immune system. It is a key signaling molecule in innate and adaptive immunity, mediating signaling from ITAM-containing (immunoreceptor tyrosine-based activation domain) receptors, including the B-cell and T-cell receptors. MALT1 has not been shown to mediate IL-17 signaling, but there has been speculation of such involvement. In situ hybridization suggests widespread MALT1 expression in mouse brain, (Allen Brain Atlas), but no physiological role in neurons has been reported. We find that C. elegans MALT-1 is expressed throughout the nervous system and forms an in vivo complex with IL-17 signaling components, namely the C. elegans homologs of Act1, IRAK and IκB/IκBNS. We show that MALT-1 acts both as a protease and a scaffold to regulate neural function. Defects in IL-17/MALT-1 signaling lead to reconfigured gene expression, and changes in behavior and physiology, including altered immunity and extended lifespan.

**Results**

**Proteomics identifies an ACTL-1–IRAK–MALT-1–NFKI-1 complex.** C. elegans IL-17 signaling components appear to be expressed predominantly in the nervous system. We epitope tagged all soluble IL-17 pathway components highlighted by genetics, immunoprecipitated them from C. elegans extracts, and identified interacting proteins using mass spectrometry (MS, Fig. 1a).

ACTL-1 and PIK-1 are C. elegans orthologs of mammalian Act1 and IRAKs, respectively, and signal downstream of the C. elegans IL-17 co-receptors ILCR-1 and ILCR-2. Genetic analysis suggests NFKI-1, a homolog of mammalian IκB and IκBNS, acts downstream of ACTL-1, PIK-1, and ILCR-1/ILCR-2 co-receptors.

We tagged endogenous ACTL-1 with a FLAG epitope, endogenous PIK-1 with a Myc epitope, and integrated an nfkj-1:gfp transgene. We showed the tagged proteins were functional (Supplementary Fig. 1), and immunoprecipitated them from C. elegans extracts. As controls, we immunoprecipitated proteins unrelated to IL-17 signaling tagged with the same epitopes. Using mass spectrometry (LC-MS/MS) we identified specific interactors for each signaling component (Fig. 1b–g and Supplementary Data 1a–c).

As expected from co-IP experiments using mammalian tissue culture cells, PIK-1 co-precipitated specifically with ACTL-1 (Fig. 1b), and reciprocally, ACTL-1 co-precipitated specifically with PIK-1 (Fig. 1c). IP of NFKI-1 also identified ACTL-1 and PIK-1/IRAK as specific interactors, suggesting these proteins form a complex in vivo (Fig. 1d). We identified other apparently specific interactors for each component. These are listed in Supplementary Data 1 as a resource.

The C. elegans ortholog of the paracaspase MALT1 consistently co-immunoprecipitated with each of ACTL-1, PIK-1 and NFKI-1 (Fig. 1b–g). MALT1 paracaspases are cysteine proteases with specificity for arginine residues. Their caspase-like protease domain is highly conserved, as is their domain organization, which consists of an N-terminal death domain (DD) followed by 2-3 Ig (immunoglobulin)-like motifs that flank the paracaspase domain (Supplementary Fig. 2a). Mammalian MALT1 signals downstream of B cell, T cell, and other cell surface receptors containing an ITAM motif, and forms a filamentous complex called the CRM signosomes that contains a CARD domain protein, BCL10, and MALT1 (Supplementary Fig. 2b). The functions of MALT1 in the immune system are under intense scrutiny, but its roles elsewhere, and in invertebrates, have not been established.

To confirm the biochemical interactions of MALT1 with C. elegans IL-17 signaling components, we expressed functional, GFP-tagged MALT1 pan-neuronally, and identified interacting partners using IP/MS of extracts from the transgenic C. elegans strain. As a control, we performed IP/MS on extracts from strains expressing GFP-tagged neuronal proteins unrelated to IL-17 signaling. ACTL-1, PIK-1, and NFKI-1 each interacted specifically with MALT1-GFP (Fig. 1h, i). We also identified other specific MALT1 interactors (Supplementary Data 1d) including the C. elegans ortholog of mammalian SARM1, called TIR-1, which is implicated in the immune response, left/right asymmetry of an olfactory neuron, and experience-dependent plasticity. MALT1 also interacted specifically with a large group of proteins implicated in RNA metabolism, including splicing factors and poly A binding proteins, suggesting it may localize to the nucleus or ribonucleoprotein particles (RNP) (Supplementary Fig. 3).

MALT1 promotes aggregation and escape from 21% O2. MALT1 has not previously been implicated in IL-17 signaling or neural function. In C. elegans, ILC-17.1 signals through the ILCR-1/ILCR-2 receptors on the RMG interneurons to increase RMG responsiveness to input from their pre-synaptic partner, the URX O2-sensing neurons (Fig. 1j). Increased RMG signaling enables C. elegans to strongly and persistently escape 21% O2 and to aggregate. To probe the functional relevance of our proteomics data we sought malt-1 alleles in a collection of 583 strains isolated in a genetic screen for aggregation-defective mutants. This collection has been subjected to whole genome sequencing, and previously yielded IL-17 pathway mutants. Four strains in...
the collection harbored malt-1 alleles; one introduced a premature stop codon; another mutated the highly conserved E464 residue (Supplementary Fig. 4a and b), which is essential for catalytic activity in mammalian MALT132. We mapped the aggregation defect of this strain to an interval containing malt-1 (Supplementary Fig. 4c). Targeted disruption of malt-1 using CRISPR/Cas9 resulted in an aggregation-defective strain whose phenotype could be rescued using a wild-type malt-1 transgene (Fig. 1k; and Supplementary Fig. 4f). These data confirm that MALT-1, like IL-17 signaling, promotes aggregation.

C. elegans aggregate to escape 21% O2, a signal of surface exposure33–35. In wild C. elegans isolates, 21% O2 evokes
sensed by URX neurons, which tonically signal to RMG hub interneurons. IL-17 signaling increases the responsiveness of RMG neurons to promote escape from 21% O₂ if allowed to settle over a 2 h period (Fig. 1l, m), consistent with increased aggregation defects of the domesticated N2 lab strain37. By contrast, we observed almost complete rescue of the O₂-sensing neurons, restored aggregation (Fig. 3b). These phenotypes recapitulate those observed in IL-17 signaling mutants (Fig. 4b). Together, these data indicate that, like ILCR-1 and ILCR-2, MALT-1 functions in both pre-synaptic and post-synaptic neurons in the O₂-sensing circuit.

The malt-1 and ilc-17.1 mutant phenotypes were not additive. Both the Ca²⁺ signaling (Fig. 4c) and behavioral response (Fig. 4d) defects of malt-1; ilc-17.1 double mutants resembled those of single mutants, suggesting MAL-T1 and ILC-17.1 function in the same pathway. Similarly, the RMG response defects of malt-1 mutants were not enhanced by defects in PIK-1/IRAK (Supplementary Fig. 4h). Together, our biochemical, genetic, behavioral and physiological data suggest that the paracaspase MAL-T1 mediates IL-17 signaling in neurons, most likely via a signaling complex made up of ACTL-1–IRAK/PIK-1–NFKI-1.

To examine if malt-1 is required developmentally, we expressed it selectively in adults using a heat-shock-inducible promoter. Without heat-shock, the phsp-16::malt-1 cDNA transgene did not...
Heat-shock-induced expression during the 4th larval stage was (Fig. 5b). Unexpectedly, overexpressing w a su n a b lor e s c u et h ep e n t y ph oo trauma of C374A rescued by pan-neuronal expression of malt-1 (Supplementary Fig. 6a). By contrast, a is expressed from both promoters simultaneously. Lines indicate average speed and shaded regions indicate SEM.

**MALT-1 functions in RMG interneurons.** a A MALT-1::mCherry translational fusion, expressed from its endogenous promoter (4 kb), is expressed in RMG interneurons. RMG is recognized by its characteristic shape, location, and using a flp-5p::gfp reporter. Similar results were obtained in 3 experiments. Scale bars: 20 μm. b Expressing malt-1 cDNA from either the flp-5 promoter (RMG, ASG, PVT, I4, M4, and pharyngeal muscle), or the gcy-32 promoter (URX, AQR and PQR) rescues the aggregation defect of malt-1 mutants. N = 4 assays. Data are presented as mean values +/- SEM. **P < 0.01, ***P < 0.001, one-way ANOVA with Tukey’s post hoc HSD. c The O2-response defect of malt-1 mutants is partially rescued by expressing malt-1 cDNA from the flp-5 promoter (RMG, ASG, PVT, I4, M4, and pharyngeal muscle), or the gcy-32 promoter (URX, AQR and PQR), and almost completely rescued when malt-1 is expressed from both promoters simultaneously. Lines indicate average speed and shaded regions indicate SEM. n = 55 animals (npr-1), n = 85 animals (npr-1; malt-1), n = 58 animals (npr-1; malt-1; gcy-32p::malt-1), n = 66 animals (npr-1; malt-1; flp-5p::malt-1), n = 46 animals (npr-1; malt-1; gcy-32p::malt-1; flp-5p::malt-1). Plots show average speed (line) and SEM (shaded regions). *P < 0.05, **P < 0.01, ***P < 0.001, two-sided Mann-Whitney U test.

**MALT-1 functions as a protease in the nervous system.** In the mammalian immune system MALT1 functions both as a scaffold and as a protease. To examine if MALT-1 acts as a protease in neurons we edited the active site cysteine of the endogenous malt-1 gene to alanine. The equivalent mutation is used in a paracaspase-dead model in mice. Endogenous malt-1, PIK-1, MALT-1, and NFKI-1 were each co-immunoprecipitated with MALT-1 in a multiple knock-in strain (Fig. 6a).

To analyze the signaling complex further we carried out IPs from strains overexpressing NFKI-1-GFP. When we quantitatively compared NFKI-1 complexes from WT, malt-1 and pik-1 mutants, using IP/MS, we found that the amount of PIK-1/IRAK co-precipitating with NFKI-1 was reduced when MALT-1 was absent (Fig. 6b). By contrast, in pik-1 mutants the interaction between MALT-1 and NFKI-1 was not significantly reduced (Fig. 6c). These data suggest that NFKI-1 recruitment to the signaling complex requires MALT-1.

To ask if MALT-1 and NFKI-1 interact directly, we expressed epitope-tagged versions of the proteins in E. coli, and performed pairwise tests for co-immunoprecipitation. MALT-1–HA immunoprecipitated NFKI-1–V5, and conversely NFKI-1–V5 immunoprecipitated MALT-1, supporting a direct physical interaction (Fig. 6d). MALT-1 also interacted directly with ACTL-1 (Fig. 6e). These complexes are structurally related filamentous oligomers that assemble in the cytosol. IkB family proteins can perform

**MALT-1 promotes assembly of IL-17 signaling complexes.** To extend our in vivo proteomic analyses we made a strain in which endogenous ACTL-1, PIK-1, MALT-1, and NFKI-1 were each tagged with different epitopes. To corroborate our LC-MS/MS data we first showed that ACTL-1, PIK-1, and NFKI-1 specifically co-immunoprecipitated with MALT-1 in a multiple knock-in strain (Fig. 6a).

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both cytoplasmic and nuclear functions downstream of signalosome assembly43. Fractionation of a C. elegans lysate by gel filtration revealed that ACTL-1 and PIK-1 exist mostly as high-molecular weight species; they eluted in the heaviest fractions, including the void, of a gel filtration column (Fig. 6g and Supplementary Fig. 7). MALT-1 and NFKI-1 ran most as smaller species (~50–200 kDa), but they were also detectable in the heavier ACTL-1-containing and PIK-1-containing fractions. The high-molecular weight species we observed may be an artifact of unsolubilized membrane or protein aggregation, or may represent interactions with additional proteins. Alternatively, they may report oligomeric complexes of ACTL-1/PIK-1/MALT-1 related to the Myddosome and the CBM signalosome44,45, although this hypothesis requires further testing.

To determine the sub-cellular localization of IL-17 signaling components, we separated the nuclear and cytosolic fractions of our lysate. ACTL-1-FLAG and MALT-1-HA were consistently detected in both cytoplasmic and nuclear fractions (Fig. 7a). NFKI-1-V5 was predominantly in nuclear fractions (Fig. 7a; five replicates), although as NFKI-1-V5 immunoreactivity in the fractions was weak we cannot rule out the possibility that NFKI-1 was also present in the cytoplasmic fractions at levels below our detection threshold. It is notable that NFKI-1 specifically co-immunoprecipitated with transcription factors and chromatin state modifiers, including CREB binding protein (CBP), a histone acetyltransferase44, suggesting that NFKI-1 regulates transcription (Supplementary Data 1c).

MALT-1 and NFKI-1 provide partially parallel IL-17 outputs. Overexpressing NFKI-1 suppresses ilc-17.1, actl-1 and pik-1 null phenotypes, suggesting NFKI-1 functions downstream of those signaling components43. Overexpressing MALT-1 also rescued the O2 arousal defects of ilc-17.1 mutants to that of controls (Fig. 7b and c). To test whether MALT-1 functions upstream or downstream of NFKI-1, we asked whether overexpressing either component rescued a null mutant of the other. Overexpressing NFKI-1 in malt-1(null) mutants, or MALT-1 in nfk-1(null) animals, fully rescued the aggregation defect but either did not restore, or only partly restored, the arousal response to 21% O2 (Fig. 7d–g). These data suggest MALT-1 and NFKI-1 provide partially parallel outputs for IL-17 signaling.

Disrupting IL-17 signaling reprograms gene expression. In mammalian tissues IL-17 acts globally to drive pro-inflammatory gene expression45. We defined a transcriptional fingerprint of C. elegans IL-17 signaling by comparing the whole-animal RNA-seq profiles of ilc-17.1, malt-1, and nfk-1 mutants to that of controls...
Mutants of the IL-17 receptor, npr-1, show defects in several behaviors, including attraction to NaCl and response to pathogenic bacteria. The role of MALT-1 in IL-17 signaling is illustrated in the following figure (Fig. 5).

**Fig. 5 MALT-1 has enzymatic roles in IL-17 signaling.**

- **a** MALT-1’s function in the nervous system requires its protease active site. Mutants expressing a catalytically inactive MALT-1 (C374A) show different responses compared to controls.
- **b** and **c** show plots of average speed (line) and SEM (shaded regions) for animals under different conditions.

MALT-1 and IL-17 signaling regulate multiple behaviors. The widespread expression of MALT-1 and other IL-17 signaling components in the nervous system suggests their involvement in various behaviors. For example, increased pathogen resistance is often associated with increased lifespan. It was observed that MALT-1 strongly and specifically inhibits aggregation behavior and escape from 21% O₂, indicating that MALT-1 acts downstream of the IL-17 signaling pathway.

Increased lifespan is observed when MALT-1 mutants are rescued by pan-neuronal expression of MALT-1 in the nervous system. This suggests that MALT-1 acts as a direct regulator of the immune system and lifespan in C. elegans.

Neural IL-17–MALT-1 signaling alters immunity and lifespan. The role of MALT-1 in the nervous system is further supported by the observation that animals lacking both MALT-1 and IL-17 receptors show significantly reduced lifespan compared to controls.

**References:**

1. Supplementary Data 3
2. NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-020-15872-y
3. FIG. 5 MALT-1 has enzymatic roles in IL-17 signaling. a-c MALT-1’s function in the nervous system requires its protease active site. a malt-1(syb296) mutants that express a catalytically inactive MALT-1 (C374A) show O₂ response defects compared to those of malt-1(null) animals. Pan-neuronal expression of malt-1 cDNA rescues this phenotype. n = 53 animals (npr-1), n = 55 animals (npr-1; malt-1(db1194)), n = 50 animals (npr-1; malt-1(syb296)), n = 29 animals (npr-1; malt-1(syb296); rab-3p::malt-1). Plots show average speed (line) and SEM (shaded regions). ***P = 2.95e−09, two-sided Mann-Whitney U test.
4. b cDNA encoding a MALT-1 C374A catalytically inactive protein, expressed from the npr-1; malt-1; rab-3p::malt-1 promoter, does not rescue the O₂ response defects of malt-1 mutants. Corresponding data to npr-1 and npr-1; malt-1; rab-3p::malt-1 in b are the same as those shown in Fig. 1m, and were obtained in parallel to the genotypes shown. n = 46 animals (npr-1), n = 74 animals (npr-1; malt-1), n = 71 animals (npr-1; malt-1; rab-3p::malt-1). Plots show average speed (line) and SEM (shaded regions). NS, P = 0.693918, two-sided Mann-Whitney U test. c Overexpressing MALT-1 C374A cDNA in npr-1 animals inhibits the arousal response to 21% O₂. n = 53 animals (npr-1), n = 87 animals (npr-1; rab-3p::malt-1 (C374A)). Plots show average speed (line) and SEM (shaded regions). ***P = 1.09e−12, two-sided Mann-Whitney U test. See also Supplementary Fig. 6.
inhibits the behavior and physiology of MALT-1 signalosome to modify neural properties and remodel resistance in malt-1 (Fig. 8i). Thus TIR-1/SARM can still promote PA14 resistance was reduced in malt-1 expression of malt-1 rescued by either intestine-specific or nervous system-specific expression of malt-1 (Supplementary Fig. 9a), and this reduction could be rescued by either intestine-specific or nervous system-specific expression of malt-1 (Supplementary Fig. 9b). However, PA14 resistance was reduced in malt-1; tir-1 double mutants compared to malt-1 (Fig. 8i). Thus TIR-1/SARM can still promote PA14 resistance in malt-1 mutants, and while overall IL-17 signaling is reduced in malt-1; tir-1 double mutants compared to wild type. NFKI-1::GFP was purified using GFP-Trap beads, and immunoprecipitated proteins labeled using tandem mass tags (TMT-labeling). The average relative abundance in two biological replicates is shown. p-values are reported by a two sample t-test. The amount of PIK-1 that co-IPs with overexpressed NFKI-1::GFP is significantly reduced in malt-1(db1194) mutants (b). The relative amount of MALT-1 that co-IPs with NFKI-1 is not significantly decreased in pik-1(tm2167) mutants (c). Peptides derived from MALT-1 and PIK-1 are shown in Supplementary Data 2. 

Discussion

Our data suggest that MALT1 modules neural circuit function in C. elegans, by acting as a protease and a scaffold. MALT1 participates in an ACTL-1-IRAK-MALT1 signaling complex that mediates IL-17 signaling. The high molecular weight of this complex in C. elegans extracts suggests it may form a structure related to the MYD88-IRAK4-IRAK2 Myddosome and CARMA1-BCL10-MALT1 CBM signalsome, although this hypothesis needs further study. MALT1 directly binds ACTL1 in vitro, and yeast two hybrid data suggest ACTL1 directly binds C. elegans IRAK4. MALT1 also interacts directly with NFKI-1, a homolog of mammalian IκB/IkBNS, and can signal through both NFKI-1-dependent

Fig. 6 MALT1 has scaffolding roles in IL-17 signaling. a Endogenous ACTL-1, PIK-1 and NFKI-1 co-IP with endogenous MALT1 in npr-1 animals. Anti-HA antibody was used to immunoprecipitate MALT1 complexes. Half of the lysate was immunoprecipitated with anti-IgG as a control. Tags were knockin by CRISPR. Similar results were obtained in 3 experiments. b and c Volcano plot showing quantitative LC-MS/MS of proteins that interact with NFKI-1::GFP in malt-1 and pik-1 mutants compared to wild type. NFKI-1::GFP was purified using GFP-Trap beads, and immunoprecipitated proteins labeled using tandem mass tags (TMT-labeling). The average relative abundance in two biological replicates is shown. p-values are reported by a two sample t-test. The amount of PIK-1 that co-IPs with overexpressed NFKI-1::GFP is significantly reduced in malt-1(db1194) mutants (b). The relative amount of MALT1 that co-IPs with NFKI-1 is not significantly decreased in pik-1(tm2167) mutants (c). Peptides derived from MALT-1 and PIK-1 are shown in Supplementary Data 2. d and e IPs of His10-tagged C. elegans ACTL-1-FLAG, MALT1-1HA, and NFKI-1-V5 recombinantly expressed in E. coli show that MALT1 can directly bind NFKI-1 (d) and ACTL1 (e). d was performed once, e was performed three times with similar results. f Interaction of the MALT1 Death Domain (1-81) with the N-terminus of NFKI1 (1-374) in a yeast two-hybrid assay using nutritional selection (ADE2). Rows show 10-fold serial dilutions of each of the seven Prey–Bait combination strains tested and shown top. Similar results were obtained in 2 experiments. g Elution profiles of ACTL1-1, PIK-1, MALT1-1, and NFKI-1 proteins in a C. elegans extract run on a Superose 6 Gel Filtration column and visualized by immunoblot. All four proteins can be found in high molecular weight complexes. Similar profiles were observed in two runs. See also Supplementary Fig. 7 and Supplementary Data 2.

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a. Immunoblot analysis of IL-17 signaling components from nuclear and cytoplasmic fractions of C. elegans lysate. I, input; C, cytosolic; N, nuclear. NFKI-1 is predominately nuclear; ACTL-1 and MALT-1 are distributed between the nucleus and cytoplasm. Similar results were obtained in 5 experiments.

b. Overexpressing malt-1 in neurons, using the rab-3 promoter, restores the arousal response to 21% O₂ to ilc-17.1 and ilcr-1 mutants (b), and actl-1 and pik-1 mutants (c). b. n = 52 animals (npr-1), n = 104 animals (npr-1; ilc-1), n = 71 animals (npr-1; ilc-1; rab-3p::malt-1), n = 66 animals (npr-1; ilc-1; malt-1), n = 61 animals (npr-1; ilc-17; malt-1), n = 46 animals (npr-1; actl-1; malt-1), n = 26 animals (npr-1; actl-1; rab-3p::malt-1), n = 23 animals (npr-1; pik-1; malt-1), n = 28 animals (npr-1; pik-1; rab-3p::malt-1). Plots show average speed (line) and SEM (shaded regions). ***P < 0.001, two-sided Mann-Whitney U test.

c. Overexpressing malt-1 gDNA also rescues the aggregation phenotype (d), but not the arousal defect (e) of nfk1-1 mutants. d. N = 7 assays (npr-1), N = 6 assays (npr-1; nfk1-1 and npr-1; nfk1-1; malt-1::gfp). ***P = 3.5e–05, one-way ANOVA with Tukey’s post hoc HSD.

d. Animals in groups (%) for npr-1, npr-1; malt-1, npr-1; malt-1; nfk1-1::gfp.

e. Animals in groups (%) for npr-1, npr-1; malt-1, npr-1; malt-1; nfk1-1::gfp.

Fig. 7 MALT-1 and NFKI-1 provide partially parallel outputs of IL-17 signaling. a. Immunoblot analysis of IL-17 signaling components from nuclear and cytoplasmic fractions of C. elegans lysate. I, input; C, cytosolic; N, nuclear. NFKI-1 is predominately nuclear; ACTL-1 and MALT-1 are distributed between the nucleus and cytoplasm. Similar results were obtained in 5 experiments. b. Overexpressing malt-1 in neurons, using the rab-3 promoter, restores the arousal response to 21% O₂ to ilc-17.1 and ilcr-1 mutants (b), and actl-1 and pik-1 mutants (c). b. n = 52 animals (npr-1), n = 104 animals (npr-1; ilc-1), n = 71 animals (npr-1; ilc-1; rab-3p::malt-1), n = 66 animals (npr-1; ilc-1; malt-1), n = 61 animals (npr-1; ilc-17; malt-1), n = 46 animals (npr-1; actl-1; malt-1), n = 26 animals (npr-1; actl-1; rab-3p::malt-1), n = 23 animals (npr-1; pik-1; malt-1), n = 28 animals (npr-1; pik-1; rab-3p::malt-1). Plots show average speed (line) and SEM (shaded regions). ***P < 0.001, two-sided Mann-Whitney U test.

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e. Animals in groups (%) for npr-1, npr-1; malt-1, npr-1; malt-1; nfk1-1::gfp.

f. Animals in groups (%) for npr-1, npr-1; malt-1, npr-1; malt-1; nfk1-1::gfp.

g. Animals in groups (%) for npr-1, npr-1; malt-1, npr-1; malt-1; nfk1-1::gfp.

and independent mechanisms to alter neuron function and change behavior. The ACTL-1-IRAK-MALT-1-NFKI-1 pathway is present in most neurons of the C. elegans nervous system, and appears to be a neuromodulatory axis impacting multiple phenotypes.

Like ILCR-1 and ILCR-2, MALT-1 functions in both URX O₂ sensors and RMG interneurons to promote escape from 21% O₂. In RMG, ILC-1/17.1/MALT-1 signaling potentiates Ca²⁺ responses to pre-synaptic input from URX O₂ sensors, which are tonically activated by 21% O₂. In URX, ILC-17.1/MALT-1 signaling does not appear to disrupt O₂-evoked Ca²⁺ responses, suggesting that it potentiates behavioral arousal to 21% O₂ by augmenting synaptic or gap junctional communication. These
different effects of IL-17 signaling may be indicative of cell-type specific effects on gene expression. Our IP/MS experiments identified transcription factors, chromatin remodeling factors and RNA binding proteins as specific interactors of NFKI-1 and/or MALT-1, but further work is needed to identify cell types in which these interactions are functionally relevant.

Neuronal MALT-1 signaling also modulates pathogen susceptibility and longevity. The nervous system plays an important and conserved role in regulating immunity35–39, and multiple neurons60–62 and secreted factors63,64 that regulate innate immune gene expression in non-neuronal tissues have been discovered. The nervous system also mediates behavioral avoidance of pathogens, by
mechanisms that can be innate or learned\textsuperscript{49,65}. Our data suggest that neuronal IL-17.1/MALT-1 signaling reduces survival on \textit{Pseudomonas aeruginosa} by non-behavioral mechanisms. A simple model is that by altering neural circuit activity ILC-17.1 can change immune gene expression, for example in the intestine. MALT1-like paracaspases are found in organisms lacking other CBM components\textsuperscript{18}, suggesting MALT1 has unknown functions that predate its coaction with Bcl10 and CARD domain proteins. Our results raise the possibility that one ancestral function was in IL-17 signaling. As IL-17Rs are found throughout metazoa\textsuperscript{66}, we speculate that the ACTL-1-IRAK-MALT1 complex we have identified is the original and primary mechanism by which IL-17Rs signal in non-aminote animals, from cnidarians to cephalochordates. In amniotes, ACT1 orthologs have lost a death domain (DD) that is present in ACT1 orthologs from most other lineages\textsuperscript{66}. DDs mediate homotypic interactions in large immune complexes such as the Myddosome\textsuperscript{67}, and are present in both MALT1 and IRAKs. The DD–SEFIR domain architecture of ACT1 is a nuclear–localized protein that acts as a transcriptional regulator, and is rapidly induced by inflammatory stimuli, including IL-17. IκBζ is a nuclear-localized protein that acts as a transcriptional regulator, and is rapidly induced by inflammatory stimuli, including IL-17. IκBζ is thought to mediate its effects on gene expression primarily by regulating chromatin structure, although how it is recruited to target genes is not completely understood since it lacks a DNA binding domain\textsuperscript{76,77}. Our IP/MS data find NFKI-1 physically
interacts with the CREB binding protein (CREBBP), cebp-1, which is a histone acetyltransferase, consistent with NFKI-1 acting to modify chromatin structure. One of the gene expression changes highlighted by our RNA-seq studies likely reflect secondary consequences of IL-17 signaling defects, although some genes may be directly regulated by NFKI-1.

Our biochemical and genetic analyses of IL-17 signaling in *C. elegans* have identified functional roles and biochemical interactions previously undescribed in mammals. An outstanding challenge is to examine which of these are conserved in mammals. Does MALTI play a role in modulating mammalian neural actions previously undescribed in mammals. An outstanding challenge is to examine which of these are conserved in mammals. Does MALTI contribute to known neuronal responses to IL-17? Does mammalian MALTI physically interact with its targets, and its advantages for genetics, biochemistry and single neuron analysis, to probe how key immune molecules signal in neurons to alter circuit function.

**Methods**

**Strains and genetics.** *C. elegans* were maintained on nematode growth medium (NGM) at room temperature (22 °C) with *E. coli* OP50 food. Strains used are provided in Supplementary Table 5.

Whole genome sequencing showed that the aggregation-defective AX3621 strain was defective in mal-1. We used SNP mapping to investigate if the aggregation defect was linked to mal-1. We crossed AX3621 animals with the AX288 (cgl-1(n1190) trp-1(ad609) str-5(ad199) fi) strain; AX288 was constructed by backcrossing lon-2 npr-1(ad609) X 16 to the CB4856 (pbyc) strain. The npr-1 (ad609) allele confers stronger aggregation than the CB4856 Hawaiian strain routinely used for mapping. We 'singlef' Z animals and scored their progeny for aggregation to determine if non-aggregating F3 lines were pooled, and their DNA extracted and sequenced. Sequencing libraries were made using the Nextera DNA Library kit, and sequenced on a HiSeq 2500 (Illumina) machine with 125 bp paired-end reads. Sequencing data were analyzed using CloudMap.

**Molecular biology.** Primers used in this study are provided in Supplementary Table 6. *C. elegans* expression constructs were generated using MultiSite Gateway Recombination (Invitrogen). To amplify the mal-1 promoter (4 kb) we used primers ggggACAACTTTGTATAGAAAAAAGCAGGCTtttcagaaaaatgaacacaaacttggcggagt tc and ggggACAACTTTGTATAGAAAAAAGCAGGCTtttcagaaaaatgaacacaaacttggcggagt tc and ggggACAACTTTGTATAGAAAAAAGCAGGCTtttcagaaaaatgaacacaaacttggcggagt tc and ggggACAACTTTGTATAGAAAAAAGCAGGCTtttcagaaaaatgaacacaaacttggcggagt.

The Q5 Site Directed Mutagenesis Kit (NEB) was used to create mal-1 (C374T) cDNA, with the following primers: TCTTGTAGTGtcCAGAAAAATTGTCTCATTATG and ggggcaagagcttggctgc gaggc

To generate delections in the mal-1 locus by CRISPR/Cas9 we expressed a gacgacuacccagcag short guide from the rpr-1 promoter. The primers used to amplify this sequence for insertion into an EcoRI-cut expression plasmid (addgene #48961) were ggcgacctgttgtgtgtgttttttgtg ggcgacctgttgtgtgttttttgtg and TCTTGTAGTGtcCAGAAAAATTGTCTCATTATG and ggggcaagagcttggctgaggc.

Expression constructs were injected at 30 ng/µl, with the exception of CRISPR-Cas9 mixtures that were prepared as previously described23. 30 ng/µl eFl-cas9, 100 ng/µl sgRNA, 30 ng/µl cspGFP.

The following alleles were generated by SunyBiotech (Fuzhou, China) using CRISPR/Cas9-based genome editing: mal-1(syb573) fi, mal-1(syb617) fi, and npr-1(syb617). We verified modified sequences using Sanger sequencing (Supplementary Table 7).

**Behavioral assays.** Behavioral assays were performed at room temperature (22 °C). Aggregation was assayed as previously described24, 60 young adults were picked onto a plate seeded with 100 µl OP50 48 h previously. Animals were left undisturbed for 2 h and then scored blind to genotype. The % of animals in groups was calculated, with a group defined as 3 or more animals in contact with one another. Statistical analysis was made using ANOVA (with RStudio v 1.2.1430)

Locomotory responses to O2 stimuli were measured as described previously25, with minor modifications. 15–25 young adults were picked onto a plate seeded with 20 µl OP50 14–18 h previously, and covered with a microfluidic PDMS chamber. Defined O2 mixtures (balance nitrogen) were bubbled through H2O and delivered to the PDMS chamber at a rate of 1.4 ml/min using a PHD 2000 infusion syringe pump (Harvard Apparatus). Video recordings were acquired at 2 fps with FlyCapture 1.0 software (FLIR Systems), using a Point Grey Grasshopper camera mounted on a Leica MZ6 microscope. Speed and reversals were measured using Zentraacker custom software (https://github.com/nectarTracker/zentraacker). To measure phenotypes associated with IL-17 signaling defects, worms were left undisturbed for 2 h on assay plates prior to recording.

To measure thrashing, single animals were placed into individual wells containing 50 µl M9 buffer. The number of complete body bends per minute was measured by a scorer blind to genotype.

**Heat-shock.** As reported previously, the hsp-16.41 heat shock promoter is leaky in animals grown at room temperature. We therefore kept animals at 15 °C until the time of heatshock (late L4). To induce heat-shock, paraflm-2 wrapped plates were submerged in a 34 °C water bath for 30 min, and then recovered at room temperature until the time of assay.

**Light microscopy.** Worms were immobilized with 25 mM sodium azide on 2% agarose pads. Z stacks from animals expressing MALTI-1::GFP and MALTI-1::RFP were acquired on an Inverted Leica SP8 confocal microscope using a x63/1.20 water objective, using the LAS X software platform (Leica). Figure panels were obtained using the Z-project (average intensity) function in FIJI (Image) v2.0.0rc-69.

We quantified GFP intensity in L4 animals expressing the sgr21p::syr2::GFP mutant15 using NIH-Elements (Nikon) and a Nikon Ti2 microscope with a ×100 Plan-Neofluar 20 µm × 20 mm nanoViper (Thermo Fisher) objective, a 10 °C stage (Olympus) and a 5 °C temperature gradient (Kodak Digital Camera) (Nikon). Whole body fluorescence was quantified using NIH-Elements. We used NIH-ImageJ for analysis.

**Calcium imaging.** Animals expressing cagmeleon YC2.60 were imaged with a ×2 AZ-Plan Fluor objective (Nikon) on a Nikon AZ100 microscope fitted with ORCA-Flash4.0 digital cameras (Hamamatsu). Excitation light was provided from a DualObjective Spectro-LED light source (Bluebox Optics, Huntingdon, UK) and a NLO stealth CMOS camera (Andor, Belfast, UK), with a ×10 objective (Nikon, Tokyo, Japan) and 50 ms exposure time.

To image neural activity in freely moving animals (Supplementary Fig. 4g), single young adults were transferred to peptone-free agar plates spotted with 4 µl of concentrated OP50 food in M9 buffer, and imaged at 2x zoom. For all other figures, 4–8 young adults were transferred to peptone-free agar plates immobilized on a 2 µl patch of concentrated OP50 in M9 buffer using Dermabond adhesive, leaving the nose exposed, and imaged at 4x zoom.

**Immunoprecipitation from C. elegans.** For co-IP experiments analyzed by LC-MS/MS, C. elegans lysis and affinity purification was performed as previously described26 with minor modification. Lysis buffer A was prepared with 50 mM HEPES (pH 7.4), 1 mM EGTA, 1 mM MgCl₂, 100 mM KCl, 1% glycerol, 0.05% NP-40, 1 mM DTT, 0.1 M PMSF and 1 complete EDTA-free proteinase inhibitor cocktail tablet (Roche Applied Science) per 12 ml. Unsonorized worms grown in liquid were washed twice in M9 and once in ice-cold lysis buffer A, then snap-frozen by doubledrop addition to LN₂ in preparation for cryogenic grinding. Worm powder was pulverized using a FreezerMill (SPI SamplePrep). Crude extract was clarified at 4 °C for 10 min at 20,000g, and again for 20 min at 100,000g with a TLA-100 rotor (Beckman Coulter). For IP, roughly equal volumes of sample and control lysate were incubated with 100 µl GFP-Trap MA (ChromoTek gma), Myc-Trap MA (ChromoTek myc), or anti-FLAG M2 magnetic beads (Sigma M8823) for 3–4 h at 4 °C, then washed twice with 50 mM HEPES, 100 mM KCl. Purified complexes were eluted in SDS-sample buffer at 95 °C and fractionated by SDS-PAGE prior to characterization by LC-MS/MS.

For co-IP experiments analyzed by Western blot, the following modifications were made. Lysis buffer B contained 50 mM HEPES (pH 7.4), 100 mM KCl, 0.05% NP-40, 1 mM DTT, 0.1 M PMSF and 1 complete EDTA-free proteinase inhibitor cocktail tablet (Roche Applied Science) per 12 ml. Crude extract was clarified at 4 °C for 30 min at 18,000g. For immunoprecipitation, half of the lysate was incubated with anti-HA agarose (Sigma A2095) for 30 min at 4 °C, then washed twice with 50 mM HEPES, 100 mM KCl. A control, the other half of the lysate was incubated with IgG-agarose (Sigma A9019).

**Identification of protein-protein interactions by MS.** Gel samples were destained with 50% v/v acetonitrile and 50% v/v ammonium bicarbonate, reduced with 10 mM DTT, and alkylated with 55 mM iodoacetamide. Proteins were digested with 6 ng/µl trypsin (Promega, UK) overnight at 37 °C, and peptides extracted in 2% v/v formic acid 2% v/v acetonitrile, and analyzed by nano-scale capillary LC-MS/MS (Ultimate 3000 HPLC, Thermo Scientific Dionex) at a flow of ~300 nl/min. A C18 Acclaim PepMap100 5 µm, 100 µm × 20 mm nanoViper (Thermo Scientific Dionex), trapped the peptides prior to separation on a C18 Acclaim PepMap100 3 µm, 75 µm × 250 mm nanoViper. Peptides were eluted with an
Quantification of NFκB-1 interacting peptides by TMT labeling. Protein samples on beads were reduced with 10 mM DTT at 56 °C for 30 min and alkylated with 15 mM iodoacetamide (IAA) in the dark at 22 °C for 30 min. Alkylation was quenched by adding DTT and the samples digested with trypsin (Promega, 1.25 μg) overnight at 37 °C. After digestion, supernatants were transferred to a fresh Eppendorf tube, the beads were extracted once with 80% acetonitrile/0.1% TFA and combined with the corresponding supernatant. The peptide mixtures were then partially dried in a Speed Vac and desalted using home-made C18 (3 M Empore) stage tip filled with 4 μl of pores R3 (Applied Biosystems) resin. Bound peptides were eluted sequentially with 30%, 50%, and 80% acetonitrile in 0.1%TFA and lyophilized.

Dried peptide mixtures from each condition were re-suspended in 40 μl of 250 mM triethyl ammonium bicarbonate. 0.8 μg of TMT 6plex reagents (Thermo Fisher Scientific) was reconstituted in 41 μl anhydrous MeCN. Twenty microliter of TMT reagent was added to each peptide mixture and incubated for 1 h at r.t. The labeling reactions were terminated by incubation with 4.4 μl of 5% hydroxylamine for 15 min. For each condition, the labeled samples were pooled, Speed Vac to remove acetonitrile, desalted and then fractionated with home-made C18 stage tip using 10 mM ammonium bicarbonate and acetonitrile gradients. Eluted fractions were acidified, partially dried down in speed vac and ready for LC-MSMS.

Peptides were separated on an Ultimate 3000 RSLC nano System (Thermo Scientific) using a reverse phase C18 column (75 μm × 15 cm) packed in-house with 2 μm C18 (2% MeCN, 0.1% formic acid) and buffer B (80% MeCN, 0.1% formic acid). Eluted peptides were introduced directly via a nanospray ion source into a Q Exactive Plus hybrid quadrupole–Orbitrap mass spectrometer (Thermo Fisher Scientific). The mass spectrometer was operated in standard data dependent mode, performed survey full-scan (MS, m/z = 350–1600) with a resolution of 70,000, and MS/MS acquisitions of the 15 most intense ions with a resolution of 35,000 and NCE of 33%. MS target values of 3E6 and MS2 target values of 1E5 were used. Dynamic exclusion was enabled for 40 s.

The acquired MSMS raw files were processed using MaxQuant83 with the integrated Andromeda search engine (v.1.5.5.1). MSMS spectra were searched against human reverse sequence database, modified peptide only, and common contaminants. Each peptide channel was normalized to the median and log2 transformed.

Protein expression in E. coli. His10-tagged C. elegans MALT-1, ACTL-1, and NFκB-1 were expressed in E. coli strain BL21(DE3) and purified using Ni-NTA agarose (Qiagen) or HiPur Cobalt resin (ThermoFisher Scientific).

Sub-cellular fractionation. Nuclear/cytoplasmic fractionation was performed as described previously84. Young adult worms were washed 3–5 times in M9, and twice in hypotonic buffer (15 mM HEPES, 10 mM KCl, 5 mM MgCl2, 0.1 mM EDTA, 350 mM sucrose). Lysis was on ice in complete hypotonic buffer plus 1 mM DTT and 1 complete EDTA-free proteinase inhibitor cocktail tablet (Roche Applied Science). Homogenized, motorized pellet pellets (Sigma Z399971, Z399947) until most worm carcasses were homogenized were kept as the input fraction. Nuclei were pelleted at 4000g (5 min), and the resulting supernatant centrifuged again at 17,000g and kept as the cytoplasmic fraction. Nuclear pellets were washed twice in complete hypotonic buffer and dissolved in complete hypotonic buffer (15 mM HEPES, 400 mM KCl, 5 mM MgCl2, 0.1 mM EDTA, 0.1% Tween 20, 10% glycerol, 1 mM DTT, complete EDTA-free proteinase inhibitor as above).

Size-exclusion chromatography. C. elegans lysate was prepared as described above for LC-MS/MS, and loaded onto a Superose 6 10/300 GL column. The column was equilibrated with lysate buffer A and 1 ml fractions were collected using Unicon 7.0 (GE Healthcare Life Sciences).

Immunoblotting. After SDS-PAGE using Bolt 4–12% Bis-Tris Plus gels (ThermoFisher Scientific), protein was transferred to PVDF membrane (0.45-micron pore size, ThermoFisher Scientific) using the XCell II Blot Module (ThermoFisher Scientific). Membranes were blocked with 5% milk for 1 h, then incubated with primary antibody overnight at 4 °C, followed by secondary antibody for 1 h at RT. Unbound antibody was washed away with PBS (T <5 min), and SuperSignal West Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific) used for detection. The following commercially available antibodies were used: anti-FLAG M2 Peroxidase (A8592, Sigma A8592-2MG, diluted 1:1000), anti-Myc (9B11, Cell Signalling #2276, diluted 1:1000), anti-HA (C29F4, Cell Signalling #3724, diluted 1:1000), anti-Histone H3 (Cell Signalling #9715, diluted 1:10000), anti-alpha tubulin (DM1A, abcam ab40742, diluted 1:4000), anti-Rabbit IgG (Bio-Rad #1706515, diluted 1:3000), and anti-Mouse IgG (Bio-Rad #1706516, diluted 1:3000). Western blots were imaged with Image Lab 4.1 (Bio-Rad).

Yeast two-hybrid assays. Yeast two-hybrid assays were performed by Panbionet (http://panbionet.com). cDNAs encoding NFκB-1 (full length) and NFκB-1 (3-374) were amplified by PCR and cloned into pGBK7 vector, and cDNAs encoding MALT-1 (1-81) and MALT-1 (248-639) were amplified by PCR and cloned into pGADT7 vector (Clontech). Plasmids were transformed into the AH109 yeast strain, which expresses HIS3 and ADE2 reporters. Transformants were dropped separately onto SD-LW, SD-LWA and SD-LWI media containing 10 mM of 3-AT (3-aminoo-1,2,3-triazole), a competitive inhibitor of the HIS3 protein (His3p).

RNA preparation. 10 Gravid adults were allowed to lay eggs for 2 h on an OP50 lawn seeded 24 h previously, before being picked away. 8–10 plates were used per replicate, and all genetic backgrounds were prepared in parallel. Once animals reached late L4 stage, they were washed twice in M9 and frozen in liquid N2. One thousand synchronized L4 animals raised on OP50 were transferred to M9 with no NaCl (mock) or 300 mM NaCl (conditioned). For each transgenic strain, which expresses npr-1, 1000 animals were scored every 12 h, and counted as dead if they did not respond to prodding. The following commercially available antibodies were used: anti-FLAG M2-Peroxidase (A8592, Sigma A8592-2MG, diluted 1:1000), anti-Myc (9B11, Cell Signalling #3724, diluted 1:1000), anti-HA (C29F4, Cell Signalling #3724, diluted 1:1000), anti-Histone H3 (Cell Signalling #9715, diluted 1:10000), anti-alpha tubulin (DM1A, abcam ab40742, diluted 1:4000), anti-Rabbit IgG (Bio-Rad #1706515, diluted 1:3000), and anti-Mouse IgG (Bio-Rad #1706516, diluted 1:3000). Western blots were imaged with Image Lab 4.1 (Bio-Rad).

ASSOCIATIVE LEARNING ASSAYS. Chemotaxis assays were performed as previously described85 with minor modifications. To establish salt gradients, 100 mM NaCl agar plugs were layered on NGM plates (no food) and incubated for 2 days. Twenty-four hours later, plugs were removed and the resulting supernatant centrifuged again at 17,000g and kept as the starting point as a control. The chemotaxis index was calculated as (A – B)/N, where A was the number of animals within 1 cm of the peak of the salt gradient, B was the number of animals with 1 cm of the control area, and N was the total number of animals. Conditioning was performed as described previously86. Synchronized young adults raised on OP50 were washed three times in CTX buffer (5 mM K2HPO4 pH 6, 1 mM CaCl2 and 1 mM MgSO4), then left for 4 h on NGM agar with no NaCl (mock) or 300 mM NaCl (conditioned). For each transgenic strain the behavior of animals bearing the transgene was compared to that of their non-transgenic siblings.

P. aeruginosa killing assays. Slow killing assays were performed with 10 μg/ml 5-fluorooxozuridine (FUDR). Synchronized L4 animals raised on OP50 were added to 0.35% peptone NGM plates, seeded the day before with PA14. Animals were scored every 12 h, and counted as dead if they did not respond to prodding. Logrank tests with Bonferroni correction were performed using OASIS (On-line Application for Survival Analysis, https://sbi.postech.ac.kr/oasis/89). Life span analyses. Lifespan assays were performed on OP50, starting on day 1 of adulthood89. Scoring and statistical analyses were performed as described above for P. aeruginosa killing assays.

Statistics. Statistical tests and n values used for experiments in this paper are indicated in the corresponding figure legend or methods section. For salt chemotaxis and aggregation behavior, statistical significance between two groups was determined using one-way ANOVA followed by Tukey’s post hoc LSD. Differentially up-regulated transcriptional response and FRET levels (yellow camelone) were evaluated by Mann-Whitney U test. Logrank tests with Bonferroni correction were used to compare
genotypes in lifespan and PA14 survival assays using OASIS (Online Application for Survival Analysis, https://sbi.postech.ac.kr/oasis/).

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

**Data availability**

Data supporting the findings of this study are provided as a Source Data file, and are available from the corresponding author. Full scans of blots are provided in Supplementary Fig. 10. RNA-seq data has been deposited in Gene Expression Omnibus (GEO) with accession number GSE144057. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository PXD018080.

**Code availability**

Zentracker, the custom MATLAB software used to analyze locomotory responses is available at https://github.com/wimtrracker/zentracker. Neuron Analyzer, the custom-written MATLAB software used to analyze FRET levels (yellow camelion), is available at https://github.com/neuronanalyser/neuronanalyser.

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Author contributions
S.F., C.C., M.A.B., and M.d.B. conceived experiments; S.F., C.C., M.A.B., and S.B. performed experiments; A.C. and G.N. performed sequence analysis; S.-Y.P.-C., F.B., and M.S. performed mass spectrometry analysis; S.F. and M.d.B. wrote the manuscript.

Competing interests
The authors declare no competing interests.

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