Insulin/insulin-like growth factor signalling (IIS) is a critical regulator of an organism’s most important biological decisions from growth, development, and metabolism to reproduction and longevity. It primarily does so through the activity of the DAF-16 transcription factor (forkhead box O (FOXO) homologue), whose global targets were identified in Caenorhabditis elegans using whole-worm transcriptional analyses more than a decade ago. IIS and FOXO also regulate important neuronal and adult behavioural phenotypes, such as the maintenance of memory and axon regeneration with age, in both mammals and C. elegans, but the neuron-specific IIS/FOXO targets that regulate these functions are still unknown. By isolating adult C. elegans neurons for transcriptional profiling, we identified both the wild-type and IIS/FOXO mutant adult neuronal transcriptomes for the first time. IIS/FOXO neuron-specific targets are distinct from canonical IIS/FOXO-regulated longevity and metabolism targets, and are required for extended memory in IIS daf-2 mutants. The activity of the forkhead transcription factor FKH-9 in neurons is required for the ability of daf-2 mutants to regenerate axons with age, and its activity in non-neuronal tissues is required for the long lifespan of daf-2 mutants. Together, neuron-specific and canonical IIS/FOXO-regulated targets enable the coordinated extension of neuronal activities, metabolism, and longevity under low-insulin signalling conditions.

The C. elegans IIS pathway acts both cell autonomously and non-autonomously to control longevity, growth, dauer formation, metabolism, and reproduction through its regulation of the nuclear localization and transcriptional activation of DAF-16 (also known as FOXO). The canonical IIS/FOXO gene set, which identified primarily intestinal and hypodermal targets (Extended Data Fig. 1a, b), has been instructive in our understanding of how insulin signalling regulates a diverse range of activities, including metabolism, autophagy, stress resistance, and proteostasis. However, IIS mutants also exhibit daf-16-dependent neuronal phenotypes, including extended positive olfactory learning, increased short- and long-term associative memory, increased thermotaxis learning, improved neuronal morphology maintenance, and improved axon regeneration. These phenotypes are unlikely to be regulated by the known intestinal and hypodermal IIS/FOXO targets. Therefore, to understand how IIS daf-2 mutant animals extend behavioural functionality, we must identify the neuronal targets of FOXO/DAF-16.

We first profiled the expression of daf-16;daf-2 mutant worms with daf-16 rescued in specific tissues (Supplementary Table 1). Intestinal daf-16 rescue correlates best with whole-worm profiles. By contrast, neuronal daf-16 rescue profiles are anti-correlated with the intestinal DAF-16 and whole-worm profiles. Surprisingly, many genes induced by neuronal DAF-16 rescue are expressed (WormBase) or predicted to be expressed in non-neuronal tissues (Extended Data Fig. 1d), and have non-neuronal functions (for example, collagens). Extended data Fig. 1b, e, Supplementary Table 2). Thus, whole-worm transcription analyses of neurally rescued DAF-16 failed to reveal targets that account for daf-16-dependent age-related behaviours of daf-2 mutants. Therefore, we needed to specifically examine transcription in IIS-mutant neurons.

The tough outer cuticle prevents dissociation of adult tissues, thus the wild-type adult neuronal transcriptome has not been described. To solve this problem, we used rapid, chilled chemomechanical disruption followed immediately by fluorescence-activated cell sorting (FACS) to isolate neurons marked with green fluorescent protein (GFP) from wild-type worms, then RNA-sequenced these isolated cells (Fig. 1a–c, Extended Data Fig. 2a–c, f, g, Supplementary Table 3). This method is gentle enough to preserve the integrity of cells and some neurites (Extended Data Fig. 2a), does not involve cell culturing before FACS, in contrast to previous methods, and does not affect transcription (as shown by actinomycin D treatment; Fig. 1b, Extended Data Fig. 2d, e, Supplementary Table 4). Downsampling analysis showed that sufficient sequencing depth was achieved (Extended Data Fig. 2h).

We compared gene expression in isolated wild-type neurons with whole-worm expression to identify genes that are enriched in neurons (Fig. 1a–c). Of the 1,507 ‘neuron-enriched’ genes (false discovery rate (FDR) < 0.1; Supplementary Table 3; Fig. 1a, b), only 4% have previously described expression patterns exclusively in non-neuronal tissues, and ‘Neuron’ is the only significantly enriched tissue (Fig. 1c, Extended Data Fig. 2i), indicating that this method is highly selective for neuronal transcripts. Gene promoters–GFP tests of previously uncharacterized genes from our neuron-enriched list confirmed neuronal expression, with no bias for particular neuron types (Extended Data Fig. 3a). We also detected genes previously reported to be expressed only in single neurons or small subsets of neurons, including glr-3 (in the RIA neuron), ttx-3 (interneuron AY/AIA) and npr-14 (neuron AY) (WormBase).

The wild-type neuron-enriched set includes synthetic machinery, ion channels, neurotransmitters, and signalling components (Supplementary Table 3), as well as >700 previously uncharacterized genes; these genes are predicted to have ‘neuronal’-like character and function (Fig. 1d). Comparison of the wild-type embryonic and larval neuronal transcriptomes with the adult neuronal transcriptome at the same FDR revealed a shift in functional categories from developmental processes to neuronal function/behaviour in the adult neuronal transcriptome (Fig. 1e, Extended Data Fig. 3b, c, Supplementary Table 5), suggesting that previous isolation methods, either due to early developmental stage isolation or to re-culturing, biased expression towards developmental genes rather than neuronal/behavioural genes.

To identify adult neuronal IIS/FOXO targets, we sequenced RNA from isolated daf-2 and daf-16;daf-2 mutant neurons on day 1 of adulthood (Fig. 2a, Extended Data Fig. 4, Supplementary Table 6, 8). The IIS/FOXO neuron-isolated gene set is enriched for neuronal expression: 86% and 92% of the up- and downregulated genes, respectively, are expressed in wild-type neurons. While several top Class I genes...
targets of DAF-16, including hil-1, sip-1, mtl-1, nmt-1, ins-6, and daf-16 itself, were upregulated in both daf-2 mutant neurons and daf-2 mutant whole worms (Group B; Fig. 2b), most of the IIS/FOXO neuronal regulated set differs from the canonical whole-worm IIS/FOXO set\(^1,8\) (Fig. 2b). Specifically, in contrast to the metabolism-dominated functions of canonical whole-worm IIS/FOXO targets\(^1,8\), the neuronal IIS set gene ontology terms reflect neuron-like functions (Extended Data Fig. 5b): serpentine receptors, G protein–coupled receptors, synaptin, globins, kinesins, insulin, ion channels, potassium channels, seven-transmembrane receptors, the NPR-1 neuropeptide receptor, and the SER-3 octopamine receptor are upregulated in daf-2 neurons (Supplementary Table 6). A few genes (fat-3 and crh-1, a CREB homologue) are upregulated in daf-2 neurons but downregulated in whole daf-2 animals.

The IIS/FOXO downregulated set includes serpine receptors, guanylate cyclases, signalling peptides and receptors (neuropeptide-like proteins, FMRF-like peptides and neuropeptides), and the vesicle trafficking G protein rab-28 (Supplementary Table 6). Expression of the sensory neuron cilia protein IFTA-2, which co-localizes with DAF-2 and whose loss increases lifespan\(^19\), is downregulated in daf-2 mutants, consistent with the longevity of daf-2 and ciliated sensory neuron mutants\(^18\). Similarly, sams-1 (S-adenosyl methionine synthetase), which is downregulated under long-lived dietary restriction conditions\(^19\), and sma-5 and dbl-1, components of TGF-beta pathways linked with IIS\(^5,20\), are downregulated, perhaps coordinating the longevity and reproductive output of these pathways.

Unlike canonical IIS/FOXO targets\(^1\), neuronal IIS/FOXO gene promoters are not enriched for the DBE (DAF-16 binding element, GTAAAt/ca), but the overlapping, upregulated (Group B) targets’ promoters contain twice as many DBEs (Extended Data Fig. 6). The bZIP transcription factor CREB, which is required for long-term memory in many organisms, including C. elegans\(^2\), is upregulated by IIS/FOXO in neurons (Supplementary Table 6), correlating with the increased long-term memory of daf-2 mutants\(^21\). However, short-term associative memory (STAM) in daf-2 mutants are unknown. While the DAF-16 non-neuronal target sod-3 had no effect on the extended STAM of daf-2 mutants (Fig. 2c, Extended Data Fig. 6b–d), knockdown of 8 of the 10 top-ranked, upregulated IIS/FOXO targets significantly decreased the STAM of daf-2.
Figure 2 | RNA-seq transcriptional profile of isolated neurons reveals IIS/FOXO neuronal transcriptome. a, Volcano plot of daf-2-regulated, daf-16-dependent up- (red) and downregulated (green) neuronal genes (P < 0.05, N = 4 biological replicates per strain). b, Comparison of whole-worm (Class I) vs neuronal-IIS/FOXO targets. P values: hypergeometric distributions. c–e, Short-term associative memory (STAM) assays. c, Schematic of STAM assay and chemotaxis profiles of daf-2 treated with sod-3 (c) or neuronal IIS/FOXO target gene RNAi (d, e). d, Learning indices relative to control RNAi at 3 h post-training of daf-2 treated with adult-only (green) or whole-life (blue) neuronal IIS/FOXO target gene RNAi. Mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, two-way repeated measures ANOVA, Bonferroni post hoc tests. At least 3 biological replicates were performed for all STAM assays.

Figure 3 | FKH-9 is a direct target of DAF-16 and is expressed in mechanosensory neurons. a, b, daf-16 is required for enhanced day 5 axon regeneration in daf-2 mutants, mean ± s.e.m., *P < 0.05, Fisher’s exact test. N = 26 (wild-type), 36 (daf-2) and 16 (daf-16;daf-2), 2 biological replicates. c, Known larval regeneration genes are significantly enriched in the day 1 adult mechanosensory transcriptome. 63 genes are both DAF-16 targets and expressed in mechanosensory neurons (FDR < 5%; 3 biological replicates). d, fkh-9 messenger RNA levels are higher in aged daf-2 compared to wild type in a daf-16-dependent manner. N = 4 biological replicates, two-way ANOVA, Bonferroni post hoc tests. e, Chromatin immunoprecipitation of DAF-16–GFP worms with and without heat shock, which mobilizes DAF-16 into the nucleus. DAF-16 binds to the sod-3 promoter but not its 3′ UTR, and to the fkh-9 promoter at multiple locations (Extended Data Fig. 8). Fold enrichment relative to wild-type (not expressing DAF-16–GFP) is shown (mean ± s.e.m., two-tailed t-test). N = 3 biological replicates. f, Neuronal FKH-9–GFP (fkh-9p::fkh-9::gfp) expression in daf-2 compared to wild type. N = 25 animals. Mean ± s.e.m., two-tailed t-test. d–f, *P < 0.05, **P < 0.01, ***P < 0.001.
FKH-9 is required for improved axon regeneration, short-term associative memory, and lifespan in daf-2 mutants. 

(a), fkh-9 knockdown reduces axon regeneration of day 5 daf-2 mutants, as does daf-16 knockdown. Mean ± s.e.m., *P < 0.05, Fisher’s exact test, N = 34 (control), 33 (fkh-9) and 31 (daf-16), 4 biological replicates.

(b), c, Neuronally-expressed fkh-9 rescues day 5 axon regeneration in daf-2;fkh-9 mutants. Mean ± s.e.m., *P < 0.05, Fisher’s exact test, N = 20 (daf-2), 19 (daf-2; fkh-9) and 35 (daf-2; fkh-9; Punc-119::fkh-9b), 2 biological replicates. d, fkh-9 is required for enhanced memory in adult-only RNAi-treated daf-2 mutant worms. e, Neuronally-expressed fkh-9 rescues extended STAM in daf-2;fkh-9 mutants with defective learning and memory. Mean ± s.e.m., **P < 0.01, ***P < 0.001, ****P < 0.0001, two-way repeated measures ANOVA, Bonferroni post hoc tests. f, Adult-specific fkh-9 RNAi treatment reduces daf-2 mutant lifespan. Median lifespan: control RNAi 42 days, fkh-9 RNAi 21 days, daf-16 RNAi 21 days. P < 0.0001 for control RNAi vs daf-16 RNAi and control vs fkh-9 RNAi, log-rank test. N = 144 worms per strain. g, Integrative Multi-species Prediction (IMP; see ref. 30) network analysis of DAF-16 neuronal target genes with STAM phenotypes (red circles).
Thus, IIS/FOXO-regulated FKH-9 function is important for both neuronal and non-neuronal growth and development, as well as adult memory and axon regeneration. Interestingly, the FKH-9 mammalian homologue FOXG1 is required for axon outgrowth, and is the most highly-induced gene in spinal cords treated with radial glial cell transplantaion following spinal cord injury.

Network analysis using fkh-9 and the other 8 neuronal DAF-16 STAM genes (Fig. 4g, Supplementary Table 10) identified ca-1, which is required for several forms of associative learning and memory. By the analysis, the C. elegans orthologue of amyloid precursor protein (APP) that can disrupt sensory plasticity, and dkk-1, which has previously been described as a regulator of age-dependent axon regeneration, are also upregulated in the dau-2 mutants. Additionally, genes involved in neuronal degeneration (mec-17), neuronal development (egl-44, sm-gnr-1, vab-9, cycf-1), synaptic regulation and function (cab-1, hbl-1, magu-4, sph-1, unc-64), and axon outgrowth (unc-14) and regeneration (egll-8, fos-1, pmk-3), were connected to the STAM genes. PQM-1 (ref. 8), whose motif (DAF) is overrepresented in neuronal IIS target promoters, and other IIS (akt-2, dct-6, hbl-30), TGF-3 (daf-14, sma-4, crm-1, sma-9, sma-1, sta-1), and MAPK pathway (vhp-1, pmk-3) components emerged in the network. Transcriptional regulation by IIS/FOXO and its targets may lead to broader, indirect transcriptional and non-transcriptional regulation of genes with important neuronal functions.

Plasticity in development, reproduction and longevity allows organisms to respond appropriately to nutrient availability and changes in their environment. The IIS pathway is a critical mediator of these decisions, and FOXO selecting transcriptional targets to execute specific biochemical functions in each tissue, including factors that maintain cognitive function with age. daf-2 mutant worms maintain neuronal behaviours with age by using a set of transcriptional targets that are distinct from previously identified metabolic and stress resistance targets expressed in other tissues. These genes may regulate additional neuronal targets through non-transcriptional mechanisms (Fig. 4g). The regulation of tissue-specific transcriptional programs is important to coordinate phenotypic responses, extending neuronal abilities in concert with the extended longevity and reproductive span of daf-2 mutants.

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Author Contributions C.T.M., R.K., V.L. and J.L. designed experiments. R.K., V.L., R.A., J.L., J.A. and C.T.M. performed experiments and analysed data. R.K., V.L. and J.L. performed tissue isolation and RNA-Seq analysis. R.K., V.L. and J.L. performed bioinformatics analysis. R.K., V.L. and R.A. performed short-term memory experiments. V.L. performed axon regeneration experiments. R.K., V.L. and C.T.M. wrote the manuscript. R.A. and A.W. contributed equally to this work.

Author Information Sequencing reads are deposited at NCBI BioProject under accession number PRJNA297798. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.T.M. (ctmurphy@princeton.edu).
METHODS

Adult cell isolation. Day 1 adult neuronally GFP-labelled worms (Punc119::GFP or Pmec-4::GFP) were prepared for cell isolation as previously described with modifications (Extended Data Fig. 2). Synchronized adult worms were washed with M9 buffer to remove excess bacteria. The pellet (~250 μl) was washed with 500 μl lysis buffer (200 mM DTT, 0.25% SDS, 20 mM HEPES pH 8.0, 3% sucrose) and resuspended in 1,000 μl lysis buffer. Worms were incubated in lysis buffer with gentle rocking for 6.5 min at room temperature. The pellet was washed 6 × with M9 and resuspended in 20 mg ml⁻¹ pronase from Streptomyces griseus (Sigma-Aldrich). Worms were incubated at room temperature (<20 min) with periodic mechanical disruption by pipetting every 2 min. When most worm bodies were dissociated, leaving only small debris and eggs, ice-cold PBS buffer containing 2% fetal bovine serum (Gibco) was added. RNA from FACS-sorted neurons was prepared for RNA-seq and subsequent analysis (see Extended Data for details).

No statistical methods were used to predetermine sample size. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

Short-term associative memory assay. Memory assays were performed as described².

Axon regeneration assays. In vivo laser axotomy of PLM neurons was performed as described²².

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Extended Data Figure 1 | Tissue-specific rescue of DAF-16 activity in daf-16;daf-2 mutants identifies distinct gene expression profiles.

a, DAF-16 tissue-specific transgenics; heatmap of all genes with expression differences ≥1.5-fold in ≥3 arrays. b, Significant gene ontology (GO) cluster terms from Punc-119::daf-16-regulated up- and downregulated genes (enrichment score >1). c, Pairwise Pearson correlations between arrays of DAF-16-upregulated or downregulated targets. The red box highlights the negative correlation between neuronal DAF-16 rescued targets (Punc-119::daf-16::gfp;daf-16;daf-2 vs daf-16;daf-2) and intestinal DAF-16 targets (Pges-1::daf-16::gfp;daf-16;daf-2 vs daf-16;daf-2), while the blue box shows the positive correlation between intestinal DAF-16 targets (Pges-1::daf-16::gfp;daf-16;daf-2 vs daf-16;daf-2) and whole-worm DAF-16 targets (Pdaf-16::daf-16::gfp;daf-16;daf-2 vs daf-16;daf-2). The green box shows the weak correlation between neuronal rescued and whole-worm DAF-16 targets. d, Tissue enrichment analysis (mean ± s.e.m.) of significant DAF-16-rescued up- and downregulated genes (Supplementary Table 1) (FDR <0.5). e, Significant GO terms (adjusted P value < 0.05) for DAF-16 upregulated and downregulated genes from whole worm, intestine-, neuron- and muscle-rescued DAF-16 strains. Genes used for GO analysis (Supplementary Table 2) were derived from SAM analysis of the microarrays in a and Supplementary Table 1.
Extended Data Figure 2 | Protocol for isolating neuron-specific targets using FACS followed by RNA-sequencing. a, Pipeline for isolation of adult cells for FACS and RNA sequencing. b, Workflow for RNA-seq data analysis of isolated neurons. c, Heatmap of wild-type neuron-expressed relative to whole-worm-expressed genes. d, Actinomycin D (transcription inhibitor) treatment (100 μg ml\(^{-1}\)) during the cell isolation process demonstrates that the neuron isolation technique induces minimal transcriptional changes in wild type animals. Gene ontology (GO) terms represent genes upregulated in the absence of actinomycin D (Fig. 1b, Supplementary Table 4). e, The 26 differentially expressed genes from actinomycin D treatment are listed. f, *Caenorhabditis elegans* tissue gene expression prediction confirms neuronal character of adult wild-type neuron-enriched genes. Neuron-enriched genes were divided among equal bins according to P value. Bin 1: FDR < 0.003%; bin 2: 0.003–0.03%; bin 3: 0.03–1.3%; bin 4: 1.3–4%; bin 5: 4–10%. g, Principal component analysis (PCA) shows a clear separation between wild-type adult neuronal and whole-worm samples. h, Downsampling of wild-type neuron sequencing reads demonstrates sufficient sampling depth. The number of genes detected at the 3 counts per million threshold (for expressed genes) with different proportions of total sequencing depth analysed.
Extended Data Figure 3 | Neuron-expressed genes identified by our method are confirmed to be expressed in adults and have adult neuronal functions. a, Promoter–GFP transcriptional fusions of candidate uncharacterized neuronal genes (day 1 of adulthood). b, Gene ontology clusters were generated from the categories in Fig. 1e. Non-overlapping GO terms suggest a transition from development-related processes in embryonic and larval animals to neuronal processes involved in behaviour in adults (Supplementary Table 5). c, Venn diagram depicting the overlap between genes classified as “expressed” among embryonic and larval neurons and adult neurons from our RNA-seq analysis (Supplementary Table 5).
Extended Data Figure 4 | Comparison of neuronal DAF-16 targets with wild-type neuronal targets and whole-worm DAF-16 targets.

a, Principal component analysis of the whole worm and isolated adult neuron samples obtained for this study. b, Venn diagram depicting the overlap of daf-2- and daf-16;daf-2-expressed genes with those expressed in wild-type adult neurons. c, Spearman correlation of whole-worm and isolated adult neuron samples. d, The DAF-16 cell-autonomous and cell-non-autonomous targets are distinct. The number of genes that overlap between neuronal DAF-16-rescued whole-worm targets (Punc-119::daf-16::gfp;daf-16;daf-2 vs daf-16;daf-2) and isolated neuron IIS targets (daf-2 vs daf-16;daf-2) is shown (Supplementary Table 8). Hypergeometric distribution analysis (P values) shows that the extent of overlap between the gene categories is not significant.
**Extended Data Figure 5 | Promoter analysis and gene ontology term analysis of neuronal IIS/FOXO genes.**

**a**, The different classes of neuronal IIS/FOXO genes shown in Fig. 2b were analysed for DBE and DAE sequences in the 1 kb upstream promoter regions. The genome-wide percentage of DBE and DAE occurrences across the 1 kb promoters of all gene-encoding regions is reported. Comparison of whole-worm (Class I) vs neuronal-IIS/FOXO-regulated targets. *P* values: hypergeometric distributions. **b**, GO terms of Class I whole worm vs neuronal-IIS upregulated genes (left) and Class II whole worm vs neuronal-IIS downregulated genes (right) (Supplementary Table 5).
Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | Short-term associative memory phenotypes obtained upon knocking down neuronal IIS genes in daf-2 mutants and wild-type animals. daf-2 is required for various forms of C. elegans associative learning. daf-16 is required for the improvements and extensions of abilities with age of daf-2 mutants. daf-2 mutants are defective for salt chemotaxis learning, and daf-16 is not involved in salt chemotaxis learning. Furthermore, salt learning utilizes a unique daf-2 isoform in a daf-16-independent manner, suggesting a learning mechanism distinct from the associative memory paradigms studied here. We are specifically interested in understanding how activation of DAF-16 results in the improved and extended abilities of daf-2 mutants to carry out olfactory associative learning, short-term associative memory, and long-term associative memory, all of which require daf-16. a. Chemotaxis index profile of wild type (N2) and daf-2 animals at time points following memory training. b. RNAi knockdown of sod-3, a non-neuronal DAF-16-regulated target that influences lifespan, has no effect on the extended

short-term associative memory (STAM) of daf-2 mutants when treated with RNAi-feeding bacteria throughout the whole life (b) or only the post-developmental (adult-only) period (c, d) of the animal. daf-2 worms treated with daf-16 RNAi have defective STAM, as previously reported. e. Knockdown of the neuronal IIS candidate genes zip-5 and best-23 does not affect STAM. Time-courses showing the chemotaxis index for each time point are shown in d and e. Learning indices are shown in b, c, f and g. b–e, Two-way repeated measures ANOVA, Bonferroni post hoc tests. f, Treatment of daf-2 worms with neuronal DAF-16 target RNAi does not affect short-term associative learning. g, Neuronal-RNAi sensitive worms (Punc-119::sid-1) in a wild-type background were treated only during adulthood with RNAi targeted against the neuronal DAF-16 target genes. Learning (0 h) and 1 h short-term associative memory time points are shown. a–g, Mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
Extended Data Figure 7 | Characterization of age-dependent axon regeneration and structural defects upon fkh-9 overexpression in mechanosensory neurons. a, Six adult mechanosensory neurons labelled by mec-4p::GFP were isolated for RNA-seq. b, Axon length from the cell body to the site of injury was measured in μm immediately after axotomy and 24 h later. Regenerative capacity of wild-type PLM axons declines from day 1 to day 5 of adulthood. c, Day 5 wild-type animals regrow axons that are significantly shorter than in day 1 animals. d, Axotomies of daf-2 mutants grown on vector control, sod-3, or daf-16 RNAi demonstrate that sod-3, a lifespan-regulating DAF-16 target, does not influence the axon regeneration capacity of daf-2 worms at day 5 of adulthood. e, fkh-9 does not affect the regenerative capacity of daf-2 axons on day 1 of adulthood. f, fkh-9 is not required for axon regeneration in day 1 adults. b–f, Mean ± s.e.m., Fisher’s exact test, *P < 0.05. g, Overexpression of the a and b isoforms of fkh-9 in wild-type animals causes axonal structural defects. Rescuing fkh-9 activity in the mechanosensory neurons of daf-2;fkh-9 mutants results in severe beading and degeneration of axons.
WormBase gene models for *fkh-9* and *sod-3* are shown with modENCODE data for DAF-16 ChIP-seq experiments.

**A** and **B**. Wormbase (http://www.wormbase.org) gene models for *fkh-9* (A) and *sod-3* (B). Primer sets for ChIP-qPCR are depicted in **A**. Posterior intestinal FKH-9–GFP expression is only modestly increased in *daf-2* compared to wild-type animals expressing *fkh-9p::fkh-9::gfp*. *N* = 25 animals.
Extended Data Figure 9 | Knocking down fkh-9 via RNAi or using mutants reduces the enhanced short-term memory of daf-2 animals. 

**a**, Whole-life RNAi of fkh-9 reduces daf-2 STAM. **c**, RNAi knockdown of fkh-9 exclusively during adulthood results in reduced daf-2 STAM comparable to daf-16 RNAi-treatment. **d**, daf-2;fkh-9 mutants have reduced learning (tested immediately following STAM training) and STAM compared to daf-2. Mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Time-courses showing the chemotaxis index for each time point are shown in **b** and **e**. Learning indices are shown in **a**, **c** and **d**.
Extended Data Figure 10 | Neuronal FKH-9 is not required for the enhanced lifespan of daf-2 mutants. a, b, Adult-only (a) or whole-life (b) fkh-9 RNAi treatment increases matricide in daf-2 worms. The cumulative percentage of animals dead as a result of bagging and/or exploding was recorded every other day. Two biological replicates were performed, with a representative experiment shown. c, Neuronal rescue of fkh-9 in daf-2;fkh-9 animals does not diminish the rate of vulval protrusions with age. N ≥ 60 per conditions for each experiment. d, Neuronal rescue of fkh-9 does not restore longevity of the daf-2;fkh-9 double mutant. daf-2 median lifespan: 41 days, daf-2;fkh-9 20 days, daf-2;fkh-9;Punc-119::fkh-9 20 days. P < 0.0001 for daf-2 vs both daf-2;fkh-9 and daf-2;fkh-9;Punc-119::fkh-9. N = 112 worms per strain. Censor rate for daf-2 19%, daf-2;fkh-9 51%, daf-2;fkh-9;Punc-119::fkh-9 56%.