Supporting Information

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SI Materials and Methods

Animal Strains. PS5511 *daf-9(dh6)*; *dhEx24* was a gift from the Antebi laboratory, Max Planck Institute for Biology of Ageing, Cologne, Germany. Strains obtained from the *Caenorhabditis* Genetics Center (CGC) include NY16 *flp-1(yn4)*, VC2324 *flp-6(ok3056)*, RB1990 *flp-7(ok2625)*, PT501 *flp-8(pk360)*, RB1989 *flp-10(ok2624)*, FX02706 *flp-11(tm2706)*, RB1863 *flp-12(ok2409)*, AX1410 *flp-18(db99)*, RB2188 *flp-20(ok2964)*, RB982 *flp-21(ok889)*, VC1982 *flp-25(gk1016)*, and VC3017 *flp-26(gk3015)*. AX1129 *flp-21(pk1601)* was a gift from the De Bono laboratory, MRC Laboratory of Molecular Biology, Cambridge, UK. MT15933 *flp-17(n4894)* and MT15973 *flp-10(n4543); flp-17(n4894)* were gifts from the Horvitz laboratory, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA. PS7112 *sbt-1(ok901)* was outcrossed six times from CGC RB987; PS7370 *flp-2(ok3351)* was outcrossed three times from CGC VC2591; PS7378 W07E11.1 and *flp-2(gk1039)* was outcrossed three times from CGC VC2490; PS7379 *flp-3(ok3265)* was outcrossed three times from CGC VC2497; PS6813 *flp-13(tm2427)* was outcrossed three times from the Mitani strain FX02427; and PS7221 *flp-34(ok3071)* was outcrossed three times from CGC RB2269.

Transcriptional Reporter Strains. Transcriptional reporter constructs were built using fusion PCR (1). The promoter regions of *srt-41* and *col-40* were fused to the *mCherry*::*unc-54* 3′ UTR (amplified from pGH8 from Addgene). The flanking sequences of the amplified *srt-41* promoter were GCACAGTTTTAAGTTTTTCTGTCTT and TGCTGCCAACCTGTTCTG. The flanking sequences of the amplified *col-40* promoter were ATGATGACCGCCTGATTTTC and AATTATTGTAGTAAAGGGGGAAGTC. Injection mixtures were prepared at a concentration of 20 ng/μL reporter construct, 50 ng/μL *unc-119(+)* rescue construct, and 130 ng/μL 1-kb DNA ladder carrier DNA. Transgenic animals were obtained by microinjecting the mixtures into the adult gonads of *unc-119(ed4)* animals (2, 3). The following fluorescent transcriptional reporter strains were generated: PS7128 *unc-119(ed4); syEx1534[srt-41p::mCherry; unc-119(+)]* and PS6727 *unc-119(ed4); syEx1338[col-40p::mCherry; unc-119(+)]*.

CRISPR-Generated Strains. Guide RNA (gRNA) target sequences of 19 bp [corresponding to sequence upstream of an NGG protospacer adjacent motif (PAM) site] were cloned into pRB1017 single-guide RNA (sgRNA) vector (Addgene). Four distinct gRNA sequences were used to target each gene. Injection mixtures were prepared at a concentration of 25 ng/μL per sgRNA expression plasmid, 50 ng/μL Cas9 plasmid (no. 46168; Addgene), 25 ng/μL *dpy-10* sgRNA plasmid (pJA58; Addgene), and 500 mM *dpy-10(cn64)* donor oligonucleotide (synthesized by Integrated DNA Technologies). Injected P_0 hermaphrodites were transferred to individual Petri plates to produce F_1 progeny. F_1 progeny exhibiting a Rol or Dpy phenotype were picked to individual Petri plates 4 d after injection. F_1s that produced Rol or Dpy F_2s were genotyped for the presence of a deletion allele. Homozygous deletion mutants were isolated from the F_2 or F_3 population, and the deletion alleles were confirmed by Sanger sequencing (Laragen).

The 1,343-bp *flp-21(sy880)* deletion is flanked by the sequences TATGTACACTATTTAAGATTTGATTGTGTA and CATTCGGGGCCACAAACTCCTGCTTCGATC. *flp-32(sy853)*, *flp-34(sy810)* and *flp-34(sy811)* deletion alleles have short DNA fragment insertions. The 460-bp *flp-32(sy853)* deletion is flanked by the sequences TATGAATATGTTCCGGAGCGCATGTCAAAC and AACTAAAGATACACCACTACCACCTGAACC, with a TAACT insertion. The 1,365-bp *flp-34(sy810)* deletion is flanked by the sequences TCAAATTTTTTGAGGAAATCCTCCTGAAAC and AATATTTTCGAGTTTCGAAACATTTCAAAT with a AATATATTTTCGAGTTTCGAAACATATTTTCGAGTTTCGAAACAC insertion. The 1,607-bp *flp-34(sy811)* deletion is flanked by the sequences TTTGTGTCTAGCAAAAGGAGATGCTCTTTA and CATAGGCGTAGGCCATAGGCGTAGGCCATA with a AATAAATTAATTAAATATCTGAAATAAAAACAAAACCTCGAGAGAGAAAATTTAGAAAAAAAAACGAGACGGCTACGGACGGGCTGACGTGATGGAATTATTTACGGCCAAATCTGAAAATAAAATGGATTATATTTTGTTTTAGGCCATAGACGTAGGTCATAGGCGTAGACCATAGGCGTAGGC insertion.

Statistical Analysis for the Dauer-Entry Assay. For each genotype, the data from all the plates tested for that genotype were pooled, and the numbers of dauers and nondauers were converted into a Boolean array (1 for dauer, 0 for nondauer). Nonparametric bootstrapping was used to sample the data array (with replacement) to calculate a corresponding dauer-entry percentage. This procedure was repeated 10,000 times to construct a dauer-entry percentage distribution from which the mean and 99% CI were calculated.

For each comparison between two genotypes, data arrays from the two genotypes were concatenated, shuffled, and split into two datasets of original size as before concatenation, and a difference of means was calculated between the two new datasets. This procedure was repeated 10,000 times to generate a distribution of differences of means that simulated the null hypothesis. The *P* value was calculated as the fraction of the distribution in which the simulated difference was greater than or equal to the observed difference.

A binomial likelihood was used with a uniform prior for values in the range [0, 1], so that the log posterior probability distribution was proportional to the log binomial distribution in the allowed range. The data for each genotype were pooled, and the posterior distribution was sampled using Markov chain Monte Carlo (MCMC). The difference between mutant and wild-type animals was computed by subtracting the respective MCMC samples.

1. Hobert O (2002) PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic C. elegans. *Biotechniques* 32:728–730.
2. Mello C, Fire A (1995) DNA transformation. *Methods Cell Biol* 48:451–482.
3. Maduro M, Pilgrim D (1995) Identification and cloning of unc-119, a gene expressed in the Caenorhabditis elegans nervous system. *Genetics* 141:977–988.

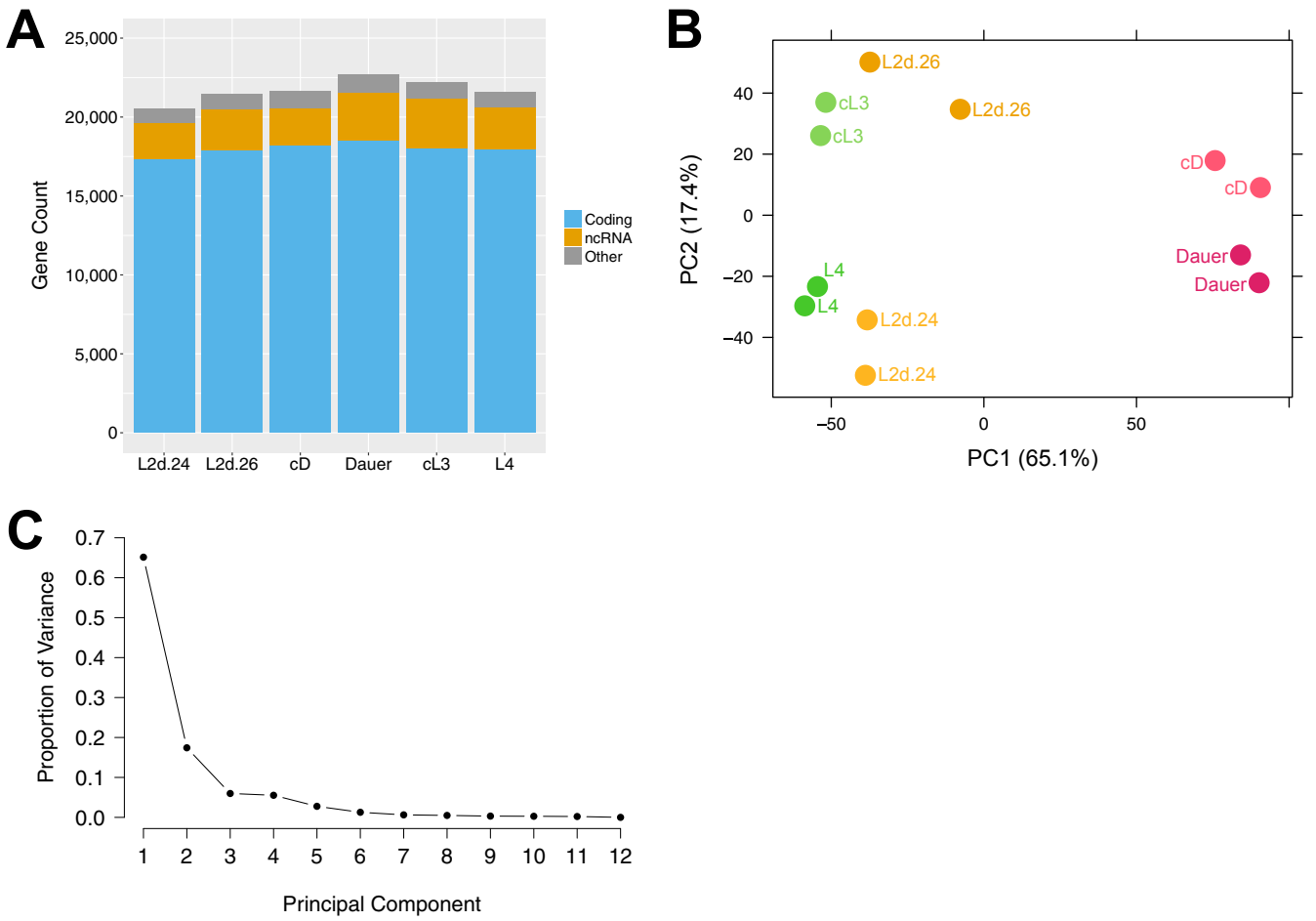


Fig. S1. *daf-9(dh6)* RNA-seq dataset summaries. (*A*) Detected gene counts from the six sequenced stages along dauer and reproductive development. (*B*) PCA plot of the variation in gene expression across the 12 sequenced samples. The proportions of total variation spanned by PC1 and PC2 are listed in parentheses. (*C*) Scree plot demonstrating the proportion of the total variation between the 12 sequenced samples that is explained by each PC in the PCA.

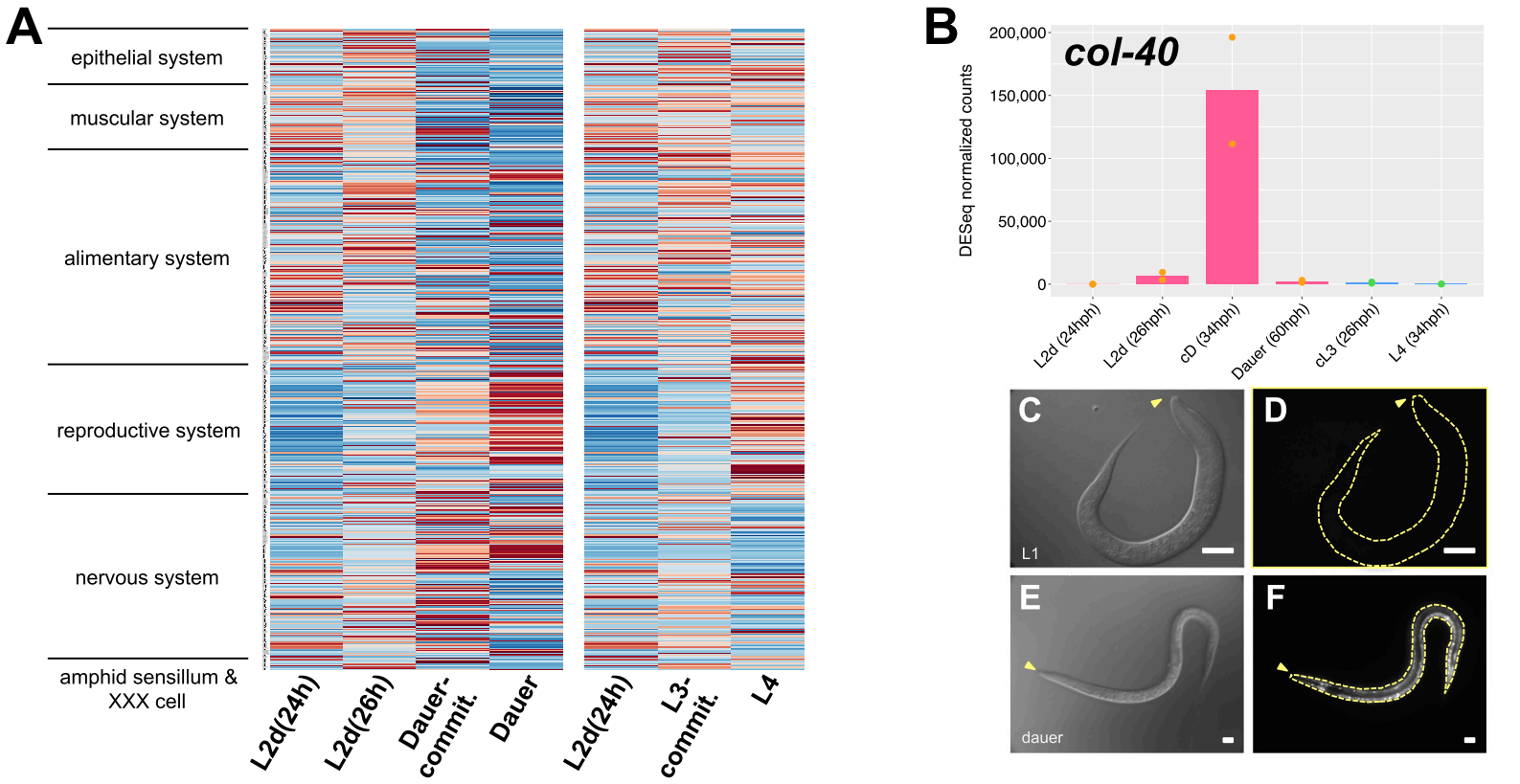


Fig. S2. Differential expression was detected with high accuracy and at single-cell resolution. (*A*) The expression profiles of differentially expressed genes with putative tissue-specific expression in the epithelial system, muscular system, alimentary system, reproductive system, nervous system, and amphid sensillum and XXX cell. The expression data were scaled and heatmapped as in Fig. 3. (*B*) Detected read counts for the *col-40* gene. Points indicate count values from each sequenced replicate. The bar height represents the mean count value for each stage. cD, dauer-committed; cL3, L3-committing; hph, hours post hatch. (*C* and *D*) Brightfield and fluorescence images of the *col-40* nondauer expression pattern (shown is an L1). The body is traced in yellow dotted lines, and yellow arrows point to the mouth for reference. (*E* and *F*) Brightfield and fluorescence images of the *col-40* dauer expression pattern. (*G*) Detected read counts for the *srt-41* gene. (*H* and *I*) Brightfield and fluorescence images of the *srt-41* nondauer expression pattern (shown is an L2). The intestine is traced in blue dotted lines for reference. (*J* and *K*) Brightfield and fluorescence images of the *srt-41* dauer expression pattern. The two fluorescence images in *I* and *K* were captured using the same imaging parameters. (*L*–*O*) *srt-41p*::*mCherry* expression in the AWC^{on} neuron. GFP expressed from the AWC^{on} marker *str-2p*::*gfp* (*M*) and mCherry from *srt-41p*::*mCherry* (*N*) colocalized in the same cell, as shown in the merged image (*O*). Pictured is a nondauer, since *str-2p*::*gfp* changes expression to the ASI neuron in dauers (1). (Scale bars: *C*–*F* and *H*–*O*, 20 μm.) (*P*) Venn diagram comparing our dataset with SAGE data published in ref. 2, and microarray data published in ref. 3, drawn using the eulerr package (4). Differential expression in our data was tested for using comparisons 2–6 and 11–12 to identify genes that were significantly up-regulated at dauer commitment and dauer relative to L2d and L4.

1. Peckol EL, Troemel ER, Bargmann CI (2001) Sensory experience and sensory activity regulate chemosensory receptor gene expression in Caenorhabditis elegans. *Proc Natl Acad Sci USA* 98:11032–11038.
2. Jones SJ, et al. (2001) Changes in gene expression associated with developmental arrest and longevity in Caenorhabditis elegans. *Genome Res* 11:1346–1352.
3. Wang J, Kim SK (2003) Global analysis of dauer gene expression in Caenorhabditis elegans. *Development* 130:1621–1634.
4. Larsson J (2016) eulerr: Area-Proportional Euler Diagrams, Version 2.0.0. Available at https://cran.r-project.org/package=eulerr. Accessed November 10, 2017.

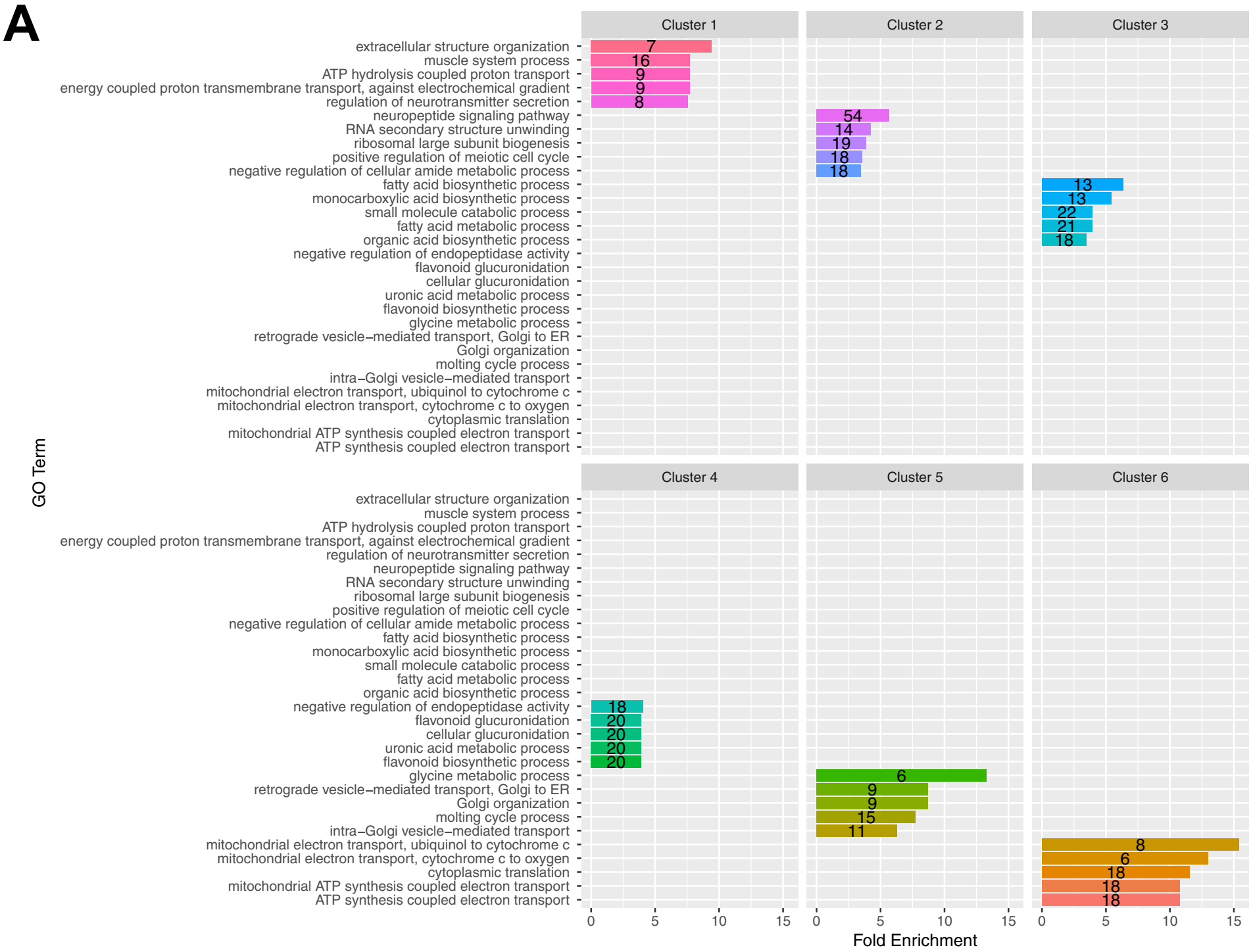


Fig. S3. Enriched GO terms and KEGG pathways in clusters 1–6. The number in each bar indicates the number of genes with that term in the cluster. (*A*) The five most enriched GO terms (based on descending fold enrichment) in clusters 1–6, using a cutoff of Bonferroni-adjusted *P* value < 0.05. (*B*) The five most enriched biochemical pathways (based on ascending *q*-value) in clusters 1–6, using a cutoff of BH-corrected *q*-value < 0.05.

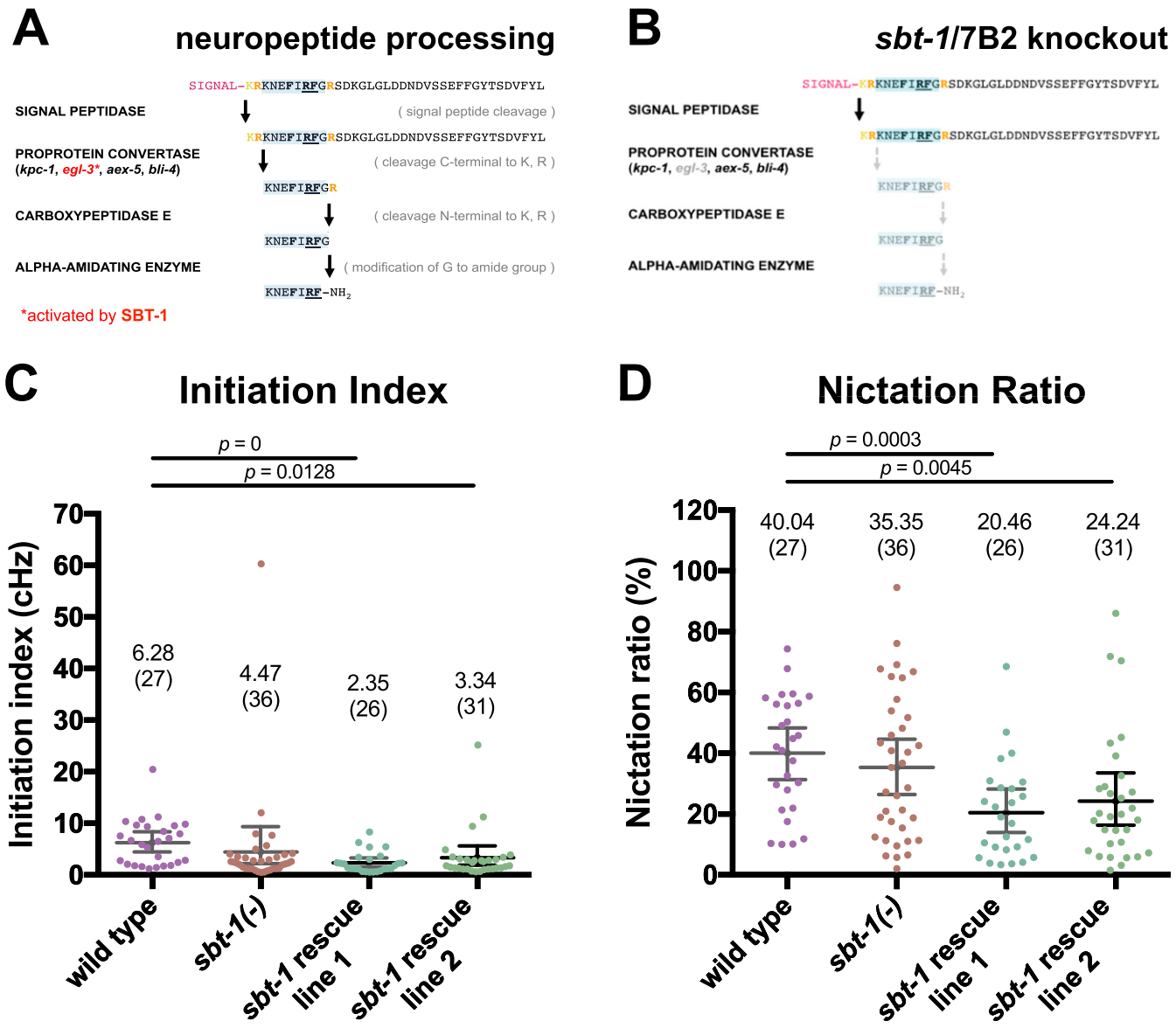
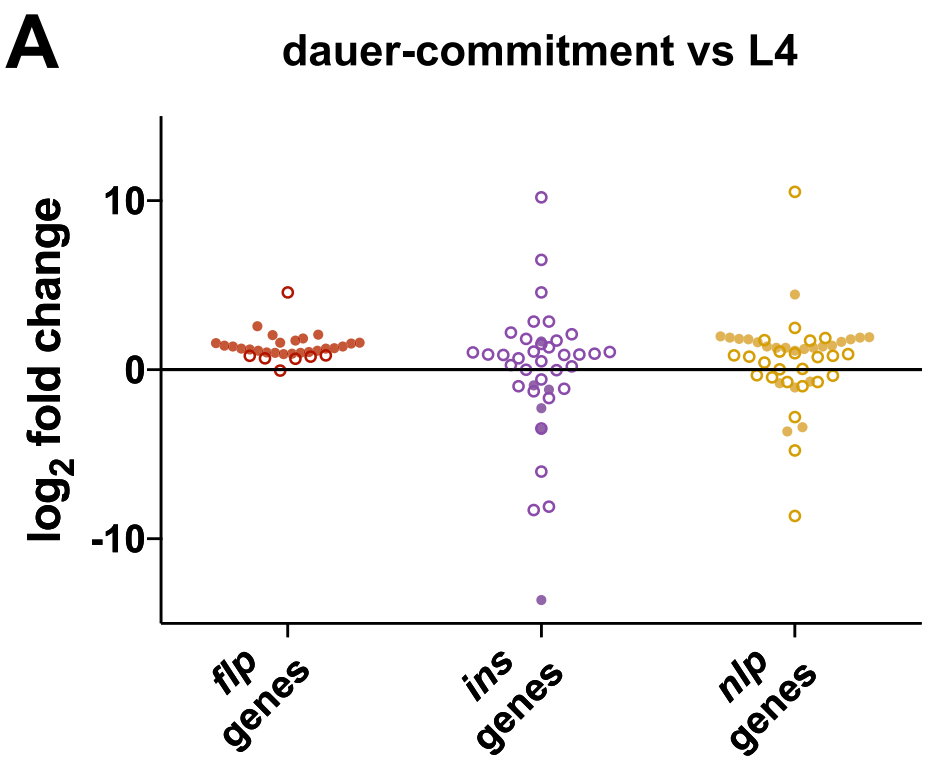
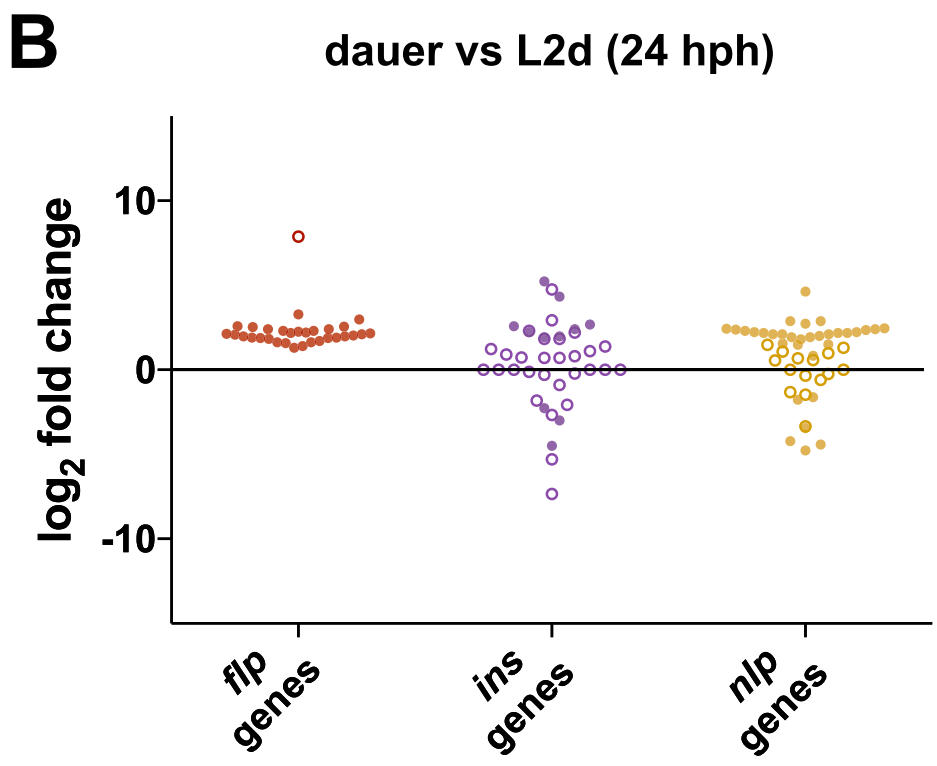
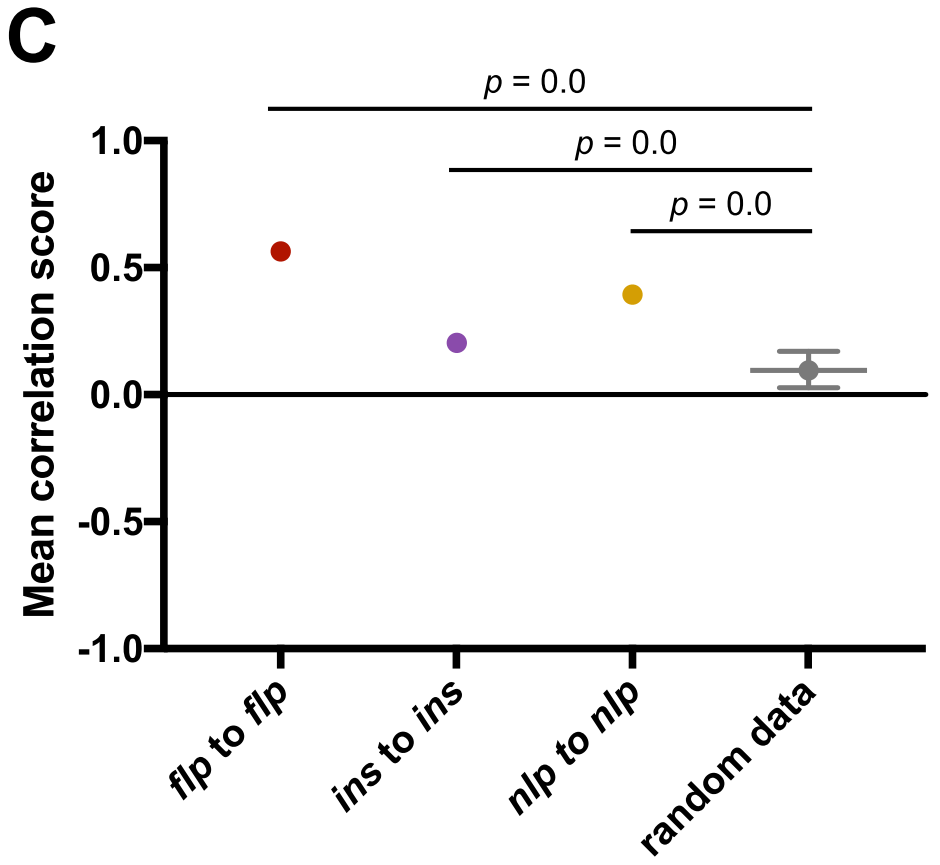
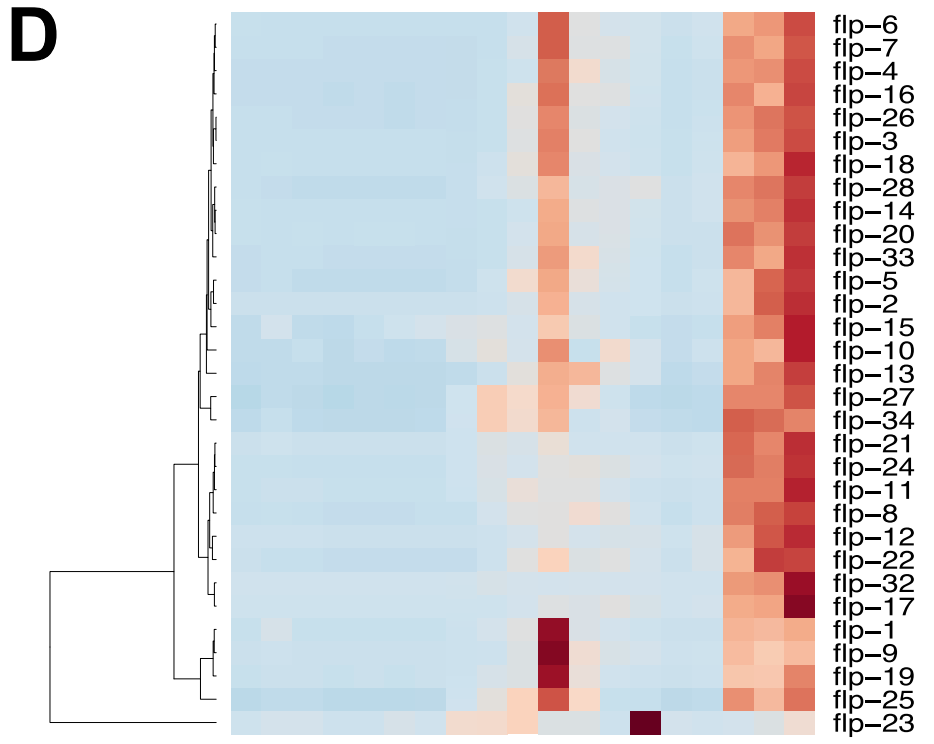


Fig. S4. Nictation initiation and the proportion of time spent nictating are not significantly affected in *sbt-1*–null mutants. (*A* and *B*) Neuropeptide processing in wild-type (*A*) and *sbt-1*–null (*B*) animals, using the FLP-8 peptide sequence as an example. (*C* and *D*) Nictation initiation (*C*) and ratio (*D*) measurements that were collected simultaneously with the nictation duration data in Fig. 4*B*. Bootstrapped means and 99% CIs are indicated. Statistic: permutation test.









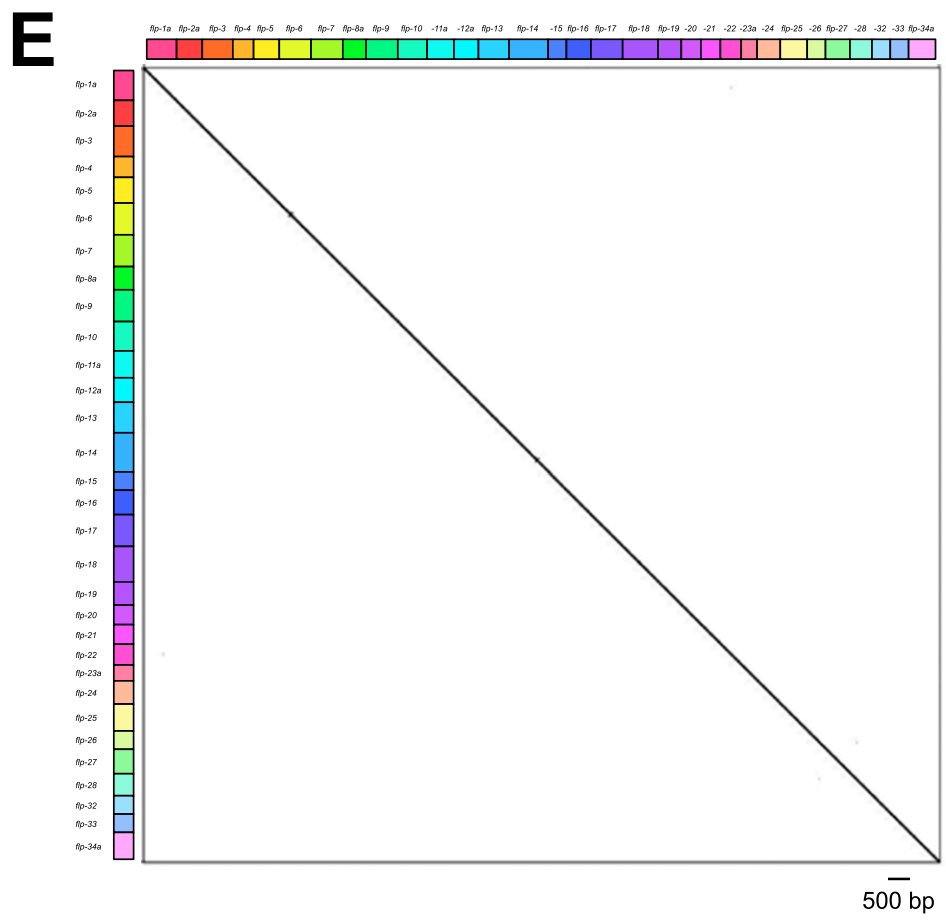
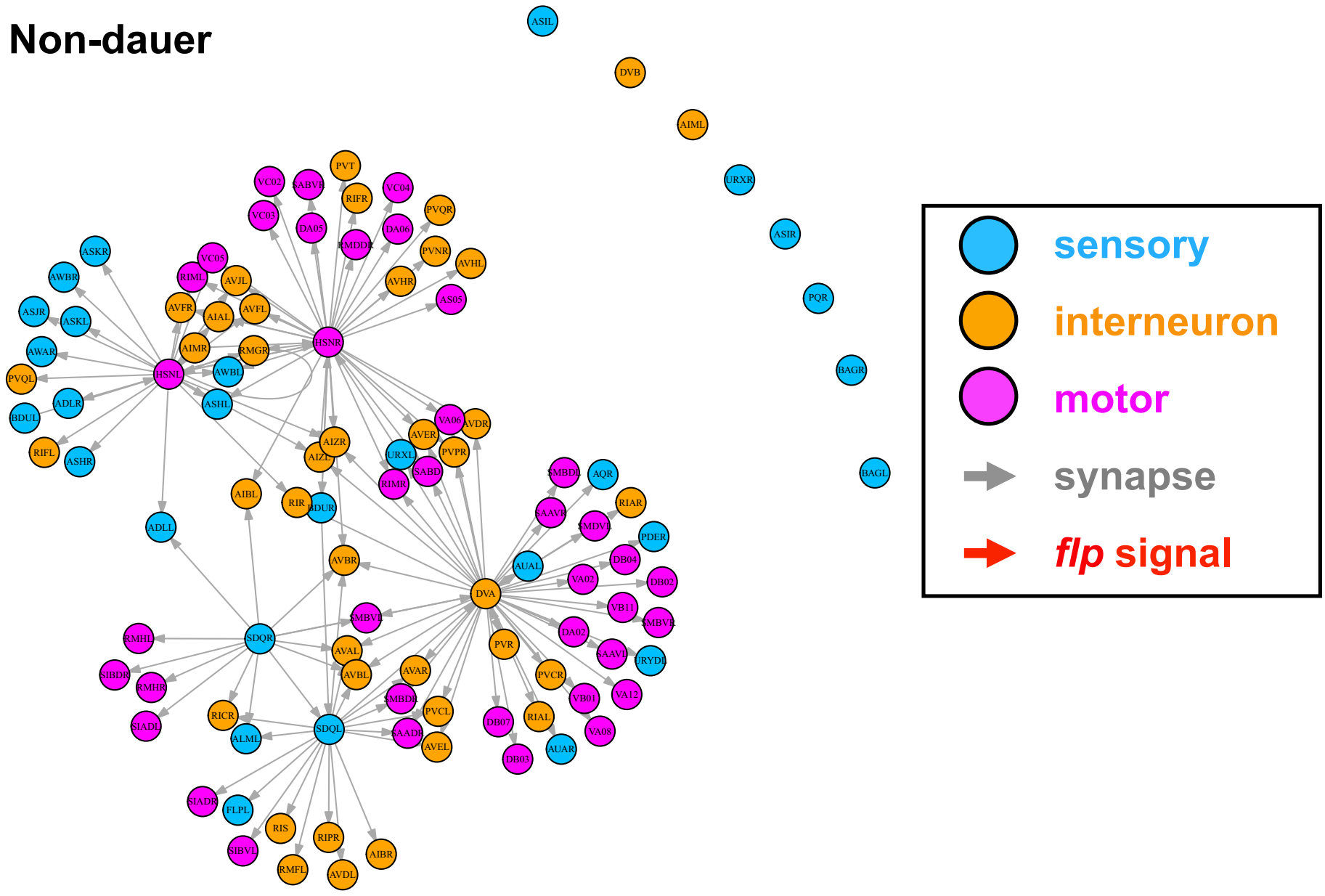


Fig. S5. FMRFamide-like peptides are coordinately up-regulated during dauer development. (*A* and *B*) Fold changes in gene expression for all 118 *C. elegans* neuropeptide genes during dauer commitment versus L4 (*A*) and dauer versus L2d (*B*). Each circle represents an individual neuropeptide gene. Closed and open circles indicate significant and nonsignificant differential expression, respectively. (*C*) Average Spearman correlation scores of genes to other genes of the same neuropeptide family, calculated across 246 publically available RNA-seq datasets describing various *C. elegans* life stages and experimental conditions, including embryos, larvae, adults, and males (1). (*D*) Heatmap of *flp* median gene expression (analyzed in TPM) across the 246 RNA-seq datasets (1). The expression data were scaled and heatmapped as in Fig. 3. (*E*) Dotplot of the coding sequences of all 31 *flp* genes, compared against each other. The *x* and *y* axes represent the concatenated coding sequences of the 31 *flp* genes (using only the a isoform if multiple isoforms exist for that gene). Regions of sequence similarity are represented as a diagonal line of hits along the alignment space; a minimum of 20 identical, consecutive nucleotides was required to generate a hit.

1. Gerstein MB, et al.; modENCODE Consortium (2010) Integrative analysis of the Caenorhabditis elegans genome by the modENCODE project. *Science* 330:1775–1787.

Non-dauer



Dauer

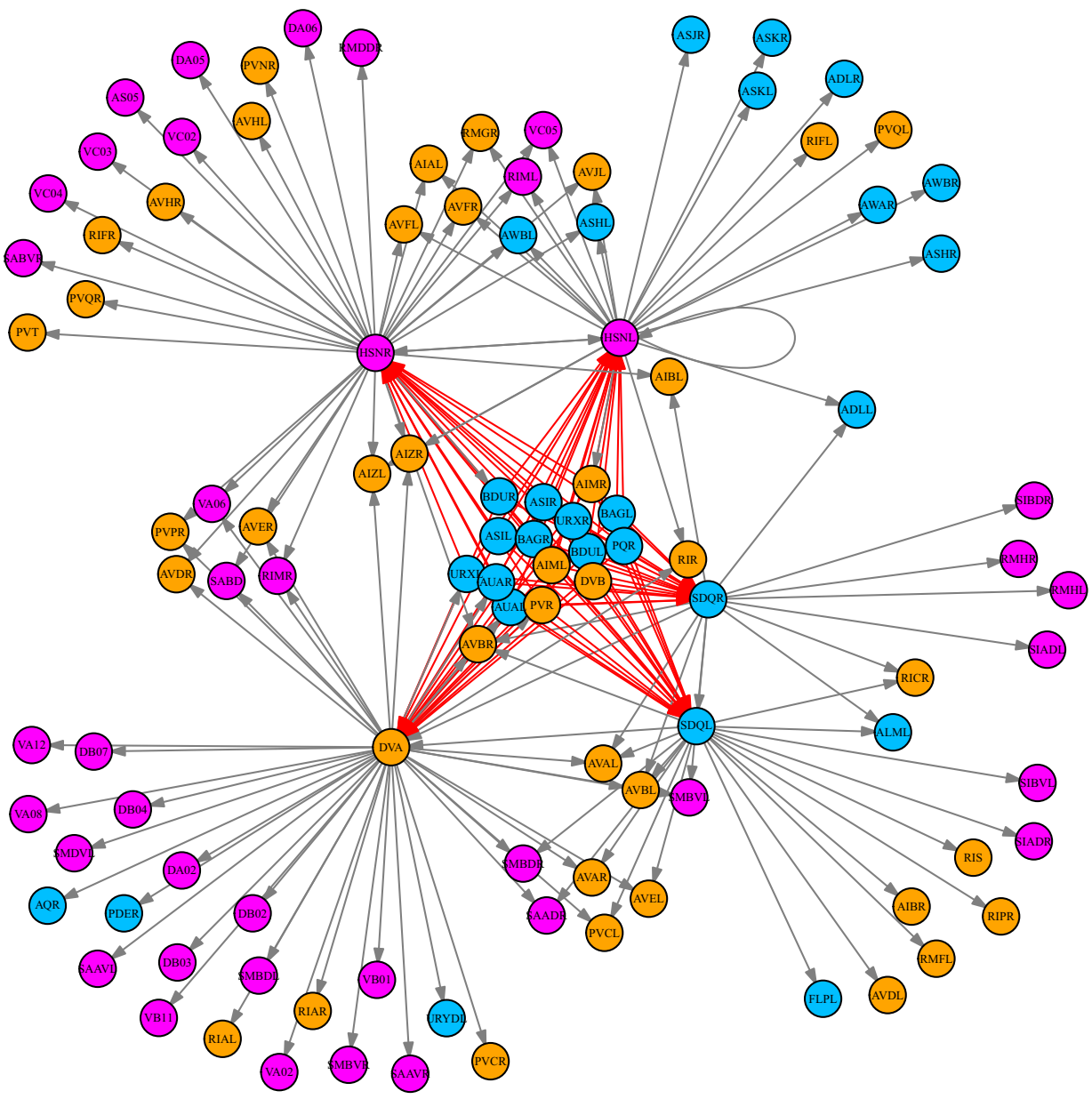
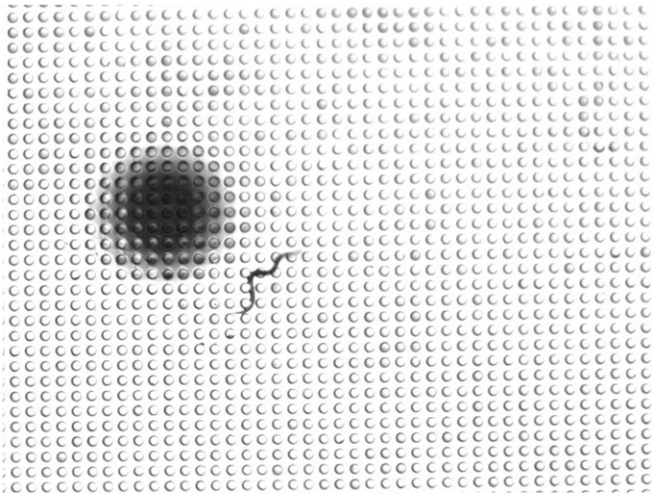


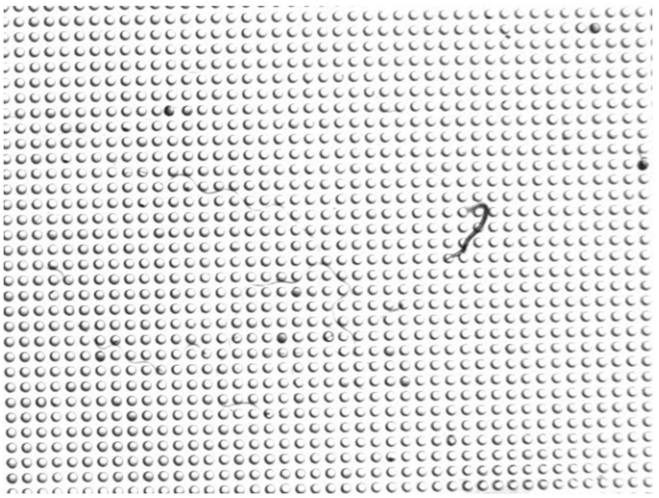
Fig. S6. Model of circuit changes during dauer development via nonsynaptic FLP signaling. The FLP-10 ligand EGL-6 receptor circuit is shown as an example. The synaptic connections that are indicated are (in presynaptic to postsynaptic order) from *flp-10*–expressing neurons to *egl-6*–expressing neurons to synaptic targets directly downstream. Expression pattern, connectomic, and biochemical data from refs. 1–3, WormWiring, and WormBase were used.

1. Li C, Kim K (2008) Neuropeptides. *WormBook*, 10.1895/wormbook.1.142.1.
2. Li C, Kim K (2014) Family of FLP peptides in Caenorhabditis elegans and related nematodes. *Front Endocrinol (Lausanne)* 5:150.
3. White JG, Southgate E, Thomson JN, Brenner S (1986) The structure of the nervous system of the nematode Caenorhabditis elegans. *Philos Trans R Soc Lond B Biol Sci* 314:1–340.



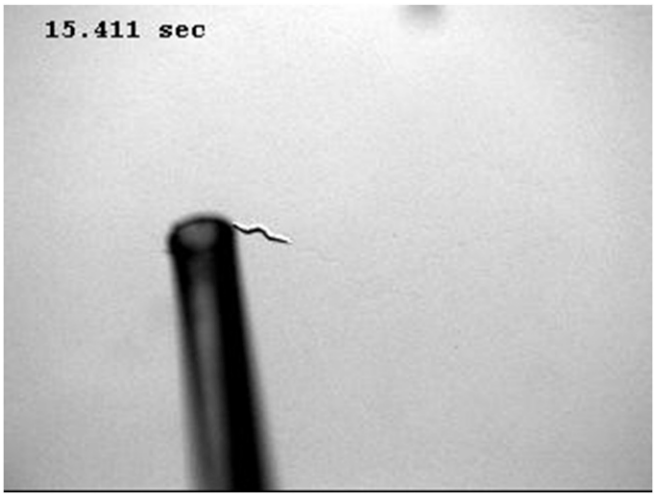
Movie S1. Wild-type nictation. A wild-type dauer nictating on a microdirt chip.

Movie S1



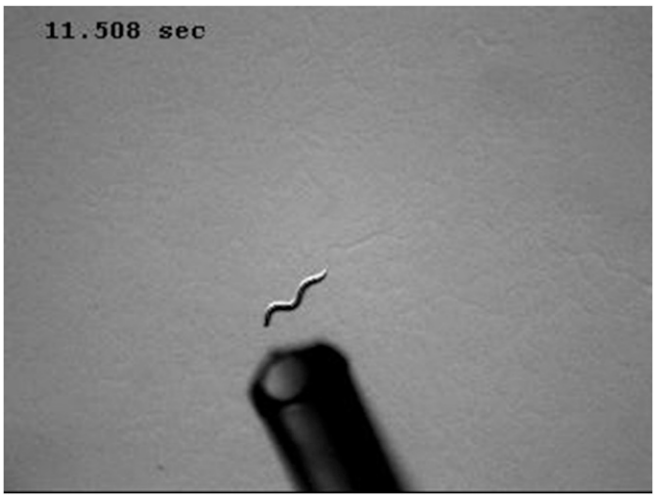
Movie S2. *sbt-1(-)* nictation. A *sbt-1(ok901)* dauer nictating on a microdirt chip.

Movie S2



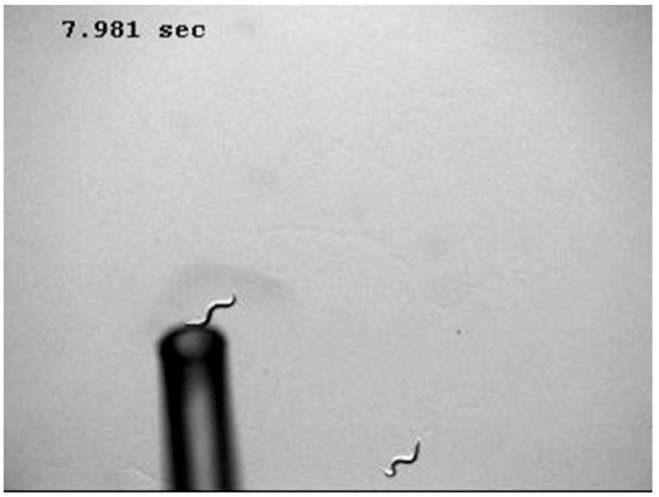
Movie S3. Wild-type CO_2 response. A wild-type dauer responding to CO_2 gas mixture.

Movie S3



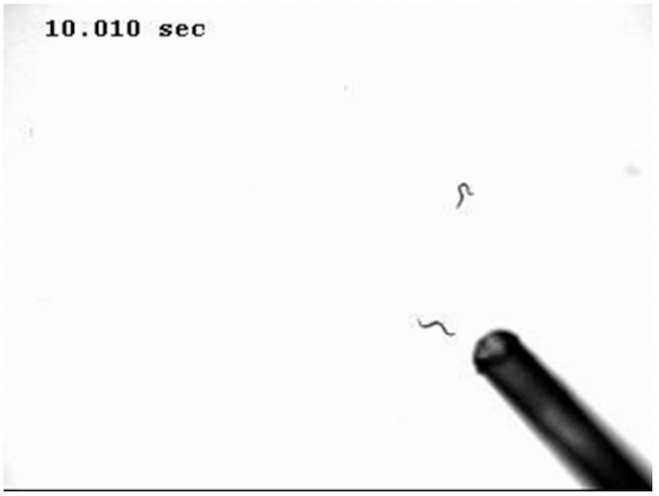
Movie S4. Wild-type response to control gas. A wild-type dauer responding to the control gas mixture.

Movie S4



Movie S5. *sbt-1(-)* CO_2 response. A *sbt-1(ok901)* dauer responding to CO_2 gas mixture.

Movie S5



Movie S6. *sbt-1(-)* response to control gas. A *sbt-1(ok901)* dauer responding to the control gas mixture.

Movie S6

Dataset S1. Metadata of our *daf-9(dh6)* RNA-seq experiment

Dataset S1

The detailed metadata, including the experimental conditions and sequencing depth, of each of our sequenced libraries. DA, dafachronic acid; hph, hours post hatch; PE, paired-end; SE, single-end.

Dataset S2. Normalized expression values for each detected gene in the RNA-seq dataset

Dataset S2

Expression values are given in DESeq-normalized read counts.

Dataset S3. Differentially expressed genes from the 12 pairwise comparisons

Dataset S3

The differentially expressed genes detected from the 12 pairwise comparisons between dauer and reproductive development time points. Differential expression testing was performed using DESeq at a Benjamini–Hochberg controlled false discovery rate cutoff of $P < 0.01$.

Dataset S4. Genes in clusters 1–6

Dataset S4

Mean expression values (in DESeq-normalized read counts) are given for each gene. Positively enriched GO and KEGG terms for each cluster are listed. Significantly enriched GO terms were selected using a cutoff Bonferroni-adjusted P value < 0.05, and significantly enriched biochemical pathways were selected using a Benjamini–Hochberg corrected q-value < 0.05 as a cutoff.

Dataset S5. Differentially expressed neuronal effector genes

Dataset S5

The 606 neuronal effector genes that are differentially expressed during dauer and reproductive development, with hypergeometric test data for over-represented neuronal gene classes in clusters 1–6.