Chapter 4

GENETIC MARKERS ENABLE THE VERIFICATION AND MANIPULATION OF THE DAUER ENTRY DECISION

(This work was done in collaboration with Shih P.Y.)

4.1 Introduction

Phenotypic plasticity enables organisms to respond to changing environments through activation of different phenotypes or alternative developmental courses (1). For example, nutritional factors contribute to the development of morphological distinct ants castes in some species (2), and also influence neuronal plasticity in human (3).

Caenorhabditis elegans can go through two different developmental trajectories depending on the conditions of the environment. In favorable environments, they proceed from L1, L2, L3, and L4 larvae stages to reproductive adults. When the animal senses harsh stimuli, including high temperature, low food, and high amount of pheromone, L1 larva can enter an alternative pre-dauer stage, L2d, and commit to become a dauer if the unfavorable conditions persist. The dauer entry decision is a whole animal decision that involves remodeling of individual tissues to transform the entire animal to have dauer-specific physiology and behaviors. The specialized physiology, the thickened cuticle for example, makes dauer more resistant to environmental insult (4, 5), and the special behaviors enable dauers to disperse to better environments and resume reproductive development (6, 7).

Genes involved in dauer development, including genes in insulin and TGF-beta signaling pathways, have been identified through intense genetic screening (8–11). However, our knowledge regarding how the dauer entry decision is made and how the decision is coordinately executed across different tissues is still limited (12). First, it is difficult to identify L2d, the stage when environmental signals are integrated and the dauer-commitment decision is made, because of its lack of

distinct features (13). Additionally, it can be labor-intensive to look for non-dauer features in dauers that fail to coordinately remodel all the tissues. SDS sensitivity and fluorescent beads are two available tools for dauer hypodermis and pharynx selection (14, 15), but not for other tissues.

We previously reported the time-resolved gene expression profiles from animals going through dauer or reproductive development (16). From the rich dataset, we were able to find genes that are specifically regulated in either of the developmental tracks as potential readouts of the decision. Here we describe four molecular markers that can track the decision at the level of different tissues, and are predictive of the decision. We verified that the markers could also be used to drive gene expression during the dauer entry decision, and to parse incomplete dauer development phenotypes. Our findings provide strong molecular tools for studying phenotypic plasticity during a whole animal decision.

4.2 Results

Dauer and reproductive markers demonstrated specific expression patterns

Unfavorable conditions promote L1 larvae to develop into pre-dauer L2d. Depending on whether the environment improves and enough dafachronic acid (DA) growth hormone gets amplified, L2d larvae can progress to either reproductive or dauer development. In our previous study, we controlled the animals' binary developmental choice by withholding or adding synthetic DA at 24 hours post hatch (hph) to *daf-9(dh6)* mutant, which lack intrinsic DA, and we profiled the transcriptional changes from animals going through dauer (L2d, dauer-committed, and dauer) or reproductive (L3-committing and L4) development (16). To find good markers for dauer, we selected candidate genes based on the following criteria: (i) genes that have high expression specifically during dauer or reproductive development; (ii) genes that are expressed in large tissues, including collagen genes, for convenient observation under low magnification; and (iii) genes that might shed light on dauer biology, including transcription factors and unknown genes.

First, 156 of 164 genes in the collagen (*col*) family were detected and differentially expressed in the RNA-seq dataset. Within those, five collagen genes (*col-2*, *col-37*, *col-85*, *col-40* and *col-183*) have the highest transcripts per million (tpm) counts at the dauer-commitment, while having low counts in other stages (**Figure 4.1**). Indeed, *col-2* and *col-40* have previously been reported to have specific expression in dauer (16, 22). We made a *col-183p::mcherry*

transcriptional reproter strain, and we observed strong mCherry expression exclusively in dauer but not other stages (**Figure 4.2A-C**). *col-85* also has similarly high dauer expression, but dauers expressing *col-85p::mcherry* were abnormally sensitive to SDS treatment (data not shown), possibly caused by promoter quenching or toxicity. Because SDS-resistant is a standard way for selecting and verifying dauers, we excluded *col-85* in further experiments.

Second, we detected 274 transcription factor genes that are differentially expressed during dauer and reproductive development. We clustered those genes by their expression profiles, looked for dauer marker candidates, and found 119 that fit our criteria (**Figure 4.3**). We chose to focus on *ets-10*, a member of the ETS-domain family of transcription factors. The *ets-10* gene had the highest tpm counts during dauer-committed and dauer relative to other stages (**Figure 4.2D**). We observed that *ets-10* is expressed in different tissues during dauer and non-dauer (**Figure 4.2E-F** and **Figure 4.4**). During dauer, *ets-10p::gfp* was expressed in two sets of neurons and the intestine (**Figure 4.2E-F**). In non-dauers, its expression was only observed in uterine cells in L4 animals and spermatheca in adults (**Figure 4.4**).

We also investigated the transcription factor *nhr-246*. The tpm counts of *nhr-246* only increased during dauer development and was at its highest level at the dauer-commitment time point (**Figure 4.2G**). Other than intestinal expression in embryo and L1 stages, *nhr-246p::gfp* was only detected in dauer in intestine and muscle (**Figure 4.2H-I** and **Figure 4.5**).

In addition to dauer-specific genes, we also looked for genes that are downregulated specifically in dauer. Out of the five genes we tested — *asp-1*, *F53F1.4*, *sqt-3*, *dpy-13* and *col-156* — *F53F1.4* marker animals were the healthiest, and had the highest tpm reads in reproductive development (**Figure 4.6** and **Figure 4.2J**). We found that *F53F1.4p::gfp* is expressed in the hypodermis at all stages (**Figure 4.2K-L**), and the fluorescence intensity was reduced in dauer (data not shown). Because of its expression profile, we propose the gene name *led-1*, which stands for “Low Expression in Dauer”.

To sum up, we have developed three dauer markers (*col-183p::mcherry*, *ets-10p::gfp*, and *nhr-246p::gfp*) that have increased expression level and distinct expression patterns in dauers. We have also detected intensity changes in *led-1p::gfp* that mark non-dauers from dauers.

col-183, *ets-10* and *nhr-246* label the dauer commitment decision

Because the dauer marker genes have high expression levels at dauer-commitment, we expected that the fluorescence of these genes might be useful for indicating the dauer-commitment event. If the markers do label the animals that are committed to dauer, then: (i) all dauers will have fluorescence expression (**Figure 4.7A**) and (ii) fluorescent animals will still become dauer even if the environment improves (**Figure 4.7B**). We found that the fluorescence markers were turned on in all the dauers examined (100% for all three markers strains, with 174-311 animals examined per marker) (**Figure 4.7C**). Moreover, after we transferred animals from unfavorable to favorable condition as soon as the

fluorescence was detected, we observed that 96% to 100% of the animals still entered dauer despite the shift to reproduction-promoting environment (*col-183p::mcherry* 100%, n=22; *ets-10p::gfp* 100%, n=18; *nhr-246p::gfp* 96%, n=26) (**Figure 4.7D**). These data suggest that *col-183* and *ets-10* label the dauer commitment decision, and *nhr-246* labels the decision or slightly before commitment.

The promoters of the dauer markers can be used to manipulate the dauer decision

Reproductive development in *C. elegans* requires the synthesis of DA, the product of DAF-9/cytochrome P450. The timing of *daf-9* expression and the amplification of DA in the hypodermis has been shown to coincide with the critical period of time when L2d animals decide to go through reproductive instead of dauer development (23) (**Figure 4.8A-B**). However, it is not known whether ectopically expressing *daf-9* during dauer-commitment can alter developmental trajectory. We therefore used the *col-183* promoter to overexpress *daf-9* in hypodermis during dauer-commitment when *daf-9* would otherwise be expressed at its lowest (**Figure 4.8C**). We then examined the animals' decision between dauer and reproductive development under dauer-inducing conditions. We observed that animals with *daf-9* overexpression were 0.5 times as likely to become dauers compared to those with control *gfp* (*col-183p::daf-9* bootstrap mean = 30%, n = 336; *col-183p::gfp* bootstrap mean = 59%, n = 262) (**Figure 4.8D**). This data suggests that the promoters of the dauer markers can be used

to drive ectopic gene expression during dauer-commitment, and that *daf-9* hypodermal expression can shift animal development from dauer to adulthood.

The dauer markers can be used to study the coordination between tissues

The dauer entry decision is a whole-animal decision, with all the tissues coordinating dauer development programs. Previous studies have identified partial dauers, where one or more of the tissues fail to coordinate and therefore exhibit non-dauer features. Known partial dauer phenotypes include continued pharyngeal pumping, indistinct dauer alae on the cuticle, and L2/L3-like pharynx, neuron, intestine, or excretory gland morphologies. For example, *daf-9(e1406)/*cytochrome P450 dauers have a non-dauer intestine, cuticle, pharynx, and neurons (24); *daf-15(m81)*/RAPTOR dauers fail to remodel the cuticle, pharynx, neurons intestine and excretory gland (24); *daf-18(e1375)*/PTEN dauers have an unremodeled, still pumping pharynx, and an intestine that is neither fully dauer nor L3 (25).

Because identifying partial dauers relies on close examination of the animal's morphology, it can be time-consuming and requires experience. We therefore utilized the dauer-specific *ets-10p::gfp* expression in neurons and intestine to pinpoint partial dauer phenotypes.

In *daf-9(e1406)* dauers, we confirmed their partial dauer phenotype in the intestine: we observed a 3-fold decrease in *ets-10p::gfp* expression in the intestine compared to wild type dauers (average intensity in wild type = 9017 arbitrary units (a.i.), n = 26; average intensity in *daf-9(e1406)* = 2998 a.i., n = 25)

(**Figure 4.9A-B** and **Figure 4.9K**), providing a clear indication of the non-dauer feature of *daf-9(e1406)* intestines.

We were also able to confirm the intestinal partial dauer phenotype of *daf-15(m81)* animals as well: we observed a 4-fold reduction in *ets-10p::gfp* intestinal expression compared to wild type (average intensity in wild type = 7166 a.i., n = 12; average intensity in *daf-15(m81)* = 1512 a.i., n = 16) (**Figure 4.9C-D** and **Figure 4.9K**). Additionally, we confirmed the neuronal partial dauer phenotype of *daf-15(m81)*, as neuronal *ets-10p::gfp* fluorescence was present in all wild type animals (n=20), but was undetectable (16 out of 20 animals) or dimly expressed (4 out of 20) in *daf-15(m81)* (**Figure 4.9G-H** and **Figure 4.9L**).

In *daf-18(e1375)*, we observed a slight increase in *ets-10p::gfp* intestinal expression (average intensity in wild type = 3299 a.i., n = 11; average intensity in *daf-18(e1375)* = 5169 a.i., n = 9) (**Figure 4.9E-F** and **Figure 4.9K**), and the disappearance of neuronal expression in most of the animals (9 out of 10) (**Figure 4.9I-J** and **Figure 4.9L**). These results not only confirmed the partial dauer characteristic of *daf-18(e1375)* intestine, but also revealed the previously unknown non-dauer characteristic of *daf-18(e1375)* neurons.

From our results, we have identified *ets-10p::gfp* as a tool for studying the execution of the dauer decision in different tissues. We propose a model for how *ets-10* expression is differentially regulated in the dauer intestine and neurons by DAF-9, DAF-15 and DAF-18 (**Figure 4.9M**). In the dauer intestine, DAF-15 and DAF-9 promote *ets-10* expression and DAF-18 inhibit *ets-10*; both DAF-15 and DAF-18, but not DAF-9, positively regulate *ets-10* expression in the dauer

nervous system. This model suggests that the same signal (*e.g.* DA produced by DAF-9/cytochrome P450) can have distinct effects on the differentiation of different tissues in dauer.

4.3 Discussion

We have described four genetic markers that label dauer or non-dauer animals, and which can be used for conveniently assaying the dauer entry decision. We demonstrated that the dauer markers in fact mark the dauer-commitment decision using condition-shift experiments. Beyond fluorescence labeling, we were able to use the promoter region to manipulate the commitment decision, and to tease apart the tissue-specific defects of partial dauer mutants.

We picked members of the hypodermis-expressed collagen gene family as one of our dauer marker candidates because they fit our criteria of being expressed at high levels and in a large tissue. In addition, they offered the opportunity to learn more about the role of hypodermal *daf-9* expression in the developmental decision. When animals commit to reproductive development, *daf-9* functions by promoting a positive feedback amplification loop in the hypodermis to lock in the decision (23). Even under dauer-inducing conditions, when we introduced *daf-9* expression under the control of *col-183* promoter, we were able to shift the animal's decision toward reproduction.

Notably, dauer-specific collagen expression has been reported before for *col-2* (22), but we are the first to connect the expression of a collagen gene with the dauer-committment decision. We speculate that the biological function of *col-183* is to shape the stress-resistance and impermeability of dauer cuticle starting from the commitment decision (4, 26).

We also looked at the transcription factor gene class for additional marker candidates. We found that both *ets-10* and *nhr-246* demonstrated dauer-specific

expression patterns during dauer-commitment, suggesting their function in execution and maintainance of the dauer program. For instance, the expression of *ets-10* and *nhr-246* in intestine might help establish the specialized intestine structure and metabolism of dauers. We speculate that they participate in remodeling the dauer intestine or switching metabolism from the TCA cycle to long-term lipid metabolism (27, 28).

The full coordination of tissue physiology and function is important for dauer survival. Using these markers, we can study how tissue-coordination is achieved during dauer development. Partial dauers represent breaks in tissue-coordination, and by using the markers we can read out their phenotypes on a molecular level. Using *ets-10* markers, we were able to not only recapitulate known partial dauer phenotypes in *daf-9*, *daf-15* and *daf-18*, but identify the previously unknown function of DAF-18 in remodeling dauer neurons. Moreover, we found that DA and insulin signals (controlled by *daf-9* and *daf-15*, *daf-18*, respectively) are combined in discrete ways to control *ets-10* expression in different tissues. It would be intriguing to figure out how different tissues might use different *cis*-regulatory elements and signaling receptors to interpret the same signal to meet their specialized needs.

We have described three dauer-specific markers and one reproductive-specific marker selected from our previously published dauer RNA-seq time course. We have demonstrated that these markers are useful for tracking the dauer-committment decision, driving gene expression during dauer-committment, and for teasing apart partial dauer phenotypes tissue by tissue. 117 transcription

factor genes and 6 collagen genes also fit the selection criteria we used to pick our markers. This selection opens up the exciting potential of using these genes for further tracking, manipulating, and parsing the dauer entry decision.

4.4 Materials and Methods

Animal strains.

C. elegans strains were grown using standard protocols with the *Escherichia coli* strain OP50 as a food source (17). The wild type strain is N2 (Bristol). Other animal strains are listed below.

Transgenic strains.

Transcriptional reporter strains. All of the transcriptional reporters were built suing fusion PCR (18). Primers used to amplify the promoter regions and the amplified promoter sizes were as follows: *col-183p* (*col-183* promoter, 1695bp)

forward- AATCGCAAACCTTCAACGAAGAG,

reverse- tcacccttgagaccattaagcGGTTGACTGGTTGCTGTTGCT;

ets-10p (1111bp)

forward- GGTTGACTGGTTGCTGTTGCT,

reverse-agtcgacctgcaggcatgcaagct GTTTGTCAGCTAGTTTGCGG;

nhr-246p (3069bp)

forward- GTTTGTCAGCTAGTTTGCGG,

reverse- agtcgacctgcaggcatgcaagctATTGTTGAAATTGAAAATTATTTTGAA;

F53F1.4p (1851bp)

forward- ATTATGTAGGCCCAATATAAAGTTTGA,

reverse- agtcgacctgcaggcatgcaagct GTTGAAAATGTTGAAAGTCAAAAGAG.

The promoter regions of *ets-10*, *nhr-246* and *F53F1.4* were fused to *gfp::unc-54* 3'UTR (amplified from pPD95_75 from Addgene), and the promoter region of

col-183 was fused to *mCherry::unc-54* 3'UTR (amplified from pGH8 from Addgene). Injection mixture was 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 strain was obtained by microinjecting the mixtures into the adult gonads of *unc-119(ed4)* animals. The *ets-10p::gfp* and *F53F1.4p::gfp* were further integrated into the genome by X-ray (19, 20). The fluorescent transcriptional reporter strains generated were as follows: PS6725 *unc-119(ed4); syEx1337[col-183p::mcherry; unc-119(+)]*; PS7127: *unc-119(ed4); syIs360[ets-10p::gfp; unc-119(+)]* (outcrossed 3 times); PS7921 *unc-119(ed4); syEx1539[nhr-246p::gfp; unc-119(+)]*; PS7920 *unc-119(ed4);* PS6724: *unc-119(ed4); syIs263[F53F1.4p::gfp; unc-119(+)]* (outcrossed 10 times).

Transcriptional reporter in partial dauer mutant backgrounds. The strains with *ets-10p::gfp* expression in *daf-15(m81)* or *daf-9(e1406)* background were generated by crossing PS7127 with DR732 *daf-15(m81) unc-22(s7)/nT1* or AA823 *daf-9(e1406) dhEx354[sdf-9::daf-9cDNA::GFP; lin-15(+)]*. The strain with *ets-10p::gfp* expression in *daf-18(e1375)* background was obtained by microinjecting the injection mixture (20 ng/μL reporter construct, 50 ng/μL *ofm-1p::rfp* coelomocyte co-injection marker, and 130 ng/μL 1-kb DNA ladder carrier DNA) into the adult gonads of CB1375 *daf-18(e1375)*.

daf-9 overexpression strain. col-183 promoter region were cloned into the pSM vector that contains *gfp* or *daf-9* cDNA. *daf-9* cDNA sequence was obtained from

Wormbase and amplified with forward primer ATGCACTTGGAGAACCGTG and reverse primer TTAGTTGATGAGACGATTTCCG. Injection mixture was prepared at a concentration of 20 ng/μL *col-183p::gfp* or *col-183p::daf-9* cDNA, 50 ng/μL *ofm-1p::rfp* coelomocyte co-injection marker, and 130 ng/μL 1-kb DNA ladder carrier DNA. Transgenic strain was obtained by microinjecting the mixtures into the adult gonads of wild type animals. The transgenic strains generated were PS7949 *syEx1628[col-183p::gfp; ofm-1p::rfp]* and PS7931 *syEx1629[col-183p::daf-9 cDNA; ofm-1p::rfp].*

Dauer induction.

The preparation of crude pheromone and the induction of dauers on pheromone plates were performed with previously described methods (16, 21). Briefly, crude pheromone plates (NGM-agar with added crude pheromone and no peptone) were used to induce synchronized dauers: For each pheromone plate, 20 μL of heat-killed OP50 (8 g/100 mL) were spotted and 12-15 young adult animals were picked onto the plate to lay eggs at 20°C for 3 (for *environmental condition shift*) or 12 hours (for examing fluorescence expression in dauer) before being removed. The plates were then moved to 25.5°C incubation for 48 hours.

Verification of dauer markers.

SDS assay on fluorescent animals

Dauers induced on pheromone plate were identified by morphology and examined for the presence of fluorescence expression. The fluorescence animals were

further transferred to unseeded plates and treated with 1% SDS. The numbers of total and survived animals were scored after 15 minutes.

Environmental condition shift of fluorescent animals.

The fluorescence expression in the transcriptional reporter strains was detectable under dissecting microscope staring around 30-32 hours after egg laid. At 33-34 hour, we transferred the fluorescent animals from dauer-inducing pheromone plates to reproduction-inducing plates, which contain high amount of bacteria and no pheromone. 24 hours after the transfer, the animals were treated with 1% SDS, and the numbers of total and survived animals were scored after 15 minutes.

Quantification of fluorescence intensity.

The fluorescence intensity of *ets-10p::gfp* was measured using ZEISS ZEN microscope software. The regions of interests were drawn on both the intestine and the background area, and the net fluorescence intensity was calculated as the subtraction of the two measurements.

Dauer formation assay.

The preparation of crude pheromone and the dauer entry assay were performed with previously described methods (16, 21). On the day of the experiment, seven to ten young adults were picked onto each pheromone plate (NGM-agar with added crude pheromone and no peptone), and allowed to lay approximately 50-60 eggs before being removed. 20 µl of heat-killed OP50 was added to the plates as

a food source for the un-hatched larvae. After 48 hours of incubation at 25.5°C, dauers and non-dauers were counted on each plate based on their distinct morphologies. The permutation test was used to calculate statistics as previously describe (16).

4.5 Figures

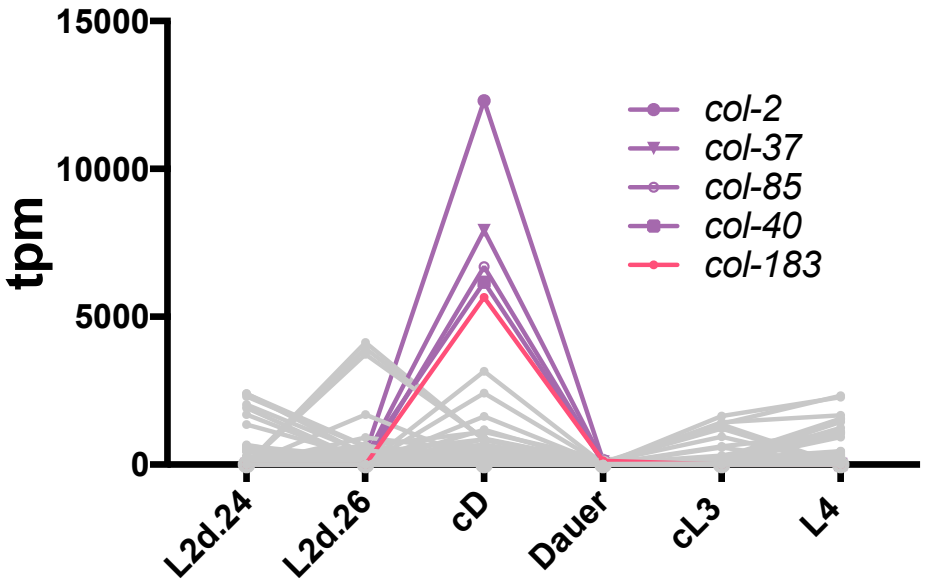


Figure 4.1. Expression profiles of collagen genes

Expression profiles of all the collagen genes detected. Each line represents one collagen gene. The top five genes with the highest expression level were highlighted in purple (*col-2*, *col-37*, *col-85*, and *col-40*) and pink (*col-183*). The rest of the genes were colored in grey for simplicity. All the expression data plotted were from our previous paper (16).

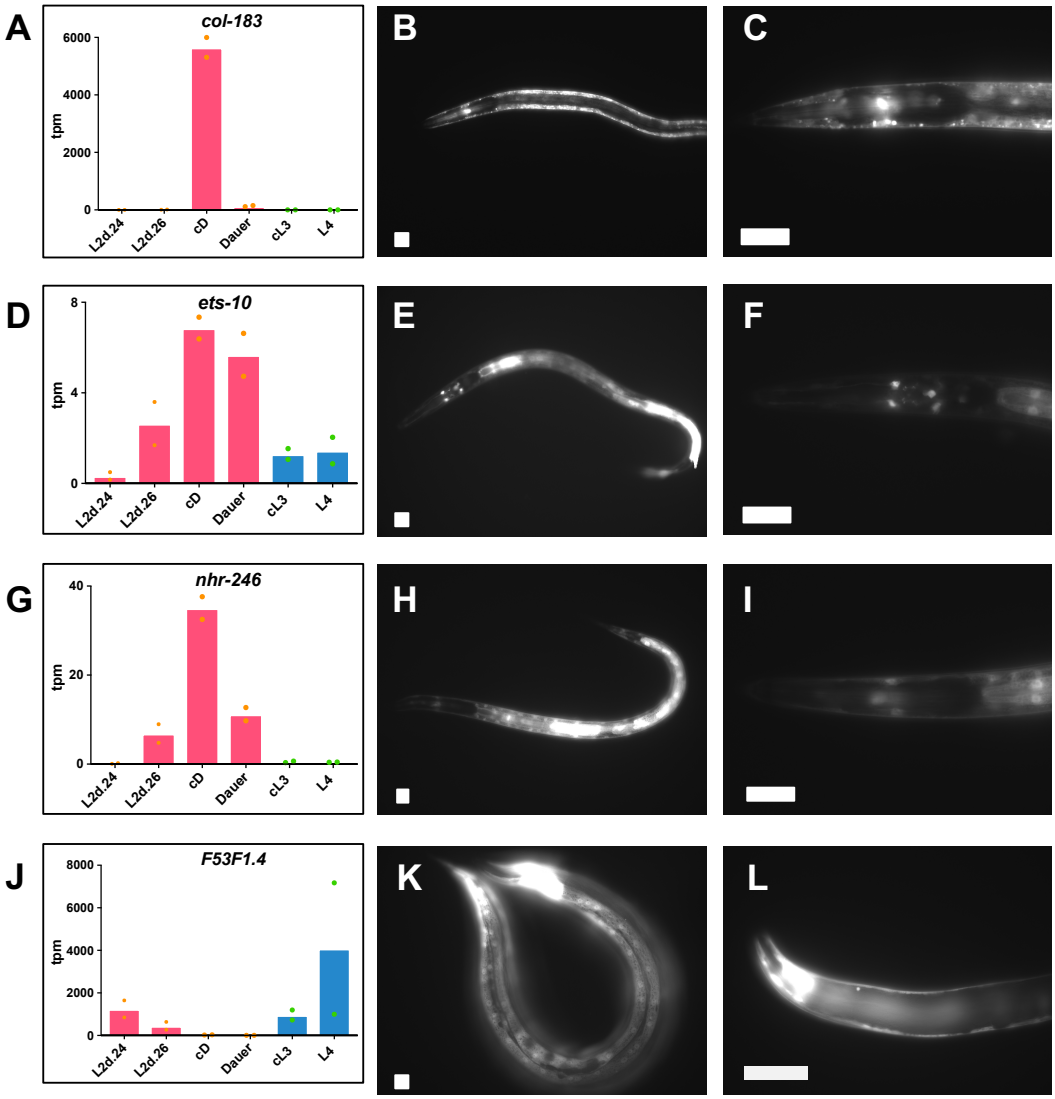


Figure 4.2. *col-183*, *ets-10*, *nrh-246* and *led-1*(*F53F1.4*) Genetic markers demonstrates dauer- or reproductive-specific expression pattern

(A-C) *col-183*: detected read counts of the *col-183* gene across developmental stages **(A)**, and fluorescence images of the *col-183* expression pattern in dauer **(B-C)**. **(D-F)** *ets-10*: detected read counts of the *ets-10* gene across developmental stages **(D)**, and fluorescence images of the *ets-10* expression pattern in dauer **(E-**

F). **(G-I)** *nhr-246*: detected read counts of the *nhr-246* gene across developmental stages **(G)**, and fluorescence images of the *nhr-246* expression pattern in dauer **(H-I)**. **(J-L)** *led-1(F53F1.4)*: detected read counts of the *F53F1.4* gene across developmental stages **(J)**, and fluorescence images of the *led-1(F53F1.4)* expression pattern in dauer **(L)**. In read count figures **(A, D, G, and J)**, points indicate the values from each sequenced replicate, and the bar height represents the mean count value for each developmental stage. Red and blue bars represent dauer and reproductive development, respectively. tpm, transcripts per million; L2d.24, L2d at 24 hours post hatch (hph); L2d.26, L2d at 26 hph; cD, dauer-committed; cL3, L3-committing. All the plotted read counts data were from Lee and Shih *et al.* (16). Scale bar: 0.1mm.

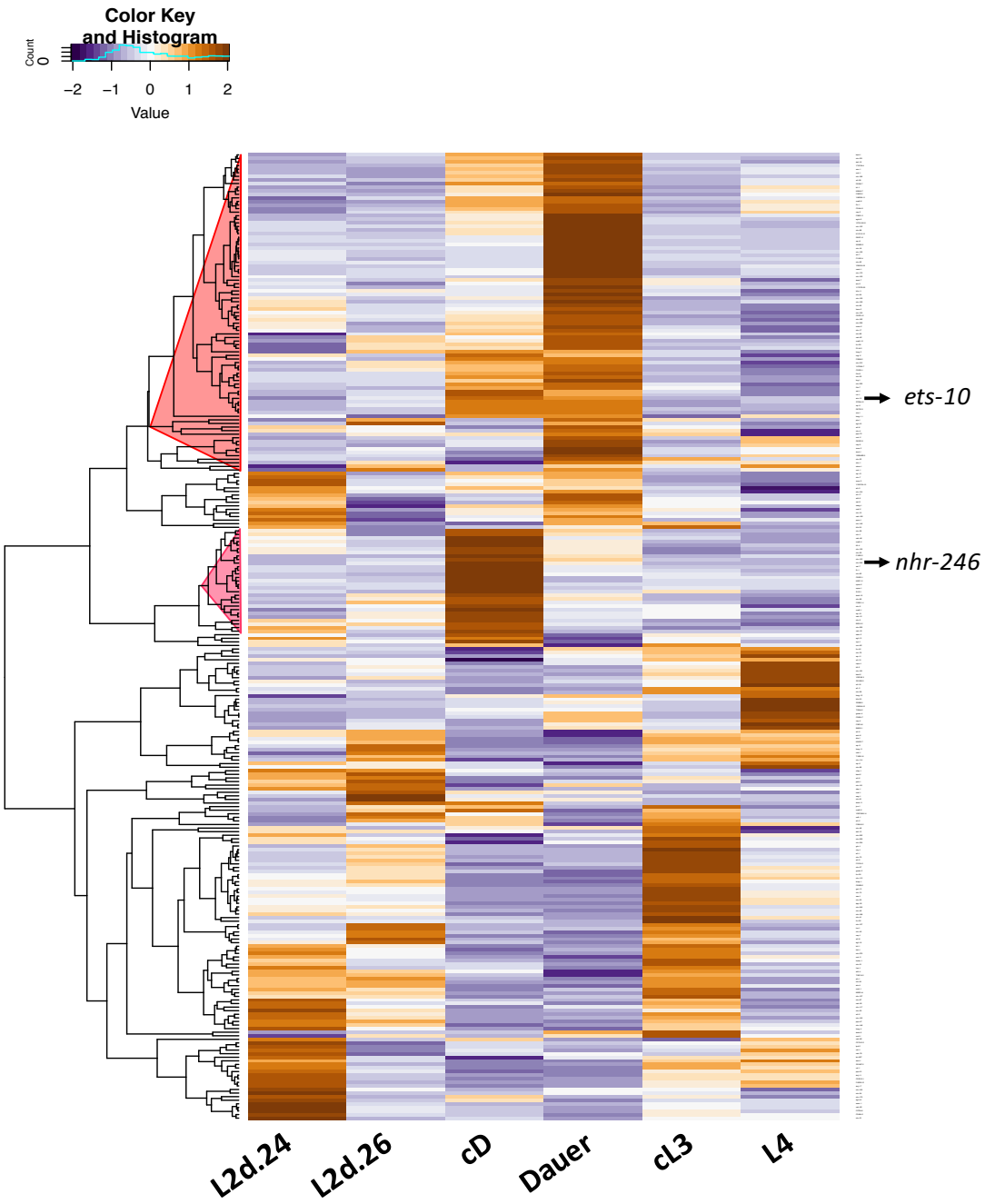

Color Key
and Histogram
Count
0
−2
−1
0
1
2
Value
ets-10
nhr-246
L2d.24
L2d.26
cD
Dauer
cL3
L4

Figure 4.3 Expression profiles of transcription factors

The expression profiles of genes encoding transcription factors were scaled and plotted on the heatmap. High and low expression level were shown in brown and purple color, respectively. Each row represents one single gene, and the genes were clustered based on their expression patterns. *ets-10* and *nhr-246* belong to the two gene clusters that have increased expression in dauer and dauer-committed (cD) stage, respectively. The heatmap was generated using packages in R, as described previously (16).

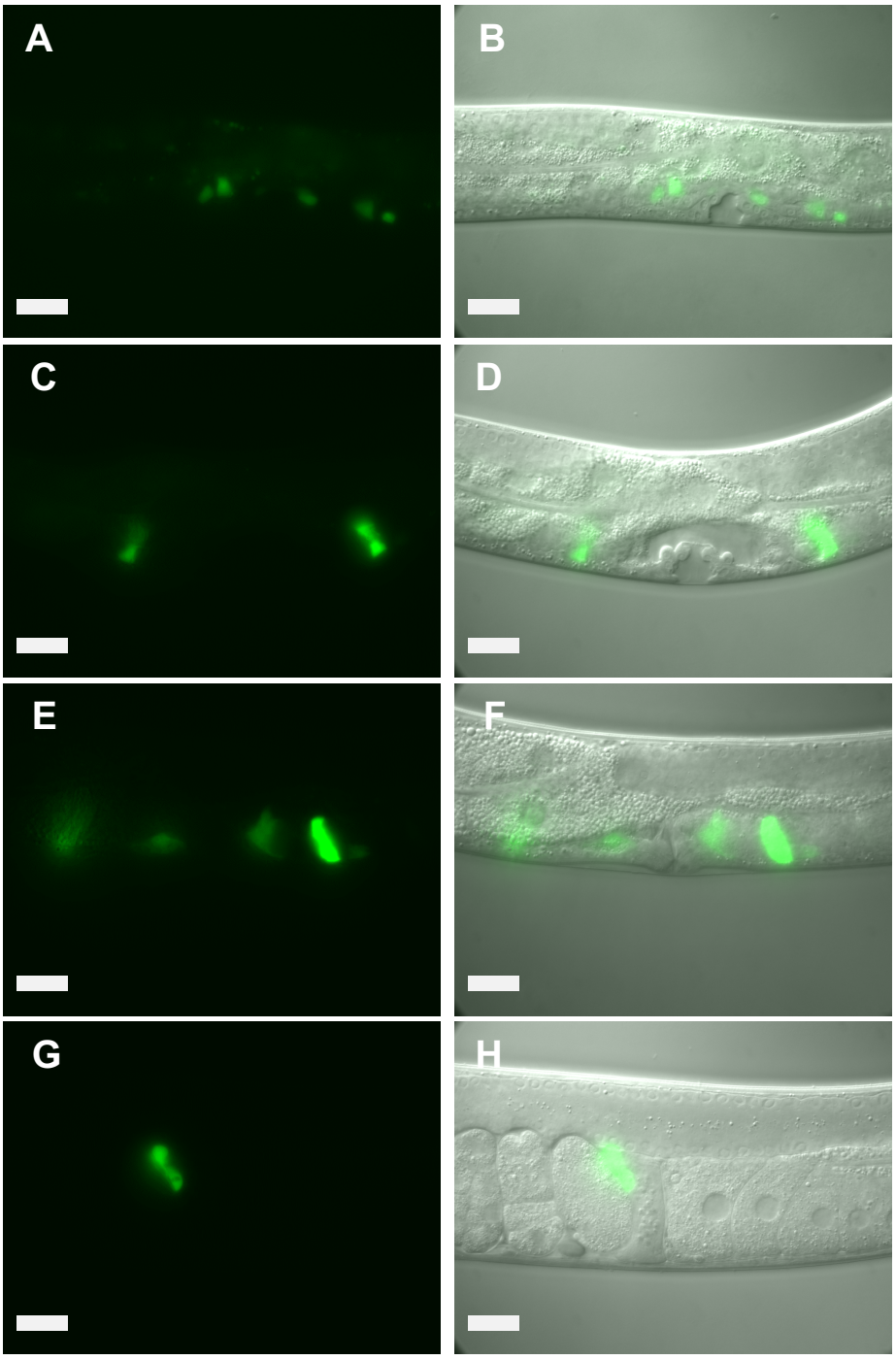

A
B
C
D
E
F
G
H

Figure 4.4. *ets-10* expression pattern in non-dauer stages (L4-adult)

Fluorescence **(A, C, E, and G)**, and the corresponding brightfiled and fluorescence merged images **(B, D, F, and H)** of *ets-10* across different life stages: early-mid L4 **(A-B)**, mid-L4 **(C-D)**, L4 lethargus **(E-F)** and adult **(G-H)**. Scale bar: 0.02mm.

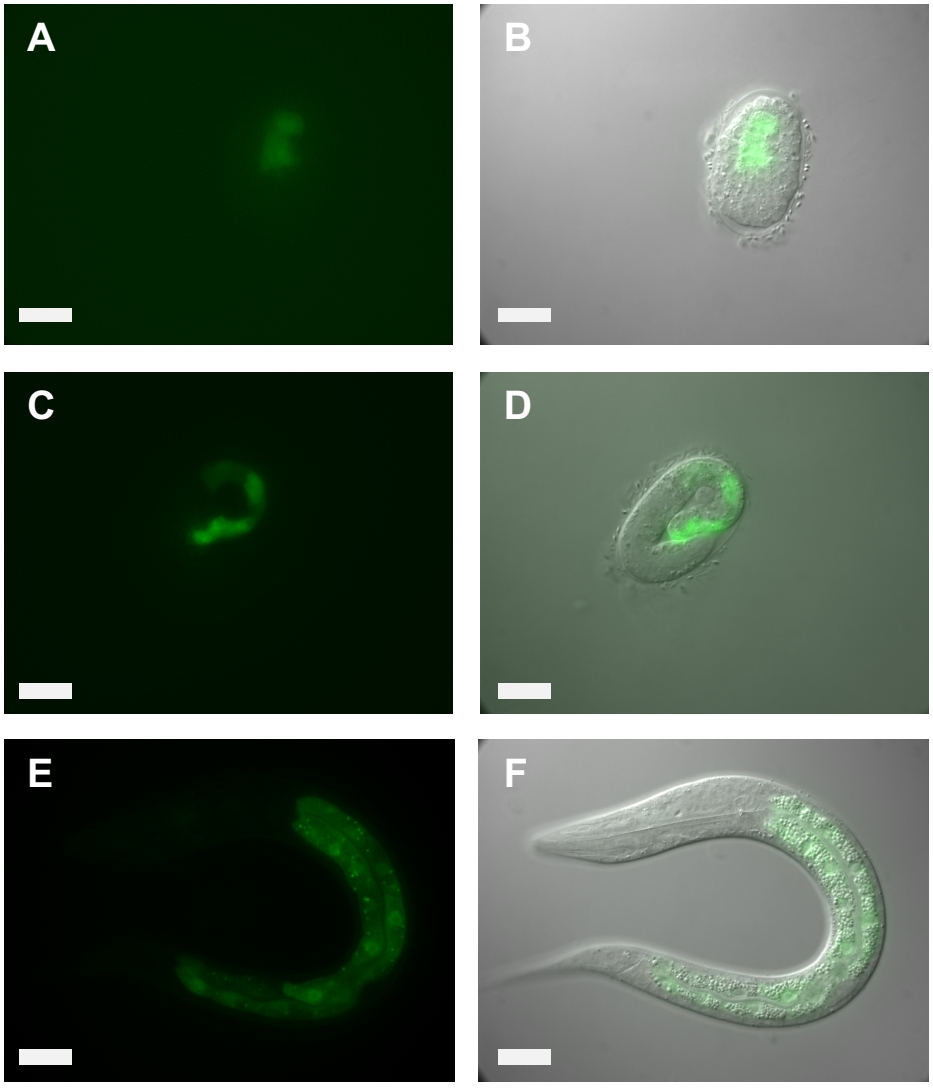


Figure 4.5. *nhr-246* expression pattern in non-dauer stage (embryo-L1)

Fluorescence **(A, C, and E)**, and the corresponding brightfiled and fluorescence merged images **(B, D, and F)** of *nhr-246* across different life stages: embryo **(A-D)** and L1 **(E-F)**. Scale bar: 0.02mm.

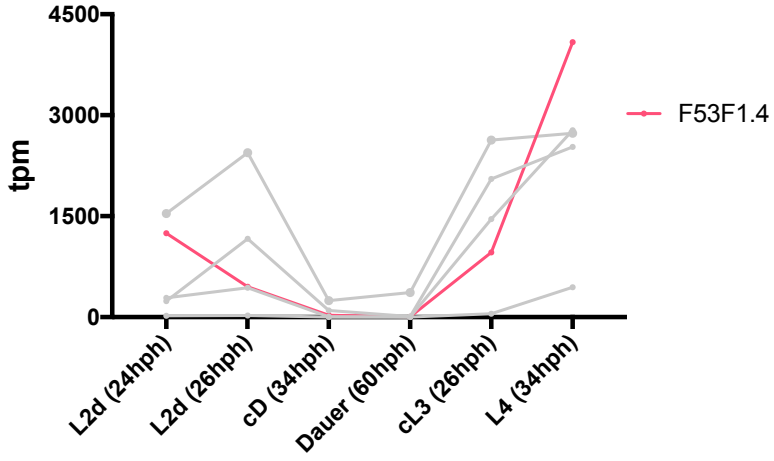
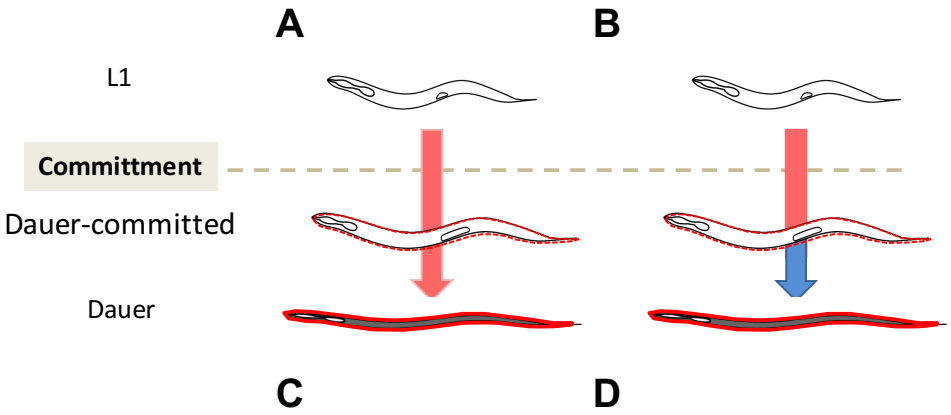


Figure 4.6. Expression profiles of genes that are down-regulated in dauer

Expression profiles of genes that are down-regulated specifically in dauer. Each line represents the average read counts of one single gene across different stages. *led-1(F53F1.4)*, the one we studied, was labeled in pink, and the rest of the genes were colored in grey for simplicity.



	dauers with fluorescence	dauers after shifted to favorable
col-183p::mecherry	100% (192/192)	100% (22/22)
ets-10p::gfp	100% (311/311)	100% (18/18)
nhr-246p::gfp	100% (174/174)	96% (25/26)

Figure 4.7. The appearance of fluorescence correlates with the dauer-commitment decision and stays on in dauer

(A-B) Cartoon diagram showing the experimental design with red hypodermal marker as an example. The red and blue arrows indicate the developmental progression in unfavorable and favorable conditions, respectivly. **(C-D)** The results from each of the marker strains. The numbers in parentheses represent the number of animals with positive results / total number of animals tested.

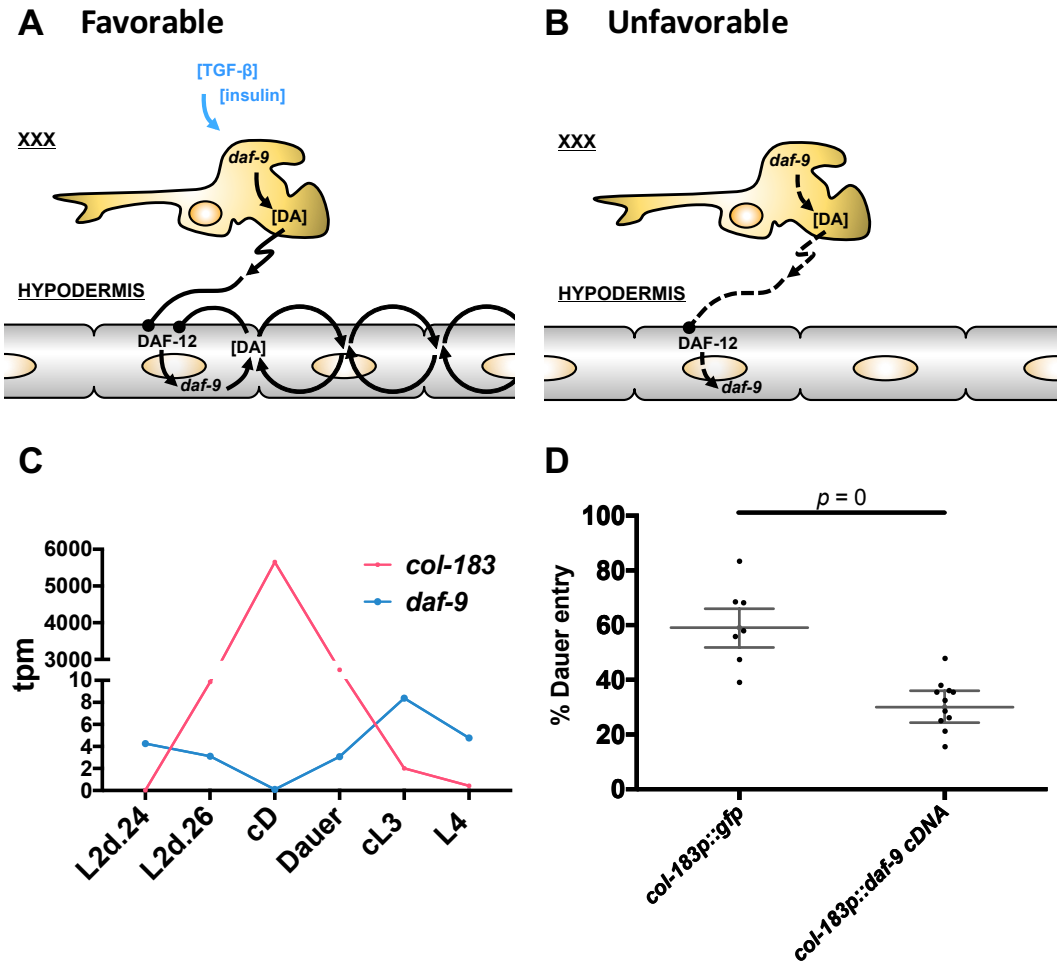


Figure 4.8. Overexpressing *daf-9* in the hypodermis during commitment increases the reproduction decision

(A-B) Cartoon diagram showing the amplification of dafachornic acid in hypodermis through TGF-beta and insulin signaling under favorable condition **(A)**, and the lack of dafachornic acid amplification in dauer-inducing environment **(B)**. **(C)** Average detected read counts of the *col-183* and *daf-9* gene across developmental stages. **(D)** Dauer entry assay on animals with *col-183* promoter driving expression of *gfp* or *daf-9* cDNA. The long horizontal line indicates the

bootstrapped mean, and the error bar shows the 99% confidence intervals. Each dot is one trial, and the data were collected from at least three different days. Statistics: permutation test. XXX: XXX cell; DA, dafachronic acid; tpm, transcripts per million.

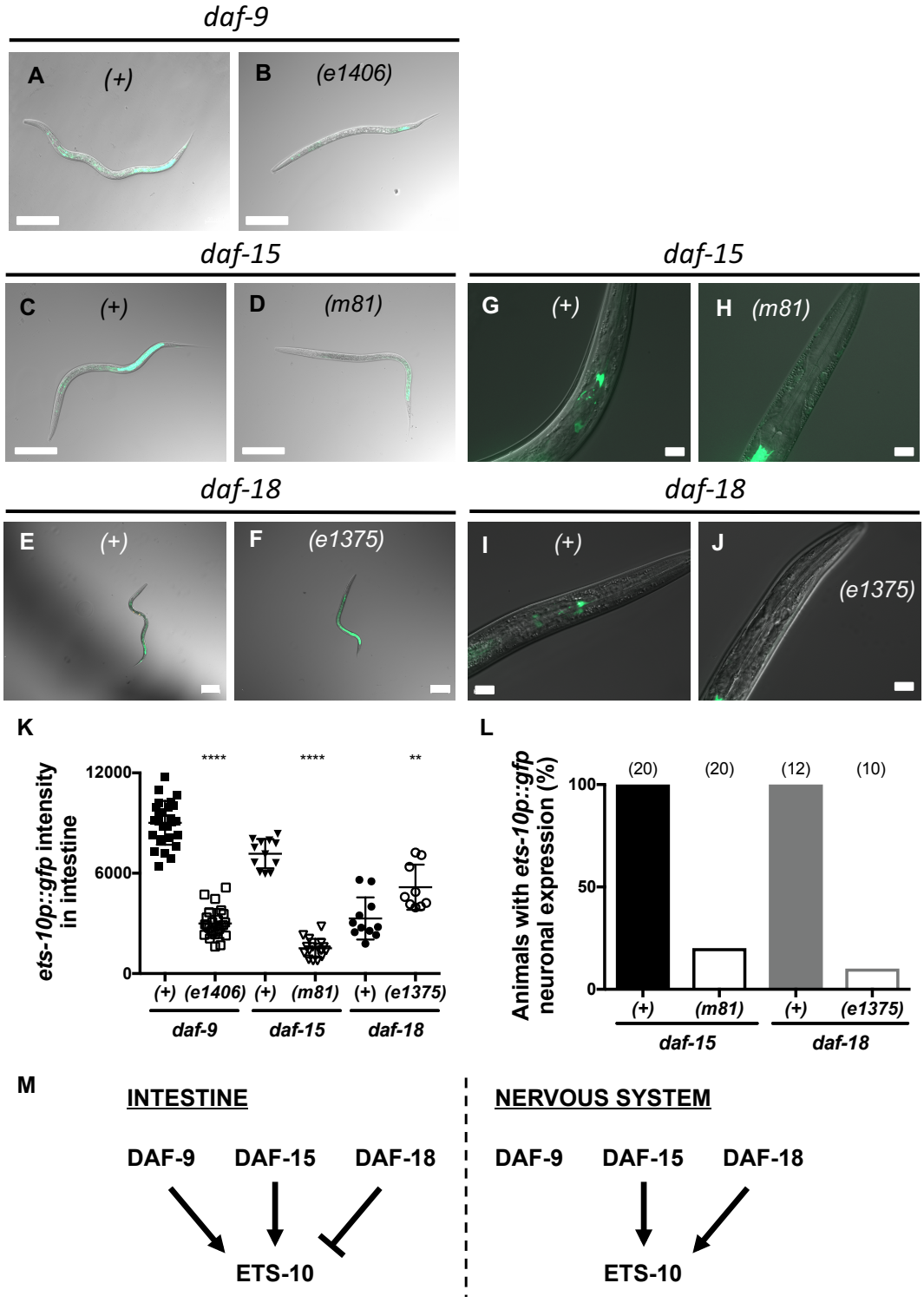

daf-9
A (+)
B (e1406)
daf-15
C (+)
D (m81)
daf-15
G (+)
H (m81)
daf-18
E (+)
F (e1375)
daf-18
I (+)
J (e1375)
K
ets-10p::gfp intensity in intestine
12000
6000
0
****
****
**
(+) (e1406) (+) (m81) (+) (e1375)
daf-9
daf-15
daf-18
L
Animals with ets-10p::gfp neuronal expression (%)
100
50
0
(20)
(20)
(12)
(10)
(+) (m81) (+) (e1375)
daf-15
daf-18
M
INTESTINE
DAF-9
DAF-15
DAF-18
ETS-10
NERVOUS SYSTEM
DAF-9
DAF-15
DAF-18
ETS-10

Figure 4.9. Partial dauers mis-express dauer markers

(A-J) Representative images of *ets-10p::gfp* expression pattern in wild type **(A, C, E, G and I)**, *daf-9(e1406)* **(B)**, *daf-15(m81)* **(D and H)**, *daf-18(e1375)* **(F and J)** animals. **(K)** Quantification of *ets-10p::gfp* intestinal fluorescence intensity in wild type, *daf-9(e1406)* and *daf-15(m81)* animals. Each dot represented one animal. The error bars showed standard deviation. Statistic: nonparametric two-tailed t test. **** $p < 0.0001$; ** $p < 0.01$ **(L)** The percentage of wild type, *daf-15(m81)* and *daf-18(e1375)* animals with *ets-10p::gfp* neuronal expression. The number in parenthesis indicates the number of animals examined. **(M)** A proposed model for how DAF-9, DAF-15, and DAF-18 influence ETS-10 expression in the intestine and nervous system. Scale bar: 100um **(A-F)** and 10um **(G-J)**.

4.6 References

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