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The Olig Family Member HLH-17 Controls Animal Behavior by Modulating Neurotransmitter Signaling in Caenorhabditis elegans

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THE OLIG FAMILY MEMBER HLH-17 CONTROLS ANIMAL BEHAVIOR

BY MODULATING NEUROTRANSMITTER SIGNALING

IN *CAENORHABDITIS ELEGANS*

by

CHAQUETTEA FELTON

Under the Direction of Casonya Johnson, PhD

ABSTRACT

In vertebrate and invertebrate systems, the role of glia-neuron interactions during development and behavior is becoming apparent. Recent studies have been aimed at characterizing glial-expressed proteins that affect the modulation of activities traditionally thought to be regulated by the neuron itself. The soil nematode *Caenorhabditis elegans* has recently emerged as an important invertebrate model to study glial roles in nervous system function and development. My dissertation work focuses on the characterization of HLH-17, a *C. elegans* basic helix-loop-helix transcription factor that is strongly and constitutively expressed in the glial cells that associate with four of the cephalic (CEP) neurons in the head of the animal. The CEP neurons are four of eight dopaminergic neurons with well characterized roles in the modulation of a number of behavioral activities in the worm. Although HLH-17 is required for neither the specification nor the development of the CEPsh glia or the CEP neurons, it does have a defined role during dopamine responses. We show that HLH-17 functions upstream of the dopamine receptors DOP-1, DOP-3 and the dopamine transporter DAT-1 to affect DA-dependent behaviors. Also, our microarray analyses provide preliminary evidence that HLH-17 targets factors responsible for receiving and transducing signaling molecules that are involved in the modulation of synaptic events in the worm nervous system. Together these results point to a role for HLH-17 in glia-neuron interactions in *C. elegans*. My dissertation studies therefore

provide further support for the role of glial-expressed proteins in the regulation of activities mediated by the nervous system.

INDEX WORDS: Dopamine signaling, Transcriptional networks, bHLH transcription factors, Cephalic sheath glia, Behavioral analysis

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DEDICATION

To my parents, Sheldon and Maudline Walker,

and my daughter, Cameron Marie

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ach	Acetylcholine
C. elegans	*Caenorhabditis elegans*
CEPsh	Cephalic sheath
DA	dopamine
GABA	□-aminobutyric acid
GPCR	G-protein coupled receptor
HLH	helix-loop-helix
IIS	insulin/IGF-1-like signaling
N2	wild-type

1 GENERAL INTRODUCTION

1.1 The C. elegans nervous system serves as a platform for neuroscience research

A fundamental challenge in the field of neuroscience is determining how a behavior is generated by the actions of individual gene products. It is extremely challenging to address this phenomenon in most vertebrates due to their large and complex nervous systems. However, the small and well-characterized nervous system of *C. elegans* gives researchers a number of unique advantages for addressing the molecular and cellular mechanisms that modulate specific behaviors. Firstly, the well-defined anatomy of *C. elegans* has provided us with a complete reconstruction of its nervous system [1, 2], aiding in the formulation of hypothetical neural circuits that are vital to understanding behaviors at both molecular and functional levels [3, 4]. Additionally, there are only 302 neurons in the nematode. Since each of these neurons has a characteristic identity/morphology and a large portion of their synaptic connections are known, genetic studies are much less tedious to conduct. Secondly, the transparency, rapid generation time and ease of genetic manipulation in *C. elegans* allows us to genetically identify genes and molecularly confirm their roles during specific behavioral processes. Lastly, well-defined behavioral assays allow us to further characterize genetic and physiological influences on specific animal behaviors after they are initially identified [5-7].

A large number of proteins with roles in nervous system development and function were first identified in the nematode. For example, UNC-17, the vesicular acetylcholine transporter [8], and ODR-10, one of the only odorant receptors with a known function [9], were both first genetically identified in *C. elegans*. Additionally, proteins previously identified in other systems

but with unknown biological functions, have been linked to specific behaviors only through functional studies conducted in the worm [10, 11]. For instance, novel roles for LIN-12/Notch signaling in mammalian development and disease [12] were identified only after studies in *C. elegans* revealed the mechanism through which LIN-12/Notch proteins transduce signals during nematode development [13, 14]. Most recently, many *C. elegans* mutants have been used as models for human disease [15, 16] and drug studies [17, 18], providing powerful advancement in these fields of genetic research. For example, there are numerous *C. elegans* models of the protein misfolding disease, Alzheimer's, in which the toxic outcomes of abnormally folded proteins have been examined [19, 20]. These models have led to the discovery of numerous Alzheimer's modulating candidates making *C. elegans* well positioned to aid in drug discoveries used to expedite the development of new therapeutics.

Despite the tremendous progress that researchers have made in the field of neuroscience, many questions still remain concerning how behaviors are modulated at the neural and molecular levels in *C. elegans*. For example, although most neuronal connections have been identified in *C. elegans*, many aspects of the connections including the neurotransmitter(s) produced by each neuron, the receptors that respond to these neurotransmitters and the spatial and temporal connections made between neurons and behaviors, are still largely unknown. One approach to begin to address these unknowns is to characterize genes with specific roles during neuronal signaling. In *C. elegans*, gene products which directly regulate neurotransmitter biosynthesis, vesicular transport and reception have been well characterized; however, an increased effort to identify the factors which work upstream of these genes would allow researchers to more specifically identify cells/tissues in the nervous system. This would ultimately lead to an increase in the specificity of techniques like neuronal labeling and immunocytochemistry that help us to

better understand different aspects of neuronal connections. Interestingly, since the characterization of *hlh-17* by our lab, reporter constructs driven by *hlh-17*'s promoter have been used as a marker for the CEPsh cells in *C. elegans* [21, 22], a technique that was previously unachievable specifically to mark the CEPsh cells only. My dissertation work therefore plays a pivotal part in helping us to better correlate the relationship between gene products and behaviors.

1.2 Glial cells are important modulators of behavior

To orchestrate the diverse range of animal behaviors, the nervous system is comprised of two major classes of cells, neurons and glia. Historically, most studies have focused on neuronal roles while more recent studies have also focused on glial roles and glia-neuron interactions that control behaviors. In general, worm glia function to (1) regulate the location and morphology of neuronal structures, (2) protect neurons from other cells by creating a barrier and (3) modulate neuronal activity [23-25]. Although glial cells have roles in nervous system development, the scope of my introduction will focus on their roles in behavior.

There are three types of glial cells, 50 ectodermally-derived sheath (sh) (24) and socket (so) (26) cells and 6 mesodermally-derived GLR (Glia-Like cells in the nerve Ring) cells. Glia and the neurons with which they are associated are arranged in groups of sense organs commonly referred to as sensilla. There are six inner labial (IL) sensilla, six outer labial (OL) sensilla, four cephalic (CEP) sensilla and two amphids positioned in the head of the animal [1]. Each sensillum consists of one or more ciliated neurons that end in a channel, enclosed by one sheath and one socket cell (Figure 1). The amphids are considered to be the main, anteriorly located, chemoreceptive organ in the animal [26]; most glia-based studies have been performed primarily in the context of this organ (some of these studies will be discussed further in

succeeding sections). The information processed by sensory organs are relayed to a central processing center, the nerve ring. The nerve ring, also known as the "brain" of the worm, is a synaptically dense region of dendrites, axon terminals and glial processes, containing synapses between sensory neurons and interneurons, and between interneurons [1]. In fact, of the 302 neurons in the adult worm, 180 project processes into the nerve ring, making it the site of most synaptic interactions in the animal [1, 2].

To facilitate the processing of information needed to regulate behavioral events, glial cells are located at three types of neuronal junctions. Firstly, sheath and socket cells associate with dendrites of sensory neurons where they can detect sensory cues from the environment [27]. Secondly, they are positioned at neuron-neuron synapses where chemical messages can be communicated between the neurons. Lastly, GLR glia are located at neuromuscular junctions (GLRs) [28] where they presumably affect neuron-muscle communication. *In vivo* studies are vital for determining glial influence on neuronal activity. Unlike vertebrates and some invertebrates where the loss of glial cells causes neuronal death, the *C. elegans* nervous system does not require glia for neuronal survival, thereby permitting studies aimed at determining glial functions *in vivo*. In fact, there have been recent successful efforts aimed at developing methods to label and manipulate glial cells [29-31] and to isolate genes that may regulate glial functions in *C. elegans* [32].

To study glial contributions to neuron function, Ohkura and Burglin (2011) set out to determine if the amphid sheath glia were required for the sensory neurons of the amphid sheath to function properly [24]. The amphid is composed of 12 sensory neurons (each is associated with one socket and one sheath glia cell) which are individually associated with specific behavior(s) [26, 27, 33, 34]. The ciliary ending of some sensory neurons that are exposed to the

environment can take up fluorescent dyes. This group showed that the amphid sheath is also stained with the dye which suggests that the sheath may interact with the sensory neurons they are associated with [24]. In a separate study, Bacaj et al, 2008, tested the consequences of ablating the amphid sheath glia following development [32]. In most cases, glia ablations caused animals to be defective in thermotaxis and chemotaxis behaviors mediated by a subset of the amphid sensory neurons. Interestingly, in some cases defects of neuronal dysfunction were also accompanied by structural defects at sites where the glial cells had been removed, while in other cases there was no evidence of structural abnormalities. These data raised the possibility that glia-secreted proteins can affect neuronal activity. This possibility was supported by the identification of *fig-1*, a glial-expressed gene with similar structure to *THBS1*, a mammalian gene with important roles during synaptogenesis [35]. Although, *fig-1* mutants did not display neuronal or glial developmental defects, they did exhibit defects in synapse formation [32]. These were the first studies that demonstrated that amphid glia play a vital role in the regulation of neuronal function in *C. elegans*.

Studies by Han et al, 2013, and by Wang et al, 2008 further supported the requirement for glial-expressed proteins in the modulation of sensory behaviors in *C. elegans* [36, 37]. These studies demonstrated that proteins which encode channels in the glia, contribute to neuronal activity that affects behavioral responses. Firstly, the DEG/ENaC (Degenerin/Epithelial Na Channel) subunits, DELM-1 and DELM-2 (Degenerin Linked to Mechanosensation) that are expressed in the glia associated with OLQ and IL1 sensory neurons, are required for the modulation of nose touch and foraging behaviors mediated by those neurons. Consistent with earlier studies, Han et al, 2013 show that these subunits are not required for the structural development nor integrity of the OLQ and IL1 neurons or their associated glia [36]. Similarly,

another member of the DEG/ENaC family, ACD-1 (Acid-sensitive channel, Degenerin-like) is required in the amphid sheath glia to regulate sensory perception [37]. From these data it is postulated that DEG/ENaC channels located in the glia are required to regulate ion concentrations in the synapse that are responsible for controlling neuron excitability. Together these data demonstrate that glial-expressed proteins can affect neuronal function and they also provide examples of the types of proteins that can facilitate these roles. Many studies have demonstrated that neuron excitability can affect behavioral events. However, the mechanisms surrounding these affects are still elusive [38-40].

1.3 The CEPsh cells are a unique set of glial cells

The sheath cells of the cephalic sensilla (CEPsh) are associated with the four cephalic (CEP) neurons and are arranged in four-fold symmetry (dorsal left/right, ventral left/right) in the *C. elegans* head. Unlike sheath cells of other sensilla, the CEPsh cells exhibit a unique bipolar morphology with each sensory cell extending a ciliated dendrite to the tip of the nose and a flat, sheet-like process into a quadrant of the nerve ring (Figure 2) [1]. Functional studies in the CEPsh are limited; however, there are three unique qualities of the CEPsh that suggests that the CEPsh function similarly to vertebrate glia. Here I discuss how researchers have begun to investigate these three characteristics.

Firstly, in *C. elegans*, UNC-6 is an evolutionarily conserved guidance cue secreted by numerous glia and neurons of the developing and mature nervous system [41, 42]; the expression of *unc-6*/netrin in the ventral left and right CEPsh suggests that this subset of CEPsh cells may be required for axon guidance and cell migration. To address this hypothesis, researchers examined the role of the CEPsh glia on axon guidance by the three sensory neurons that enter the nerve ring through the ventral ganglion, AWC, AFD and ADF neurons [30]. They demonstrated

that ablation of the ventral, but not the dorsal, CEPsh glia precursors caused pronounced defects in axon guidance and morphology of the AWC and AFD neurons, but not the more distally located ADF neuron. This result suggested that the CEPsh glia produce short-range signals that regulate axonal guidance during neurogenesis. Further analysis demonstrated that this glia-derived signal requires UNC-6 to control AWC guidance. Together these preliminary studies strongly suggest that the CEPsh cells play spatial and neuron-specific roles during axon guidance and that these roles are at least partially mediated by UNC-6 in *C. elegans*.

Secondly, during synapse formation in vertebrate and invertebrate systems, glia secrete proteins required for the establishment of postsynaptic connections [43]. It has been suggested that the CEPsh glia may be required for synapse formation due to its unique location enveloping synapse-rich regions of the nerve ring. The expression of *unc-6* in the ventral CEPsh cells also supports this hypothesis since recent evidence shows that UNC-6 has a dual role in axon guidance and synaptogenesis [44]. In addition to axon guidance defects, *unc-6* mutants also display defects in its synapse formation between the AIY amphid interneuron and the RIA interneuron of the nerve ring [31]. During development when AIY-RIA innervation takes place, *unc-6* expression is required exclusively in the ventral CEPsh cells to regulate the pattern of the AIY presynapses [41]. To determine if the physical overlap between the CEPsh glia and the AIY-RIA synapses is important for mediating AIY-RIA innervation, Colon-Ramos et al, 2007, isolated mutants with altered CEPsh morphology and showed that the repositioning of the CEPsh by *unc-34*/enabled, a regulator of actin cytoskeleton, affects RIA axon guidance and AIY presynapses. These studies provide evidence that UNC-6/Netrin effects synaptogenesis from the ventral CEPsh glia cells.

Lastly, the CEPsh in *C. elegans* are regulated similarly to oligodendrocytes in vertebrates [30]. The Olig2 transcriptional regulator is important for oligodendrocyte development in vertebrates and its expression is required in discrete domains in ventral and dorsal regions of the developing spinal cord [45, 46]. In the ventral domain, the homeodomain transcription factors Nkx6.1/2 and Pax6 are required, while dorsal expression requires Pax7 (Figure 3) [47, 48]. Interestingly, the development of the CEPsh glia are regulated similarly to Olig2, the ventral CEPsh requires the Nkx/Hmx-related genes *mls-2* and the dorsal CEPsh requires the Pax6/7-related gene *vab-3* (Figure 3) [30]. Additionally, Olig2 is most similar in sequence to *hlh-17*. My dissertation provides evidence that like Olig2, *hlh-17* also plays a vital role in the support cells of the nervous system. These data show that there is a strong molecular similarity between vertebrate and nematode glia development.

The data obtained provide molecular and functional support to the hypothesis that the CEPsh are the astrocytes of the worm. Due to the major roles of astrocytes in vertebrate systems, it is crucially important to better understand the roles of the CEPsh in the *C. elegans* nervous system. My dissertation work therefore provides much needed insight into the functional roles of these unique glial cells.

1.4 My dissertation studies show that HLH-17 modulates behaviors regulated by the CEP neuron, possibly from the CEPsh glia

In spite of the many roles that glia play in the nervous system, few glia-specific proteins have been functionally characterized. My dissertation studies help to support the importance of glia-neuron interactions in the nervous system and my work provides exciting new evidence that the expression of *hlh-17* in the CEPsh is important for modulating behaviors mediated by the

CEP neurons in the *C. elegans* hermaphrodite. Similarly to other glia-specific proteins important for neuronal function, HLH-17 does not seem to be required for the development of the CEP neuron nor the CEPsh glia [30]. In chapter 2, we describe our characterization of HLH-17. We show that *hlh-17* mutants are defective in behaviors mediated by the CEP dopaminergic neurons. These studies were the first piece of evidence that a factor expressed in the CEPsh glia could affect behaviors mediated by the CEP neuron. In Chapter 3, we further analyzed DA dependent behaviors in *hlh-17* mutants. We show that HLH-17 modulates some of these behaviors by working upstream of the dopamine receptors DOP-1 and DOP-3 and the dopamine transporter DAT-1. We also show through rescue experiments that these behaviors are a direct result of the loss-of HLH-17. In chapter 4, we take a global look at how the loss-of HLH-17 affects gene expression in the hermaphrodite. Our microarray analyses show that HLH-17 regulates mostly membrane bound proteins responsible for signal transduction events that affect the animal's ability to respond to stimuli. The identification of putative HLH-17 targets that are responsible for receiving (receptors) and transducing (transporters, enzymes) signaling molecules helped us to better understand how HLH-17 may modulate behaviors from the glia. In the last chapter, I focus on the foundation that my work has set for future studies aimed at further characterizing the CEPsh and HLH-17 in *C. elegans*.

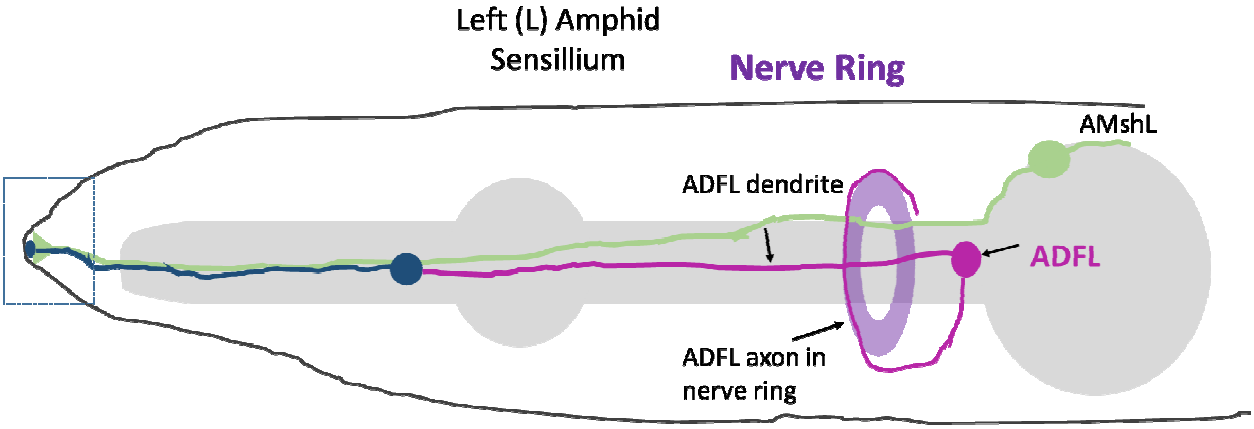

Figure 1 Amphid Sensilla
Each amphid contains twelve sensory neurons, this image is simplified and only shows ADFL. The dendrite of ADFL (dark purple) penetrates the tubular ending made by the sheath cell (green). The socket cell (blue) wraps itself around the end of the amphid channel. Picture adapted from Altun, Z. F. and Hall, D. H. 2005. Handbook of *C. elegans* Anatomy. In *WormAtlas*.

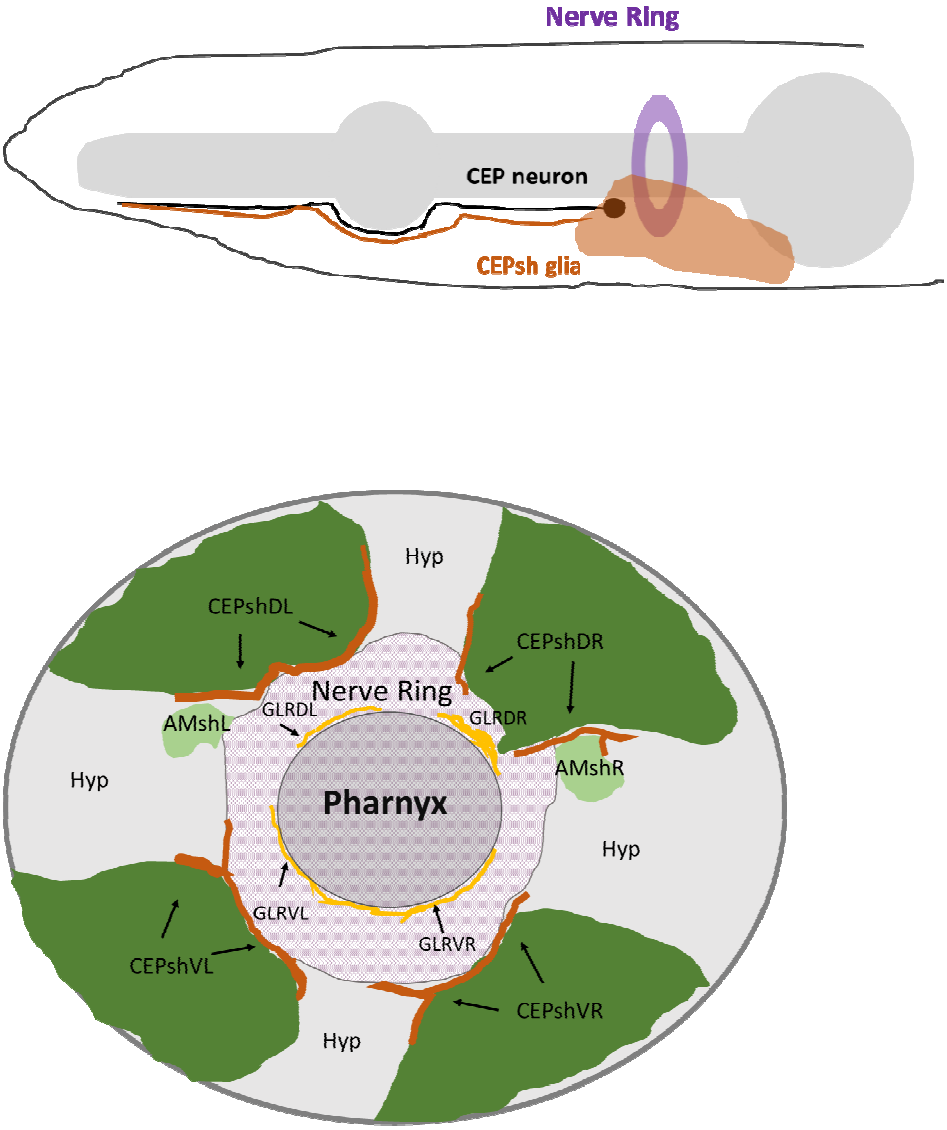

Figure 2 Cephalic Sensilla
A. Cephalic Sensilla. Simplified representative of CEP neuron and a CEPsh cell. Projections from the sheath cell are shown around the nerve ring. Socket cell and axons of the CEP neuron are omitted. **B.** Cross section of CEPsh cells. CEPsh (DL, VL, DR, VR) separate the hypodermis from muscle and separate the hypodermis and muscle from the nerve ring. Also shown are the GLR (DL, VL, DR, VR)

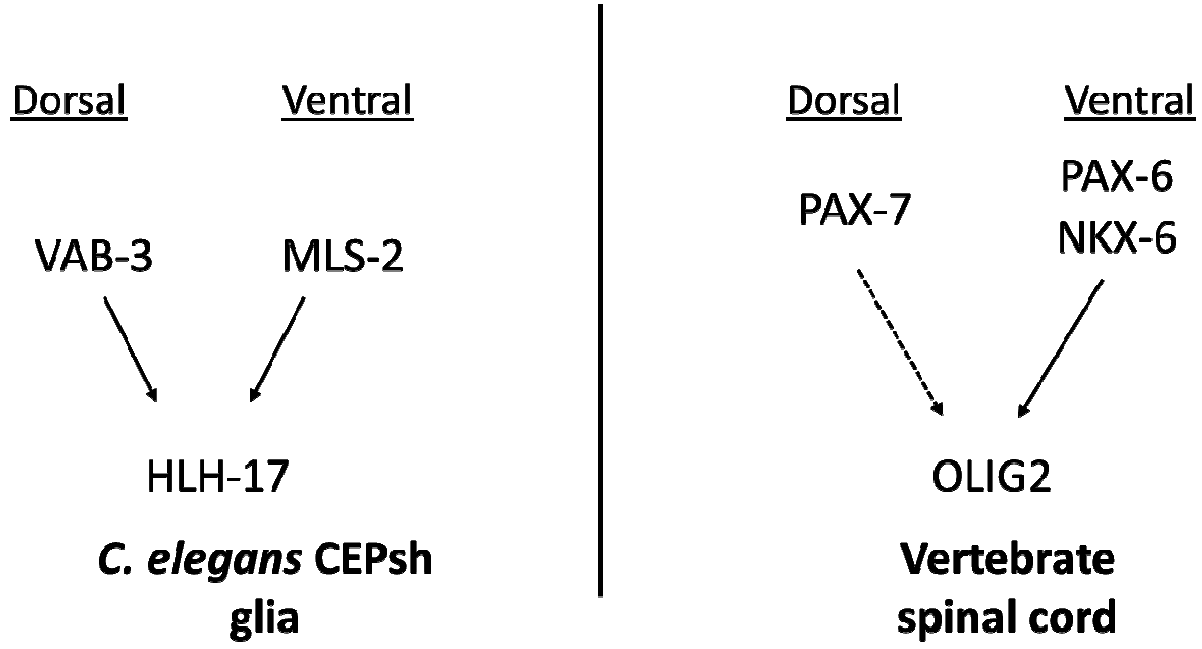

Figure 3 Transcriptional regulation of glial formation
In ventral *C. elegans* CEPsh glia and in ventral spinal cords, NKx family and Pax6-related proteins regulate *hlh-17* and Olig2 expression. In dorsal *C. elegans* CEPsh glia and in dorsal vertebrate spinal cords, a Pax7-related protein regulate *hlh-17* and Olig2 expression. Dashed line represents a mode of regulation which has not yet been confirmed. Figure adapted from Yoshimura et al, 2008.

2 MODULATION OF DOPAMINE-DEPENDENT BEHAVIORS BY THE *C. ELEGANS* OLIG HOMOLOG HLH-17

2.1 Introduction

In humans and other vertebrates, a critical balance of dopamine signaling is required for normal physical and behavioral functions. Loss of dopamine signaling in humans is associated with Parkinson's disease [49, 50] and is thought to affect various depressive states [51], levels of energy and activity [52] and sleep disorders [53-55]. At the other extreme, hyperactive dopamine signaling is associated with schizophrenia [56], Tourette's syndrome[57], attention deficit hyperactivity disorder [58, 59] and addictive behaviors [60-62]. These disorders are mimicked in animal models with altered dopamine signaling. Dopamine-deficient mice lack energy, do not display any environmentally stimulated, explorative behaviors, and are not motivated to engage in goal-directed activities [63, 64]. Likewise, when rats are treated with dopamine uptake inhibitors, which would result in hyperactive dopamine signaling, they display symptoms associated with schizophrenia and ADHD [65, 66]. Finally, dopamine signaling affects learning, memory, neural plasticity [67, 68] and adaptation in both vertebrates and invertebrates [69-71].

The nematode *Caenorhabditis elegans* is a suitable model for understanding dopamine signaling and DA dependent behaviors. DA is synthesized in eight, well-characterized sensory neurons in both *C. elegans* sexes and in six additional sex-specific neurons in the *C. elegans* male (reviewed in[72]). The dopaminergic neurons arise in pairs, and are protected by glial

support cells that act, in part, to provide substrates needed for guiding axonal processes during early development [73]. The anterior deirid (ADE) and posterior deirid (PDE) neuron pairs are each supported by their own glia. The two pairs of cephalic neurons found in both sexes (CEP), and one pair found only in males (CEM) are supported by the sheath and socket cells of the cephalic sensilla. Genes required for DA synthesis and transport in *C. elegans* have been identified, along with genes that encode D1-like, D2-like, and invertebrate specific DA receptors. Furthermore, the dopamine signaling pathway has been extensively reconstructed from the synthesis and release and subsequent re-uptake of the neurotransmitter by the pre-synaptic neurons, to its activation of transmembrane receptors, some of which are G-protein coupled receptors, located on post-synaptic and extrasynaptic neurons [74]. Signals from the activated DA receptors are then transduced to second-messenger molecules inside the cell, through the Gαo and Gαq subunits, to activate adenylate cyclase and inositol triphosphate, respectively [75]. Disruption of dopamine signaling at any of these levels has stereotypical effects on synaptic transmission, sensory plasticity, locomotion, defecation, and egg-laying [76-78]. DA also modulates behavioral responses in *C. elegans*, most notably mechanosensory locomotory responses to food and adaptive responses to chemical cues [79]. Recently, *C. elegans* has been used to model human and mammalian behaviors and conditions in response to altered dopamine signaling, including Parkinson's disease, schizophrenia, cocaine and other drug dependencies, memory and behavioral adaptation [80-86].

In a previous study, we found that the *C. elegans* gene for the basic helix-loop-helix transcription factor, HLH-17, is expressed at all developmental stages in the glial-like cells that ensheath the CEP and CEM dopaminergic neurons [87]. The functional role of HLH-17 in the cephalic sheath cells is unclear. Reducing or eliminating *hlh-17* expression resulted in delayed

egg-laying and slightly defective chemosensory phenotypes. However, despite bearing significant homology to Olig2, a vertebrate protein required for axon guidance, the HLH-17 protein is not required for the development and proper morphology of the dopaminergic neurons [30]. Here we show that behaviors controlled by DA are altered in *hlh-17* (ns204) animals. We show that mutations in the *hlh-17* gene disrupts dopamine signaling at least in part by altering expression of the dopamine receptor genes, *dop-1*, *dop-2*, and *dop-3*, and the RGS protein gene, *egl-10*, which selectively inhibits G-protein signaling. Finally, we show that the HLH-17 paralogs, HLH-31 and HLH-32, do not function redundantly with HLH-17 in the DA dependent behaviors that we analyzed.

2.2 Materials and Methods

2.2.1 Nematode Strains and Maintenance

The wild-type strain used in this work was the strain Bristol N2. Other strains used were LX645 [*dop-1*(vs100); [88], LX703 [*dop-3*(vs106); [88], OS2649 [*hlh-17*(ns204); [30], and OS2929 [*hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) ; [30]. Standard methods used for culturing *C. elegans* were as described by Lewis and Fleming, 1995. Strains were maintained at 20°C and all assays were at 22°C.

2.2.2 Behavioral Assays

For all assays except gustatory plasticity assays, cultures were synchronized by hypochlorite as previously described [87]. Measurements of rates of egg-laying behavior in response to DA and 5HT were as previously described [89]. Newly hatched L1 animals were allowed to feed on OP50 for 72 hours prior to the assay. Individual animals were then rinsed

briefly in M9 and transferred into a well of a 48-well microtiter plate containing 50 µL of M9 buffer +/- the appropriate drug. DA was used in these assays at 3 mg/ml; serotonin (5HT) was used at 5 mg/ml.

Immobilization assays were used as measurements of resistance to exogenous DA as described previously [88]. Young adult animals were transferred to NGM plates containing 0, 5, 10, or 20 mM DA and incubated at 22°C for 40 minutes. Animals were scored by visual inspection as being mobile if they initiated spontaneous body bends within 20 seconds. Animals that did not move spontaneously, but responded to gentle prodding with a platinum wire were scored as immobile; those that did not respond to prodding were scored as paralyzed.

Basal slowing response was measured using young adults as previously described [88]. Prior to the assay, animals were washed in sterile deionized water, placed on assay plates either with or without a fresh lawn of OP50, and allowed to recover for 2 minutes. The number of body bends made by each animal (n=10 animals/strain/feeding condition) was counted for three consecutive 20-second intervals.

Gustatory plasticity was measured as the response to 25 mM NaCl after pre-exposure to 100 mM NaCl and was performed essentially as described previously [90, 91]. Mixed-staged cultures of *C. elegans* were enriched for gravid adults by incubating in ice-cold chemotaxis (CTX) buffer (minus NaCl) and aspirating away animals that were not heavy enough to sink to the bottom. Naïve animals were rinsed three times in CTX buffer (without NaCl). Pre-exposure to 100 mM NaCl was performed in CTX buffer for 20 minutes in the absence of food. Animals were scored after 30 minutes on chemotaxis plates, and the chemotaxis index was calculated using the formula (A-C)/(A+C) where A represents the number of animals in the quadrant containing NaCl, and C represents the number of animals in the quadrant without NaCl [91].

Statistical significance was calculated using single factor ANOVA, with Bonferroni correction. As described previously [92], significance was determined by comparing the response of mutant animals to the response of wild-type animals under the same conditions. Error bars represent the 95% confidence interval.

2.2.3 Analysis of Gene Expression

The extraction of total RNA from L1 stage populations was performed using the RNeasy Plus Micro Kit (Qiagen, Inc.) essentially as directed by the manufacturer for purifying RNA from animals and cells. Worms were collected from two 100 mM NGM-agar plates, rinsed twice in sterile, deionized water, and pelleted by centrifugation at 1,000 rpm for 1 min in a 15 ml conical tube. The samples were transferred to 1. 5 ml microcentrifuge tubes, and frozen on dry ice. Samples were subjected to two additional freeze/thaw cycles, resuspended in 0.1 ml of STE buffer (0.5% SDS, 5% 2-mercaptoethanol, 10 mM EDTA, 10 mM Tris-Cl pH 7.5) containing 0.5 mg/ml proteinase K, and then incubated at 55°C for 30 minutes. After incubation, the samples were mixed with 0.35 ml of RTL buffer (provided in RNeasy Plus Micro Kit, contents proprietary; Qiagen #1053393) and homogenized by two passages through a Qiashredder column (Qiagen, Inc) and were then processed as directed by the manufacturer. For the final elution step, the total RNA was recovered in 20 μL of sterile, nuclease-free water. RNA purity and concentration was determined by UV spectroscopy at 260, 280, and 230 nm. RNA samples were only used if 260/230 ratios were equal to or greater than 1.7. RNA quality was determined by denaturing gel electrophoresis. Finally, we tested each RNA for genomic DNA contamination by performing PCR in the absence of reverse transcription with the endogenous control primer pairs. cDNA synthesis reactions were performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), as directed by the manufacturer, with each 20 μL

reaction containing 2.0 μg of total RNA. Real-time PCR was performed on the 7500 Fast Real-Time PCR System® using either the Fast Sybr Green or the Taqman Universal Master Mix (Applied Biosystems) and the appropriate gene specific primer/probes listed in Table 1. Each reaction was performed in quadruplicate, and three biological replicates were tested for each gene. Assays were performed using relative quantitation, with normalization against two different endogenous control genes. The endogenous control genes were *pmp-3 and cdc-42* for the Sybr green reactions and were *pmp-3* and *rrc-1* for the Taqman reactions. Relative fold changes in expression were determined by setting the wild-type to an arbitrary level of one. In the final determination of genes whose expression was significantly altered by loss-of *hlh-17*, we considered only those genes whose expression changed for an average of at least 1.5 fold over the course of three experiments and that showed a *P*-value less than 0.05 when evaluated by single factor ANOVA.

2.3 Results

2.3.1 HLH-17 Animals Are Resistant To Exogenous Dopamine

C. elegans have 24 sheath cells that act with socket cells to enclose sensilla endings within a protected environment (for review, see www.wormatlas.org). Though they do not form myelin like the glia in vertebrates, the *C. elegans* sheath cells are considered glia [93], and play roles in the regulation of dendrite extension and axon guidance. In *C. elegans*, anterior processes of cephalic sheath cells enclose the dopaminergic CEP and CEM neurons of the cephalic sensilla. The cephalic sheath also projects posterior processes that wrap around the outside of the nerve ring, the neural ganglion of the head. This portion of the CEPsh act to facilitate assembly and maintenance of the nerve ring (www.wormatlas.org; [72]). We previously

demonstrated that the *hlh-17* gene is expressed in cephalic sheath cells [87]. More recently, two other genes, HLH-32 and HLH-31, were identified in the genome that show 95% and 83% sequence similarity to HLH-17, respectively [30]. Thus far, expression of *hlh-31* has not been confirmed, and expression of *hlh-32* has only been detected in two unidentified neurons of the head [30]. Nevertheless, the strong degree of sequence similarity, and the inability to rule out a more extensive expression pattern for all three proteins, suggested that HLH-17, HLH-31, and HLH-32 could act redundantly though the extent of their functional overlap is unclear. Because the cephalic sheath cells act as glia for the CEP/CEM neurons, we wondered if loss of *hlh-17* or loss of *hlh-17*, *hlh-31*, and *hlh-32* affects DA sensitive behaviors in *C. elegans*.

Egg-laying in *C. elegans* is an established paradigm for the characterization of neurotransmitter pathways [94]. Egg-laying occurs through the contraction of muscles of the vulva, and wild-type animals regulate this behavior based on the availability of food. When food is plentiful, wild-type animals lay eggs at an average rate of 10-12 eggs/hour (reviewed in [95]). Under conditions where food is less plentiful, wild-type animals lay eggs less frequently, retaining their eggs in the uterus until food becomes available. Modulation of egg-laying behavior in response to food is mediated by antagonism between the neurotransmitters serotonin (5HT) and dopamine (DA). As shown previously [79, 94, 96] and in Fig. 4, in wild-type animals exogenous DA significantly inhibits egg-laying, while 5HT significantly stimulates egg-laying when compared to egg laying in the absence of either neurotransmitter. Also, as shown previously, DA can inhibit 5HT stimulation so that animals treated with both drugs lay eggs at the same rate as those not treated with neurotransmitters. We found that in assays of egg-laying, both *hlh-17* (ns204) and *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals are insensitive to exogenous DA, but remain sensitive to 5HT. DA still inhibits 5HT stimulation of egg laying in

both strains; however, DA could only partially block the response to 5HT in *hlh-17* (ns204) animals. Finally, we noted that *hlh-17* (ns204) animals lay eggs at a slower rate than wild-type animals. The loss of *hlh-31* and *hlh-32* compensated for the defective egg-laying rates of *hlh-17* (ns204) animals, suggesting that, in the regulation of egg-laying, these proteins do not function redundantly with HLH-17.

DA also regulates the locomotion circuit in *C. elegans*. Endogenous DA is required for a phenomenon known as the basal slowing response (see below; [97]). Additionally, wild-type animals become paralyzed when exposed to exogenous DA in a manner that is both dose-dependent and exposure time-dependent [96]. This paralysis is thought to be the result of hyper-activation of dopamine signaling and requires a functional D2-like receptor, DOP-3 [88]. We used this trait to measure the sensitivity of wild-type and mutant animals to increasing concentrations of exogenous DA. In this immobilization assay, we scored animals as mobile if they moved within 20 seconds of observation, as immobile if they did not move on their own but were responsive to gentle prodding, or as paralyzed if they did not move even when prodded.

As shown in Fig. 5, wild-type and mutant animals show a dose dependent sensitivity to exogenous DA. All three groups of animals tested in our study were affected by concentrations of DA as low as 5mM; however, *hlh-17* (ns204) animals were the least sensitive, with almost 60% of the population remaining mobile after 40 minutes (Fig. 5A). Wild-type animals were either immobilized or paralyzed after exposure to 10 mM DA; however, 48% of *hlh-17* (ns204) animals and 18% *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals were still mobile. In fact, at least 15% of both *hlh-17* (ns204) animals and *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals were moving even after exposure to 20 mM DA. By comparing the fraction of animals in each of the three responsive states, we were able to differentiate between the

behaviors of the *hlh-17* (ns204) animals and *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals. After 40 minutes of exposure to 10 mM DA, for example, 100% of wild-type animals were motionless on plates, with a distended body posture. As shown in Fig 5B, approximately 78% of those motionless animals responded to gentle prodding and were scored as immobile. The remaining 22% did not respond to prodding and were considered paralyzed. At 20 mM DA, greater than 50% of wild-type animals were completely paralyzed after 40 minutes. The responses of *hlh-17* (ns204) animals were strikingly different from wild-type animals. First, some fraction of *hlh-17* (ns204) animals was mobile at all concentrations tested. Second, only 30% of *hlh-17* (ns204) animals were paralyzed, even at the highest concentrations of DA tested. It was particularly interesting that the triple mutant animals shown an intermediate phenotype between the phenotypes of the wild-type and *hlh-17* (ns204) animals. First, a small fraction of *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals were paralyzed at 5 mM DA. Second, greater than 30% of *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals were paralyzed by 10 mM DA. Third, the percentage of *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals that were paralyzed at 20 mM DA (approximately 48%) was not significantly different from the percentage of paralyzed wild-type animals (approximately 57%) at the same concentrations of DA. Together, these results underscore our earlier observations that *hlh-17* alters sensitivity to exogenous DA, and that HLH-31 and HLH-32 are not functionally redundant to HLH-17.

2.3.2 HLH-17 Is Required For Dopamine Dependent Behaviors

Wild-type, well-fed animals significantly slow their forward locomotion rate when they encounter a bacterial lawn [97]. This response is known as the basal slowing response, and has been shown to be mechanosensory in nature [79, 97]. Endogenous DA is thought to be released

by dopaminergic neurons when the animal contacts food [97, 98], and so the basal slowing response can be considered an assay for responsiveness to endogenous DA. Basal slowing is measured by comparing the frequency of sinusoidal body bends in the presence of food to the frequency in the absence of food. As shown previously [88, 97], and in our assays (Figure 6), wild-type animals slow by as much as 40% in the presence of food. Interestingly, *hlh-17* (ns204) animals do not alter their locomotion rates in the presence of food, an indication that dopamine signaling may be compromised. Animals with mutations in *hlh-17, hlh-31, hlh-32* show less of a response than wild-type animals; however, the *hlh-17* phenotype is rescued in these animals as they still slow significantly (21%) in the presence of food.

DA modulates several modes of behavioral plasticity in *C. elegans*, including gustatory plasticity, which is the ability to associate levels of salt in the environment with either negative or positive food cues [91, 99]. Gustatory plasticity can include a distinct behavioral change, from active attraction to active avoidance. Naïve, wild-type animals are strongly attracted to low levels of sodium chloride, but are repulsed by higher concentrations. This stereotypical response is altered when wild-type animals are pre-exposed to high concentrations of sodium chloride, and is more dramatically altered when the sodium chloride is presented with a chemical that is normally repulsive to them. For example, wild-type animals that were pre-exposed to 100mM sodium chloride are less attracted to 25 mM NaCl thirty minutes later [91]. Likewise, wild-type animals that were pre-exposed to 100 mM NaCl in the presence of 500 mM glycerol avoided 25 mM sodium chloride in chemotaxis assays [91].

Gustatory plasticity involves signals from four pairs of chemosensory neurons [92] and requires DA, 5HT, and glutamate [91]. Naïve animals with mutations in either the DA receptor genes or in genes required for DA synthesis are attracted to 25 mM sodium chloride;

however, DA defective animals that are pre-exposed to 100 mM sodium chloride do not avoid 25 mM sodium chloride as rigorously as wild-type animals [91]. Prolonged starvation, a negative food cue, together with pre-exposure to 100 mM sodium chloride, enhances gustatory plasticity in wild-type animals. Animals with defects in DA or 5HT signaling still display enhanced gustatory plasticity, while animals with defects in glutamate receptors do not.

To determine if *hlh-17* (ns204) animals and *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals are defective in gustatory plasticity, we tested the attraction of naïve and pre-conditioned animals to 25 mM sodium chloride. As shown in Fig. 7, naïve *hlh-17* (ns204) animals, as well as naïve animals with mutations in the D1-like receptor gene, *dop-1*, or the D2-like receptor gene, *dop-3*, show normal attraction to sodium chloride; however, these animals show significantly reduced levels of gustatory plasticity when compared to wild-type animals. In agreement with the results from the basal slowing assays, gustatory plasticity is restored in *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals. Taken together, these data are consistent with a model in which HLH-17 regulates the DA dependent behaviors in *C. elegans.*

2.3.3 HLH-17 Regulates Expression of the Dopamine Receptor Genes

In *C. elegans*, DA synthesis starts from the conversion of tyrosine into levadopa by the *cat-2* gene product, tyrosine hydroxylase (reviewed in [74]). Subsequently, the aromatic amino acid decarboxylase, BAS-1, converts levadopa into DA which is then packaged into synaptic vesicles for release from pre-synaptic neurons by the vesicular monoamine transporter homolog, CAT-1 [100]. The neurotransmitter then binds to receptors either post-synaptically or extrasynaptically, or is returned to the pre-synaptic neuron by the DA transport protein, DAT-1.

C. elegans has one D1-like receptor, encoded by the *dop-1* gene [101], and two D2-like G-protein coupled receptors, encoded by the *dop-2*, and *dop-3* genes [98, 102]. In *C. elegans,* the DOP-3 receptor is coupled to the Gαo homolog GOA-1, and is negatively regulated by the regulator of G protein signaling (RGS) homolog, EGL-10 [103]. Activities and behaviors mediated by the DOP-3 receptor can be antagonized by DOP-1 receptor signaling, which is coupled in *C. elegans* to the Gαq homolog, EGL-30. The RGS protein EAT-16 inhibits signaling by EGL-30 [103].

The results from our behavioral analysis suggest that *hlh-17* influences dopamine signaling. Using reverse transcription, quantitative PCR (RT-qPCR), we measured the expression of *cat-2*, *dop-1*, *dop-2*, *dop-3*, *eat-16*, *egl-10*, *goa-1*, and *egl-30* in wild-type, *hlh-17* (ns204), and *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals. As shown in Table , the expression of the DA receptor genes *dop-1*, *dop-2*, and *dop-3*, and the RGS protein gene, *egl-10*, is significantly down-regulated in *hlh-17* (ns204) animals (fold change >1.5, *P*-value < 0.05). This down-regulation was partially rescued in *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals. Expression of *dop-3* was not significantly different from wild-type in these animals (fold change = 1.25, *P*-value 0.09), and expression of *dop-1*, *dop-3*, and *egl-10* was down-regulated only slightly (fold change < 1.5, *P*-value < 0.05). The expression of the genes *cat-2*, *egl-30*, *goa-1*, and *eat-16* was not significantly altered in either single or triple mutant animals.

Based on these data, loss of *hlh-17* activity would result in decreased signaling through the *dop-1*, *dop-2*, and *dop-3* receptors and the *hlh-17* (ns204) animals would be expected to phenocopy animals with mutations in the DA receptor genes. Locomotion in *C. elegans* is directly controlled by the ventral cord motor neurons, which express both *dop-1* and *dop-3*. Previous studies have shown that exogenous DA-induced paralysis is dependent upon DOP-3

and that the *dop-1* phenotype is epistatic to the *dop-3* phenotype [88]. As shown in Fig. 8, 35% of wild-type animals are completely paralyzed after 40 minutes of exposure to 10 mM DA. Under the same conditions, *dop-1* animals are no more resistant to exogenous DA than are wild-type animals; however, only 6.7% of *dop-3* animals become paralyzed. Interestingly, the phenotype of *hlh-17* animals more closely resembles the phenotype of *dop-3* animals than *dop-1* animals; approximately 8% of *hlh-17* (ns204) animals become paralyzed. The loss of *hlh-31* and *hlh-32* partially rescued the *hlh-17* phenotype (13% paralyzed), though this difference was not statistically significant (p-value = 0.35). Failure of the *hlh-31* and *hlh-32* mutations to enhance the resistance of *hlh-17* animals to DA further supports our findings that *hlh-31*, *hlh-32*, and *hlh-17* are not redundant.

2.4 Discussion

DA is one of four biogenic amines that act in *C. elegans* to modulate environmentally responsive behaviors, including egg-laying, locomotion, and foraging. Here we provide behavioral and molecular evidence that the transcriptional regulator HLH-17 mediates DA signaling in *C. elegans* by regulating the expression of the DA receptor genes and of the RGS protein gene, *egl-10*. Levels of *dop-1*, *dop-2*, and *dop-3* mRNA are reduced by at least 2 fold in *hlh-17* (ns204) animals; *egl-10* mRNA is reduced by approximately 1.5 fold. As would be expected from these expression studies, *hlh-17* (ns204) animals phenocopy *dop-1* and *dop-3* animals and are less responsive to exogenously applied DA and more defective in DA responsive behaviors than wild-type animals [88, 91, 97]. Exogenous DA does not inhibit basal egg-laying in *hlh-17* (ns204) animals, and *hlh-17* (ns204) animals are more resistant to DA-induced paralysis than wild-type animals. Furthermore, *hlh-17* (ns204) animals are defective in both basal slowing and gustatory plasticity, behaviors that are modulated, in part, by endogenous DA.

Here we also demonstrate that the paralogous genes *hlh-31* and *hlh-32* do not enhance *hlh-17* phenotypes. The ability of DA to inhibit basal egg-laying and to induce paralysis in *hlh-17* (ns204) animals is partially restored in *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals. Likewise, *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals' show near normal gustatory plasticity and a significant basal slowing response. Taken together with the reduced effect on *dop-1*, *dop-2*, *dop-3*, and *egl-10* gene expression, our data suggest that *hlh-17*, *hlh-31* and *hlh-32* do not function redundantly in DA signaling.

The partial rescue of *hlh-17* (ns204) animals by mutations in *hlh-31* and *hlh-32* raises questions about the putative roles of HLH-31 and HLH-32 in dopamine signaling. The inability to detect expression of *hlh-31* [30] suggests that only *hlh-32* contributes to the dopamine response. While a mutant allele of *hlh-32* is currently available, it is a different allele from the one used in this study and not appropriate for direct comparison. We have not looked at the DA dependent behaviors in those strains; however, our current efforts are focused on making the appropriate single and double mutant strains by transgenic rescue of *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32* (ns223) animals. Future efforts will then include further characterization of HLH-32 interactions in dopamine signaling and with HLH-17.

Genetic analyses of DA signaling have revealed both separate and overlapping roles for the DA receptors in regulating DA dependent behaviors. All three of the DA receptors are involved in gustatory plasticity [91]; only DOP-3 is required for the basal slowing response [79, 88]. Previous studies have also suggested that DOP-1 and DOP-3 play antagonistic roles in controlling locomotion [88] and the mechanosensory response to food [104]. This antagonism is believed to occur in part through the coupling of DOP-1 and DOP-3 to different Gα subunits and their respective RGS proteins [88]. The RGS protein EGL-10 enhances the GTPase activity of

GOA-1, effectively reducing both Gαo signaling [103, 105, 106] and, presumably, DA signaling through the DOP-3 receptor [88]. This relationship between EGL-10 and the DA receptor genes may explain why, in our assays of DA-induced paralysis, *hlh-17* (ns204) animals more closely resemble *dop-3* animals than they do *dop-1* or *dop-1*; *dop-3* animals [88]. The combined decrease in *dop-3* activity and the decreased inhibition of DA signaling through DOP-1 would be consistent with the DA-induced paralysis phenotypes that we see here. This process of coupling the DA receptors to antagonistic Gα subunits is not unique to *C. elegans*, and is particularly prevalent in the mammalian brain [107-110].

In *C. elegans*, egg-laying, food response, and gustatory plasticity are regulated by both DA and 5HT [91, 94, 97]. Glutamate is also involved in food response in *C. elegans*, and is required for both the attraction to NaCl and the enhanced gustatory plasticity responses after extended periods of starvation [91]. Though future studies will address the roles of *hlh-17* in serotonin and glutamate signaling, the behavioral data presented here indirectly suggest that HLH-17 acts through DA signaling and not through 5HT or glutamate signaling. First, exogenous 5HT stimulates egg-laying in wild-type and *hlh-17* (ns204) animals; exogenous DA inhibits the 5HT stimulation of egg-laying even in *hlh-17* (ns204) animals which are otherwise unresponsive to DA. The ability of dopamine to block 5HT stimulation is dependent on MOD-1, an ionotropic 5HT receptor that is sensitive to DA [89]. Thus, it is likely that DA is acting in this instance through MOD-1 or some other 5HT receptor. Second, *hlh-17* (ns204) animals do not show a basal slowing response in the presence of food. Though 5HT modulates *C. elegans* behavior in response to food, DA mediates the basal slowing response which is thought to be mechanosensory in nature [97]. Serotonin is required for an enhanced, DA-independent slowing response that is not triggered by the mechanosensory neurons [97]. The expression of *hlh-17* in

glial cells that support neurons that are mechanosensory in nature (see below) is consistent with our interpretation that DA signaling is affected in this assay. Third, *hlh-17* (ns204) animals show *reduced* gustatory plasticity when compared to wild-type animals but still show significantly different responses to 25 mM NaCl when naïve versus when pre-exposed to 100 mM NaCl (*P*-value = 3.4 8 10^{-6}). However, *hlh-17* (ns204) animals are not defective in starvation-enhanced gustatory plasticity (Felton and Johnson, unpublished data) or in the attraction to NaCl. These findings suggest that the behavioral defects that we see in *hlh-17* (ns204) animals are not the result of altered glutamate signaling.

hlh-17 is strongly expressed in the cephalic sheath cells throughout development [87], and also in the sheath or socket cells of the inner labial (IL) and outer labial (OL) [30]. While it is likely that the full expression profile for HLH-17 has not yet been determined, our findings raise rather intriguing questions about how HLH-17 regulates expression of the DA receptor genes and of *egl-10* in wild-type animals. The *dop-3* gene is expressed in the neuronal support cells of the head, which includes the IL, OL, and CEP sensilla [88]; however, expression patterns for the *dop-1*, *dop-2*, and *egl-10* genes do not overlap with the known expression of *hlh-17*. Yoshimura and others also detected weak and transient expression of *hlh-17* in the commissures of some ventral cord motor neurons [30]. As *dop-1* and *dop-3* both are expressed in the GABAergic and cholinergic motor neurons [88], this finding raises the possibility that HLH-17 functions within the glial cells and transiently within ventral motor neurons to regulate dopamine signaling, however, that HLH-17 functions primarily in glial cells to regulate expression of the DA receptors. The involvement of glia cells in controlling DA signaling is not unprecedented. In mammals, for example, glia helps to clear extracellular DA from the synapse via DA

transporters [111]. Furthermore, functional DA receptors have been identified on vertebrate astrocytes and Muller cells [112-114].

The bHLH proteins have been previously implicated in controlling the differentiation, specification, and maintenance of neuronal cells in vertebrate and invertebrate systems. In vertebrates for example, Olig1 and Olig2 control the glia versus neuron cell fate decision [115] and act with the inhibitory HLH protein Id to maintain a balance between astroglia and oligodendrocytes during development [116]. Likewise, the bHLH protein Ngn2 controls differentiation of dopaminergic neurons in mice [117] and is also required for the specification of motor neurons [118]. Recently, it has been shown that Hand2 expression in postmitotic, differentiated sympathetic neurons is required to maintain noradrenergic properties of those neurons [119]. This role for Hand2 appears to be conserved in vertebrates, as mutations in Hand2 results in reduced levels of tyrosine hydroxylase and dopamine α-hydroxylase in the developing sympathetic ganglia of zebrafish, mice, and chick.

Despite bearing significant sequence similarity to Olig and being primarily expressed in the *C. elegans* glia, HLH-17 does not affect glial development, and only weakly affects axon guidance of the non-dopaminergic, chemosensory neuron, ADF [30]. HLH-17 is also not likely to be a pro-neural gene; there is no indication that HLH-17 is required for either specification or the differentiation of dopaminergic neurons in *C. elegans*. The data presented here suggest a role for HLH-17 in DA signaling, by controlling the production of proteins required for transducing the neurotransmitter signals. Bröhl and others (2008) recently described a transcriptional network involving the bHLH protein Ptf1a that coordinates the inhibitory neurotransmitter phenotype of dorsal horn neurons of the spinal cord. Ptf1a acts through the of paired homeodomain protein, Pax-2 and of the Lim homeodomain proteins, Lhx1/5, to activate the gene for the inhibitory

neuropeptide protein NPY [120]. Our gene expression studies show that HLH-17 works upstream of the DA receptor genes. These results do not eliminate the possibility that HLH-17 acts in a manner similar to Ptf1a to regulate an unidentified gene whose product subsequently activates each of the DA receptor genes. The existence of such a gene would provide a mechanism for the regulation of *dop-1* and *dop-3* by *hlh-17* in a non-cell autonomous fashion. Future studies will perhaps identify other transcriptional elements required for HLH-17 dependent regulation of DA response in *C. elegans*.

Table 1 Taqman probe ID

Gene Name	Taqman Probe ID	Amplicon (bp)
cat-2	CE02426732_m1	74
dop-1	CE02494345_m1	71
dop-2	CE02479829_m1	66
dop-3	CE02496464_m1	88
egl-10	CE02482855_m1	68
goa-1	CE02409649_m1	71
pmp-3	CE02485188_m1	71
rrc-1	CE02499261_m1	65

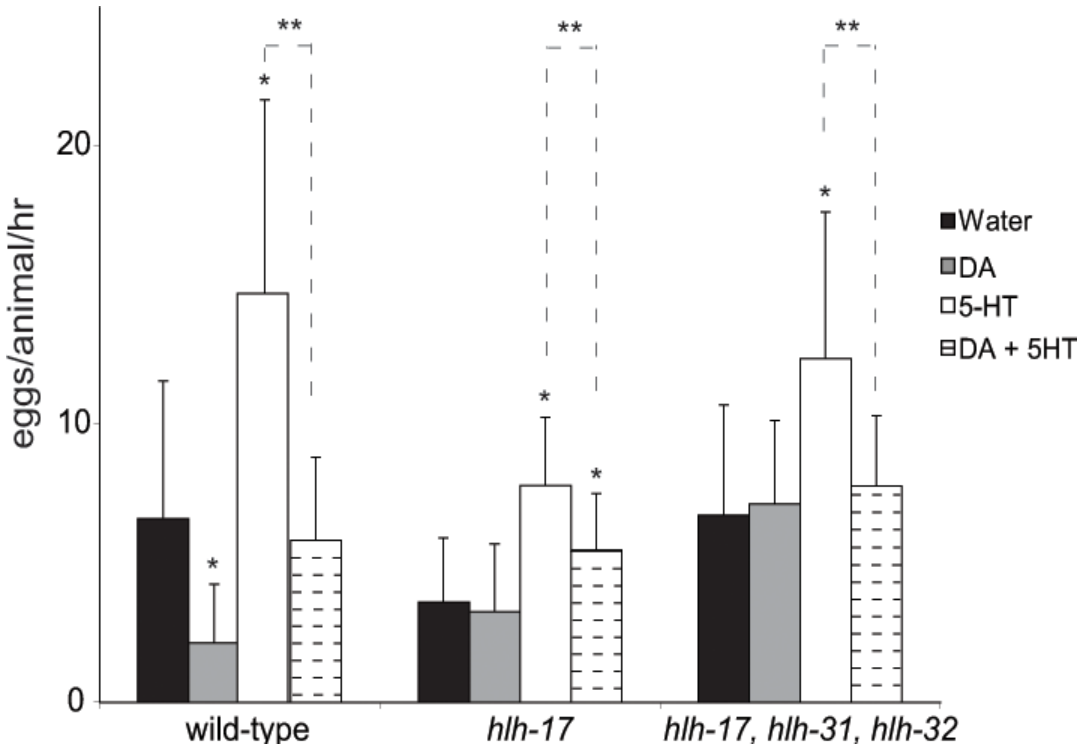

Figure 4 Inhibition of egg-laying by DA
5HT (5mg/ml) stimulates egg-laying in wild-type and *hlh-17* (ns204) animals. DA (3 mg/ml) inhibits both basal egg-laying and 5HT-stimulated egg-laying in wild-type animals, but does not inhibit basal egg-laying in *hlh-17* (ns204) animals. Graph shows the average of 30 observations. Error margins indicate 95% confidence intervals. Single astericks (*) indicate significant (single-factor ANOVA, P-value <0.05) differences in egg laying rates in water (black bars) when compared to rates in the neurotransmitter(s) indicated; double astericks (**) indicate significant differences in egg laying rates in 5HT (white bars) compared to rates in 5HT + DA (striped bars).

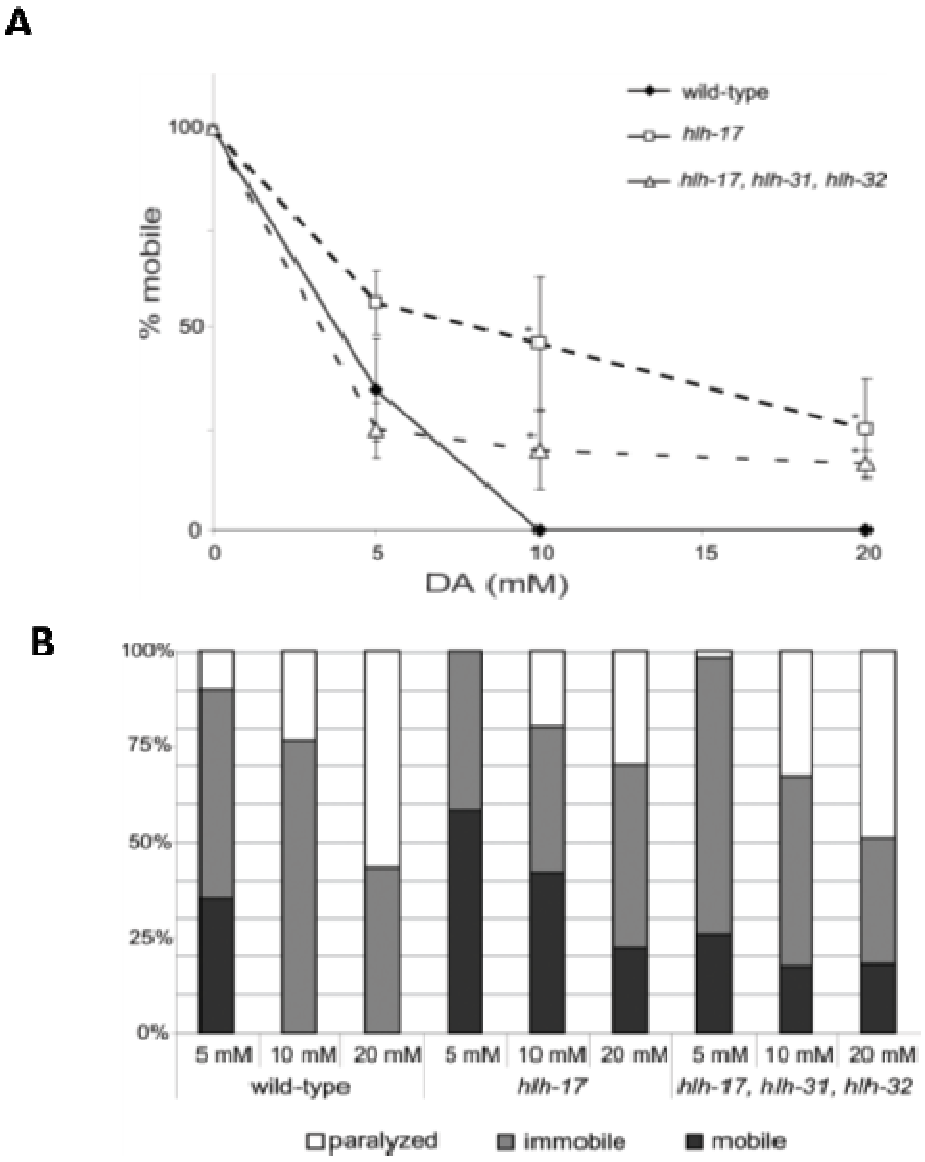

Figure 5 DA-induced paralysis in *hlh-17* (ns204) animals
A. Dose response curve measuring the percentage of animals that remain mobile after 40 minutes at the indicated concentration of DA. Each data point represents the mean for three trials totaling 60 animals. Error bars represent 95% confidence intervals. Statistical significance was measured using single factor ANOVA (P-value < 0.01). **B.** Fraction of worms for each DA concentration that remained mobile, were immobilized, or were paralyzed by exogenous DA. For each strain, this graph represents the distribution of at least 60 animals per DA concentration.

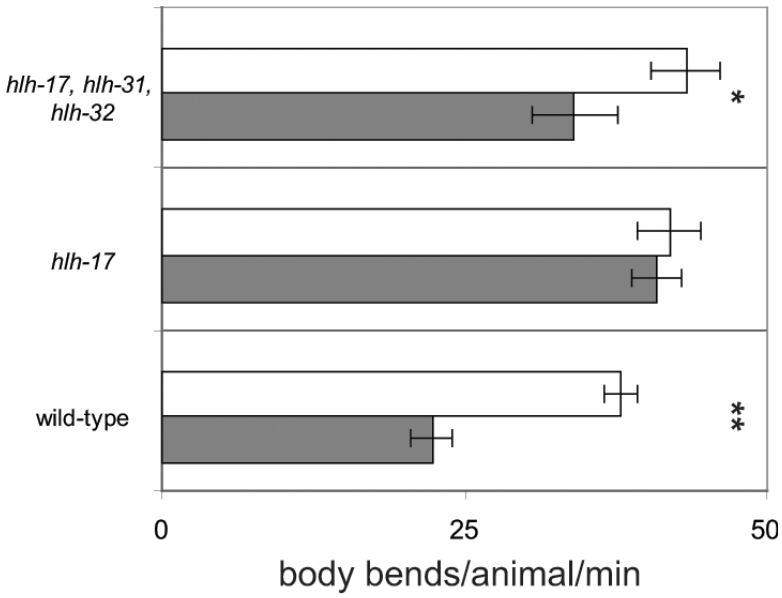

Figure 6 Basal slowing response
Analysis of the basal slowing response in *hlh-17* (ns204) animals. Locomotion rates were calculated in the absence (white bars) and presence (black bars) of bacteria and represent the average of 10 one-minute observations. Error bars represent 95% confidence intervals. Statistical significance was measure using single factor ANOVA
(* P-value < 0.05; ** P-value < 0.0001).

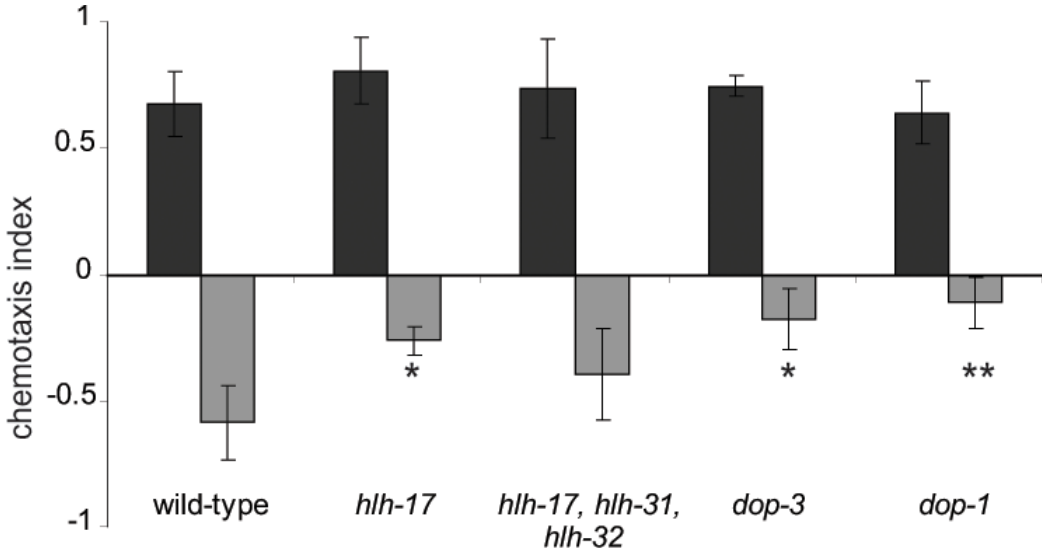

Figure 7 Gustatory plasticity
Analysis of gustatory plasticity in *hlh-17* (ns204) animals. Responses to 25 mM NaCl after mock treatment (black bars) or after pre-exposed (gray bars) to 100 mM NaCl. Error bars represent 95% confidence intervals. Significance was determined by comparing to wild-type animals under the same conditions (two-factor ANOVA with Bonferroni correction, * P-value <0.05, ** P-value < 0.01).

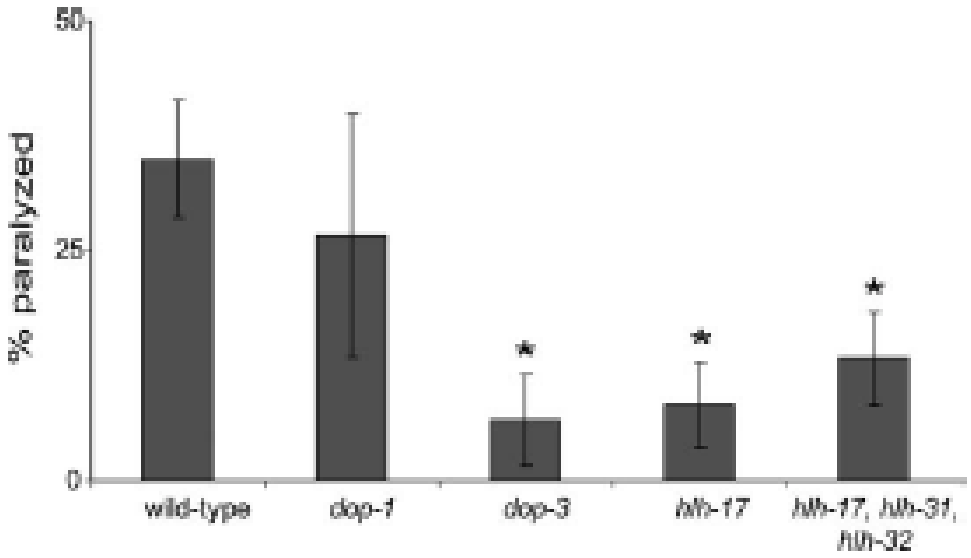

Figure 8 DA-induced paralysis in DA mutants
Analysis of DA-induced paralysis in DA-receptor mutants and *hlh-17* (ns204) animals. Animals were assayed as in Figure 2-2, except at a single concentration of DA (10mM). Each data point represents the mean for at least three experiments. Error bars represent 95% confidence intervals. Statistical significance was measured using single factor ANOVA (*P<0.005).

3 DOPAMINE SIGNALING IN *C. ELEGANS* IS MEDIATED IN PART BY HLH-17 DEPENDENT REGULATION OF EXTRACELLULAR DOPAMINE LEVELS

3.1 Introduction

In *C. elegans* and other multicellular organisms, basic helix-loop-helix (bHLH) proteins coordinate a number of developmental events, including myogenesis [121] organ morphogenesis [122] and mesodermal development [123]. These proteins also play vital functions during neurogenesis [124, 125]. For example, the proneural gene *hlh-14* is required to generate multiple neurons stemming from a variety cell lineage types, while HLH-3 is needed for the differentiation of hermaphrodite-specific motor neurons [126-128]. HLH-17 is the *C. elegans* homolog of the mammalian proneural family Olig [115, 129], but does not appear to play a role in neuronal specification during embryogenesis [30]. Our previous studies instead demonstrated that HLH-17 is required for normal behavioral responses to dopamine signaling [130].

In vertebrates and invertebrates, dopamine signaling is associated with motivation, recognition and reward, memory and adaptation, hormonal regulation, and motor control [131-134]. In humans, imbalances in dopamine signaling are associated with many neurological diseases, including Parkinson's, Alzheimer's, ADHD and substance abuse [135-137]. Dopamine signaling in *C. elegans* involves many of the same molecules as in mammals [74]. For example, dopamine is synthesized by the tyrosine hydroxylase enzyme CAT-2. Upon synthesis, dopamine is sequestered in presynaptic storage vesicles by the vesicular monoamine transporter CAT-1, where it remains until being released into the presynaptic cleft in response to a stimulus. Once in

the synapse, dopamine binds to and activates D1-like (DOP-1) and D-2 like receptors (DOP-2 and DOP-3) that are positioned either pre-, post-, or extra-synaptically. Unbound dopamine is taken back up into the presynaptic cell via reuptake by the dopamine transporter DAT-1.

HLH-17 is expressed in the glia-like cells surrounding the CEP dopaminergic neurons [87] and in the sheath or socket cells of the inner labia and outer labia [30]. Our previous data revealed that HLH-17 affects dopamine signaling through the DOP-1, DOP-2 and DOP-3 receptors as shown by the impaired response of *hlh-17(ns204)* animals to endogenous and exogenous dopamine. *hlh-17 (ns204)* animals also have reduced levels of the *dop-3* and *dop-1* mRNAs, and phenocopy *dop-3* hypomorhs [88, 130]. Together, these data suggest that HLH-17 functions upstream of the dopamine receptor genes and that the loss-of *hlh-17* causes a reduction in dopamine receptor activity.

Here we continue our characterization of the role of HLH-17 in dopamine signaling. Our data suggest that HLH-17 influences DA dependent behaviors by regulating genes that mediate levels of extracellular dopamine. *dat-1* mRNA levels are reduced, but not eliminated, in *hlh-17(ns204)* animals. Furthermore, *hlh-17(ns204)* animals display SWIP behavior in water that is an intermediate between the behavior in *dat-1* animals and in wild-type animals and that is enhanced by treatment with the dopamine reuptake inhibitor, bupropion. We show that a null allele of *dop-3* completely suppresses the SWIP phenotype of *hlh-17* animals, supporting previous data that HLH-17 acts upstream of DOP-3. Surprisingly, the SWIP phenotype of *hlh-17* animals is unaffected by treatment with the VMAT inhibitor reserpine or with the serotonin reuptake inhibitor, fluoxetine; however, this unresponsiveness is not due to reduced acetylcholine signaling. Taken together our results suggest that HLH-17 influences extracellular

dopamine levels in *C. elegans*, in part by its regulation of the dopamine receptors and the dopamine transporter.

3.2 Materials and methods

3.2.1 Nematode Strains and Maintenance

The following strains were used in this study: wild-type: Bristol strain (N2); RM2702 [*dat-1(ok157)*]; OS2649: [*hlh-17(ns204*)]; and LX705 [*dop-1 (vs100); dop-3 (vs106)*]. OS2649 was a gift from Dr. S. Shaham. The strains CMJ2003 [*hlh-17(ns204); dat-1(ok157)*] and CMJ2004 [*hlh-17(ns204); dop-1 (vs100); dop-3 (vs106)*] were generated using traditional crossing techniques and the genotypes were confirmed by PCR. To generate CMJ2004, *hlh-17 (ns204)* males were crossed with *dop-1 (vs100); dop-3(vs106)* hermaphrodites, and the F1 males were backcrossed to *dop-1 (vs100); dop-3(vs106)*. F2 hermaphrodites were separately cloned, and their progeny were genotyped by PCR. The strain CMJ2005 [*hlh-17(ns204); dat-1(ok157); dop-1 (vs100); dop-3 (vs106)*] was generated by crossing CMJ2003 males with CMJ2004. F1 hermaphrodites were separately cloned, and their progeny were genotyped by PCR for *hlh-17* and *dat-1*. The progeny of hermaphrodites that were confirmed to be homozygous for both *hlh-17* and *dat-1* were then subcloned and their progeny screened for homozygosity for *dop-3* by PCR and for rescue of SWIP behavior.

The transgene, cmjEx22, is a 6.2 kb genomic fragment consisting of 2 kbp upstream of the *hlh-17* translational start site, the entire *hlh-17* coding region, the SV40 nuclear localization signal (NLS) and 850 bp of the sequences coding for green fluorescent protein (GFP). The GFP sequences were amplified from pPD95.67 (a gift from A. Fire) using serial overlap PCR. Transgenic lines to rescue loss-of *hlh-17* were produced by microinjecting the final PCR product

(cmjEx22) into *hlh-17(ns204)* animals, along with the pCFJ90 [P*myo-2*::mCherry::unc-54utr] co-injection marker, using standard microinjection techniques[138], and is designated as CMJ2002 [*hlh-17(ns204)*; cmjEX22, pCFJ90(P*myo-2*::mCherry::unc54utr)] .

Three separate lines (15.1, 15.3, and 3.1) were tested for rescue of SWIP, basal slowing response, and dopamine paralysis. All three lines were able to at least partially rescue each of the phenotypes tested; however, the degree of rescue for each line was specific to the phenotype tested.

Unless otherwise noted, strains were cultured on solid nematode growth media (NGM) containing OP50 at 20°C using standard methods and synchronized cultures were prepared by hypochlorite treatment of gravid adults, as previously described [139]. The following primers were used for genotyping: HLH17F: 5'-TCCCTGGGGACTCTCCTCG-3'; HLH17R: 5'-CGATTTTTGCTGCTAATGGGCAACAC-3'; DAT1F: 5'-CTATTCGGATATCTTGCCAATGCTATACC-3'; DAT1R: 5'-CTATTCGGATATCTTGCCAATGCTATACC-3'; DOP3F: 5'-CTATTCGGATATCTTGCCAATGCTATACC-3'; and DOP3R: 5'-CTAACTCACCAGAAAATCAGAAACTGC-3'.

3.2.2 Gene Expression Analysis

Synchronized populations were collected at the L4 stage, pelleted and frozen at -80° C. Total RNA, cDNA synthesis, and real-time PCR were performed as previously described [130], except the cDNA was amplified from 1 µg of total RNA in 20 µL reactions. Real-time PCR was performed with Taqman Gene Expression Assays (Applied Biosystems/Invitrogen) using relative

quantitation against glyceraldehyde 3-phosphate dehydrogenase (*gpd-3*) (Ce02616909_gH) as the endogenous control. The probe sets used were: *hlh-17*(Ce02616669_m1), *dat-1*(Ce02450896_g1), *cat-1*(Ce02495610_m1), *mod-5* (Ce02415245_m1), *dop-1* (Ce02494345_m1), *dop-2* (Ce02479824_m1) *dop-3*(Ce02496462_m1), *lev-8* (Ce02501240_g1), and unc-43 (Ce02458977_m1). Gene expression assays were done in triplicate, for at least three biological replicates.

3.2.3 Behavioral Assays

Assays for dopamine paralysis and basal slowing response were as previously described [130], except animals were assayed at late L4 stage. For Swimming Induced Paralysis (SWIP), approximately ten L4 stage animals were placed in 150 µL of water in a single well of 48-well tissue culture plate (CELLSTAR, Cat # 677180). After 20 minutes animals were categorized as paralyzed if they failed to exert the normal thrashing behavior within a 20 second time frame [140]. For the SWIP assays conducted with inhibitors, animals were grown on NGM plates containing the appropriate drug (reserpine [0.6mM] (Cat#S1601), fluoxetine [145 µM] (Cat# S1333) and bupropion [10mM] (Cat#S2452)) and then analyzed in water. All inhibitors were obtained from Selleckchem. Aldicarb-induced paralysis and levamisole-induced paralysis assays were conducted using standard protocols [141-143] with some modifications. Plates containing aldicarb [1.0 mM] (FisherSci#US-PST-940) or levamisole [0.2 mM] (FisherSci#ICN15522805) were prepared one hour before use. Drugs were prepared as 100 mM stocks in 70% ethanol, diluted in sterile M9 buffer, added to NGM plates already seeded with OP50 to the appropriate concentration, and allowed to diffuse into the media for one hour. L4 stage animals were

manually selected to confirm their age and moved to plates using a platinum wire, and examined every hour for a 5 to 6 hour period. Animals were categorized as paralyzed if they failed to move after prodding with a platinum wire.

3.3 Results

3.3.1 HLH-17 functions upstream of the D2-like dopamine receptor DOP-3 to regulate behavioral responses to dopamine

The effects of dopamine signaling in *C. elegans* are mediated by the three heterotrimeric G-protein receptors, DOP-1, DOP-2 and DOP-3 [144]. Our previous studies demonstrated that the mRNA levels of these three receptors are reduced in *hlh-17(ns204)* animals and that *hlh-17(ns204)* animals phenocopy those carrying loss-of-function alleles of *dop-3*[130]; thus, we decided to conduct our behavioral analyses with *dop-1 (vs100); dop-3(vs106)* animals to account for the possible antagonism between *dop-1* and *dop-3* during HLH-17 regulation. As shown in Figure 9A, and in our previous studies, fewer *hlh-17(ns204)* and *dop-1 (vs100); dop-3(vs106)* animals than wild-type animals were paralyzed after 40 min exposure to 10 mM of exogenous DA, and both well-fed *hlh-17(ns204)* animals and well-fed *dat-1(vs106)* animals failed to exhibit the basal slowing response (BSR) when encountering a bacterial lawn (Figure 9B). In this study, we used an extragenic, translational reporter for *hlh-17* to rescue the dopamine paralysis and basal slowing phenotypes of transgenic *hlh-17(ns204)* animals. This reporter was able to restore dopamine sensitivity and to enhance BSR, showing that the previously reported phenotypes are indeed a result of loss of HLH-17 (Figure 9C). We previously reported that a transcriptional reporter for *hlh-17* drives expression in the glial-like,

cephalic sheath cells of the dopaminergic neurons [87], and others have detected weak *hlh-17* expression in the sheath or socket cells of the inner labia and outer labia [30]. The translational reporter used in this study was driven by the same promoter sequences used in the previous study, and was similarly expressed (data not shown). This expression pattern weakly correlates with expression of the dopamine receptors in neuronal support cells of the head [88], and so we looked for genetic interactions between *hlh-17* and *dop-3*. As shown in Figure 9, the resistance to dopamine-induced paralysis and the basal slowing response of *hlh-17(ns204)*; *dop-1 (vs100); dop-3(vs106)* is not significantly different from the resistance and slowing response phenotypes of *dop-1 (vs100); dop-3(vs106)* and *hlh-17(ns204)* animals (Figure 9), and is consistent with a model in which HLH-17 is functioning in the same genetic pathway as DOP-3 to modulate these behaviors. Taken together, we conclude that HLH-17 influences behaviors that are mediated by dopamine through the transcriptional regulation of *dop-3*. Our existing data suggest that this regulation is indirect; however, it is possible that the transcriptional and translational constructs used in our studies do not fully report the wild-type expression pattern for *hlh-17*. In fact, recent gene expression profiles from FACS sorted cells point to overlapping expression of *hlh-17* and *dop-3* in the dopamine neurons of late embryos, in panneuronal cells and the glutamate receptor neurons of L2 stage animals, and in the cephalic sheath of young adult animals [145] http://www.vanderbilt.edu/wormdoc/wormmap/WormViz.html. Additionally, dopamine receptor genes are expressed in mammalian glial cells [146, 147] and further support the possibility that HLH-17 directly regulates *dop-3* expression in the cephalic sheath and in selected neurons during *C. elegans* development.

3.3.2 hlh-17 mutants are defective in clearing dopamine from the synaptic cleft

In our previous studies, the mRNA levels of genes required for dopamine synthesis, those encoding tyrosine hydroxylase gene (*cat-2*) and the aromatic amino acid decarboxylase (*bas-1*), were not affected by loss of *hlh-17* [130]. This suggested that the presynaptic synthesis of dopamine is not compromised in *hlh-17(ns204)* animals. Additionally, exogenous dopamine failed to repress egg-laying in naive *hlh-17(ns204)* animals; however, exogenous dopamine was able to repress the stimulation of egg-laying by the neurotransmitter, serotonin. Though we did not further address the serotonin-responsiveness of *hlh-17(ns204)* animals, this result suggested the ability of *hlh-17(ns204)* animals to respond to exogenous dopamine may be mediated by the binding of the neurotransmitter to other non-dopaminergic receptors. For example, dopamine can bind with low affinity to a number of the neurotransmitter receptors involved in serotonin-stimulated egg-laying, including MOD-1, SER-1, SER-2, and SER-7 [74, 89].

To further define the role of HLH-17 during dopamine signaling, we measured the mRNA levels of the genes encoding the vesicular monoamine transporter (VMAT), *cat-1*, and the dopamine reuptake transporter, *dat-1,* in *hlh-17(ns204)* animals. As shown in Figure 10A, *dat-1,* but not *cat-1,* mRNA levels, are decreased in *hlh-17(ns204)* animals. We also found that the mRNA levels for the dopamine receptor genes, *dop-1*, *dop-2*, and *dop-3*, are down regulated in L4 stage animals, confirming that the decreased levels previously reported in L1 stage animals [130] remain low in animals at the stage used for our behavior assays.

Like the mammalian VMATs, CAT-1 mediates the packaging and transport of the biogenic amines into synaptic vesicles and is required for proper release of dopamine from

presynaptic neurons in *C. elegans* [100]. The dopamine transporter, DAT-1, is localized to the synapses of all dopaminergic neurons of *C. elegans* males and hermaphrodites [140], and is responsible for neurotransmitter clearance from the synaptic cleft [148, 149]. In otherwise WT animals, loss of *dat-1* leads to increased activation of the DOP-3 receptors located on cholinergic motor neurons [88]. Consequently, *dat-1* animals are paralyzed in water as a result of DOP-3 hyperactivation; this behavior can be measured using a swimming induced paralysis (SWIP) assay [74, 140]. Swimming induced paralysis does not occur in *cat-1* animals because dopamine is not efficiently packaged or subsequently released into the synaptic cleft. We reasoned that if *hlh-17(ns204)* animals synthesize and release normal levels of dopamine, but produce less DAT-1, then they would be less efficient than wild-type animals at clearing extrasynpatic dopamine from the synaptic cleft. To test this hypothesis, we conducted SWIP assays with wild-type, *hlh-17(ns204)* and *dat-1(ok157)* animals. As reported previously [140], and shown in Figure 10B, *dat-1(ok157)* animals, but not wild-type animals, have a strong SWIP response after 20 minutes in water. The SWIP response of *hlh-17(ns204)* animals was an intermediate response, with approximately 40% of the animals becoming paralyzed after 20 minutes in water. This phenotype was rescued by transgenic expression of HLH-17. The result suggests that loss of HLH-17 compromises the ability of mutant animals to clear dopamine from the synaptic cleft and could be interpreted as representing a partial, rather than complete, loss of *dat-1* activity.

The SWIP phenotype seen in *dat-1* animals is completely rescued by loss of DOP-3 [102]; hence, we reasoned that the reduced SWIP response of *hlh-17(ns204)*animals, which is an intermediate of the responses of wild-type and *dat-1* animals, may be the result of having decreased levels of both *dop-3* and *dat-1*. To test this hypothesis, we compared the SWIP phenotypes of *hlh-17(ns204)*; *dat-1(ok157)* animals and of *hlh-17(ns204)*; *dop-1 (vs100); dop-*

3(vs106) animals to those of *dat-1(ok157)* and *dop-1 (vs100); dop-3(vs106)* animals, respectively. As shown in Figure 10B, complete loss of *dat-1* activity enhanced the SWIP response of *hlh-17(ns204)* animals while complete loss of *dop-3* activity significantly decreased the SWIP response. Furthermore, the SWIP phenotypes of *hlh-17(ns204)*; *dop-1 (vs100); dop-3(vs106)* animals and *hlh-17(ns204)*; *dop-1 (vs100); dop-3(vs106); dat-1(ok157)* animals were not significantly different from that of *dop-1 (vs100); dop-3(vs106)* animals (p-values = 0.574 and 0.265, respectively). Interestingly, loss of *hlh-17* and *dat-1* appears to be an additive effect: a comparison of the differences of the means shows that the difference for wild-type versus *hlh-17*; *dat-1* is equal to the sum of the differences for wild-type versus *hlh-17* and wild-type versus *dat-1*. These results underscore the dependence of the SWIP phenotype on DOP-3. Furthermore, the results suggest that the SWIP response is not mediated solely through *dat-1*, and that *hlh-17* may affect the SWIP phenotype through both *dat-1*-dependent and *dat-1*-independent mechanisms. A *dat-1*-independent, *dop-3* dependent mechanism for the SWIP phenotype is consistent with the results of a previously reported forward genetics screen [150] and suggests that the HLH-17 transcriptional network may include genes that act in parallel to *dat-1*.

Our results from the dopamine paralysis assays and the egg-laying assays suggest that *hlh-17(ns204)* animals are less sensitive to exogenous dopamine, a result that is consistent with reduced *dop-3* activity. The results from assays for BSR and SWIP, both of which rely on normal synthesis and release of endogenous dopamine from presynaptic neurons, suggest that *hlh-17(ns204)* animals produce normal amounts of dopamine but are deficient in the ability to transport the dopamine. This result is also consistent with reduced *dop-3* activity. Likewise, a failure in the ability to transport dopamine from the synaptic cleft is consistent with reduced *dat-1* activity, as is the SWIP phenotype of *hlh-17(ns204)* animals. From these results, we conclude

that HLH-17 functions to control extrasynpatic dopamine levels, in part by its regulation of *dop-3* and *dat-1*.

3.3.3 hlh-17 mutants are responsive to reuptake inhibitors that are selective for dopamine, but not for serotonin

Bupropion is a selective norepinephrine and dopamine reuptake inhibitor commonly used in mouse and human studies [151-153] and in the treatment of ADHD [154, 155] and depression [156, 157]. Reuptake inhibitors block the ability of a transporter to move a neurotransmitter from the synapse to the presynaptic neuron or the surrounding glial cells, thereby increasing extracellular concentrations which ultimately increase neurotransmission. We reasoned that the intermediate SWIP behavior of *hlh-17(ns204)* animals is because these animals still produce a small amount of functional DAT-1, and that treatment with bupropion would increase SWIP in *hlh-17(ns204)* animals. As expected, pretreatment of *hlh-17(ns204)* animals with bupropion increased their SWIP response to that of untreated *dat-1(ok157)* animals (Figure 11A), supporting our mRNA studies showing that *dat-1* expression is reduced but not completely eliminated in *hlh-17(ns204)* animals. It has been shown previously that SWIP can be rescued in *dat-1(ok157)* animals by pretreatment with the dopamine antagonist reserpine [140], an antipsychotic drug that depletes vesicular dopamine stores by blocking the vesicular monoamine transporter (VMAT). As shown in Figure 11B, pretreatment with reserpine reduced the SWIP responses of *dat-1(ok157)* animals, but did not affect SWIP in wild-type animals or in *hlh-17(ns204)* animals. This result was unexpected because *cat-1* mRNA levels are not affected in *hlh-17(ns204)* animals; however, others have reported reserpine insensitive mutants that show

SWIP behavior in a *dat-1* dependent manner [150]. Bupropion pretreatment also increased SWIP in *dat-1(ok157)* animals, *hlh-17(ns204)*; *dat-1(ok157)* animals; and *hlh-17(ns204)*; *dat-1(ok157)*; *dop-1 (vs100); dop-3(vs106)* animals (Figure 11A). Together, these results further emphasize that SWIP behavior may not be mediated solely through dopamine reuptake by DAT-1. The ability to induce SWIP behavior in *dop-1 (vs100); dop-3* animals suggests that the mechanism may occur through a dopamine-independent mechanism.

Although a role for 5HT during SWIP has not been reported to date, we measured the SWIP response of WT, *hlh-17(ns204)* and *dat-1(ok157)* animals after exposure to fluoxetine to test the possibility that the SWIP phenotype is also modulated through serotonin. Fluoxetine blocks the function of SERT/MOD-5, the serotonin reuptake transporter [158, 159]. As seen in Figure 11C, the SWIP response phenotype increased in WT animals that were pretreated with fluoxetine, but decreased in similarly treated *dat-1*(ok157) animals. These results can be explained by the action of fluoxetine, which is known to increase extracellular concentrations of dopamine [160, 161]. The excess dopamine in treated wild-type animals would phenocopy mutants that have increased extracellular levels of dopamine and have an increased SWIP response. Fluoxetine can also aggressively inhibit any transport of dopamine by the serotonin transporters [161] so that treated *dat-1* animals would show a reduced SWIP response, analogous to the reduced response of *dat-1; dop-3* animals [140]. Interestingly, *hlh-17(ns204)* animals were insensitive to fluoxetine, though they have normal levels of *mod-5* mRNA (see Figure 10A) and respond to exogenous serotonin in egg-laying assays [130]. Fluoxetine has previously been shown to act via both serotonin-dependent and serotonin-independent mechanisms in *C. elegans* [159, 162]. In future studies we will further explore the role of HLH-17 in serotonin signaling which may also address the mechanisms of fluoxetine resistance in *hlh-17(ns204)* animals.

3.3.4 hlh-17 animals are not defective in acetylcholine release

It is possible that the SWIP response of *hlh-17(ns204)* animals is insensitive to both reserpine and fluoxetine because HLH-17 influences the activity of *C. elegans* biogenic amines in a manner that, with the exception of dopamine, does not involve the regulation of genes directly involved in neurotransmitter synthesis, packaging, or transport. A more attractive, alternative possibility is that HLH-17 influences acetylcholine release, as the phenotypic effects of both reserpine [163] and fluoxetine [164, 165] are dependent on acetylcholine. In support of this possibility, the inhibitory effect of fluoxetine on acetylcholine release in rats is dependent on activity of the dopaminergic D2 receptors [165]. Furthermore, loss of *dop-3* activity in *C. elegans* has recently been shown to increase acetylcholine release, while null alleles of genes required for acetylcholine release have been shown to rescue the SWIP phenotype in *dat-1(ok157)* animals [166].

We used aldicarb and levamisole sensitivity assays to examine acetylcholine release and acetylcholine reception, respectively in *hlh-17(ns204)* animals. Aldicarb is an acetylcholinesterase inhibitor and thereby increases the concentration of acetylcholine in the neuromuscular junction. Animals with reduced acetylcholine release are resistant to aldicarb-induced paralysis, while those with increased acetylcholine release are more sensitive [166, 167]. As shown in Figure 12A, *hlh-17(ns204)* animals are more sensitive to aldicarb than wild-type and *dat-1(ok157)* animals (p-value = 0.0428). This result is consistent with the weak effects of the *dop-3(v106)* mutation on aldicarb sensitivity that was previously reported, and suggests that

acetylcholine release is otherwise normal in *hlh-17(ns204)* animals. We also found that *hlh-17(ns204)* animals are more sensitive to levamisole (p-value = 0.0002) (Figure 12B), a cholinergic agonist that binds selectively to acetylcholine receptors in body wall muscles [167]. We are able to tentatively explain this increased sensitivity based on our unpublished microarray analysis which indicates that the activity of the nicotinic acetylcholine receptor gene, *lev-8*, is upregulated in *hlh-17(ns204)* animals. Interestingly, our microarray data indicated that the gene encoding the calcium/calmodulin-dependent protein kinase UNC-43 is also upregulated. Mutants carrying gain-of-function alleles of *unc-43* have previously been reported to have increased resistance to fluoxetine. As shown in Figure 10A, we were able to validate these results using RT-qPCR analysis. mRNA levels of *unc-43* and *lev-8* are increased in *hlh-17* animals, while the level of *mod-5*, a gene that was not differentially affected in our microarray analysis, remained unaffected. To our knowledge, loss of dopamine receptor activity, in particular *dop-3,* has not previously been tested; however, animals that are defective in dopamine synthesis display normal sensitivity to levamisole [168]. Taken together our results suggest that neither acetylcholine release nor acetylcholine reception is compromised in *hlh-17(ns204)* animals, and that the resistance to reserpine and fluoxetine may be mediated through other genes in the HLH-17 transcriptional network.

3.4 Conclusion

The Olig sub-family of bHLH transcription factors influences the specification of oligodendrocytes, myelin formation and axon pathfinding of motorneurons in both invertebrates and vertebrates [115, 169-171]. In *C. elegans*, HLH-17 is an Olig homolog that is expressed in sheath cells of the dopaminergic neurons; however, this protein has no known role in glial cell specification, neurite extension, or axon guidance [30]. The work presented here and in previous

studies points to a role for HLH-17 in controlling dopamine-dependent behaviors. Specifically, our work suggests that HLH-17 is needed to clear extracellular dopamine from the synaptic cleft. First, *hlh-17(ns204)* animals have reduced mRNA levels for *dat-1*, *dop-3*, *dop-2*, and *dop-1,* but maintain normal levels of *cat-1* and *cat-2*. Second, the SWIP response of *hlh-17(ns204)* animals is consistent with reduced levels of *dat-1* and *dop-3*, and is rescued when *dop-3* activity is completely eliminated. Third, *hlh-17(ns204)* animals are not defective in acetylcholine release, and in fact, show an increased sensitivity to aldicarb that is consistent with the increased acetylcholine release that occurs in animals with reduced *dop-3* activity.

The bHLH transcription factor family has well established roles in neurogenesis and the specification and maintenance of neuronal identity. In Drosophila, for example, the bHLH gene, lethal of scute, is required for cell-specific transcription of the dopaminergic H-cell neuron of the ventral nerve cord, and for specification of the non-midline dopaminergic neurons [170, 172] In zebrafish, olig2 regulates expression of the gene encoding Sim1, a bHLH-PAS protein that drives specification of the diencephalic dopaminergic neurons [173]. It is less clear, however, is whether HLH-17 plays a conserved role in the regulation of genes required for neurotransmitter signaling, in general, and dopamine signaling, in particular. The gene encoding the human dopamine reuptake transporter is regulated by the hairy/enhancer of split-like bHLH protein, HesR1. HesR1 represses activity of the human DAT1 gene in cell culture by binding to sequences in the 3' UTR [174]. HesR1 also affects dopamine receptor expression in mice, and hesr1 mutant mice show defects in dopamine dependent behaviors [175]. Although both are basic helix-loop helix proteins, HLH-17 shows no sequence similarity to HesR1, and is most similar to the human Olig-related proteins, bHLHb5/Beta3 and bHLHb4. Neither of these proteins has been shown to directly regulate expression of the dopamine transporter or dopamine

receptor genes. However, both proteins are part of the bHLH transcriptional network that drives retina development [176-178], and the dopamine receptors are critical for normal retinal function [142, 179-182]. Our own transgenic expression data shows strong expression of *hlh-17* in the cephalic sheath cells of wild-type animals, which does not support the direct regulation of *dat-1* and *dop-3* by HLH-17. However, mRNA for both *dop-3* and *hlh-17* was recently detected in glutamate receptor neurons of L2-stage animals and in the cephalic sheath cells of young adult animals [145]. Furthermore, mRNA for *dop-3*, *dat-1*, and *hlh-17* was detected in the dopamine neurons and pan-neuronal neurons of late embryos and L2-stage animals, respectively. Taken together with the epistasis analysis presented in this study, these data support the possibility that HLH-17 is a direct regulator of *dop-3* and *dat-1*. However, further studies are in progress to confirm that prediction. Additionally, future studies are aimed at determining if HLH-17 affects *dop-1* similarly to *dop-3* by conducting behavioral analyses with *hlh-17* (ns204); *dop-1* (vs100) and *hlh-17* (ns204); *dop-3* (vs106) animals.

Interestingly, the SWIP response in *hlh-17(ns204)* animals is enhanced by pre-treatment with bupropion, an anti-depressant and DAT inhibitor that is used to treat attention deficit hyperactivity disorder (ADHD) in adults and children [183, 184], but is unaffected by the antidepressant fluoxetine and the dopamine antagonist, reserpine. This finding underscores the need to develop animal models of dopamine signaling that accurately reflect the effects of reduced expression of multiple neurotransmitter signaling pathway genes, rather than complete loss of function of a single gene. Our future studies are aimed at exploiting *hlh-17(ns204)* for this purpose.

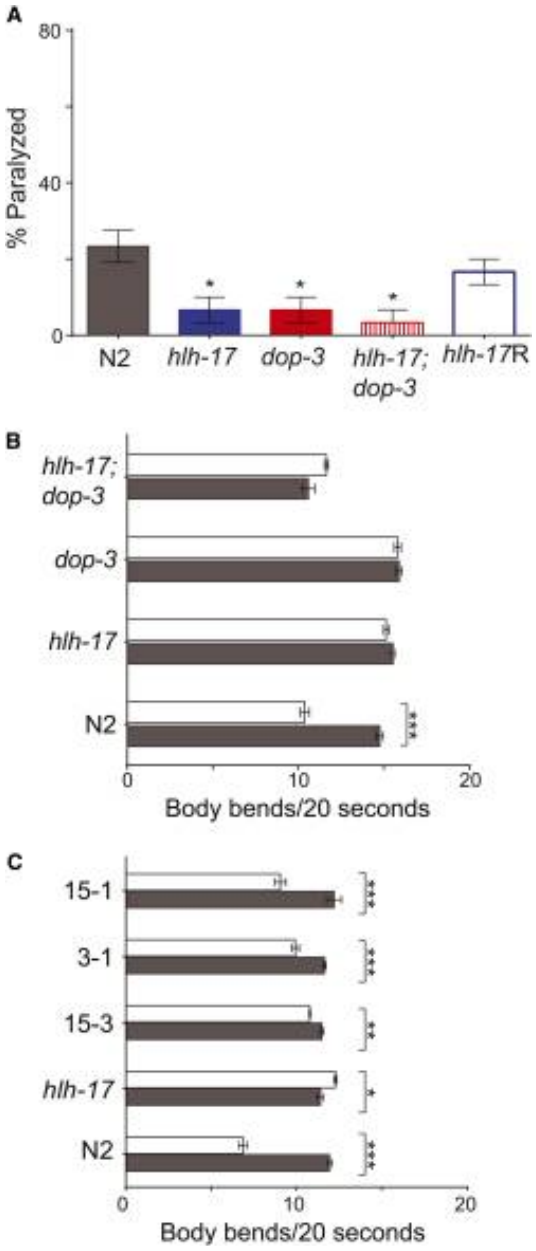

Figure 9 HLH-17 functions upstream of *dop-3* to regulate DA signaling
A. DA-induced paralysis: *hlh-17(ns204)*, *dop-1 (vs100); dop-3 (vs106)*, and *hlh-17(ns204)*; *dop-1 (vs100); dop-3 (vs106)* animals are less sensitive than wild-type (N2) animals to 10 mM DA. Transgenic expression of HLH-17:: GFP in *hlh-17(ns204)* animals rescues the DA induced paralysis phenotype. The bar for *hlh-17*R represents the average measurements from three biological replicas of three independent lines. * represents statistical significance when compared to wild-type, n=10 animals/strain/rep for three biological replicas. **B.** Basal slowing response: Well-fed wild-type animals (N2) , but not *hlh-17(ns204)*, *dop-1 (vs100); dop-3 (vs106)*, or *hlh-17(ns204)*; *dop-1 (vs100); dop-3 (vs106) animals,* move significantly slower in the presence of food (white bars) than in the absence of food (grey bars).

C. Transgenic expression of HLH-17:: GFP rescues the basal slowing response of *hlh-17(ns204)* animals. Three independent lines, 15.3, 15.1, and 3.1, were assayed. In (B) and (C), five animals/rep/strain for a total of three biological replicas were assayed. Each animal was analyzed for three separate 20 second intervals, so that the total number of observations was 15 observations/rep/strain. *, $P<0.05$; **, $P<0.005$; ***, $P<0.0005$.

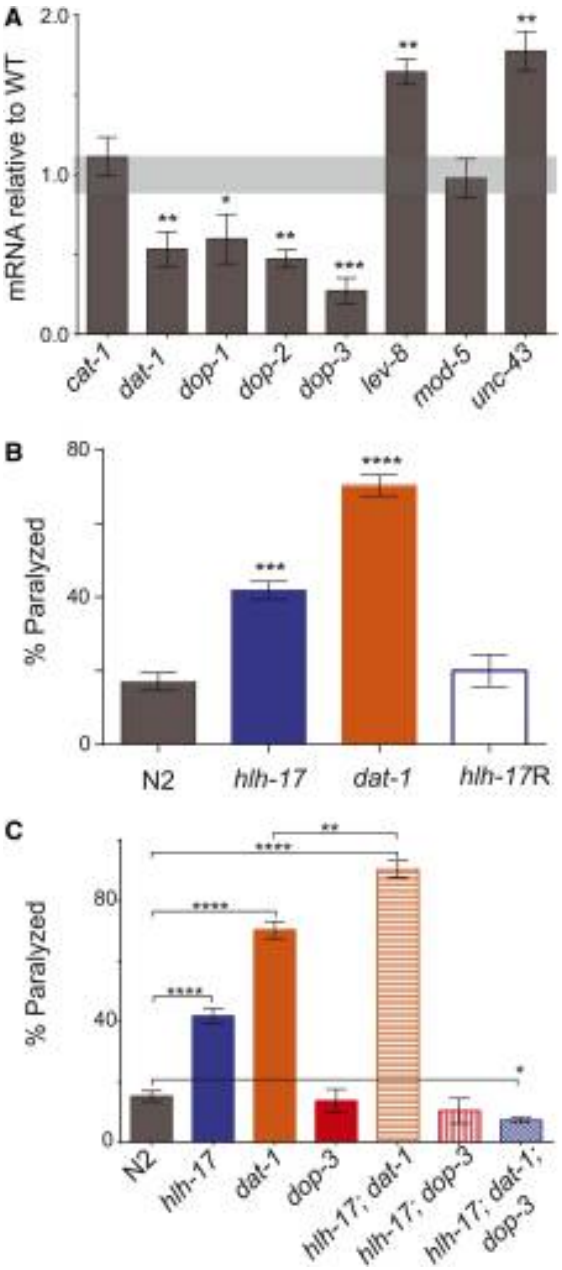

Figure 10 Loss-of *hlh-17* affects extracellular DA levels
A. mRNA levels in L4-stage *hlh-17(ns204)* animals when normalized against mRNA levels in age-matched wild-type (N2) animals. Light grey shading represents wild-type range of expression (1.0 +/-0.115). The levels of *cat-1 and mod-5* mRNA are not significantly affected in *hlh-17(ns204)* animals. **B.** *hlh-17(ns204)* animals demonstrate a SWIP behavior that is an intermediate of the behavior in N2 and *dat-1(ok157)* animals, and that is rescued by transgenic expression of HLH-17:: GFP. The bar for *hlh-17*R represents the average measurements from three biological replicas of three independent lines. For all strains except hlh-17R, n = 30 animals/rep/strain. For hlh-17R, n was equal to an average of at least 15 animals/line/biological rep (ranges was from 12 to 26) because of differences in transmission frequency of the transgene.

C. SWIP phenotype in double mutant *hlh-17(ns204)*; *dat-1(ok157)* and *hlh-17(ns204)*; *dop-1 (vs100); dop-3(vs106)* animals is more similar to the phenotype in *dat-1(ok157)* and *dop-1 (vs100); dop-3(vs106)* animals, respectively, than in wild-type animals. The SWIP phenotype of *hlh-17(ns204)*; *dat-1(ok157)*; *dop-1 (vs100); dop-3(vs106)* animals is not significantly different from *dop-1 (vs100); dop-3(vs106)* or *hlh-17(ns204)*; *dop-1 (vs100); dop-3(vs106)* animals. n = 30 animals/rep/strain for three biological replicas. *, $P<0.05$; **, $P<0.005$; ***, $P<0.0005$; ****, $P<0.0001$.

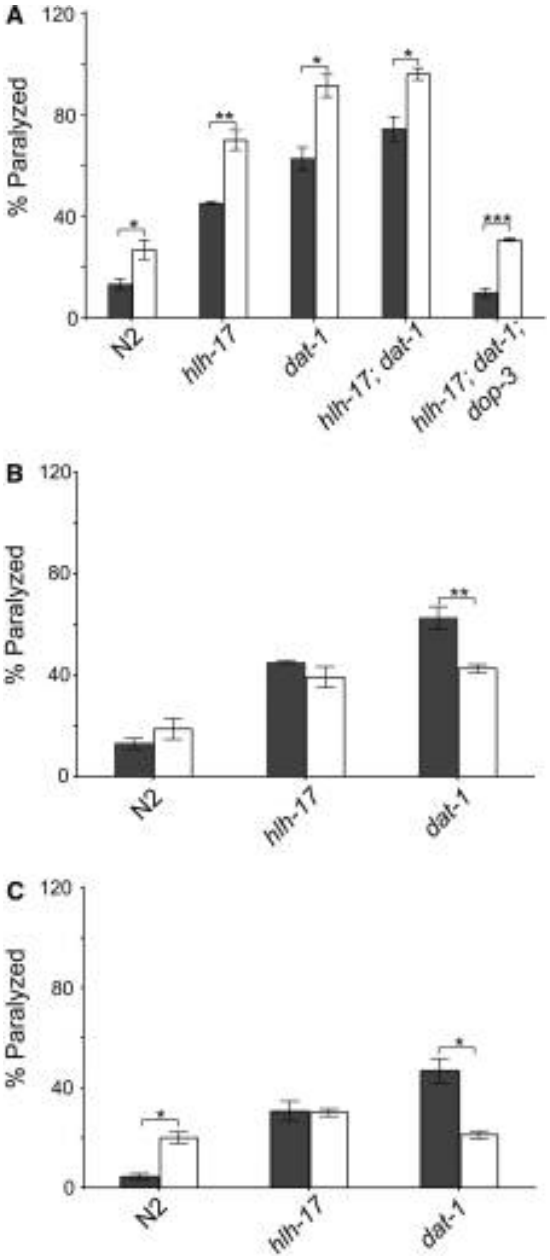

Figure 11 *hlh-17* animals respond selectively to reuptake inhibitors
A. Pretreatment with the DAT reuptake inhibitor, bupropion, increases the SWIP phenotype of N2, *hlh-17(ns204)*, *dat-1(ok157)* and *hlh-17(ns204)*; *dop-1 (vs100); dop-3(vs106)* animals. The ability of bupropion to enhance SWIP behavior is not dependent on DOP-3. Assay shows results using a final concentration of 100 mM of bupropion; results with 10 mM of bupropion showed the same trend. **B, C.** The SWIP phenotype in *hlh-17* (ns204) animals is unaffected by pretreatment with reserpine (B) or fluoxetine (C). In all panels, n = 30 animals/rep/strain, dark bars = minus inhibitor, light bars = plus inhibitor. *, P<0.05; **, P<0.005; ***, P<0.0005.

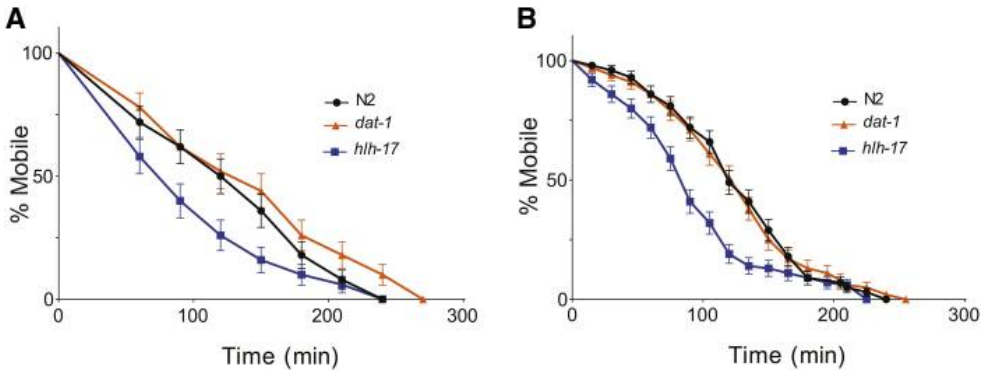

Figure 12 *hlh-17* animals do not have reduced acetylcholine signaling
A. *hlh-17(ns204)* animals are more susceptible to aldicarb induced paralysis than wild-type (p-value=0.0428) and *dat-1(ok157)* animals (p-value=0.1319). B. *hlh-17(ns204)* animals are more susceptible to levamisole induced paralysis than wild-type (p-value=0.0002) and *dat-1(ok157)* animals (p-value=0.0002). In all panels n = 30 animals/rep/strain.

4 ANALYSIS OF DIFFERENTIAL GENE EXPRESSION PROFILES FOR HLH-17 IN *C.ELEGANS*

4.1 Introduction

Living organisms continuously adapt to changing conditions in their environment in an effort to carry out normal cellular functions and to maintain system homeostasis. This is accomplished when cells interact with their environment, most often by reacting to chemicals found in their internal or external space [185]. The process by which the chemical signals are received and processed is termed signal transduction. Signal transduction occurs in a sequence of phases that allows the cells to transmit information that ultimately leads to specific responses, such as changes in enzymatic or ion-channel activity. The first phase in a signal transduction pathway is reception and consists of ligand-receptor binding [186]. Signaling molecules act as ligands that bind to receptors of their target cells that can either be positioned on the cell membrane or located intracellularly in the cytoplasm or nucleus [187]. There are many different types of signaling molecules that are involved in the transmission of information between cells including, but not limited to, neurotransmitters and peptide hormones. Neurotransmitters carry signals between neurons or from neurons to other targets such as muscle cells [188]. Hormones can act at the synapse or travel to more distant sites to reach their hormonally responsive target cells [188].

After reception, the next phase is transduction. In this phase the activated receptor triggers a cascade of gene-protein and protein-protein interactions within the cell that function to transmit the signal. In the final phase (response), the activation of specific target gene(s) allows the animal to respond to the stimulus [189]. A stimulus can lead to the activation of a large set of genes and can cause a number of different downstream events. These events are dependent upon

the specific set of genes that are activated and the order in which they are activated in response to the stimulus [189].

Transcriptional regulatory networks are the central effectors needed to generate a response to a stimulus and consist of a collection of regulatory proteins that associate with genes across the genome [190]. The process of gene expression controls how the information within the genome is interpreted and how it is subsequently used to produce the proteins required for a specific response. Transcriptional regulatory networks generally consist of proteins that (1) provide structural components of the cell, (2) help to catalyze reactions such as the breakdown of food sources and (3) control the expression of genes needed to regulate specific processes in the cell [191]. The central machinery in a regulatory network are the transcription factors that work to activate and repress gene expression in a spatial and temporal specific manner, allowing the cell to respond to its environment and perform complex biological functions.

Members of the bHLH (basic helix-loop-helix) transcription factor family act in a number of transcriptional regulatory networks required during embryonic development including networks that drive neurogenesis, cardiogenesis, myogenesis and hematopoiesis [192-195]. For example, the neurogenesis network that includes the proneural HLH gene *lin-32* as the central effector is required for the development of touch sensory and male sensory neurons in *C. elegans*. Inputs from genes and proteins that affect the production, migration and outgrowth of the touch sensory neurons [196, 197] and those that are required for male sex organ determination [198] and anterior-posterior patterning during male sensory neuron development [199] represent two network motifs within the *lin-32* network.

In our lab, we are interested in constructing the transcriptional regulatory network which includes a recently characterized member of the bHLH family, HLH-17*,* as the central effector.

HLH-17 is weakly expressed at the L4 larval stage in the four cephalic (CEP) dopaminergic neurons and is strongly expressed at all developmental stages in the cephalic sheath (CEPsh) glia which surround these neurons in the head of the animal [30, 87]. The loss-of *hlh-17* does not affect CEPsh generation nor differentiation; however, persistent expression in these cells suggests that HLH-17 may regulate CEPsh function post-developmentally [30]. Many of our initial studies were geared at addressing this possibility. We conducted behavioral analyses in *hlh-17* mutants and identified a role for HLH-17 during the modulation of stimuli regulated by the CEP dopaminergic neurons in the hermaphrodite *C. elegans* [130, 200].

Our most recent efforts to better understand the *hlh-17* transcriptional regulatory network have been aimed at identifying targets of HLH-17 regulation. We hypothesized that some of the signaling pathways that are required for the animals' ability to respond to stimuli may be altered in *hlh-17* (ns204) mutants. In support of this hypothesis, we show here that the targets of HLH-17 include genes whose products function in G-protein and peptide hormone signaling pathways. These factors mostly include membrane bound receptors, channels/transporters, enzymes and cell-adhesion molecules that allow G-protein and hormone signals to be transduced during the regulation of developmental and behavioral responses in *C. elegans*. Because we have previously shown that *hlh-17* (ns204) mutants have altered responses during DA signaling, a G-protein signaling pathway, in this study, we decided to analyze the role of HLH-17 in the IIS (Insulin/IGF-1 signaling) pathway, a peptide hormone signaling pathway. We show here that *hlh-17* (ns204) mutants have a reduced lifespan and defective responses to oxidative stress, which suggests that some outputs of insulin signaling are mediated by HLH-17. Together, our studies suggest that HLH-17 functions as a central effector in transcriptional regulatory networks

that modulate behavioral and developmental responses regulated specifically by the dopaminergic and IIS signaling pathways in *C. elegans*.

4.2 Materials and Methods

4.2.1 C. elegans growth and culture conditions

The following strains were used: N2 Bristol wild-type; OS2649, *hlh-17*(ns204); OS2929, *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223). OS2649 and OS2929 was a gift from Dr. S. Shaham. Unless otherwise noted strains were cultured on solid nematode growth media (NGM) containing Escherichia coli strain OP50 at 20°C using standard methods [139]. Synchronous cultures of N2, OS2649 and OS2929 were prepared by hypochlorite treatment of gravid adults. Embryos were allowed to develop and hatch on NGM plates lacking peptone. L1 stage animals were subsequently transferred to NGM plates seeded with OP50 and harvested at the L4 stage for use in behavioral assays. RNAi against *daf-16* was administered by feeding as previously described [201].

4.2.2 Gene Expression Analysis

Synchronized populations were collected at the L4 stage, pelleted and frozen at -80° C. Total RNA, cDNA synthesis, and real-time PCR was performed as previously described [130], except the cDNA was amplified from 1 µg of total RNA in 20 µL reactions. Real-time PCR assays were performed with SYBR Green and TaqMan Gene Expression Assays (Applied Biosystems/Invitrogen). The endogenous control genes were *pmp-3* and *cdc-42* for the SYBR green reactions and glyceraldehyde 3-phosphate dehydrogenase (*gpd-3*) (Ce02616909_gH) for the TaqMan reactions. The primer sequences for the SYBR Green probes and the ID numbers for

the TaqMan probes used in this study are listed in Tables 2 and 3, respectively. All gene expression assays were done in triplicate, for at least three biological replicates.

4.2.3 Microarray Analysis

A whole genome microarray using the strain OS2649 was conducted in the Genome Technology Access Center (GTAC) at the Washington University in St. Louis School of Medicine. Signal intensity data was extracted using Agilent Feature Extraction software (v11.5). Expression data was normalized using the quantile method. The ANOVA test was applied to identify differential probes between mRNA from OS2649 and N2 animals, and 2880 probes were significantly up-/down-regulated with a p-value of $p<0.01$ and a False Discovery Rate of $FDR<0.01$. Gene Ontology (GO) was performed using the Gene Set Enrichment Analysis (GSEA) tool provided by the Broad Institute at MIT [202].

A whole genome microarray using the OS2929 strain was performed in the DNA/Protein Core Facility at Georgia State University. The GeneChip *C. elegans* Genome Array (Affymetrix) was used to compare global gene expression between mRNA from OS2929 and N2 animals. Data analysis was performed using the GeneSpring GX 11 software (Agilent). Probe intensity values were normalized using Robust Multichip Average (RMA)-algorithm. Statistical analysis was conducted using the Student's T-test ($p<0.05$) and genes with significant expression were further filtered by fold change. A total of 4,518 probes were significantly up-/down-regulated. Gene Ontology (GO) analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID), version 6.7 [203, 204].

4.2.4 Behavioral Assays

Lifespan assay. One hundred L4 stage animals/strain were selected and added to NGM plates containing 0.5mg/ml of 5-fluoro-2'-deoxyuridine (FUdR) to inhibit egg-laying and eliminate the need to separate offspring from the reproductive adult [205]. Animals were scored as live, dead or missing and transferred to new plates every other day. Animals were scored as dead if they failed to move in response to prodding with a platinum wire. All lifespan tests were repeated three times. Statistical analyses and survival curved were conducted using GraphPad Prism 6.

Oxidative stress assay. Thirty animals/strain/rep were added to an NGM plate containing the appropriate drug and kept at 20 °C until survival was calculated either every hour or every day for juglone (60 µg/ml) and paraquat (3.7 µg/ml), respectively [206, 207]. Plates were prepared under a ventilated hood and each chemical was added separately to 100 µl of 100% ethanol. After the NGM was cooled, the liquid culture containing the appropriate drug was added and mixed gently just before pouring. Animals cultured on plates containing paraquat were transferred to freshly poured plates every two days. Each assay was conducted for three biological replicates. Survival comparisons were conducted as a compilation of all three replicates using GraphPad Prism 6.

4.3 Results and Discussion

4.3.1 Identifying HLH-17 transcriptional targets using two hlh-17 mutant strains

The goal of this work was to better understand the HLH-17 transcriptional regulatory network in the *C. elegans* hermaphrodite. Our approach was to identify targets of HLH-17

transcriptional regulation using gene expression microarray analysis. Previous functional studies conducted in our laboratory show that *hlh-17* (ns204) mutants are defective in larval development, reproduction and in behaviors that require proper synaptic signaling [87, 130, 200]. We hypothesized that genes targeted by HLH-17 would be connected with the phenotypic defects displayed by the *hlh-17*(ns204) mutants. In *C. elegans*, HLH-17 shares sequence similarity to the uncharacterized HLH factors, HLH-31 and HLH-32. These three factors may therefore regulate some or all of the same transcriptional targets. To account for the potential functional similarities between these genes, we conducted two independent microarray experiments, separately comparing gene expression profiles of *hlh-17* (ns204) mutants or *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) mutants to wild-type (N2) animals.

We found that the expression of 1553 genes was affected by the loss of HLH-17 alone, of which 906 were up-regulated and 646 were down-regulated (Fig. 13, Appendix Table A.1). We assigned genes to functional groups using a gene ontology (GO) analysis, with the Gene Set Enrichment Analysis (GSEA) tool provided by the Broad Institute at MIT [202, 208]. There were a total of 68 clusters and many of the genes were clustered under 'Integral to Membrane' and 'Growth' (Fig. 14, Table 4). We also found that the expression of 2274 genes, of which 971 were up-regulated and 1302 were down-regulated by the loss of HLH-17, HLH-31 and HLH-32 (Fig. 15, Appendix Table A.2). We used the Database for Annotation, Visualization and Integrated Discovery (DAVID), version 6.7, to cluster related genes based on enriched Gene Ontology (GO) terms. There were a total of 59 clusters with many of the genes clustered under 'Embryonic development ending in birth or hatching' and 'Nematode larval development' (Fig. 16, Table 5).

We used RT-qPCR to measure changes in mRNA levels of 32 genes that were identified in the *hlh-17* (ns204) and the *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) arrays (Table 6).

Because we initially expected HLH-31 and HLH-32 to function redundantly to HLH-17, we initiated this study using the *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) array and validated seven well-characterized putative targets from that analysis. We also validated ten additional genes using mRNA from both *hlh-17* (ns204) and *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) mutants. However, because this analysis and the behavior analyses described in previous chapters suggest that HLH-31/HLH-32 function antagonistically to HLH-17, we repeated the microarray analysis using *hlh-17* (ns204) mutants and validated four additional genes that were differentially expressed in the *hlh-17*(ns204) array (indicated in bold in Table 6). We also conducted RT-qPCR for seven genes that were related to the previously studied behaviors in *hlh-17* (ns204) animals. Finally, we selected four genes that were uncharacterized to determine if HLH-17 regulated some genes whose functions are currently unknown. During our validation of the microarray results, we found that 84% of the 32 genes were significantly affected either in *hlh-17* (ns204) mutants only, in *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) mutants only or in both strains (Table 6). Additionally, the direction of regulation (up-regulated versus down-regulated) for 76% of the validated genes correlated with the results from the respective array. For example, the mRNA levels of *ama-1* were found to be down-regulated by at least two-fold in both the *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) microarray and in the RT-qPCR analysis. For the remaining 24% of the validated genes, the RT-qPCR results compared to the microarray results either showed an opposite change or no change in expression, as in the case of *cat-1* which was down-regulated by two fold in the *hlh-17* microarray but unaffected in the RT-qPCR analysis.

4.3.2 HLH-17 targets are membrane bound proteins with roles during signal transduction

Genes clustered under 'Integral to membrane' account for the largest group (489) of genes whose expression was significantly affected in the *hlh-17*(ns204) microarray (Figure 14). Proteins positioned within the membrane are important because they facilitate functions that are vital to the survival of an organism. The process of signal transduction is an example of a molecular event that requires membrane bound proteins; therefore, it was not surprising that many of the genes clustered under 'Integral to membrane' exhibit signal transduction activity. For example, in addition to *dop-3*, the gene encoding the D2-like dopamine receptor, the HLH-17 network includes *gbb-2* which encodes a subunit of the GABA B receptor [209]. As indicated in Appendix Table A.1, some of the proteins that cluster under 'Signal transduction', and also cluster under 'Integral to membrane' account for another 153 of the genes affected by the loss-of HLH-17. This result, together with the previously identified role for HLH-17 in the nervous system, led us to focus our analysis primarily on putative HLH-17 targets that are membrane bound: (1) receptors, (2) transporters and channels, (3) enzymes and (4) cell adhesion molecules with roles in signal transduction.

Most of the genes clustered under 'Signal transduction' function in G-protein, steroid, or peptide hormone signaling pathways, though genes involved in Wnt, RTKRas/MAP kinase and TGF-β signaling were also included. In this section of this chapter, we will focus on genes involved in G-protein and steroid signaling; the succeeding section will emphasize genes involved in peptide hormone signaling, particularly involved in insulin/IGF1-like signaling.

Genes involved in the HLH-17 transcriptional network underscore the influence of HLH-17 on animal behavior through its action within signal transduction pathways. Firstly, receptors

play a crucial role in the initiation of signal transduction cascades. For example, binding of the neurotransmitter GABA (gamma-aminobutyric acid) to the G-protein coupled neuropeptide receptor NPR-1 initiates transcriptional changes in the neuromodulatory network needed to regulate social feeding behavior in *C. elegans* [210, 211]. Our *hlh-17*(ns204) microarray results identified 108 genes with receptor activity including the uncharacterized neuropeptide genes *npr-12*, *npr-16* and *npr-31*. Of those 108 genes, 43 encode G-protein coupled receptors. We previously reported that the genes *dop-3*, *ser-7* and *gar-1*, functioning during dopamine [88] serotonin [212] and acetylcholine [213] signaling, respectively, are down-regulated in *hlh-17*(ns204) mutants [200]. Additionally, within this subgroup of 43 genes, 23 were members of the Serpentine GPCR family. Although mostly uncharacterized, members of this family are widely expressed in chemosensory neurons and have a probable chemosensory function [214]. Gustatory plasticity is regulated, in part, by at least four pairs of chemosensory neurons in *C. elegans* [92]. Interestingly, we previously demonstrated that the loss-of HLH-17 compromises gustatory plasticity, a phenotype that is also seen in animals with reduced dopamine signaling [91, 130]. Possibly this phenotype is also a result of altered expression of the serpentine GCPR proteins in the HLH-17 network.

Secondly, the 'Signal transduction' cluster included 28 genes involved in steroid hormone receptor signaling. Small lipophilic molecules, which include steroids, are able to diffuse across the plasma membrane and bind to hormone receptors located in the cytosol or nucleus. In *C. elegans*, proteins in this family are called nuclear hormone receptors (NHRs) [215]. Only 20 of the 284 NHR genes present in the *C. elegans* genome have been genetically characterized, to date; mutations in those genes affect developmental timing, diapause, sex determination, neural development, axon outgrowth and neuronal identity [216, 217]. Therefore,

it is not surprising that the 28 NHR genes affected by the loss-of HLH-17 are not characterized. Nevertheless, these data suggest that HLH-17 is a central effector in transcriptional regulatory networks in which NHRs play unidentified roles during developmental and behavioral events.

Thirdly, following ligand-receptor binding, transporters and channels function to transduce signals by moving specific molecules across the plasma membrane. For example, sodium and potassium voltage gated ion channels in nerve cells passively transport ions across the membrane after ion binding triggers the channels to open, a vital action during signaling within these cells [218, 219]. *kcc-2* and *klp-11* are two genes in the HLH-17 network that fit under this category. *kcc-2* encodes a channel that is required during GABA signaling to generate the cellular chloride gradient needed for synaptic transmission [220]. Likewise, *klp-11* encodes an essential kinesin motor protein that is required to stabilize protein transport through the ciliary membranes of neurons [221, 222].

Fourthly, membrane bound enzymes are important during signal transduction because they metabolize membrane components like lipids, proteins and cell wall oligosaccharides responsible for mediating the effects of specific cellular responses. This includes enzymes located within the subsynaptic membrane that control neurotransmitter levels by inactivating them. Sixty-four putative HLH-17 targets are membrane bound enzymes, most of which belong to the metallopeptidase family (23). Although most targets in this collection are uncharacterized, we surmise that HLH-17 influences signal transduction events that require enzymatic activity at the cell surface. In support of this, one well characterized metalloprotease that is also affected by the loss-of HLH-17 is the ADMATS protease, ADT-1. Loss-of ADT-1 compromises the regulation of extracellular matrix components possibly by inhibiting the binding of necessary

substrates needed during remodeling. Animals without functional ADT-1 develop a twisted pharynx as a result of the deficit [223].

Fifthly and lastly, genes that encode cell adhesion molecules were also affected by the loss-of HLH-17. These molecules regulate embryonic development and morphogenesis by transducing signals from extracellular matrix components like collagen and fibronectin. HLH-17 transcriptional targets linked to cell adhesion include *deb-1*, *spon-1*, *unc-44, vha-12* and *Y43F4A.1*. The gene *vha-12* encodes an ortholog of subunit B of the cytoplasmic (V1) domain of vacuolar proton-translocating ATPase (V-ATPase) and is required maternally for early embryonic development [224]. Animals lacking *vha-12* are uncoordinated due to decreased neurotransmission and have a dumpy phenotype often associated with defects in cuticle formation [225]. That *vha-12* and other cell adhesion genes are part of the HLH-17 transcriptional network underscores a probable role for HLH-17 during tissue remodeling and organogenesis, of which a number of HLH factors already have well characterized roles [226, 227].

The loss-of HLH-17 affects the expression of membrane bound proteins involved in signal transduction events that regulate development and behavior. These microarray data, together with our behavioral studies, provide molecular and genetic support for our hypothesis that HLH-17 is required for *C. elegans* to respond to changes in the environment. Furthermore, these data highlight the potential impact that HLH-17 may have on multiple signal transduction pathways including the recently described role in dopamine signaling.

4.3.3 HLH-17 targets play significant roles in the Insulin/IGF-1 Signaling (IIS) Pathway

Previous studies in our lab suggested that HLH-17 may influence the response to the insulin/IGF-1-like signaling (IIS) pathway [87]. In support of this possibility, genes related to insulin signaling were identified in our GO analysis and were clustered under 'Determination of adult lifespan', 'Metabolic processes', 'Signal transduction', 'Reproduction' and 'Positive regulation of growth'. These targets included, but were not limited to, nine insulin-like peptides (ILPs): *ins-7*, *ins-11*, *ins-12*, *ins-24*, *ins-25*, *ins-31*, *ins-36*, *ins-38* and *ins-39*. This group of targets also included four other neuropeptide-like genes, *nlp-17*, *nlp-30*, *flp-10* and *flp-20*, that, like the ILPs, act as neuromodulators to regulate locomotion, social behavior, dauer formation and egg-laying [228-230].

In *C. elegans*, IIS is mediated by the effects of insulin and ILPs that act through the DAF-2 receptor. DAF-2 is responsible for activating a conserved PI3K (Phosphoinositide 3-kinase) pathway required for the regulation of the FOXO transcription factor, DAF-16 [231, 232] (Figure 17). DAF-16 activity mediates most outputs of the IIS pathway, regulating genes that play key roles in developmental timing, metabolism, reproduction, stress responses and lifespan [233-236]. However, increased stress resistance and lifespan resulting from reduced IIS are also regulated by the Nrf family transcription factor SKN-1 [237]. SKN-1 also functions downstream of DAF-2 but in parallel to DAF-16 in the p38 MAPK pathway [238, 239], targeting genes involved in Phase II detoxification [240] (Figure 17).

To determine if HLH-17 functions in the insulin signaling pathway, we measured the mRNA levels of IIS-related genes in *hlh-17* (ns204) mutants. We first found that *daf-2* and *skn-1*

expression were up-regulated and that *daf-16* expression was unaffected by the loss-of HLH-17 (Fig. 18). We then measured *skn-1* mRNA and the mRNAs which encode the proteins responsible for the state (normal/unstressed vs stressed) dependent regulation of SKN-1, including *wdr-23*, *cul-4*, *gsk-3*, *pmk-1* and *sek-1* in stressed and unstressed animals (Fig. 19). In normal animals, GSK-3 inhibits SKN-1 activity in the cytoplasm [237, 241] while nuclear SKN-1 is recruited by WDR-23 to the CUL-4 ubiquitin ligase complex and is subsequently degraded [242]. In stressed animals PMK-1 and SEK-1 phosphorylate cytoplasmic SKN-1 so that it bypasses WDR-23 mediated degradation in the nucleus and activates genes involved in detoxification [238]. As indicated in Figure 19, *wdr-23* and *cul-4* mRNA levels were up-regulated in both unstressed and stressed *hlh-17(ns204)* mutants. This suggests that HLH-17 regulates genes whose products negatively regulate SKN-1 activity in normal cells. Currently we do not know if this increase in *wdr-23* and *cul-4* transcripts are indicative of increased WDR-23 and CUL-4 protein levels. Future studies will determine if the increased *wdr-23* and *cul-4* mRNAs lead to increased protein and ultimately to increased SKN-1 degradation in *hlh-17* (ns204) animals.

We next measured two IIS signaling outputs, lifespan and oxidative stress response, to validate the role of HLH-17 in IIS. We first analyzed lifespan. Reduced IIS in *daf-2* mutants increases lifespan [243]; therefore, we hypothesized that increased *daf-2* expression would decrease lifespan in *hlh-17* (ns204) mutants. As expected, we found that the mean lifespan of *hlh-17* (ns204) mutants was shorter (p-value 0.0076) than that of wild-type animals (Figure 20). This suggested that HLH-17 is required for lifespan in *C. elegans*. Previous studies suggest that neurons are the central lifespan-determining tissue; neuronally, not intestinally, expressed DAF-2 is responsible for the decreased lifespan in *daf-2* mutants [244]. To date HLH-17 expression has

only been identified within the nervous system. Therefore, HLH-17 may be critical regulator of lifespan in these tissues. Although it remains to be determined whether HLH-17 influences lifespan from within the glia or within the neurons.

Next, to determine if HLH-17 functions in the same pathway as DAF-16 or in a parallel pathway to DAF-16 we measured the lifespan of *hlh-17* (ns204) mutants subjected to *daf-16* RNAi (Figure 20). We found that the lifespan of N2/*daf-16* RNAi animals was not significantly different from *hlh-17* (ns204)/*daf-16* RNAi animals (p-value 0.1495). This data suggests that *daf-16* is in fact epistatic to HLH-17 in the IIS pathway required for lifespan.

Lastly, we analyzed the oxidative stress response. Because reduced IIS in *daf-2* animals promotes oxidative stress resistance [245], we hypothesized that the increased levels of *daf-2* would cause *hlh-17* (ns204) animals to be less resistant to oxidative stress. To test this we treated young adult *hlh-17(ns204)* mutants and wild-type animals with juglone and paraquat, and calculated their survival rates in hours and days, respectively (Fig. 21). Contrary to our hypothesis, mean survival after exposure to both oxidants was significantly increased in *hlh-17* (ns204) animals (p-value <0.0001, <0.0001) indicating that like *daf-2* animals, *hlh-17 (ns204)* animals are more resistant to oxidative stress than wild-type. This suggests that IIS is reduced in *hlh-17* (ns204) animals despite the increase in *daf-2* expression. Reduced IIS generally causes increases in both lifespan and oxidative stress resistance [246, 247]. In *hlh-17* (ns204) animals these phenotypes are uncoupled, suggesting that HLH-17's role in the IIS pathway is complicated. Future studies are aimed at determining how HLH-17 may affect both of these IIS outputs in a manner that would allow this uncoupling of phenotypes. Alternatively, other genes with well characterized roles in stress responses are also up-regulated in *hlh-17* (ns204) animals. Interestingly, *bli-3* is required for cuticle integrity and increased levels of this protein may

further account for reduced sensitivity to juglone and paraquat [248]. Thus HLH-17 probably normally functions to regulate a number of genes that affect the animals' response to the environment and the decrease in sensitivity to juglone and paraquat seen in *hlh-17* (ns204) animals is the result of a number of factors.

The increased stress resistance seen in *hlh-17* (ns204) animals suggests an increase in SKN-1 activity. Our studies therefore do not fully explain the biological relevance of HLH-17 dependent regulation of *wdr-23* and *cul-4,* two genes which encode proteins responsible for the negative regulation of SKN-1. However, a recent study has shown that increases in SKN-1 activation result in defects in neuromuscular function; WDR-23 and CUL-4 can influence synaptic function by negatively regulating SKN-1 in the intestine [249], suggesting that stress detected by cells in the intestine can affect function of distant neuronal tissues. HLH-17 may be required in the nervous system for this detection. Likewise, the ability of HLH-17 to regulate the ILPs and other neuropeptides, as well as the NHRs that mediate endocrine control in *C. elegans*, (see above) support the requirement for HLH-17 in endocrine control in *C. elegans*. Together these data allude to the possible crosstalk between the nervous and endocrine systems and suggest that HLH-17 may play a major role in this interaction. Identifying functionally relevant targets that mediate the effects seen in *hlh-17* mutants will be important to our understanding of how HLH-17 may affect behaviors regulated by the intestine from the CEPsh.

Together, our data suggest that HLH-17 functions upstream of the DAF-2 transcriptional network to regulate IIS outputs. In one motif of this network, HLH-17 functions upstream of DAF-16 to regulate lifespan. Currently, we don't know if HLH-17 affects the oxidative stress response through DAF-16, SKN-1 or other stress response factors. However, our data do suggest that HLH-17 functions from the nervous system to regulate these processes. Our current studies

are preliminary; there are still many unknown mechanisms and regulatory factors that may affect the behavioral defects that we see in *hlh-17* (ns204) animals. It is also important to keep in mind that neuropeptide genes like ILPs in general are difficult to characterize because they have overlapping functions and many bind to some of the same receptors. Although many ILPs signal through the DAF-2 receptor, others signal through non-DAF-2 receptors. Thus, validating and characterizing the HLH-17 transcriptional regulatory network that involves ILPs will require the examination of subtle defects in *hlh-17* (ns204) animals, the generation of animals with multiple deletions and the elimination of receptors to which these neuropeptides bind.

4.3.4 HLH-17, HLH-31 and HLH-32 share some similar targets

To construct the transcriptional network in which HLH-17 is the central effector, it was important for us to make a distinction between the genes that are regulated by HLH-17 only and those that are synergistically regulated by HLH-17, HLH-31 and HLH-32. To address this goal, we compared the two gene sets represented by the *hlh-17* (ns204) and the *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) microarray analyses and compiled a list of genes that were significantly affected in both. As indicated in Figure 22, a total of 1553 genes that were significantly affected by the loss-of HLH-17, and 2274 genes affected by the loss HLH-17; HLH-31; HLH-32. A total of 173 genes were found to be present in both microarrays. Using DAVID, we found that these 173 genes clustered under categories that were almost identical to the top 10 GO terms for the *hlh-17* (ns204) microarray (Table 7).

To identify targets that may be regulated either redundantly or antagonistically by HLH-17 and HLH-31/HLH-32 we compared the direction (up-regulated versus down-regulated) of transcriptional regulation in *hlh-17* (ns204) mutants to the direction in *hlh-17*(ns204); *hlh-*

31(ns217); *hlh-32*(ns223) mutants (Table 8). Most genes (67%) showed opposite changes in expression, suggesting an antagonistic relationship between HLH-17 and HLH-31/HLH-32. For example, *apl-1* was up-regulated in the *hlh-17* (ns204) microarray but down-regulated in the *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) microarray. Similarly, *clec-107* is down-regulated by ~ 32 fold in *hlh-17* (ns204) animals but only down-regulated by ~ 7 fold in *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) animals. These findings correlate with our previous studies that show that these factors regulate DA-dependent behaviors in an antagonistic manner [130]. Likewise, our RT-qPCR results also provides support for this functional relationship. In most cases a gene up-regulated by the loss-of HLH-17 was down-regulated by the loss-of expression of all three ; *sdc-2* was up-regulated by ~2 fold in *hlh-17* (ns204) animals but down-regulated by ~2 fold in *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) animals. The remaining 33% of the genes showed the same direction of transcriptional change and very little difference in fold change, suggesting that neither HLH-31 nor HLH-32 affected the expression of those targets. For instance, *kqt-2* was up-regulated by ~2 fold in *hlh-17* (ns204) animals and by ~3 fold in *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) animals. Currently we are most interested in the genes that are uniquely targeted by HLH-17.

4.4 Summary and Future Implications

The data obtained from our microarray analyses of *hlh-17* mutants suggest that HLH-17 functions to regulate behaviors mediated by G-protein and IIS pathways in the hermaphrodite *C. elegans*. We've previously shown that HLH-17 works upstream of DOP-1, DOP-3 and DAT-1 during dopamine signaling. We show here that HLH-17 affects behaviors regulated by the IIS pathway and have roles that are distinct from HLH-31 and HLH-32. Our future studies will be geared to better understanding the mechanisms and behaviors regulated by the HLH-17

transcriptional regulatory network. Likewise, our future studies will incorporate the *hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) animals to help better determine how HLH-31 and HLH-32 may aid HLH-17 during the regulation of some of these behaviors.

Table 2 SYBR green primer sequences

WormBase ID	Gene	Primer Sequence
WBGene00000123	*ama-1*	5'-TTCCAAGCGCCGCTGCGCCATTGTC-3' (forward)
		5'-CAGAATTTCCAGCACTCGAGGAGCG-3' (reverse)
WBGene00000390	*cdc-42*	5'-CTGCTGGACAGGAAGATTACG-3' (forward)
		5'-CTCGGACATTCTCGAATGAAG-3' (reverse)
WBGene00000478	*cfz-2*	5'-GTTATGGACATGAAAAACAGGAAGAA-3' (forward)
		5'-CTCCACCAGCGGATAAAATTG-3' (reverse)
WBGene00001031	*dnj-13*	5'-GAATAAGGAAGCTGGAGCTGAGAA-3' (forward)
		5'-TCATCGGAAAGAACATCGTAAGC-3' (reverse)
WBGene00003911	*pak-1*	5'-CCAGCACCACCAATTCGTTT-3' (forward)
		5'-GCCGGTTTTTGGGTCATCT-3' (reverse)
WBGene00004060	*pmp-3*	5'-GTTCCCGTGTTCATCACTCAT-3' (forward)
		5'-ACACCGTCGAGAAGCTGTAGA-3' (reverse)
WBGene00004746	*sdc-2*	5'-CTCGACCGTGATCGTAAAAGG-3' (forward)
		5'-CAGTCAAACATGGATGCAAAATC-3' (reverse)
WBGene00016925	*srb-18*	5'-GTTCAGTCGTTCCAACTATTTTTCAA-3' (forward)
		5'-AAGAATCCTGATAACATGTCCCATTT-3' (reverse)
WBGene00006003	*srx-112*	5'-TTCCACCCGAATTGTTGGA-3' (forward)
		5'-TTATCAGAAACCCGACGAATGA-3' (reverse)
WBGene00006816	*unc-84*	5'-GCGACTTACCATACGCAACCA-3' (forward)
		5'GGCAAGGATGGATCGTAGATTT-3' (reverse)
WBGene00012882	*Y45F10D.1*	5'-GTCGCTTCAAATCAGTTCAGC-3' (forward)
		5'-GTTCTTGTCAAGTGATCCGACA-3' (reverse)

Table 3 Taqman probe ID numbers

WormBase ID	Gene	Probe ID
WBGene00000102	*akt-1*	Ce02421048_m1
WBGene00000295	*cat-1*	Ce02495610_m1
WBGene00000296	*cat-2*	Ce02426732_m1
WBGene00000839	*cul-4*	Ce0243482_g1
WBGene00000912	*daf-16*	Ce02422843_m1
WBGene00000898	*daf-2*	Ce02444348_m1
WBGene00000934	*dat-1*	Ce02450896_g1
WBGene00001052	*dop-1*	Ce02494345_g1
WBGene00001053	*dop-2*	Ce02479829_m1
WBGene00020506	*dop-3*	Ce02496462_m1
WBGene00009266	*F30A10.9*	Ce02412279_g1
WBGene00001397	*fat-5*	Ce02488494_m1
WBGene00001648	*goa-1*	Ce02489649_m1
WBGene00001685	*gpd-3*	Ce02616409_gH
WBGene00001527	*gcs-1*	Ce02436725_g1
WBGene00001752	*gst-4*	Ce02458728_g1
WBGene00002206	*kin-24*	Ce02458320_g1
WBGene00003387	*mod-5*	Ce02415245_m1
WBGene00004055	*pmk-1*	Ce02456381_g1
WBGene00004060	*pmp-3*	Ce02485188_m1
WBGene00004758	*sek-1*	Ce02491657_m1
WBGene00004804	*skn-1*	Ce02407446_m1
WBGene00004932	*sod-3*	Ce02404517_g1
WBGene00006539	*tbb-6*	Ce02482610_si
WBGene00006876	*vab-10*	Ce02423625_m1
WBGene00008419	*wdr-23*	Ce02424745_m1
WBGene00022201	*Y71H10B.1*	Ce02492998_m1
WBGene00013593	*Y87G2A.1*	Ce02425354_m1

Table 4 Gene Ontology (GO) terms for the *hlh-17* (ns204) microarray analysis

Gene Ontology Terms	Count of Gene Symbol
GO:0016021(integral to membrane)	489
GO:0040007(growth)	153
GO:0005634(nucleus)	143
GO:0030246(carbohydrate binding)	134
GO:0009792(embryo development ending in birth or egg hatching)	123
GO:0008270(zinc ion binding)	113
GO:0007165(signal transduction)	153
GO:0040011(locomotion)	91
GO:0002119(nematode larval development)	74
GO:0005524(ATP binding)	71
GO:0010171(body morphogenesis)	67
GO:0040035(hermaphrodite genitalia development)	60
GO:0005515(protein binding)	53
GO:0019915(lipid storage)	53
GO:0055114(oxidation-reduction process)	52
GO:0008340(determination of adult lifespan)	51
GO:0019915(lipid storage)	42
GO:0003700(sequence-specific DNA binding transcription factor activity)	40
GO:0043565(sequence-specific DNA binding)	40
GO:0005622(intracellular)	33
GO:0005737(cytoplasm)	33
GO:0040035(hermaphrodite genitalia development)	33
GO:0055085(transmembrane transport)	31
GO:0010171(body morphogenesis)	29
GO:0007186(G-protein coupled receptor signaling pathway)	28
GO:0003824(catalytic activity)	27
GO:0018991(oviposition)	27
GO:0055114(oxidation-reduction process)	27
GO:0006898(receptor-mediated endocytosis)	26
GO:0006468(protein phosphorylation)	25
GO:0016491(oxidoreductase activity)	25
GO:0006810(transport)	24
GO:0003707(steroid hormone receptor activity)	23
GO:0004222(metalloendopeptidase activity)	23
GO:0004672(protein kinase activity)	23
GO:0005576(extracellular region)	23
GO:0043401(steroid hormone mediated signaling pathway)	23
GO:0040018(positive regulation of multicellular organism growth)	22
GO:0002009(morphogenesis of an epithelium)	20

GO:0004713(protein tyrosine kinase activity)	19
GO:0008237(metallopeptidase activity)	19
GO:0016787(hydrolase activity)	19
GO:0005975(carbohydrate metabolic process)	18
GO:0006915(apoptotic process)	18
GO:0018996(molting cycle, collagen and cuticulin-based cuticle)	18
GO:0003677(DNA binding)	16
GO:0005886(plasma membrane)	16
GO:0007165(signal transduction)	16
GO:0040017(positive regulation of locomotion)	15
GO:0005215(transporter activity)	14
GO:0004674(protein serine/threonine kinase activity)	13
GO:0005509(calcium ion binding)	13
GO:0007126(meiosis)	13
GO:0003676(nucleic acid binding)	12
GO:0005506(iron ion binding)	12
GO:0005525(GTP binding)	12
GO:0042302(structural constituent of cuticle)	12
GO:0003924(GTPase activity)	11
GO:0016758(transferase activity, transferring hexosyl groups)	11
GO:0030259(lipid glycosylation)	11
GO:0003674(molecular_function)	10
GO:0006184(GTP catabolic process)	10
GO:0006811(ion transport)	10
GO:0007264(small GTPase mediated signal transduction)	10
GO:0009055(electron carrier activity)	10
GO:0020037(heme binding)	10
GO:0040039(inductive cell migration)	10
GO:0043025(neuronal cell body)	10

Table 5 Gene Ontology (GO) terms for the *hlh-17; hlh-31; hlh-32* microarray analysis

Gene Ontology (GO) Terms	Count of Gene Symbol
GO:0009792 embryonic development ending in birth	617
GO:0002119 nematode larval development	457
GO:0045927 positive regulation of growth	392
GO:0043169~cation binding	364
GO:0040007 growth	348
GO:0000166 nucleotide binding	259
GO:0003006~reproductive developmental process	256
GO:0006355~regulation of transcription, DNA-dependent	137
GO:0043232~intracellular non-membrane-bounded organelle	118
GO:0007610~behavior	118
GO:0019098~reproductive behavior	116
GO:0018991~oviposition	113
GO:0042303~molting cycle	112
GO:0007049~cell cycle	110
GO:0006508~proteolysis	104
GO:0008104~protein localization	97
GO:0040012~regulation of locomotion	90
GO:0003723~RNA binding	90
GO:0008340~determination of adult life span	90
GO:0015031~protein transport	89
GO:0005856~cytoskeleton	75
GO:0007242~intracellular signaling cascade	73
GO:0005525~GTP binding	62
GO:0051276~chromosome organization	58
GO:0007369~gastrulation	41
GO:0005794~Golgi apparatus	40
GO:0016477~cell migration	35
GO:0006457~protein folding	35
GO:0007264~small GTPase mediated signal transduction	35
GO:0019842~vitamin binding	33
GO:0008219~cell death	31
GO:0040024~dauer larval development	30
GO:0006511~ubiquitin-dependent protein catabolic process	29
GO:0007155~cell adhesion	28
GO:0046903~secretion	28
GO:0006119~oxidative phosphorylation	27
GO:0010629~negative regulation of gene expression	26
GO:0009566~fertilization	25
GO:0030182~neuron differentiation	24
GO:0000785~chromatin	23

GO:0046983~protein dimerization activity	21
GO:0005874~microtubule	19
GO:0045333~cellular respiration	18
GO:0031982~vesicle	17
GO:0019899~enzyme binding	17
GO:0065004~protein-DNA complex assembly	17
GO:0006323~DNA packaging	17
GO:0030117~membrane coat	16
GO:0007588~excretion	16
GO:0007409~axonogenesis	16
GO:0031072~heat shock protein binding	15
GO:0030421~defecation	15
GO:0006096~glycolysis	13
GO:0007498~mesoderm development	13
GO:0016055~Wnt receptor signaling pathway	12
GO:0009994~oocyte differentiation	11
GO:0006898~receptor-mediated endocytosis	10
GO:0005643~nuclear pore	10
GO:0005773~vacuole	10
GO:0009798~axis specification	10

Table 6 RT-qPCR validations

Gene	Description	*hlh-17* (ns204)		*hlh-17* (ns204) ; *hlh-31* (ns217); *hlh-32* (ns223)	
		Microarray	RT-qPCR	Microarray	RT-qPCR
ama-1	subunit of RNA pol. II			-3.66	-2.13
cdc-42	RHO GTPase			-2.84	-1.89
fat-5	delta-9 fatty acid desaturase			3.44	-2.47
kin-24	protein kinase			2.12	1.99
pmp-3	ABC transporter			-3.43	-3.01
sod-3	iron/magnese superoxide dismutase	-1.62		-3.67	2.01
vab-10	spectraplakin			-4.79	-2.73
cfz-2	frizzled homolog		1.11	-2.8	-1.63
cul-4	cullin		1.92	-4.44	-2.38
dnj-13	prokaryotic heat shock protein		1.29	-3.73	-1.64
pak-1	p21-Activated kinase	-1.62	1.22	-5.71	-3.39
sdc-2	sex determination and dosage compensation		1.49	-12.32	-2.47
skn-1	bZIP transcription factor		1.32	-7.97	-2.78
srb-18	serpentine receptor, class B (beta)		1.04	3.19	2.16
srx-112	serpentine receptor, class X		1.11	2.44	1.99
unc-84	inner nuclear membrane		1.33	-4.21	-2.08
wdr-23	WD40 repeat containing protein		1.92	-5.97	-2.11
akt-1	serine/threonine kinase		2.53	-3.87	
cat-1	vesicular monoamine transporter	-2.36	1.01		
daf-16	forkheadbox O (FOXO) homolgue		1.05	-2.84	

daf-2	receptor tyrosine kinase		3.47	2.79
dop-1	D1-like DA receptor		-1.76	2.69
dop-2	D2-like DA receptor		-2.29	2.13
dop-3	D2-like DA receptor	1.39	-3.09	
F30A10.9	uncharacterized		2.98	2.01
gcs-1	gamma glutamyl cysteine synthetase		1.09	-3.79
goa-1	G-protein, O, Alpha subunit		1.47	17.37
gst-4	glutathione-requiring prostagladin D synthase		1.13	-2.28
tbb-6	tubulin, beta		1.28	2.23
Y45F10D.1	Transposon (uncharacterized)	1.8	-1.13	
Y71H10B.1	uncharacterized	1.19	1.5	-5.24
Y87G2A.1	uncharacterized		-2.03	2.49

†Genes validated using mRNA from *hlh-17* (ns204) animals only
*Uncharacterized genes validated following *hlh-17* (ns204) microarray

Table 7 Top 10 GO terms for the microarray analyses

Probe Count	*hlh-17* (ns204) GO Terms	Probe Count	Similarly expressed GO Terms	Probe Count	*hlh-17* (ns204); *hlh-31* (ns217); *hlh-32* (ns223) GO Terms
489	**GO:0016021 integral to membrane**	**63**	**GO:0016021 integral to membrane**	**617**	**GO:0009792 embryonic development**
153	**GO:0040007 growth**	**43**	**GO:0009792 embryo development ending in birth**	**457**	**GO:0002119 nematode larval development**
143	GO:0005634 nucleus	**34**	**GO:0002119 nematode larval development**	392	GO:0045927 positive regulation of growth
134	GO:0030246 carbohydrate binding	**33**	**GO:0040011 locomotion**	364	GO:0043169~cation binding
123	**GO:0009792 embryo development**	**27**	**GO:0040007 growth**	**348**	**GO:0040007 growth**
113	**GO:0008270 zinc ion binding**	23	GO:0005515 protein binding	259	GO:0000166 nucleotide binding
153	GO:0007165 signal transduction	**23**	**GO:0005524 ATP binding**	256	GO:0003006~reproductive developmental process
91	**GO:0040011 locomotion**	22	GO:0040010 positive regulation of growth rate	137	GO:0006355~regulation of transcription, DNA-dependent
74	**GO:0002119 nematode larval development**	17	GO:0040035 hermaphrodite genitalia development	118	GO:0043232~intracellular non-membrane organelle
71	**GO:0005524 ATP binding**	**13**	**GO:0008270 zinc ion binding**	118	GO:0007610~behavior

*Similar GO terms among the three gene sets are indicated in bold font.

Table 8 Similarly expressed genes

		hlh-17 (ns204) microarray		*hlh-17*(ns204); *hlh-31*(ns217); *hlh-32*(ns223) microarray	
	Gene	**Fold Change**	**Direction**	**Fold Change**	**Direction**
*	*B0222.10*	26.80	down	2.80	up
	bli-5	**2.67**	**down**	**3.29**	**down**
	C06E1.1	**2.21**	**down**	**2.13**	**down**
	C07A12.2	**1.62**	**down**	**2.19**	**down**
	C16C8.17	**7.67**	**down**	**2.70**	**down**
*	*C28C12.3*	21.48	down	2.57	up
	cdk-9	**1.31**	**down**	**2.06**	**down**
*	*clec-106*	17.85	down	5.65	down
*	*clec-107*	31.79	down	6.54	down
*	*clec-130*	66.16	down	2.27	up
*	*clec-183*	30.89	down	3.28	down
*	*clec-193*	14.71	down	3.77	down
	clec-203	**2.81**	**down**	**2.89**	**down**
	clec-208	**22.03**	**down**	**20.45**	**down**
*	*clec-94*	46.94	down	2.32	up
	cpt-3	**5.27**	**down**	**2.18**	**down**
	cyk-1	**1.09**	**down**	**2.79**	**down**
*	*cyk-4*	1.36	down	2.50	up
*	*D1022.2*	36.42	down	2.08	down
	dcr-1	**1.11**	**down**	**7.05**	**down**
*	*ddx-23*	1.05	down	3.05	up
	dut-1	**3.14**	**down**	**3.56**	**down**
	F21F3.6	**1.12**	**down**	**4.47**	**down**
*	*F31F7.1*	1.91	down	2.22	up
*	*F35C5.3*	23.09	down	3.27	down
	F49C12.9	**1.57**	**down**	**3.33**	**down**
*	*F56C3.8*	22.27	down	2.14	up
	F57A10.2	**5.61**	**down**	**3.70**	**down**
	F58E10.3	**1.20**	**down**	**5.21**	**down**
	hrp-1	**1.15**	**down**	**3.28**	**down**
	ins-24	**1.80**	**down**	**2.67**	**down**
*	*K11D12.6*	38.72	down	2.37	down
	knl-1	**1.22**	**down**	**4.40**	**down**
	lips-14	**2.07**	**down**	**2.73**	**down**
*	*lst-3*	1.12	down	2.60	up
*	*lst-4*	1.44	down	2.42	up

*	*M6.4*	1.56	down	2.67	up
*	*M70.1*	1.38	down	2.96	up
*	*mel-11*	1.20	down	2.63	up
	mop-25.2	**1.19**	**down**	**2.50**	**down**
*	*msh-6*	1.23	down	2.02	up
	npp-10	**1.13**	**down**	**4.95**	**down**
*	*npp-14*	1.21	down	3.69	up
*	*pabp-2*	1.40	down	2.10	up
	par-2	**1.31**	**down**	**5.23**	**down**
	pdk-1	**1.62**	**down**	**8.56**	**down**
	pis-1	**1.21**	**down**	**3.09**	**down**
*	*pnk-1*	1.26	down	3.01	up
	pqe-1	**1.16**	**down**	**2.31**	**down**
*	*pqn-84*	14.20	down	2.23	up
*	*R05H10.1*	2.54	down	2.01	up
*	*R53.8*	22.36	down	3.24	down
	rad-26	**1.14**	**down**	**4.64**	**down**
	rpa-2	**1.29**	**down**	**3.26**	**down**
*	*rsa-2*	1.30	down	3.59	up
*	*sax-2*	1.57	down	2.29	up
*	*sel-10*	1.27	down	3.18	up
*	*set-17*	1.37	down	2.52	up
*	*snr-3*	1.29	down	2.10	up
*	*spe-8*	1.33	down	2.29	up
	srsx-34	**1.78**	**down**	**3.55**	**down**
*	*syp-4*	1.36	down	2.56	up
*	*T02H6.9*	36.95	down	3.12	down
*	*T22G5.1*	45.88	down	2.11	down
	tag-312	**1.35**	**down**	**4.25**	**down**
*	*tag-329*	25.57	down	2.89	down
*	*try-8*	44.97	down	3.47	down
*	*W02B3.7*	8.14	down	2.35	up
*	*Y49F6B.6*	48.77	down	2.21	up
	Y49G5A.1	**2.71**	**down**	**2.61**	**down**
*	*Y74C10AR.2*	32.62	down	2.35	down
*	*aco-1*	1.56	up	3.56	down
	acs-17	**1.39**	**up**	**3.37**	**up**
*	*afd-1*	1.23	up	2.46	down
*	*ain-1*	1.41	up	4.77	down

*	*bli-3*	1.54	up	8.78	down
*	*C03B1.2*	3.28	up	3.68	down
*	*C05D10.4*	1.60	up	2.83	down
	C06G4.6	**4.50**	**up**	**2.27**	**up**
	C08G9.1	**1.24**	**up**	**2.32**	**down**
*	*C17E7.13*	2.81	up	3.08	down
	C18B12.4	**1.25**	**up**	**3.05**	**up**
*	*C24A3.4*	1.30	up	2.54	up
*	*C30G4.4*	1.66	up	2.64	down
	calu-1	**1.52**	**up**	**2.02**	**up**
*	*cdr-1*	1.72	up	2.25	down
*	*ceh-37*	1.39	up	3.66	down
*	*clh-4*	1.51	up	2.94	down
*	*cnb-1*	1.34	up	3.55	down
	col-152	**2.48**	**up**	**2.46**	**up**
	cutl-4	**2.16**	**up**	**2.48**	**up**
	cyp-34A6	**1.38**	**up**	**2.23**	**up**
*	*D2096.6*	3.01	up	3.94	down
*	*dgk-2*	1.57	up	2.44	down
*	*egl-44*	1.28	up	4.63	down
*	*F01G4.6*	1.16	up	6.62	down
*	*F09F7.4*	1.14	up	6.48	down
*	*F18F11.4*	8.35	up	3.48	down
	F38E9.4	**2.15**	**up**	**2.04**	**up**
	F38E9.5	**1.65**	**up**	**2.12**	**up**
	F48G7.10	**1.77**	**up**	**2.68**	**up**
	gap-1	**1.63**	**up**	**3.01**	**up**
*	*gar-1*	1.52	up	3.80	down
*	*gcy-3*	4.07	up	2.79	down
	gob-1	**1.31**	**up**	**2.16**	**up**
*	*grl-22*	2.27	up	2.27	down
*	*grl-27*	3.01	up	3.62	down
*	*grl-28*	2.47	up	4.18	down
*	*gst-19*	1.76	up	2.08	down
*	*hbl-1*	2.47	up	2.34	down
	hum-4	**1.78**	**up**	**2.98**	**up**
*	*K02E10.7*	1.56	up	4.55	down
	klp-11	**1.46**	**up**	**2.19**	**up**
	kqt-2	**1.44**	**up**	**2.68**	**up**
	ldb-1	**1.39**	**up**	**2.30**	**up**
*	*lec-1*	2.00	up	2.85	down

	let-721	**1.09**	**up**	**2.82**	**up**
	let-756	**1.97**	**up**	**2.05**	**up**
	lim-6	**1.59**	**up**	**2.13**	**up**
	lim-9	**1.17**	**up**	**2.34**	**up**
*	*lips-11*	1.79	up	2.27	down
*	*mab-31*	1.41	up	4.26	down
	miz-1	**1.89**	**up**	**2.13**	**up**
	nas-12	**2.44**	**up**	**2.63**	**up**
*	*nhr-116*	1.36	up	7.08	down
	nhr-34	**1.24**	**up**	**2.01**	**up**
*	*nkat-1*	1.46	up	2.15	down
	nlp-17	**1.30**	**up**	**2.38**	**up**
	nlp-30	**3.70**	**up**	**2.22**	**up**
	nmy-1	**1.47**	**up**	**2.43**	**up**
	nucb-1	**1.52**	**up**	**2.24**	**up**
	nuo-4	**1.34**	**up**	**2.16**	**up**
*	*oga-1*	152.20	up	2.23	up
	pms-2	**1.37**	**up**	**2.98**	**up**
*	*pqn-26*	3.12	up	3.36	down
*	*pqn-89*	1.68	up	4.87	down
	R07G3.8	**1.66**	**up**	**2.56**	**up**
	R11G1.6	**1.40**	**up**	**3.71**	**up**
*	*R151.2*	1.34	up	2.43	down
*	*rhi-1*	1.14	up	3.58	down
	sar-1	**1.13**	**up**	**2.39**	**up**
	sft-4	**1.19**	**up**	**2.86**	**up**
*	*skr-19*	1.20	up	2.35	down
	soc-2	**1.65**	**up**	**2.98**	**up**
*	*sptf-1*	1.33	up	4.67	down
	srd-74	**1.45**	**up**	**2.12**	**up**
	srh-179	**4.03**	**up**	**2.51**	**up**
	str-131	**2.98**	**up**	**3.36**	**up**
	str-180	**2.04**	**up**	**2.65**	**up**
*	*str-7*	1.48	up	3.01	down
*	*syd-9*	1.61	up	3.91	down
*	*T04B8.5*	2.92	up	2.41	down
*	*T06D8.3*	3.00	up	3.34	down
*	*T09B4.7*	5.86	up	4.75	down
*	*T15D6.8*	4.68	up	2.35	down
	T21D11.1	**2.52**	**up**	**2.53**	**up**
	T23B3.2	**1.53**	**up**	**2.40**	**up**

*	*tat-4*	1.33	up	2.48	down
	tmd-2	**1.60**	**up**	**2.90**	**up**
	tre-3	**1.53**	**up**	**2.05**	**up**
	tsp-14	**2.08**	**up**	**2.18**	**up**
*	*ucr-2.1*	1.47	up	2.03	down
*	*ugt-50*	1.50	up	2.59	down
	unc-43	**1.67**	**up**	**2.24**	**up**
	unc-44	**1.24**	**up**	**3.11**	**up**
*	*unc-62*	1.39	up	9.08	down
*	*vab-19*	2.64	up	3.17	down
	vha-12	**1.39**	**up**	**2.18**	**up**
*	*vha-13*	1.34	up	3.36	down
*	*vha-14*	1.27	up	4.02	down
	vha-16	**1.29**	**up**	**2.23**	**up**
	vha-8	**1.34**	**up**	**2.19**	**up**
	Y22D7AL.14	**2.30**	**up**	**2.04**	**up**
*	*Y23B4A.2*	1.73	up	2.56	down
	Y55F3AM.14	**3.38**	**up**	**2.18**	**up**
	ZC434.7	**1.11**	**up**	**2.09**	**up**
*	*zig-1*	1.49	up	4.03	down
*	*ZK909.3*	2.41	up	2.16	down

Genes in **bold** are putative targets of HLH-17 only.
Genes with an asterisk (*) are presumably regulated antagonistically by HLH-17 and HLH-31/HLH-32.

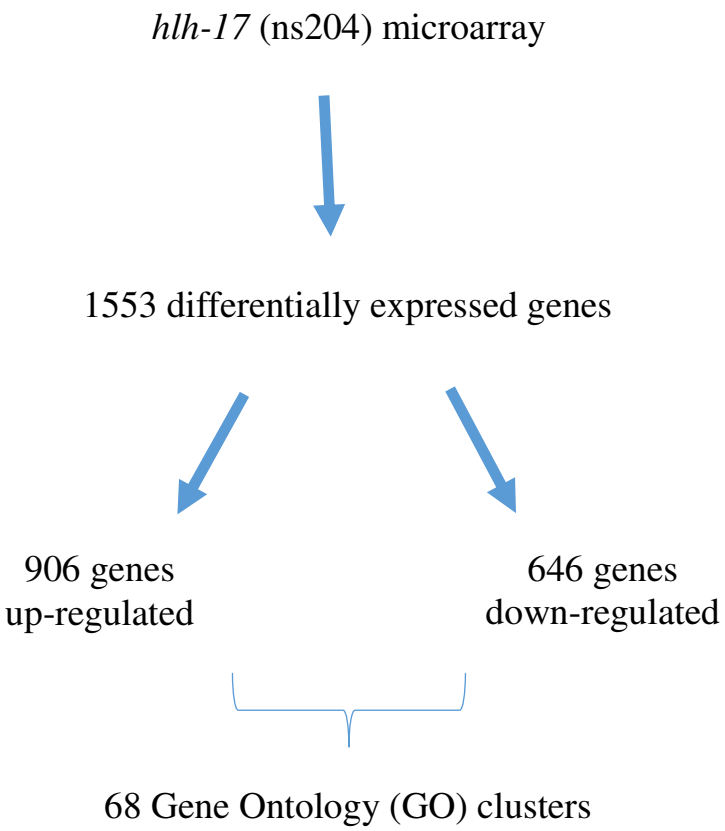

Figure 13 Flowchart of *hlh-17* microarray

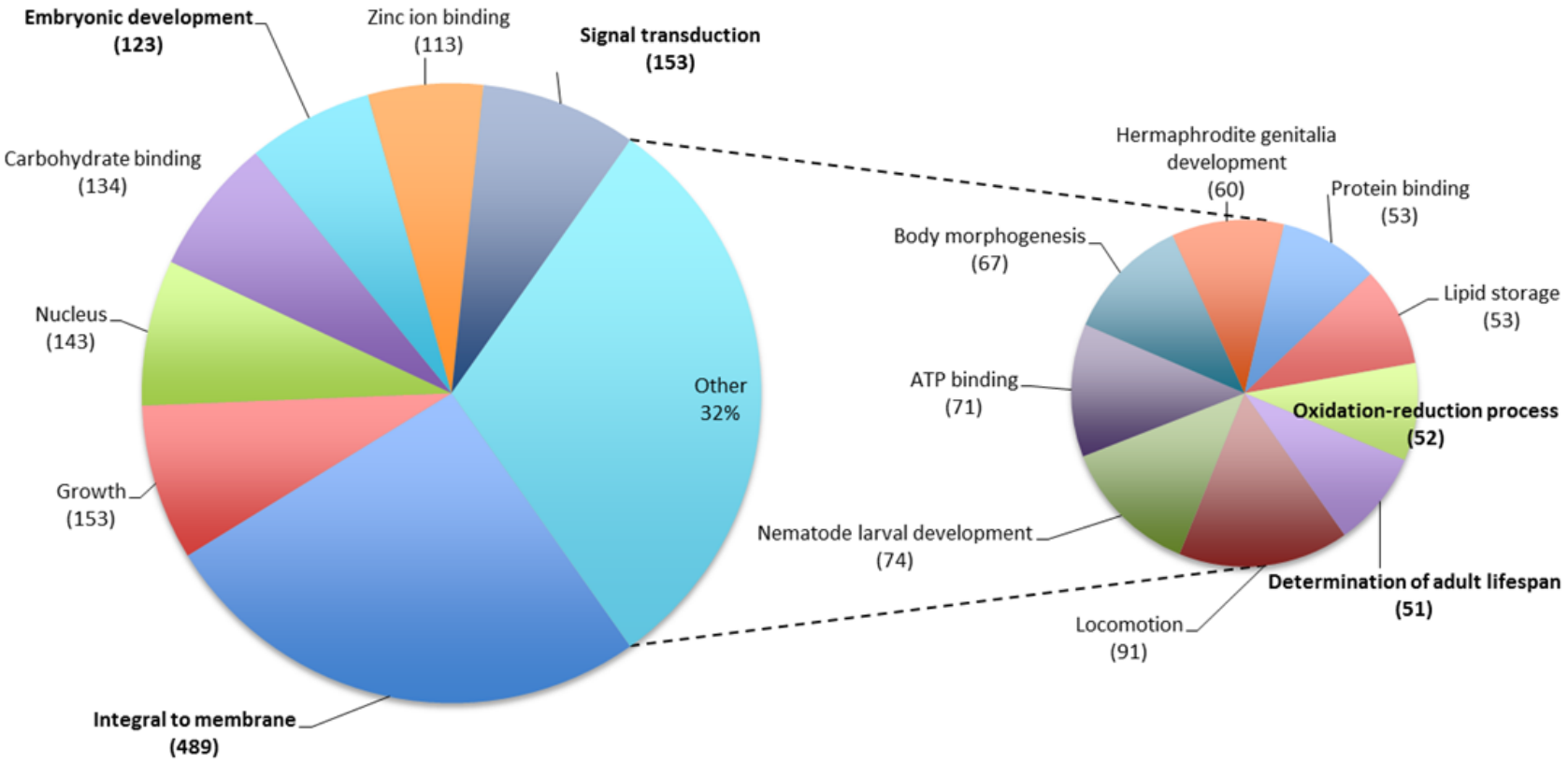

Figure 14 Top 15 GO terms for *hlh-17* microarray
Pie chart showing the distribution of *hlh-17*-regulated genes represented in the top 15 GO clusters. Gene annotations were derived from the Gene Set Enrichment Analysis (GSEA) tool provided by the Broad Institute at MIT. Number of genes represented in each cluster is indicated under each GO term. GO terms emphasized in text are indicated in bold font.

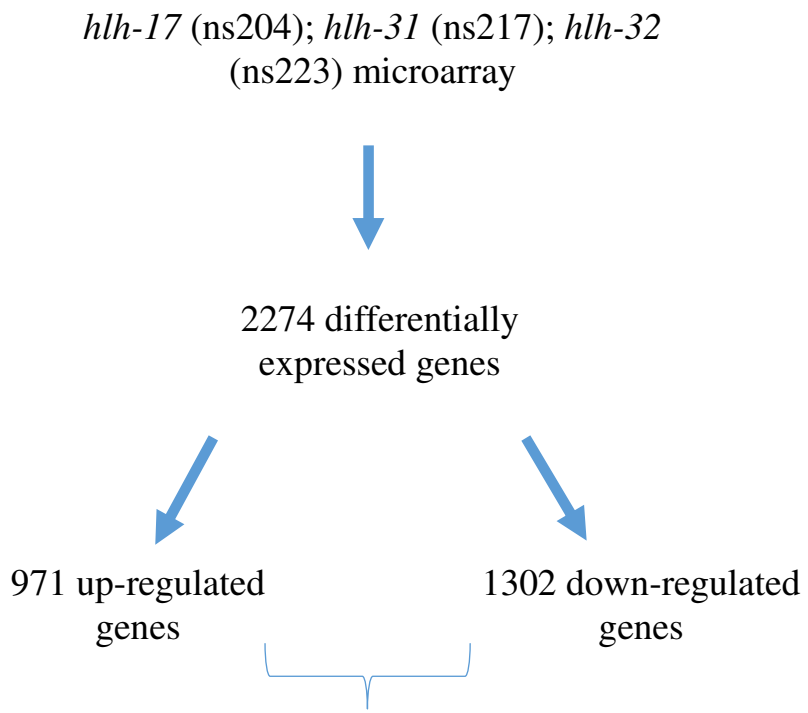

Figure 15 Flowchart of *hlh-17; hlh-31; hlh-32* microarray

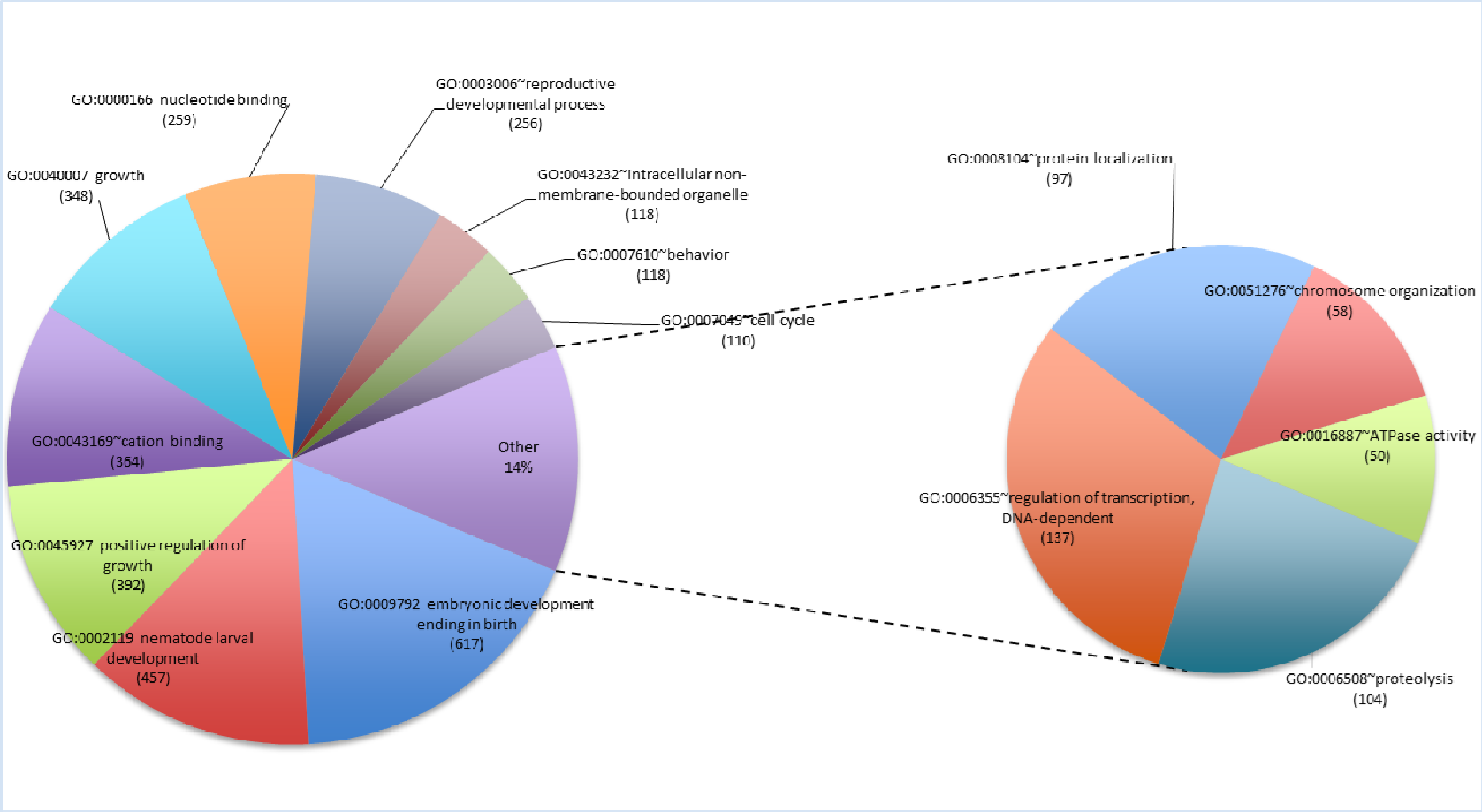

Figure 16 Top 15 GO terms for *hlh-17; hlh-31; hlh-32* microarray
Pie chart showing the distribution of *hlh-17; hlh-31; hlh-32*-regulated genes represented in the top 15 GO clusters. Gene annotations were derived from the Database for Annotation, Visualization and Integrated Discovery (DAVID), version 6.7. Number of genes represented in each cluster is indicated under each GO term.

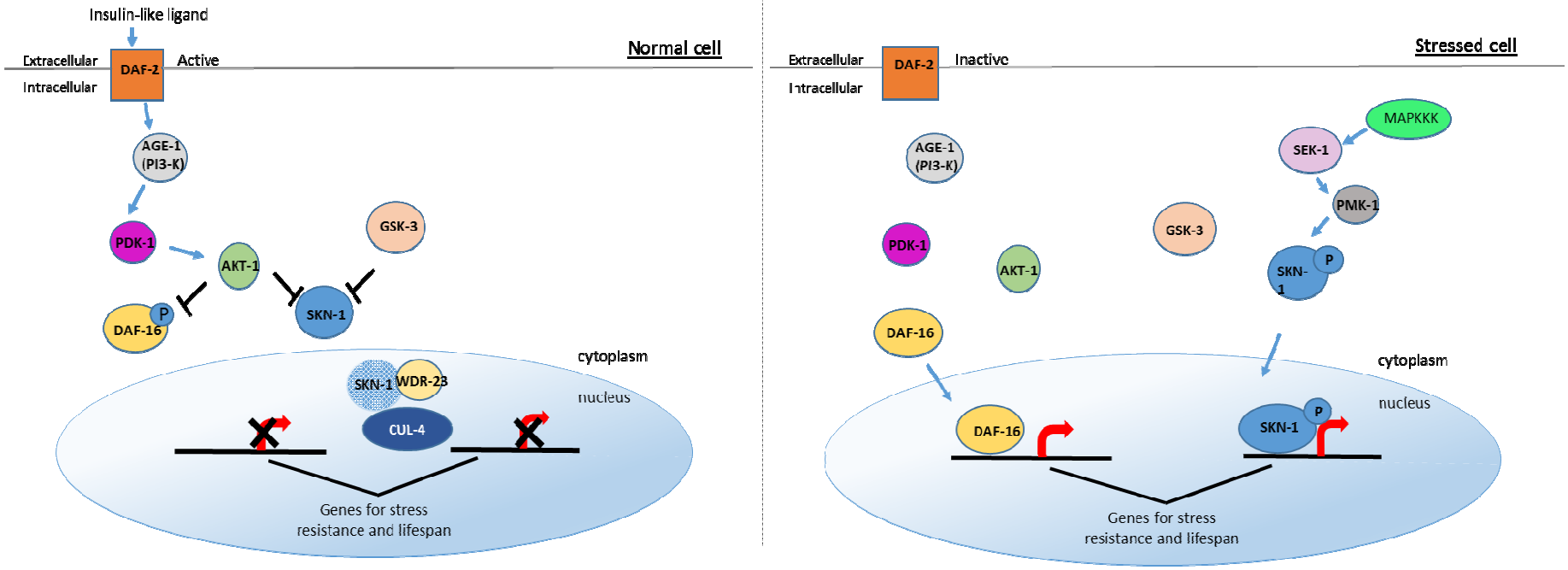

Figure 17 Regulation of genes required for stress resistance and lifespan in *C. elegans*
In the presence of an insulin-like ligand, the DAF-2 receptor is activated and in turn activates the PI3 kinase AGE-1. The phosphorylation of AGE-1 leads to the activation of PDK-1 first, then to the activation of AKT-1. AKT-1 in turn phosphorylates the transcription factor DAF-16, causing its cytoplasmic retention. AKT-1 along with GSK-3 also inhibit SKN-1 activity in the cytoplasm. Constitutively expressed SKN-1 in the nucleus is recruited by WDR-23 to the CUL-4 ubiquitin ligase complex where it is presumably targeted for degradation by the proteasome. Genes regulated by DAF-16 and SKN-1 required for lifespan and the stress response are not activated when DAF-2 is active. In cells exposed to oxidants, phosphorylation of SKN-1 by the p38 MAPK pathway (SEK-1 and PMK-1) allows SKN-1 to bypass WDR-23 mediated inhibition and therefore activate the expression of genes needed for detoxification. Likewise, an inactive DAF-2 receptor also inhibits the phosphorylation of DAF-16. This allows DAF-16 to enter the nucleus and also regulate genes required during the stress response.

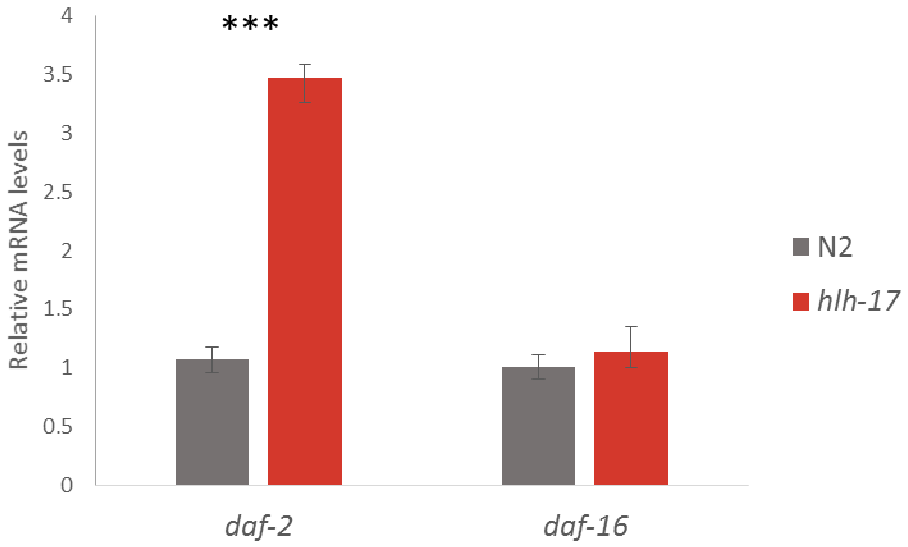

Figure 18 *daf-2* and *daf-16* expression levels
Total RNA was extracted from L4 animals grown on plates containing OP50 at 16 °C. Bars represent the mean +/- the standard errors of at least three independent RT-qPCR reactions. Astericks indicate values where differences are statistically significantly (**** P<0.0001).

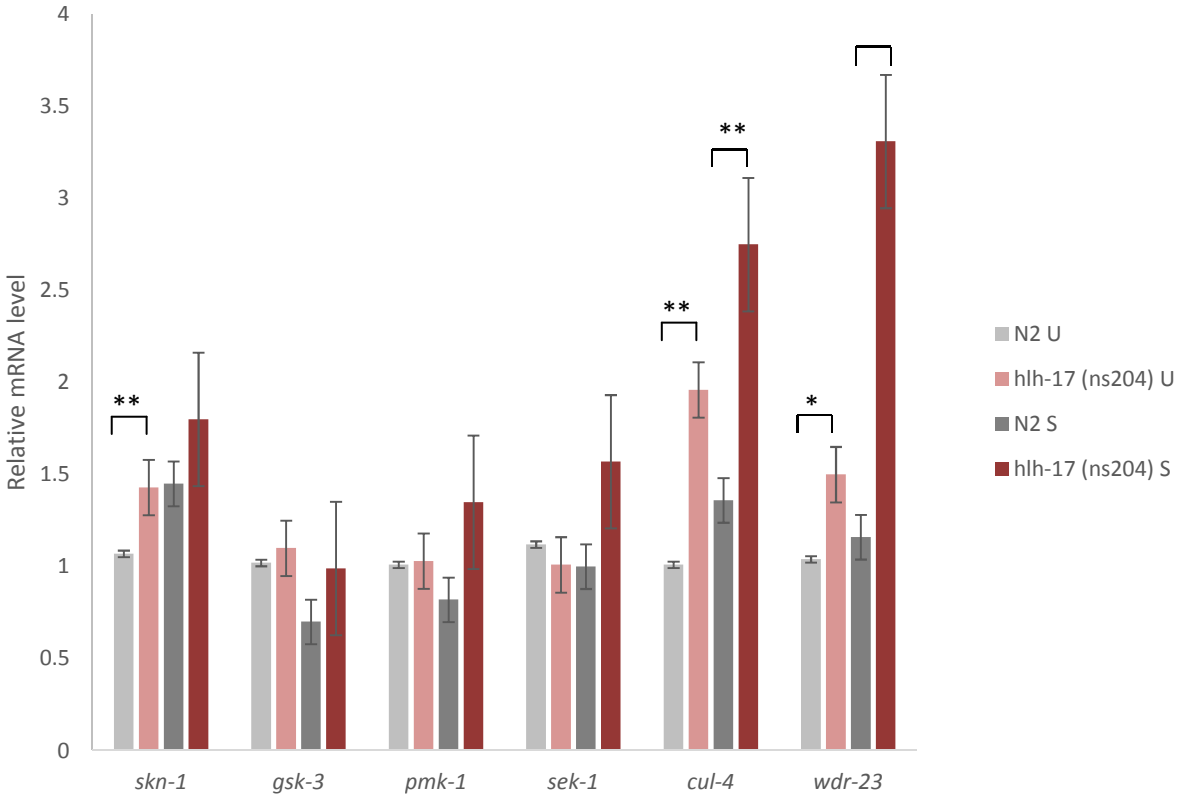

Figure 19 Expression levels of SKN-1 mediated pathway genes
Following exposure to juglone (60 µg/ml) for 1.75 hours, total RNA was extracted from L4 animals grown on NGM plates containing OP50. Bars represent the mean +/- the standard errors of at least three independent RT-qPCR reactions. Astericks indicate values where differences are statistically significantly (* P<0.05, ** P<0.01, *** P<0.001).

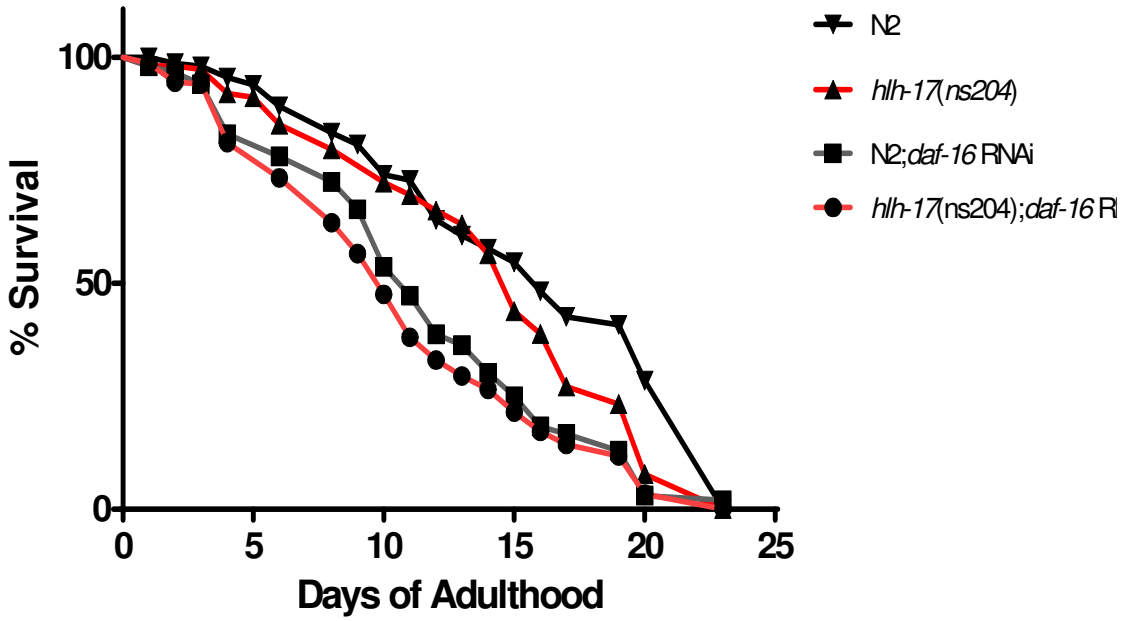

Figure 20 Lifespan assay
The shortened lifespan of *hlh-17* animals requires *daf-16.*
Survival curves of N2 (control), *hlh-17* (ns204) animals, and the same indicated strains eating bacteria expressing *daf-16* dsRNA. *hlh-17* (ns204) animals have a reduced lifespan (p-value 0.0076). *hlh-17* (ns204);*daf-16* RNAi animals do not have altered responses to *daf-16* RNAi (p-value 0.1495). Day zero corresponds to animals at the first day of adulthood. (n= 3, 1200 worms).

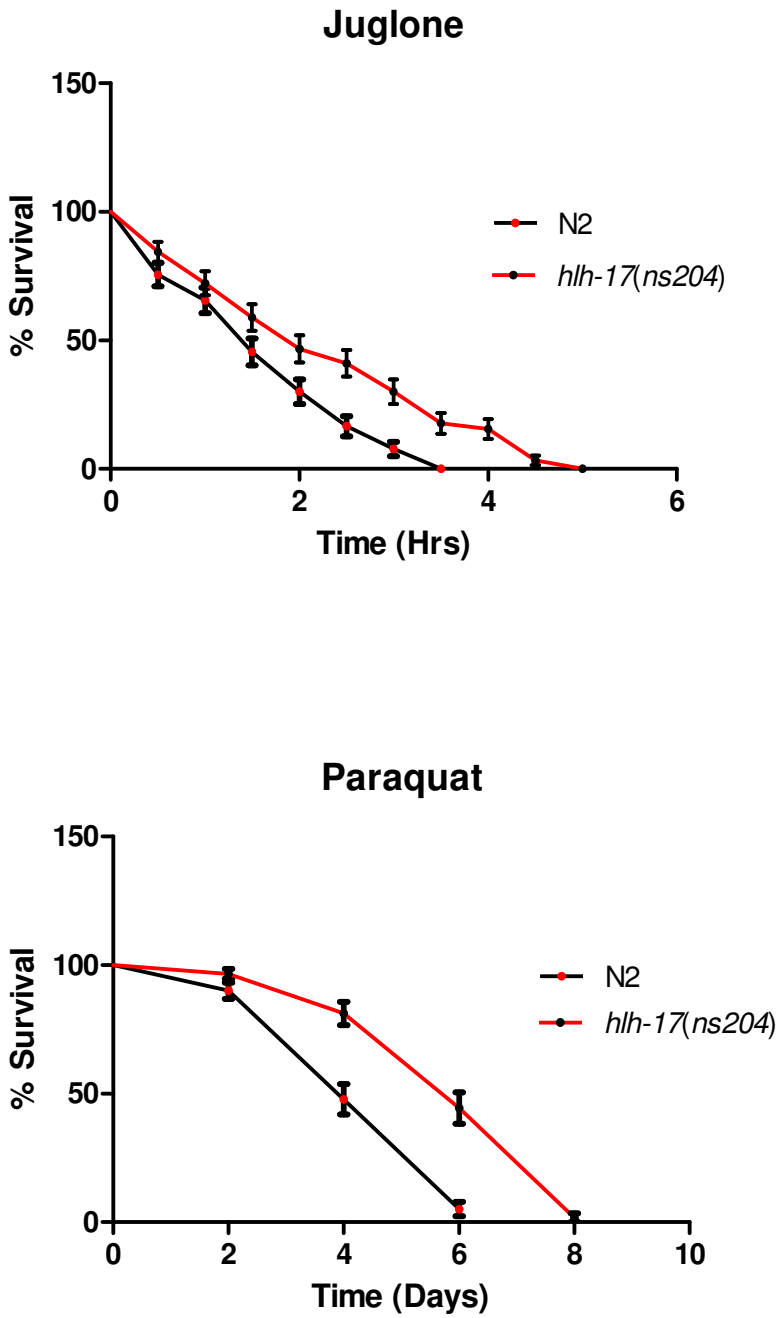

Figure 21 Oxidative stress assays
N2 and *hlh-17* (ns204) animals were scored for survival after being placed on NGM plates containing either juglone or paraquat for hours or days, respectively. **A.** Median survival was 1.5 hours for N2 animals and 2 hours for *hlh-17* (ns204) animals (****, p<0.0001). **B**. Median survival was 4 days for N2 animals and 6 days for *hlh-17* (ns204) animals (****, p<0.0001).

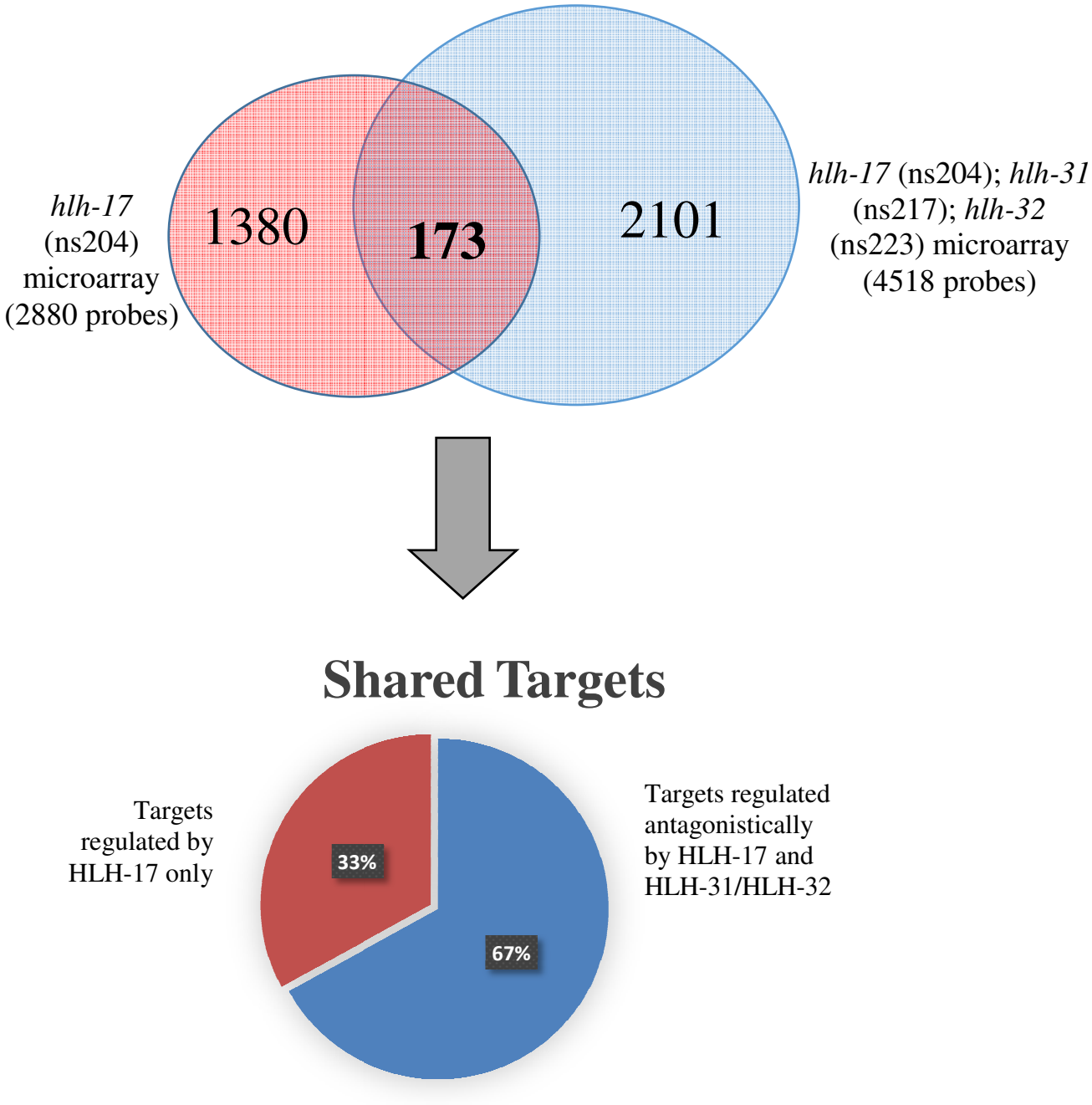

Figure 22 Shared targets in the *hlh-17* microarray analyses

5 GENERAL DISCUSSION

The integrated actions of multiple signaling pathways contribute to the regulation of developmental and behavioral events in animal systems. Understanding the molecular mechanisms that govern these processes is a major goal of scientific research. Therefore, extensive efforts have been made to identify and characterize transcription factors which function in these pathways. Despite ongoing efforts made to better understand the molecular mechanisms underlying the DA signaling pathway in *C. elegans* [250-252], previous studies have not included a role for the bHLH factor HLH-17. The goal of my dissertation has been to determine if and how this protein could affect events mediated by DA signaling. My studies have led to the identification of a single protein, HLH-17, putative HLH-17 protein networks and a glia (CEPsh)-neuron (CEP) interaction that may not only affect DA signaling but may also affect multiple signaling pathways that contribute to development and behavior in *C. elegans*. In this concluding chapter I provide a summary of my major findings, discuss the similarities between HLH-17 and a functionally comparable protein in the mouse and briefly introduce future research goals that will help to further our understanding of HLH-17's role(s) in the hermaphrodite nervous system.

Many members of the bHLH transcription factor family play vital roles in signaling pathways that regulate development and behavior. For instance, during neuronal differentiation, human TCF4 regulates genes involved in TGF-β and NF-kB signaling [253], while, in *Arabidopsis*, the bHLH-type factors MYC2, MYC3 and MYC4 function together to negatively regulate two converging pathways of Jasmonates (JAs) signaling required during stress response and plant development [254, 255]. Interestingly, a study conducted in mice provides an example of a bHLH protein that functions similarly to HLH-17 [256] . In this study, Brunskill et al sought

to characterize mice deficient in Npas3 and demonstrated that it affects dopamine, serotonin and glutamate neurotransmitter signaling during normal brain development and function [256]. Similarly, my studies provide evidence that HLH-17 may also function in multiple signaling pathways to regulate comparable processes. Since the Brunskill et al study provides the most support for my data, my discussion will largely focus on functional similarities between these two bHLH factors.

HLH-17 is important for normal growth and development

My dissertation studies have helped to identify a developmental role for HLH-17 in the *C. elegans* nervous system. To begin with, three of the largest clusters in my microarray analyses are 'Growth', 'Embryo Development' and 'Larval Development', suggesting a regulatory role for HLH-17 in transcriptional networks that regulate these events. In support of this finding, many of the genes represented in these clusters function in signal transduction pathways. For example, *nas-36* expression is affected by the loss-of HLH-17 and functions as an enzymatic regulator in the TGF-Beta signaling pathway during molting, a process that occurs at the end of each larval stage in development [257, 258]. Furthermore, since lifespan assays can be used as a means of measuring normal growth rate and development, the shortened lifespan of *hlh-17* (ns204) animals provides functional evidence that HLH-17 may be required to mediate these types of events in the worm. In addition, my molecular (RT-qPCR analyses) and behavioral studies show that HLH-17 functions upstream of the DAF-2 transcriptional network during the normal life cycle (lifespan) in *C. elegans*.

Like HLH-17, Npas3 is also important during development. Loss-of Npas3 causes growth and brain development abnormalities in the mouse [256]. In *C. elegans*, the nerve ring is most similar by structure and function to the human brain. Although my studies did not include

an anatomical analysis of the nerve ring, there are three key points that suggest HLH-17 may play a part in nerve ring development. Firstly, HLH-17 is expressed in the CEPsh, the only set of glial cells that completely innervate the nerve ring. The unique morphology of the CEPsh suggests that these cells have specialized roles in nerve ring formation and function. Secondly, HLH-17 is expressed during nervous system development but is not required for the development of the CEPsh nor the dopaminergic neurons that the CEPsh surrounds [30, 87]. Therefore, it can be postulated that HLH-17 functions to regulate the development of the nerve ring from these unique glial cells. Likewise, loss-of Npas3 does not affect development of the neuronal structure in which it is expressed [259]. Future studies will be aimed at determining if nerve ring development is normal in *hlh-17* (ns204) animals. A better defined spatial and temporal expression pattern of HLH-17 is also vital to determining if HLH-17 expression correlates with the expression of genes required for nerve ring development.

HLH-17 is important for behavior

My dissertation studies have also identified a role for HLH-17 during behavior. I have confirmed roles for HLH-17 in behaviors mediated by dopamine and the DAF-2 receptor. I showed that *hlh-17* (ns204) animals have defective responses during egg-laying, basal slowing, locomotion following exposure to dopamine, SWIP, gustatory plasticity, lifespan and oxidative stress. Through RT-qPCR analyses, I also showed that HLH-17 works upstream of factors that help to mediate the effects of these behaviors. Likewise, Brunskill et al, 2005 show that Npas3 is required for DA dependent behaviors, however, they determined that DA receptor levels were normal in the brains of Npas3 mice [256]. This highlights two pitfalls of my study. First, I only analyzed mRNA expression levels in *hlh-17* (ns204) animals, not protein levels. Second, I

conducted whole animal genetic studies and did not account for the possibility of structure specific differences in expression levels. Future goals are aimed at analyzing protein levels of DA specific proteins factors in the nerve ring of *hlh-17* (ns204) animals.

Although most of my studies centered on the role of HLH-17 during dopamine and DAF-2 mediated behaviors, some of my assays also tested the possibility that HLH-17 functions in other signaling pathways. For instance, egg-laying, food response and gustatory plasticity are regulated by dopamine and 5-HT, while gustatory plasticity is mediated by dopamine and glutamate in *C. elegans* [91, 94, 97]. Although my studies suggest that *hlh-17* (ns204) animals are defective in their responses mediated only by dopamine during these behaviors, it is possible that they also have altered responses to behaviors that are specific to 5-HT and glutamate. Preliminary gene expression analyses suggest that the loss-of *hlh-17* does not affect the expression of the serotonin receptor MOD-5 [260]. However, future studies will be done to test these possibilities at the behavioral levels.

Next, my pharmacological studies show that *hlh-17* (ns204) animals have abnormal responses to the reuptake inhibitors, reserpine and fluoxetine, suggesting that HLH-17 may play a regulatory role in the pathways that respond to these inhibitors [260]. Similarly, Brunskill et al, 2005 tested the effects of inhibitors on Npas3 mice and demonstrated that they have abnormal responses to inhibitors that affect the dopaminergic, serotonergic and glutamatergic signaling pathways [256]. Future work is aimed at determining specific roles of HLH-17 in multiple signaling pathways.

Collectively, my studies suggest that HLH-17 is a transcriptional regulator required for the modulation of behavior from multiple signal transduction pathways in *C. elegans*. To date, we are uncertain about the exact mechanism by which HLH-17 may affect behavior from these

multiple inputs. An attractive possibility is that HLH-17 regulation may be specific to a protein that affects similar outputs of multiple signaling pathways. In support of this, Brunskill et al, 2005 suggest that the synaptic protein PSD-95 may be a key determinant of the Npas3 mechanism. They show that PSD-95 levels are reduced in Npas3 deficient mice and that this reduction is specific to PSD-95 because no other synaptic protein levels were altered [256]. This finding suggests that Npas3 is able to indirectly affect multiple pathways through its regulation of post-synaptically located PSD-95. It will be important to search for a similar mode of action for HLH-17 in *C. elegans*. My microarray studies have provided prioritized candidate genes and pathways for these future mechanistic studies.

In conclusion, my dissertation studies have begun to identify HLH-17 as an important transcriptional regulator in the *C. elegans* nervous system. Better understanding the molecular mechanisms underlying development and behavior will give researchers more insight into how diseases and behavioral defects like Schizophrenia, Parkinson's disease, ADHD, and drug-addiction are manifested. Recent data demonstrate that major human behavioral defects are the result of dysfunctions in multiple signaling pathways and suggest that there is extensive cross-talk between most pathways leading to the notion of signaling networks. For instance, autism has been linked to defects in serotonin [261, 262] and WNT signaling [263] while other studies suggest that proteolytic and protein kinase C (PKC) pathways, underlie the pathogenesis of Huntington's disease [264-267]. My work and the strong functional similarity between HLH-17 and Npas3 suggest that bHLH transcription factors may play important roles in these signaling networks. My dissertation studies therefore provide a sound framework for future studies aimed at better understanding the role of HLH-17 in *C. elegans*.

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APPENDICES

Appendix A: Microarray Tables

Appendix A.1 Genes differentially expressed in the hlh-17(ns204) microarray analysis

Gene (down-regulated)	Fold Change	Gene (up-regulated)	Fold Change
F56A4.3	-196.60	*oga-1*	152.20
srbc-15	-184.45	*flp-20*	50.33
F26D10.13	-145.24	*F46F5.6*	39.33
srbc-15	-117.13	*C25F9.1*	15.09
clec-217	-116.49	*gmd-2*	9.26
clec-134	-113.33	*Y51A2A.3*	9.17
F49F1.11	-108.96	*ZC449.6*	8.95
F26D10.13	-107.16	*Y46B2A.2*	8.43
R11G10.4	-101.56	*E03H4.4*	8.38
F43C11.1	-100.96	*F18F11.4*	8.35
ZK1290.11	-100.36	*TC207055*	7.80
Y75B12B.13	-99.65	*T26C5.2*	7.47
F26D10.13	-96.68	*clec-21*	7.04
F46A8.3	-91.88	*C40H5.3*	6.90
clec-181	-89.54	*Y57G11C.42*	6.84
F49F1.9	-88.89	*F59H6.5*	6.56
F46A8.8	-87.92	*K08B12.1*	6.53
R11G10.4	-87.19	*nhr-41*	6.39
K03B8.11	-86.43	*C02F5.14*	6.29
nas-19	-86.13	*CB394306*	6.26
C08E8.3	-85.46	*T26C5.2*	6.04
CB393178	-84.24	*lact-7*	6.03
clec-135	-83.67	*F58H1.2*	5.97
Y6G8.5	-82.61	*osm-8*	5.94
R11G10.4	-82.36	*nas-23*	5.93
C33G3.5	-79.30	*ins-11*	5.90
C48B4.12	-79.01	*T09B4.7*	5.86

Y75B12B.13	-78.56	*F21G4.3*	5.66
F46A8.5	-76.76	*F38A1.13*	5.58
F09C6.10	-75.69	*C30B5.6*	5.52
F26D2.16	-74.86	*D2023.1c*	5.44
F56A4.3	-73.48	*CB394306*	5.36
R11G10.4	-72.49	*col-172*	5.29
C16C8.8	-71.20	*D1014.7*	5.28
nas-17	-70.40	*F16G10.9*	5.19
M7.9	-69.46	*C47F8.5*	5.16
F49F1.9	-68.51	*nas-13*	5.14
C16C8.10	-67.12	*C30B5.6*	5.00
TC205608	-66.93	*Y75B12B.11*	4.99
Y75B12B.13	-66.68	*srd-8*	4.99
clec-130	-66.16	*T15D6.11*	4.96
F26C11.4	-65.53	*F46F3.3*	4.94
K12H6.4	-64.97	*col-56*	4.93
F28B1.9	-64.17	*Y53F4B.26*	4.91
T19B10.12	-63.83	*nas-23*	4.90
CB393178	-63.38	*F07H5.8*	4.90
clec-161	-63.12	*C26B9.7*	4.87
F01D4.10	-63.00	*Y51H7C.10*	4.83
R11G10.4	-62.82	*his-9*	4.83
F01D4.10	-62.57	*ZK131.5*	4.82
hpo-33	-61.61	*ZC434.3*	4.80
CB393178	-59.84	*D1037.2*	4.78
C16C8.7	-59.70	*F07H5.8*	4.75
ins-31	-59.49	*C26B9.7*	4.73
Y49F6B.13	-59.43	*his-9*	4.70
CB393178	-59.38	*T15D6.8*	4.68
Y47D7A.11	-59.36	*ZK131.5*	4.68
TC199291	-59.26	*F46F3.3*	4.66
Y51B11A.1	-59.24	*alh-13*	4.63
F43C11.12	-58.70	*nas-23*	4.58

Y49F6B.13	-57.90	*C26B9.7*	4.57
F26C11.4	-57.48	*cuti-1*	4.54
F58F9.8	-57.10	*Y51H7C.10*	4.51
T26E3.6	-57.09	*F16G10.8*	4.50
TC186275	-55.94	*C06G4.6*	4.50
Y47D7A.11	-55.76	*B0454.8*	4.45
F09C6.10	-55.18	*cuti-1*	4.43
F58F9.8	-54.94	*F46F3.3*	4.37
F17E9.3	-53.57	*nas-14*	4.36
C06E2.9	-53.52	*col-172*	4.33
K12H6.5	-53.43	*C13C12.2*	4.31
C16C8.9	-52.06	*his-9*	4.31
F01D4.10	-51.83	*ZC513.2*	4.29
Y110A2AL.6	-51.71	*grd-12*	4.29
T10D4.15	-51.33	*alh-13*	4.28
F09C6.10	-51.17	*F13C5.3*	4.25
hpo-2	-51.08	*svh-1*	4.20
C06E2.9	-50.37	*TC207055*	4.14
Y51H4A.26	-50.28	*lpr-7*	4.12
F43C11.1	-49.85	*nas-13*	4.12
ZK1290.1	-49.59	*grl-28*	4.10
F46B6.13	-49.36	*F15B9.8*	4.10
F59A1.16	-49.13	*ZK131.5*	4.09
C06E2.9	-48.82	*C25H3.15*	4.08
C16C8.19	-48.81	*gcy-3*	4.07
Y49F6B.6	-48.77	*srh-179*	4.03
R05H10.7	-48.54	*lips-3*	3.97
C06E2.9	-48.46	*F13C5.3*	3.97
F28B1.9	-48.08	*R05A10.7*	3.94
K12H6.9	-48.01	*lpr-7*	3.94
T16G12.10	-47.79	*mec-1*	3.92
clec-197	-47.75	*K01D12.8*	3.88
F58F9.6	-47.38	*M01B2.8*	3.88

F09C6.10	-47.37	*K02C4.2*	3.85
clec-110	-47.33	*Y53F4B.26*	3.85
T19B10.12	-47.23	*lips-3*	3.84
clec-219	-47.09	*T16G1.2*	3.82
clec-137	-47.09	*F46C8.8*	3.81
clec-94	-46.94	*D2005.6*	3.80
T16G12.10	-46.66	*Y53F4B.26*	3.80
F40G9.7	-46.49	*Y53F4B.7*	3.79
F59B2.12	-46.36	*F23B2.3*	3.79
F01D4.10	-46.33	*srw-145*	3.78
T22G5.1	-45.88	*F08G2.1*	3.78
Y47D7A.11	-45.77	*col-131*	3.77
F46B6.13	-45.33	*ZC84.1*	3.77
try-8	-44.97	*F35A5.4*	3.77
clec-95	-44.90	*dsl-5*	3.75
Y46G5A.23	-44.83	*F01D5.6*	3.74
F09C6.10	-44.83	*F15B9.8*	3.70
T19B10.12	-44.57	*nlp-30*	3.70
Y110A2AL.7	-44.04	*F38A6.4*	3.70
Y47D7A.11	-43.96	*Y37D8A.16*	3.67
C44B12.9	-43.78	*ZK662.6*	3.66
C14F11.7	-43.36	*K01D12.8*	3.65
C17H12.11	-42.46	*Y44E3A.1*	3.62
clec-110	-42.16	*nep-16*	3.62
scl-8	-42.16	*F42F12.12*	3.61
F40G9.15	-42.01	*TC201207*	3.60
ZC328.5	-41.96	*H10E21.4*	3.59
C35E7.7	-41.73	*ttr-52*	3.56
C06E2.9	-41.46	*B0454.8*	3.55
F54B8.13	-41.43	*sel-7*	3.54
NP022293	-41.39	*R52.2*	3.54
Y43F8C.16	-41.31	*Y53F4B.7*	3.54
C05B5.9	-41.26	*clec-206*	3.53

Y47D7A.2	-40.99	*gly-1*	3.53
clec-110	-40.55	*ND3*	3.51
T19B10.12	-40.39	*F44A6.3*	3.50
F26C11.4	-39.39	*Y51H7C.10*	3.49
F20A1.8	-38.99	*pqn-90*	3.49
scl-15	-38.90	*clec-230*	3.48
Y18D10A.2	-38.76	*W04G3.12*	3.47
K11D12.6	-38.72	*npax-2*	3.47
C09G12.5	-38.65	*ptr-17*	3.47
T10B10.9	-38.63	*Y53F4B.7*	3.47
clec-96	-38.55	*T01B6.1*	3.47
F43C11.2	-38.43	*M153.2*	3.45
F17B5.7	-37.93	*ins-11*	3.43
K01D12.2	-37.85	*C49F8.1*	3.42
Y47D7A.11	-37.66	*K01D12.9*	3.41
F59A1.6	-37.64	*Y55F3AM.14*	3.38
F43C11.1	-37.61	*K09C8.7*	3.38
C44B12.9	-37.38	*F59H6.5*	3.36
K03D3.5	-37.36	*W04G3.7*	3.36
ZK39.9	-37.33	*ZK250.9*	3.35
EB996415	-37.25	*F33H12.6*	3.34
F01D4.10	-37.24	*col-97*	3.34
K12H6.8	-36.96	*Y75B12B.11*	3.34
T02H6.9	-36.95	*C09B8.3*	3.33
fipr-18	-36.86	*C28H8.5*	3.33
F42A6.2	-36.61	*mec-1*	3.32
D1022.2	-36.42	*C02E7.6*	3.32
Y116F11A.3	-36.31	*ZK470.6*	3.31
ZC204.17	-36.25	*ptr-1*	3.31
C35B1.3	-36.24	*F07H5.8*	3.31
F25E5.7	-36.16	*lips-7*	3.30
E02H9.1	-36.06	*Y47D3B.10.2*	3.29
Y64G10A.2	-36.04	*M03F4.6*	3.29

clec-141	-36.01	*ZK250.10*	3.28
clec-232	-35.92	*C03B1.2*	3.28
F30A10.11	-35.92	*lgc-21*	3.28
ZC178.2	-35.75	*jud-4*	3.27
E02H9.1	-35.72	*lys-10*	3.27
cwp-1	-35.56	*clec-230*	3.27
F46B6.13	-35.46	*mec-1*	3.27
TC202044	-35.36	*clec-230*	3.26
E02H9.9	-35.22	*T25E4.1*	3.26
M176.10	-35.18	*C02E7.7*	3.26
D2096.5	-35.09	*C38C6.6.2*	3.25
Y47D7A.11	-35.00	*F13D2.4*	3.25
F46B6.13	-34.97	*lips-7*	3.24
C44B12.9	-34.87	*fip-6*	3.24
B0228.8	-34.52	*gon-1*	3.23
T19B10.12	-34.08	*lgc-21*	3.22
T02E9.6	-33.92	*F20B10.3*	3.22
clec-263	-33.83	*grl-27*	3.21
F17B5.7	-33.81	*clec-230*	3.21
R03H10.4	-33.61	*jud-4*	3.20
clec-158	-33.47	*mab-7*	3.19
F54D12.9	-33.00	*C03F11.2*	3.19
clec-92	-32.84	*Y65B4BL.6*	3.19
Y74C10AR.2	-32.62	*nas-23*	3.18
fip-7	-32.57	*pes-8*	3.18
W02D7.12	-32.57	*F41G3.3*	3.16
clec-138	-32.34	*agmo-1*	3.16
F41D3.12	-32.24	*F16H9.2*	3.16
TC208547	-32.17	*M02G9.2*	3.15
Y43F8C.16	-32.12	*F26F12.4*	3.15
clec-207	-32.08	*C56C10.4*	3.14
clec-158	-32.02	*C40H5.3*	3.13
TC190647	-31.87	*alh-13*	3.13

clec-107	-31.79	*Y18H1A.9*	3.13
Y71G12B.5	-31.79	*zig-3*	3.13
C06A12.8	-31.74	*pqn-26*	3.12
Y87G2A.12	-31.64	*C25B8.8*	3.12
Y47D7A.11	-31.62	*Y65B4BL.6*	3.12
fipr-27	-31.52	*lgc-21*	3.11
F26C11.4	-31.36	*K02C4.2*	3.10
TC208547	-31.02	*Y46G5A.36*	3.10
clec-183	-30.89	*F23F1.2*	3.10
TC208547	-30.50	*T17H7.7*	3.10
nas-24	-30.31	*ZK180.5*	3.09
F56D2.8	-30.12	*clec-230*	3.09
clec-136	-29.98	*Y65B4BL.6*	3.09
lips-2	-29.83	*F26G1.10*	3.08
F45D11.14	-29.75	*C15C6.1*	3.07
F40G9.15	-29.57	*F01G10.10*	3.07
Y87G2A.12	-29.30	*suro-1*	3.05
Y6G8.5	-29.00	*D2092.8*	3.04
C01G10.18	-28.91	*col-70*	3.03
F34D6.8	-28.84	*ptr-22*	3.02
Y47D7A.9	-28.82	*F40H3.3*	3.02
F34D6.8	-28.80	*Y51H7C.1*	3.02
ZC204.6	-28.79	*F41F3.8*	3.02
B0207.5	-28.76	*Y46B2A.2*	3.02
C44B12.9	-28.65	*grl-27*	3.01
clc-4	-28.60	*D2096.6*	3.01
Y50E8A.8	-28.33	*C31B8.12*	3.01
Y47D7A.11	-27.94	*T06D8.3*	3.00
EB996415	-27.77	*clec-230*	2.99
F17B5.7	-27.66	*C25F6.8*	2.99
F32B4.6	-27.54	*F13H8.5*	2.99
F34D6.8	-27.52	*F26A1.9*	2.99
F41D3.13	-26.80	*jud-4*	2.98

B0222.10	-26.80	*str-131*	2.98
clec-130	-26.79	*C30H6.5*	2.98
flp-23	-26.74	*R10E11.9*	2.97
T10D4.15	-26.43	*C10A4.9*	2.97
Y116A8C.44	-26.43	*C30A5.10b*	2.96
clec-261	-26.41	*cutl-5*	2.96
E02H9.1	-26.32	*T24A6.21*	2.96
F25C8.1	-25.67	*F13E9.8*	2.96
T02D1.7	-25.63	*suro-1*	2.95
scl-9	-25.57	*C10A4.9*	2.95
tag-329	-25.57	*F41F3.8*	2.95
scl-24	-25.51	*C30H6.5*	2.95
F34D6.8	-25.34	*C35A5.10*	2.95
F54B8.13	-25.16	*C02E7.6*	2.95
C49C3.11	-25.14	*C53A3.1*	2.94
C44B12.9	-25.10	*F10D11.6*	2.93
F19H6.5	-25.06	*nas-13*	2.93
tag-329	-25.05	*Y47D3B.6*	2.93
scl-25	-24.95	*F41D3.5*	2.93
clec-157	-24.83	*cutl-5*	2.93
clec-181	-24.50	*T04B8.5*	2.92
TC208547	-24.22	*TC178098*	2.91
scl-24	-24.14	*C10A4.9*	2.91
Y47D7A.7	-23.89	*K01D12.5*	2.90
Y47D7A.6	-23.73	*T22B2.6*	2.90
T02E9.6	-23.50	*phat-1*	2.90
F46B6.13	-23.28	*C10A4.9*	2.90
F58A4.1	-23.16	*B0403.5*	2.89
W09G12.6	-23.13	*B0212.6*	2.89
M7.10	-23.12	*Y43F4A.1*	2.89
F35C5.3	-23.09	*R09H10.8*	2.89
clec-99	-22.84	*jud-4*	2.89
F13E9.10	-22.81	*TC187546*	2.89

F28B1.2	-22.59	*C15C8.8*	2.89
Y46D2A.3	-22.57	*T27A10.5*	2.89
R53.8	-22.36	*M195.2*	2.89
F56C3.8	-22.27	*T22B2.6*	2.88
W07G1.7	-22.18	*C50F7.9*	2.88
fipr-17	-22.15	*swt-5*	2.88
F33E2.7	-22.10	*C02E7.6*	2.88
R09E10.8	-22.03	*F13B12.4*	2.88
clec-208	-22.03	*F46F5.6*	2.87
cwp-2	-22.03	*C15C8.8*	2.87
fipr-17	-21.99	*C25F6.8*	2.87
TC208547	-21.91	*F41F3.8*	2.86
T10D4.7	-21.77	*C26F1.1*	2.86
K09C8.2	-21.64	*C17F4.12*	2.86
C05B5.9	-21.60	*T22B2.6*	2.86
F34D6.7	-21.57	*C36E6.8*	2.86
C28C12.3	-21.48	*suro-1*	2.86
clec-133	-21.37	*F41F3.8*	2.86
fipr-17	-21.34	*adt-1*	2.85
F34D6.8	-21.27	*C40H5.4*	2.85
T24D8.6	-21.18	*C35A5.10*	2.85
tag-329	-21.08	*M153.3*	2.84
Y37F4.3	-21.03	*F53H4.3*	2.84
clec-129	-21.00	*Y61A9LA.7*	2.84
clec-104	-20.97	*K09B3.1*	2.84
ZK39.9	-20.96	*Y51H7C.10*	2.84
clec-159	-20.91	*pqm-96*	2.83
fipr-17	-20.83	*T24C12.4*	2.83
clec-109	-20.66	*C06G1.1*	2.83
Y48G9A.6	-20.66	*T05H4.7*	2.83
Y26D4A.16	-20.32	*ZC21.9*	2.83
T22C1.12	-20.14	*C25F6.8*	2.82
F11C7.6	-20.02	*M110.9*	2.82

F28C6.10	-19.84	*T22B2.6*	2.82
F01D4.1	-19.45	*R09H10.8*	2.82
F13E9.4	-19.42	*C25B8.8*	2.82
F16G10.10	-19.28	*C17E7.13*	2.81
flp-23	-18.95	*T05H4.7*	2.81
cwp-2	-18.90	*mec-1*	2.81
flp-23	-18.72	*grl-28*	2.81
EB996415	-18.26	*Y61A9LA.7*	2.81
clec-130	-18.25	*T22B2.6*	2.80
clec-108	-18.21	*clec-144*	2.80
flp-23	-18.19	*C02F5.14*	2.79
Y25C1A.2	-18.16	*ZC334.7*	2.79
scl-7	-18.05	*T14E8.4*	2.78
F47C12.6	-17.98	*nas-12*	2.78
T23G4.5	-17.96	*clec-258*	2.78
clec-106	-17.85	*nas-29*	2.77
abf-2	-17.79	*pqm-96*	2.77
clec-125	-17.57	*lin-32*	2.77
flp-23	-17.52	*Y65B4BL.6*	2.76
clec-216	-17.26	*T22B2.6*	2.76
F19H6.6	-17.18	*srz-78*	2.76
nep-25	-17.12	*F07H5.8*	2.76
flp-23	-17.03	*Y57G11C.42*	2.76
F49C5.10	-16.96	*F54B11.9*	2.75
F33E2.6	-16.89	*F53A9.3*	2.75
F58F9.9	-16.87	*T22B2.6*	2.75
clec-103	-16.84	*nas-29*	2.75
F17B5.7	-16.64	*K03D7.3*	2.75
flp-23	-16.32	*T20B6.3*	2.75
K04F1.8	-16.12	*F46F5.6*	2.74
Y62H9A.8	-16.03	*oac-40*	2.74
F20B6.6	-15.96	*F35A5.4*	2.74
F38E1.10	-15.75	*nas-12*	2.74

C45G9.10	-15.64	*nas-38*	2.73
F41B5.6	-15.54	*E01G6.1*	2.73
abf-1	-15.50	*F46F5.6*	2.73
Y51A2D.8	-15.49	*ets-10*	2.73
clec-126	-15.41	*F46F5.6*	2.72
try-5	-15.36	*F53H4.3*	2.72
K08C9.6	-15.09	*TC182040*	2.71
K08C9.6	-15.08	*F46F5.6*	2.71
K04H8.3	-15.01	*F17E9.9*	2.70
TC202044	-14.92	*B0238.13*	2.70
F17B5.7	-14.86	*C36E6.8*	2.69
F56A4.9	-14.80	*F33A8.10*	2.69
clec-193	-14.71	*fip-6*	2.69
clec-130	-14.50	*F46F5.6*	2.69
fip-3	-14.48	*clec-229*	2.68
fipr-16	-14.42	*ptr-9*	2.68
Y64G10A.10	-14.41	*F53H4.3*	2.68
clec-124	-14.39	*bli-5*	2.67
pqn-84	-14.20	*nas-29*	2.66
K07E8.1	-14.08	*T23F6.1*	2.66
clec-110	-13.69	*C04G6.13*	2.65
F58F9.9	-13.62	*aat-8*	2.64
clec-181	-13.36	*vab-19*	2.64
F20B6.6	-13.15	*T14E8.4*	2.64
F41D3.13	-12.93	*Y48G8AL.1*	2.64
F41D3.13	-12.85	*TC207055*	2.64
ZK39.9	-12.74	*R13H4.8*	2.64
F49C5.10	-12.67	*F35A5.4*	2.63
clec-109	-12.48	*F41F3.8*	2.63
clec-193	-12.45	*TC186189*	2.63
clec-216	-12.11	*nas-13*	2.63
clec-221	-11.80	*W08E12.6*	2.62
flp-23	-11.74	*F59A1.5*	2.62

F41D3.13	-11.69	*C46H11.7*	2.62
C35B1.3	-11.68	*Y48G8AL.1*	2.61
K08C9.6	-11.61	*ZK909.3*	2.61
F30H5.5	-11.31	*T23F6.1*	2.61
C35B1.3	-11.29	*F39D8.3*	2.60
F25E5.3	-11.22	*wrt-8*	2.60
F18G5.5	-11.17	*C39B10.6*	2.60
W02B3.5	-11.09	*nas-12*	2.59
C35B1.3	-10.85	*lys-5*	2.59
Y116A8A.1	-10.83	*Y47D7A.15*	2.59
C25F9.8	-10.80	*B0403.5*	2.59
flp-23	-10.78	*ifp-1*	2.58
K08C9.6	-10.60	*ZC21.9*	2.58
Y51A2D.1	-10.45	*R10D12.1*	2.58
Y71G12B.5	-10.39	*Y47D7A.15*	2.58
F41D3.13	-10.27	*T23F6.1*	2.58
F45D11.2	-10.14	*T13C2.3*	2.58
F59A6.3	-10.04	*F46G11.2*	2.57
W06D12.6	-9.96	*sdha-1*	2.57
F13E9.9	-9.95	*nas-36*	2.57
F46A8.4	-9.79	*F09B12.3*	2.56
C35B1.3	-9.77	*M03E7.4*	2.56
T12A2.5	-9.72	*F47B7.4*	2.55
F58E6.4	-9.69	*F49E10.4*	2.55
F47C12.12	-9.47	*CELE_Y57G11C.42*	2.54
F15E11.15	-9.41	*Y47D7A.15*	2.54
K10D11.4	-9.36	*C26B2.8*	2.53
Y70G10A.2	-9.27	*T21D11.1*	2.52
F30H5.5	-9.23	*nas-12*	2.52
K02B12.9	-9.15	*col-58*	2.52
F23A7.1	-9.11	*T23F6.1*	2.52
T04A6.2	-9.11	*C26B2.8*	2.52
clec-233	-9.04	*F56F12.1*	2.51

F26C11.3	-8.94	*C47E8.9*	2.51
C35B1.3	-8.92	*F32D8.3*	2.51
Y73F8A.10	-8.90	*M153.5*	2.51
Y39B6A.9	-8.89	*ZK909.3*	2.50
CELE_F46B3.14	-8.83	*nas-29*	2.50
K02B12.9	-8.83	*C47E8.9*	2.50
K02B12.9	-8.62	*F14B8.5*	2.49
K02B12.9	-8.56	*ZK909.3*	2.49
K02B12.9	-8.50	*mam-8*	2.49
F47C12.7	-8.45	*Y39B6A.8*	2.49
clec-132	-8.40	*col-152*	2.48
Y46H3C.7	-8.35	*F16H9.2*	2.48
B0286.6	-8.34	*C27A2.5*	2.48
NP206106	-8.25	*F14B8.5*	2.48
Y26D4A.17	-8.20	*ZK909.3*	2.47
clec-216	-8.17	*hbl-1*	2.47
K08C9.6	-8.16	*grl-28*	2.47
W02B3.7	-8.14	*fip-6*	2.46
F35C5.4	-8.11	*egl-15*	2.46
F46B3.14	-7.98	*ZK131.3*	2.46
C10G8.2	-7.88	*TC209251*	2.46
F30H5.5	-7.80	*D32358*	2.45
CELE_F49F1.3	-7.79	*M01H9.5*	2.45
F15H9.7	-7.77	*ZK105.5*	2.45
C16C8.17	-7.67	*B0334.13*	2.45
F25D7.5	-7.65	*C47A10.13*	2.44
C04B4.6	-7.62	*K09B3.1*	2.44
T01A4.3	-7.61	*C26B2.8*	2.44
F46B3.14	-7.48	*C04B4.1*	2.44
clec-93	-7.44	*F49E12.8*	2.44
Y64G10A.10	-7.43	*nas-12*	2.44
clec-259	-7.43	*cutl-14*	2.43
F30H5.5	-7.38	*Y47D7A.15*	2.43

fipr-16	-7.38	*ZK909.3*	2.41
F02H6.6	-7.37	*pqm-96*	2.41
Y38H6C.18	-7.35	*T05D4.4*	2.41
Y7A5A.3	-7.20	*nas-8*	2.40
F49C5.7	-7.09	*ptr-17*	2.40
fipr-16	-7.07	*C02F5.14*	2.40
C55C3.7	-7.04	*sel-7*	2.40
C35B1.8	-6.82	*cnp-2*	2.40
ZK39.9	-6.82	*cut-4*	2.40
K02B12.6	-6.80	*clec-228*	2.39
F16G10.2	-6.79	*T05A7.9*	2.38
T04A6.2	-6.78	*K03B8.6*	2.38
F18E9.7	-6.74	*Y39B6A.8*	2.37
Y18H1A.8	-6.73	*sri-14*	2.37
C16C10.9	-6.72	*F39D8.3*	2.37
set-15	-6.70	*grl-1*	2.37
F15E11.12	-6.66	*pqn-29*	2.37
Y7A5A.3	-6.65	*pxl-1*	2.36
Y43F8C.23	-6.61	*F49E10.4*	2.36
Y67A10A.11	-6.60	*Y39B6A.8*	2.36
C28H8.7	-6.59	*Y41E3.22*	2.36
F40E12.1	-6.45	*D2045.9*	2.36
F49F1.10	-6.40	*Y39B6A.8*	2.35
C24A3.9	-6.35	*K10B3.1*	2.35
Y67D8C.7	-6.27	*D65308*	2.35
Y67D8C.7	-6.23	*C26F1.1*	2.35
lov-1	-6.21	*trx-3*	2.35
F09E10.1	-6.12	*ZK596.3*	2.35
C49C8.6	-6.12	*F25G6.7*	2.34
F11A3.4	-6.10	*C12D12.3*	2.34
F07G6.8	-6.03	*ceh-5*	2.34
xtr-2	-5.97	*haf-9*	2.34
Y67D8C.7	-5.94	*cnp-2*	2.34

F11A3.4	-5.89	*TC202604*	2.34
F28B1.3	-5.84	*B0454.8*	2.33
her-1	-5.79	*C52A10.2*	2.33
NP180076	-5.79	*let-756*	2.33
F46B3.14	-5.75	*col-75*	2.33
pkd-2	-5.69	*F38B7.2*	2.33
Y43F8C.15	-5.67	*hum-9*	2.32
F07G6.8	-5.67	*F17C11.1*	2.31
F46B3.14	-5.66	*Y54F10AM.8*	2.31
tyr-5	-5.65	*Y22D7AL.14*	2.30
clec-147	-5.64	*haf-9*	2.30
F57A10.2	-5.61	*cutl-4*	2.30
F59A6.3	-5.49	*ugt-51*	2.30
C24A3.9	-5.46	*Y41E3.22*	2.30
F13A2.5	-5.33	*T05A8.6*	2.30
trf-1	-5.32	*F38B7.2*	2.29
cpt-3	-5.27	*H08J11.2*	2.29
T05A8.7	-5.24	*T19D7.6*	2.29
R01E6.5	-5.16	*icl-1*	2.29
F59A6.3	-5.15	*C49A9.5*	2.29
Y64G10A.10	-5.03	*del-3*	2.29
dgn-3	-5.01	*F49E10.4*	2.28
Y48G8AR.3	-4.96	*ccb-2*	2.28
C08E3.14	-4.95	*C47A10.13*	2.28
Y17G7B.23	-4.93	*Y54F10AM.8*	2.28
F59D6.1	-4.93	*ugt-51*	2.28
Y57E12AL.4	-4.90	*grl-22*	2.27
her-1	-4.90	*srx-13*	2.27
F10G2.2	-4.88	*aakg-1*	2.26
F15E11.13	-4.84	*icl-1*	2.26
EB996415	-4.76	*F47B8.13*	2.26
F16G10.6	-4.75	*haf-9*	2.26
clec-74	-4.73	*Y41E3.22*	2.25

Y7A5A.3	-4.73	*F44G3.2*	2.25
C08E3.14	-4.71	*icl-1*	2.24
ZK39.9	-4.71	*col-2*	2.24
F09C6.13	-4.69	*dao-6*	2.23
Y110A2AL.1	-4.68	*C45G9.6*	2.23
ZK177.3	-4.67	*snet-1*	2.23
F36H1.12	-4.67	*cog-1*	2.22
F25D7.5	-4.67	*clec-21*	2.22
M163.9	-4.66	*F16H9.2*	2.22
F15H9.7	-4.65	*cog-1*	2.21
Y54G2A.38	-4.63	*K02A2.5*	2.21
F07G6.8	-4.61	*F14B8.5*	2.21
T12A7.3	-4.55	*F17C11.1*	2.21
T04A6.2	-4.55	*R04B3.3*	2.21
F14H12.6	-4.54	*D65291*	2.20
C32H11.4	-4.53	*dapk-1*	2.20
E01G4.5	-4.47	*C30H6.12*	2.20
C69955	-4.46	*T24A11.3*	2.19
Y19D10B.7	-4.44	*ZK131.1*	2.19
M163.9	-4.40	*ugt-51*	2.19
Y70G10A.2	-4.38	*cpz-2*	2.19
Y75B8A.33	-4.37	*sox-2*	2.18
T08D10.4	-4.33	*Y51H7C.10*	2.18
F02H6.6	-4.32	*gst-23*	2.18
F46B3.15	-4.28	*TC181947*	2.17
E01G4.5	-4.28	*T26E4.10*	2.17
clec-74	-4.26	*Y39B6A.8*	2.17
F11A3.4	-4.21	*T09B9.2*	2.16
ZC376.1	-4.18	*cutl-4*	2.16
M02D8.7	-4.14	*F56F12.1*	2.16
Y47D7A.12	-4.13	*rap-3*	2.15
F07G6.8	-4.10	*sem-2*	2.15
Y70G10A.2	-4.08	*F38E9.4*	2.15

F29A7.3	-4.08	*sox-2*	2.14
C07G3.10	-4.07	*haf-9*	2.13
M199.9	-4.05	*F56F12.1*	2.13
fipr-16	-4.02	*C34E11.2*	2.13
F11A3.4	-4.02	*F27D9.7*	2.12
Y51H7C.15	-3.99	*clec-52*	2.12
T01B6.4	-3.98	*Y54E10BL.1*	2.12
F07G6.8	-3.95	*C14A11.1*	2.11
T28A8.2	-3.94	*catp-3*	2.11
F59A6.11	-3.89	*F26G1.11*	2.11
C24A3.9	-3.86	*dyf-7*	2.10
F45D11.4	-3.83	*egl-15*	2.10
W08F4.7	-3.82	*lat-2*	2.10
Y13C8A.2	-3.82	*M03E7.2*	2.10
F15E11.1	-3.81	*catp-2*	2.10
NP176938	-3.80	*TC196650*	2.10
acp-6	-3.79	*R09H10.5*	2.09
M163.9	-3.77	*D2045.8*	2.09
F11A3.4	-3.77	*tsp-14*	2.08
C17G10.6	-3.73	*F26A10.2*	2.08
clec-176	-3.66	*Y37D8A.16*	2.08
str-55	-3.65	*ugt-25*	2.08
C32H11.3	-3.61	*C53A3.2*	2.07
W08F4.7	-3.60	*F01D4.8*	2.07
M01B12.4	-3.58	*Y37D8A.16*	2.07
Y24F12A.3	-3.53	*Y38F1A.8*	2.07
Y68A4A.13	-3.52	*F53C3.8*	2.07
C50H11.8	-3.50	*inx-16*	2.06
F07G6.8	-3.48	*TC207352*	2.06
C18F10.2	-3.47	*nas-7*	2.06
ZK792.1a	-3.46	*C33A12.6*	2.05
T02G5.4	-3.45	*C33E10.10*	2.05
Y50E8A.14	-3.43	*paqr-3*	2.05

W04A4.3	-3.43	*Y113G7B.12*	2.05
H19N07.3	-3.42	*C47A10.13*	2.04
T24E12.12	-3.39	*K10H10.6*	2.04
Y13C8A.1	-3.39	*sox-2*	2.04
K10B4.1	-3.38	*str-163*	2.04
NP024506	-3.37	*str-180*	2.04
F09C6.13	-3.37	*Y41E3.22*	2.04
T24C2.2	-3.34	*dyf-7*	2.03
M199.9	-3.26	*F31D4.5*	2.02
dut-1	-3.14	*nas-7*	2.02
TC201003	-3.12	*F13H10.1*	2.02
F49C12.7	-3.10	*F07A5.4*	2.02
T24E12.12	-3.05	*T24A11.3*	2.02
F22B7.3	-3.05	*dyf-7*	2.02
dut-1	-3.03	*C33E10.10*	2.02
F16H6.6	-3.01	*F02D8.1*	2.01
Y106G6H.10	-3.00	*cdf-2*	2.01
F47C12.11	-2.97	*lec-1*	2.00
dod-24	-2.95	*C53A3.2*	2.00
C14C6.13	-2.94	*dyf-7*	2.00
sri-20	-2.93	*best-23*	2.00
H37A05.4	-2.91	*F07A5.4*	2.00
tba-7	-2.89	*R09H10.7*	1.99
C31H5.7	-2.89	*sox-2*	1.99
C40H1.9	-2.89	*Y43F8B.2*	1.99
C26E1.1	-2.89	*sams-1*	1.99
T04A6.2	-2.87	*F17C11.1*	1.99
T26H5.9	-2.86	*F47E1.2*	1.99
C18A11.4	-2.85	*K08C7.4*	1.99
H37A05.4	-2.84	*T24A11.3*	1.98
C17F4.11	-2.83	*T11F9.6*	1.98
clec-203	-2.81	*ZC123.4*	1.98
oac-31	-2.81	*nhr-77*	1.98

Y11D7A.16	-2.80	*K09H11.7*	1.98
F02H6.6	-2.80	*F54D5.15*	1.98
T26H5.9	-2.79	*let-756*	1.97
grd-3	-2.79	*ugt-51*	1.97
D1086.2	-2.77	*gon-1*	1.97
cwp-3	-2.77	*C15H9.2*	1.96
M02D8.7	-2.76	*nas-7*	1.96
ZC53.6	-2.73	*sox-2*	1.96
Y49G5A.1	-2.71	*F25E5.8*	1.96
C29F9.5	-2.70	*F17C11.1*	1.96
Y11D7A.16	-2.68	*ugt-8*	1.96
H37A05.4	-2.68	*R05H5.7*	1.95
nspe-1	-2.68	*sox-2*	1.95
K07A1.1	-2.66	*EF491744*	1.95
pyc-1	-2.65	*F34D6.6*	1.94
T26C11.8	-2.64	*W02F12.2*	1.93
F14D7.11	-2.64	*col-102*	1.93
tdc-1	-2.63	*T24A11.3*	1.93
F28H6.7	-2.62	*F13D2.4*	1.93
D2096.10	-2.62	*mnm-2*	1.93
srw-33	-2.62	*hlh-32*	1.93
TC204851	-2.61	*ZK675.3b*	1.93
mab-23	-2.60	*F19C6.5*	1.93
K07A1.1	-2.59	*egl-15*	1.92
T24E12.12	-2.59	*T11F9.6*	1.92
mab-23	-2.58	*dgk-2*	1.92
clec-100	-2.57	*zig-6*	1.92
R05H10.1	-2.54	*F16F9.4*	1.92
Y7A5A.3	-2.53	*C17B7.9*	1.91
H15N14.4	-2.51	*best-23*	1.91
Y11D7A.16	-2.51	*T24A11.3*	1.91
nspd-9	-2.49	*C14H10.2*	1.91
F25B3.2	-2.46	*col-34*	1.91

nspe-1	-2.45	*C14A11.1*	1.91
mab-23	-2.45	*ifd-1*	1.90
nspe-1	-2.45	*lin-44*	1.90
fbxb-7	-2.44	*H23N18.4*	1.90
nspe-1	-2.42	*fkh-7*	1.89
Y24F12A.3	-2.42	*chw-1*	1.89
ZK829.1	-2.41	*miz-1*	1.89
K07A1.13	-2.40	*Y77E11A.14*	1.89
T21C9.11	-2.39	*R05H5.7*	1.89
F31B9.2	-2.37	*zig-6*	1.89
C43D7.4	-2.37	*T11F9.6*	1.89
F47C12.11	-2.36	*eff-1*	1.88
cat-1	-2.36	*sox-2*	1.88
F40H6.5	-2.34	*F46C3.3*	1.88
F28F9.2	-2.33	*ZK593.3*	1.87
sdz-6	-2.32	*T11F9.6*	1.87
C01B4.6	-2.31	*gln-3*	1.87
ZK829.1	-2.30	*lin-44*	1.87
F46B3.14	-2.30	*C25H3.15*	1.87
dmsr-2	-2.29	*F23F1.4*	1.87
clec-6	-2.28	*ZK675.3b*	1.87
F59B2.11	-2.27	*R102.11*	1.87
F45D11.1	-2.26	*lin-44*	1.86
ins-25	-2.25	*fbxa-6*	1.86
swt-6	-2.24	*Y65B4A.2*	1.86
Y43B11AR.1	-2.23	*K09H11.7*	1.86
C06E1.1	-2.21	*gst-23*	1.86
R04A9.1	-2.20	*T25D10.4*	1.86
ssp-31	-2.20	*crb-1*	1.85
C43D7.4	-2.20	*W02B12.13*	1.85
Y38E10A.2	-2.19	*K07E8.6*	1.85
mab-23	-2.19	*R07G3.8*	1.85
K07A1.1	-2.15	*TC201289*	1.85

C06E1.1	-2.14	*R07E5.4*	1.85
C01B4.9	-2.13	*TC192520*	1.85
F46B3.15	-2.12	*unc-129*	1.85
ser-7	-2.12	*lin-44*	1.85
T24E12.12	-2.11	*F52E1.9*	1.84
ZC168.2	-2.11	*H23N18.4*	1.84
F20B6.1	-2.11	*ZK1307.7*	1.84
aqp-6	-2.11	*gst-23*	1.84
F45D11.1	-2.10	*nac-2*	1.83
T06G6.4	-2.10	*Y57G11B.2*	1.83
faah-3	-2.09	*Y57E12AR.1*	1.83
lips-14	-2.07	*ugt-1*	1.83
swt-6	-2.06	*nhr-277*	1.83
clec-112	-2.05	*Y55D5A.4*	1.83
T28D6.3	-2.05	*C52E2.4*	1.82
ins-25	-2.04	*R12E2.6*	1.82
Y7A5A.5	-2.03	*lin-44*	1.82
F55G11.5	-2.03	*fbxa-6*	1.82
ins-25	-2.02	*zig-4*	1.82
T02G5.3	-2.02	*F18E9.4*	1.82
tph-1	-2.01	*ZK470.2*	1.82
C06E1.1	-2.01	*zig-6*	1.82
tph-1	-2.01	*T12A7.6*	1.81
T13F3.7	-2.00	*Y45F10D.14*	1.81
Y71F9AL.4	-2.00	*gsto-3*	1.81
F01D5.2	-1.99	*F23C8.11*	1.81
Y26D4A.19	-1.98	*str-168*	1.81
Y38C1AA.12	-1.98	*faah-2*	1.81
F07C3.3	-1.96	*npa-1*	1.80
TC194584	-1.96	*ZK180.2*	1.80
ssp-31	-1.95	*TC197696*	1.80
W03G9.7	-1.95	*T05E7.4*	1.80
Y40A1A.3	-1.95	*T02D1.5*	1.80

F47G9.6	-1.93	*C06E2.1*	1.80
ins-39	-1.92	*lam-1*	1.80
ser-7	-1.92	*M88.4*	1.80
K07A1.17	-1.91	*Y6G8.1*	1.80
F31F7.1	-1.91	*K02E11.7*	1.79
Y110A2AL.4	-1.90	*ZK54.3*	1.79
F31F7.1	-1.90	*Y62H9A.12*	1.79
cat-1	-1.89	*F30F8.5*	1.79
C15A11.7	-1.88	*Y39A3CR.5*	1.79
hpo-15	-1.88	*AC3.5.3*	1.79
Y75B8A.33	-1.88	*lips-11*	1.79
F59B8.1	-1.87	*Y17D7C.1*	1.78
TC199465	-1.87	*C24H10.1*	1.78
K08D8.5	-1.87	*EF491744*	1.78
F08H9.2	-1.87	*unc-78*	1.78
K01D12.2	-1.86	*str-7*	1.78
F42H11.2.2	-1.86	*Y57E12AR.1*	1.78
F20B6.7	-1.85	*C45B2.2*	1.78
ugt-3	-1.85	*col-50*	1.78
nep-18	-1.84	*C23H4.7*	1.78
F55G7.1	-1.84	*hum-4*	1.78
T25B9.10	-1.83	*Y57G11A.3*	1.77
W09C3.3	-1.83	*C05A9.2*	1.77
K02A2.7	-1.83	*F46C5.1*	1.77
TC203195	-1.83	*K11E4.2*	1.77
nep-18	-1.82	*F48G7.10*	1.77
K08D8.5	-1.82	*C06B3.7*	1.77
F59B8.1a	-1.82	*W02B12.13*	1.77
F19C6.4	-1.81	*F08A8.3*	1.77
oac-33	-1.81	*F28H7.7*	1.77
K10C2.3	-1.80	*gst-19*	1.76
TC203195	-1.80	*pqn-89*	1.76
ins-24	-1.80	*ZC373.5*	1.76

riok-1	-1.79	*F14E5.1*	1.76
K08D8.5	-1.79	*Y57E12AR.1*	1.76
nep-18	-1.79	*acp-5*	1.76
K08D8.5	-1.79	*nhr-156*	1.76
		EF491744	1.75
C52672	-1.79	*NP023739*	1.75
F11D5.7	-1.78	*nhr-58*	1.75
C32H11.1	-1.78	*ugt-34*	1.74
srsx-34	-1.78	*T07E3.4*	1.74
T28D6.3	-1.78	*far-3*	1.74
F01D5.3	-1.77	*C13C4.8*	1.74
T12B5.12	-1.77	*T27E4.8*	1.74
K08D8.5	-1.75	*Y57E12AR.1*	1.73
clec-12	-1.75	*str-168*	1.73
T28D6.3	-1.75	*H08J11.2*	1.73
TC203195	-1.74	*M162.5*	1.73
T13A10.2	-1.74	*F44A2.5*	1.73
B0238.9	-1.74	*Y23B4A.2*	1.73
gad-2	-1.74	*C01B10.10*	1.72
F31F7.1	-1.74	*C30G4.4a.1*	1.72
ins-25	-1.74	*T05E11.2*	1.72
TC203195	-1.73	*R07G3.8*	1.72
C07B5.3	-1.73	*str-168*	1.72
ZK896.4	-1.72	*pmp-5*	1.72
F59B8.1a	-1.72	*TC207352*	1.72
K10D11.3	-1.72	*ZK1307.7*	1.72
T23B7.3	-1.72	*cdr-1*	1.72
F55G7.1	-1.71	*ugt-34*	1.72
F21C10.11	-1.71	*C36B7.5*	1.71
ins-7	-1.68	*zig-9*	1.71
snb-7	-1.68	*glt-5*	1.71
F11D5.7	-1.68	*K09C4.6*	1.71
Y110A7A.2	-1.67	*C13F10.1b*	1.71

snb-5	-1.67	*ifb-1*	1.70
Y19D10A.8	-1.67	*T04F8.6*	1.70
B0310.3	-1.65	*mef-2*	1.70
col-40	-1.65	*lys-6*	1.70
F56A4.10	-1.65	*srab-12*	1.69
T27E7.1	-1.65	*npa-1*	1.69
Y50D4B.2	-1.64	*T28H10.3*	1.69
Y45G12C.12	-1.64	*dgk-2*	1.69
C29E4.13	-1.63	*nlp-23*	1.69
B0507.8	-1.62	*ZK1086.2*	1.69
pdk-1	-1.62	*unc-49*	1.69
C08A9.1.2	-1.62	*F18E9.4*	1.69
C01G6.10	-1.62	*C30G4.4*	1.69
Y19D10A.11	-1.62	*R10H1.1*	1.69
C07A12.2	-1.62	*T02D1.5*	1.69
gpa-13	-1.61	*spp-4*	1.69
C54C6.7	-1.61	*ZC373.5*	1.69
ncx-6	-1.60	*R11F4.3*	1.68
C53156	-1.60	*mef-2*	1.68
F11D5.7	-1.59	*K07D4.5*	1.68
W03B1.6	-1.59	*TC200301*	1.68
C01B4.8	-1.58	*dnj-24*	1.68
R05G6.1	-1.58	*F13D2.4*	1.68
fog-1	-1.57	*TC204135*	1.68
F49C12.9	-1.57	*B0034.5*	1.68
sax-2	-1.57	*F13B12.2*	1.68
F07B7.2	-1.57	*C30G4.4*	1.68
ZK896.1	-1.57	*pqn-89*	1.68
C03C10.7	-1.57	*T02D1.5*	1.68
C30276	-1.56	*T11B7.2*	1.67
C39H7.1	-1.56	*F59B10.6*	1.67
T23B7.3	-1.56	*C45B2.2*	1.67
ZC196.4	-1.56	*K05B2.3*	1.67

T01D3.6	-1.56	*php-3*	1.67
M6.4	-1.56	*snt-7*	1.67
Y57A10A.1	-1.55	*F46C5.2*	1.67
Y11D7A.3	-1.55	*pkc-2*	1.67
C60074	-1.55	*ins-12*	1.67
C56250	-1.54	*clec-170*	1.67
C38687	-1.54	*unc-49*	1.67
D33204	-1.53	*T07F10.6*	1.67
frpr-1	-1.53	*T04C12.8*	1.67
Y26D4A.17	-1.53	*R07G3.8*	1.67
F31D5.2	-1.53	*unc-43*	1.67
C23H4.6	-1.53	*C30G4.7*	1.67
kcc-3	-1.52	*C30G4.4*	1.66
C60074	-1.52	*R07G3.8*	1.66
TC208147	-1.51	*T04F8.6*	1.66
TC187462	-1.51	*ZK470.2*	1.66
C17D12.3	-1.51	*F38E9.5*	1.65
C33D9.9	-1.51	*ZK652.8*	1.65
K08D8.4	-1.51	*C38H2.3*	1.65
H12D21.13	-1.50	*ilys-3*	1.65
R02F2.8	-1.50	*soc-2*	1.65
C56250	-1.50	*F20D6.10*	1.65
fog-1	-1.50	*F13B12.2*	1.65
C53318	-1.50	*K11G9.5*	1.65
C15F1.8	-1.49	*aakb-1*	1.65
cyc-2.2	-1.49	*C55C3.1*	1.65
R102.1	-1.49	*gnrr-3*	1.65
D33390	-1.49	*C36B7.6*	1.65
D32534	-1.49	*W07G4.5*	1.65
C27A7.5	-1.49	*oac-54*	1.64
D33478	-1.48	*srg-48*	1.64
TC178793	-1.48	*K12C11.3*	1.64
TC191869	-1.48	*ifb-1*	1.64

D33478	-1.48	*ins-38*	1.64
D32534	-1.47	*Y45G12C.2.2*	1.64
ksr-2	-1.47	*ZC373.5*	1.64
clec-4	-1.47	*pmp-5*	1.64
W04A8.2	-1.47	*T28H10.3*	1.64
rab-3	-1.46	*T14G8.4*	1.64
C36759	-1.46	*gap-1*	1.63
D32534	-1.46	*F13G3.12*	1.63
Y50D4B.3	-1.46	*elo-9*	1.63
tni-3	-1.45	*K12C11.6*	1.63
C06A5.12	-1.45	*dve-1*	1.63
fbxa-190	-1.45	*C36B7.6*	1.63
lst-4	-1.44	*T28H10.3*	1.63
W04A8.2	-1.44	*math-50*	1.62
spe-15	-1.44	*lim-6*	1.62
C28A5.2	-1.43	*ZC15.4*	1.62
W02B12.15	-1.43	*H36L18.2*	1.62
F27D4.1	-1.43	*D71242*	1.62
C10385	-1.43	*unc-45*	1.62
C06A5.12	-1.42	*T28H10.3*	1.62
K08D8.6	-1.42	*C23H4.3*	1.62
C10385	-1.42	*inx-20*	1.62
fbxa-189	-1.42	*nhr-284*	1.62
C09B9.4	-1.41	*B0302.5*	1.62
cwp-4	-1.41	*unc-44*	1.62
C01B4.7	-1.41	*zip-9*	1.61
Y116A8A.10	-1.40	*ifb-1*	1.61
pabp-2	-1.40	*ifb-2*	1.61
K08D8.4	-1.40	*F43G6.4*	1.61
D33478	-1.40	*pfn-2*	1.61
ZK512.4	-1.40	*Y37A1B.11*	1.61
D2096.12	-1.40	*mpz-2*	1.61
C53134	-1.39	*C35B8.4*	1.61

F19F10.11	-1.39	*F22H10.1*	1.61
TC178793	-1.39	*M04C7.4*	1.61
F08F3.9	-1.39	*syd-9*	1.61
W04A8.2	-1.39	*C36B7.6*	1.61
F41G3.10	-1.38	*Y58A7A.1*	1.61
ced-5	-1.38	*C49F5.5*	1.60
F09E5.16	-1.38	*Y77E11A.12*	1.60
C10385	-1.38	*lev-8*	1.60
M70.1	-1.38	*C06B8.2*	1.60
D33204	-1.38	*clec-54*	1.60
ave-1	-1.38	*C06B8.2b*	1.60
W04A8.2	-1.38	*C05D10.4*	1.60
rod-1	-1.38	*F09A5.3*	1.60
C03H5.4	-1.38	*F28F5.6*	1.60
Y119D3B.12	-1.37	*kel-8*	1.60
F55G11.2	-1.37	*ZC196.9*	1.60
C29951	-1.37	*ceh-18*	1.60
set-17	-1.37	*TC188135*	1.60
ptc-1	-1.37	*tmd-2*	1.60
spe-15	-1.37	*K03E6.1*	1.59
dnj-22	-1.37	*W05H9.3*	1.59
dnj-28	-1.37	*dct-15*	1.59
C14A11.6	-1.36	*glt-3*	1.59
F59E12.1	-1.36	*lim-6*	1.59
F09E5.16	-1.36	*inx-15*	1.59
T27E4.1	-1.36	*npr-31*	1.59
H12D21.13	-1.36	*twk-13*	1.59
clec-179	-1.36	*K12C11.6*	1.59
cyk-4	-1.36	*col-84*	1.59
F27D4.1	-1.36	*ckb-2*	1.59
syp-4	-1.36	*ifb-1*	1.59
tag-312	-1.35	*gst-29*	1.59
C17H12.6	-1.35	*K09H11.6*	1.59

Y116F11B.10	-1.35	*W03F8.10*	1.58
R10D12.8	-1.35	*R09A8.5*	1.58
pnk-1	-1.35	*twk-13*	1.58
Y57G11C.9	-1.35	*vab-15*	1.58
C29951	-1.35	*H40L08.2*	1.58
C09E7.8	-1.35	*cor-1*	1.58
hcp-2	-1.35	*prl-1*	1.58
Y54G2A.41	-1.35	*sgk-1*	1.58
T27E4.1	-1.35	*aco-1*	1.58
D33204	-1.34	*W03F8.10*	1.58
F37C4.5	-1.34	*F13H6.1b*	1.58
C56318	-1.33	*prl-1*	1.58
K11D12.8	-1.33	*K02E10.6*	1.57
TC207889	-1.33	*T18D3.7*	1.57
C10C6.7	-1.33	*C35B8.4*	1.57
C56318	-1.33	*ZC334.12*	1.57
F37C4.5	-1.33	*rgl-1*	1.57
K07D4.1	-1.33	*gar-1*	1.57
F09E5.16	-1.33	*C14A4.7*	1.57
madf-7	-1.33	*aakb-1*	1.57
D33204	-1.33	*T03F7.1.2*	1.57
spe-8	-1.33	*zip-9*	1.57
B0564.11	-1.33	*mpz-2*	1.57
C37017	-1.33	*dve-1*	1.57
C30884	-1.32	*dgk-2*	1.57
set-23	-1.32	*C14F5.4*	1.57
Y54F10BM.9	-1.32	*K02E10.7*	1.56
K03D7.1	-1.32	*unc-89*	1.56
EC035882	-1.32	*gsto-3*	1.56
Y32B12B.4	-1.32	*col-153*	1.56
C55286	-1.32	*cars-1*	1.56
pnc-1	-1.32	*lgc-31*	1.56
ZK1098.1	-1.32	*prl-1*	1.56

ZK686.4	-1.32	*mef-2*	1.56
dom-3	-1.31	*F58F12.4*	1.56
W02D7.6	-1.31	*F52H2.6*	1.56
Y54G2A.50	-1.31	*C14A4.7*	1.56
par-2	-1.31	*F21D12.3*	1.56
sid-1	-1.31	*H36L18.2*	1.55
F54D12.4	-1.31	*cwn-1*	1.55
cdk-9	-1.31	*T05C3.6*	1.55
F57G4.9	-1.31	*NP488953*	1.55
C56318	-1.31	*B0554.7*	1.55
ess-2	-1.31	*umps-1*	1.55
F37C4.5	-1.31	*R03G5.1b.2*	1.55
ncs-7	-1.30	*K10B3.6*	1.55
rsa-2	-1.30	*R05H5.7*	1.55
F42G4.5	-1.30	*C55F2.1*	1.55
Y54G11A.9	-1.30	*C46G7.5*	1.55
Y20F4.5	-1.30	*snt-7*	1.55
Y54G2A.50	-1.30	*F19C6.2*	1.55
cat-4	-1.30	*prl-1*	1.55
ZK550.4	-1.30	*zig-1*	1.55
T10D4.6	-1.30	*C18B2.3*	1.55
F09E5.16	-1.30	*fah-1*	1.54
hpo-38	-1.30	*Y44E3A.1*	1.54
H35B03.1	-1.29	*lbp-3*	1.54
C06A5.5	-1.29	*F59F5.5*	1.54
C55286	-1.29	*ztf-16*	1.54
ZK381.1	-1.29	*B0462.4*	1.54
rpa-2	-1.29	*bli-3*	1.54
swp-1	-1.29	*twk-13*	1.53
D33246	-1.29	*F39C12.4*	1.53
F52C9.7.2	-1.29	*ZK154.6*	1.53
TC207889	-1.29	*T01E8.8*	1.53
snr-3	-1.29	*dod-3*	1.53

Y116A8C.16b	-1.29	*ndnf-1*	1.53
pabp-2	-1.29	*F19C6.2*	1.53
glh-4	-1.28	*ZC334.12*	1.53
Y48A5A.1	-1.28	*F19C6.2*	1.53
ppw-1	-1.28	*flr-2*	1.53
Y80D3A.9	-1.28	*C34F6.5*	1.53
K06A5.1	-1.28	*ztf-16*	1.53
C44C1.6	-1.28	*F19C6.2*	1.53
Y67H2A.2	-1.28	*F58F12.4*	1.53
npp-16	-1.28	*tre-3*	1.53
frpr-14	-1.28	*TC208144*	1.53
Y71H2B.4	-1.28	*C15H9.5*	1.53
K09C4.1	-1.27	*kel-8*	1.53
C55A6.9	-1.27	*C27B7.7*	1.53
pme-3	-1.27	*K12C11.6*	1.53
sel-10	-1.27	*T23B3.2*	1.53
ZK897.1	-1.27	*nhr-105*	1.52
Y116A8C.16a	-1.27	*nhr-97*	1.52
rad-50	-1.26	*nucb-1*	1.52
ufd-3	-1.26	*kel-8*	1.52
pnk-1	-1.26	*F39C12.4*	1.52
swp-1	-1.26	*T28H11.8*	1.52
rsd-2	-1.26	*sto-5*	1.52
ZK1025.1	-1.26	*calu-1*	1.52
Y48G1BL.4	-1.25	*F39C12.4*	1.52
C06E1.9	-1.25	*D71847*	1.52
C55286	-1.25	*F36H1.6*	1.52
Y67D2.4	-1.25	*ugt-5*	1.52
cpb-1	-1.25	*TC209250*	1.52
snr-3	-1.25	*F32D8.10*	1.52
mage-1	-1.24	*kel-8*	1.52
math-43	-1.24	*ttr-38*	1.52
T07A9.10	-1.24	*unc-44*	1.52

W04D2.6	-1.24	*F41G3.18.1*	1.52
T28D6.5a	-1.24	*tag-253*	1.52
D33246	-1.24	*ceh-37*	1.52
Y43C5A.3	-1.23	*gar-1*	1.52
Y49E10.4	-1.23	*mboa-7*	1.52
C26E6.9a	-1.23	*nhr-105*	1.52
cux-7	-1.23	*F58F12.4*	1.51
msh-6	-1.23	*eat-16*	1.51
K08H10.9	-1.23	*ZC317.2*	1.51
ubxn-4	-1.23	*F08G12.11*	1.51
T20D3.8	-1.23	*clh-4*	1.51
F56D12.6	-1.23	*shl-1*	1.51
mdt-26	-1.23	*K07C11.8*	1.51
ceh-48	-1.23	*kel-8*	1.51
pme-3	-1.22	*kel-8*	1.51
ZK370.7	-1.22	*C07B5.4*	1.51
hpo-10	-1.22	*Y50E8A.1*	1.51
C41D11.3	-1.22	*F41G3.18.2*	1.51
knl-1	-1.22	*dhs-2*	1.50
eri-3	-1.22	*C46C2.6*	1.50
C23G10.7	-1.21	*Y44E3A.1*	1.50
B0495.2	-1.21	*prx-5*	1.50
strd-1	-1.21	*ugt-50*	1.50
pis-1	-1.21	*apl-1*	1.50
npp-14	-1.21	*F55F3.2*	1.50
ZC477.3	-1.21	*acs-5*	1.50
ZK652.6	-1.21	*C05D12.7*	1.50
B0035.16	-1.21	*R05A10.4*	1.50
TC200571	-1.20	*nhr-97*	1.50
F58E10.3	-1.20	*F16H11.4*	1.50
mel-11	-1.20	*F16H11.4*	1.50
K01G5.3	-1.20	*T02D1.5*	1.50
mop-25.2	-1.19	*nhr-105*	1.50

C08B6.7	-1.19	*mpz-2*	1.50
Y37E11B.6	-1.19	*F41B4.3*	1.50
ZK1010.3	-1.19	*nipi-3*	1.50
lact-3	-1.19	*Y43B11AR.6*	1.50
T12E12.4a.1	-1.19	*T11B7.4b*	1.50
B0001.3	-1.18	*prx-3*	1.50
H34C03.2	-1.18	*C34E7.3*	1.50
F43G6.11	-1.18	*glr-8*	1.50
C15H11.4	-1.18	*Y54E10A.16b*	1.49
C09G9.1	-1.18	*cft-1*	1.49
F10G7.9	-1.18	*eat-16*	1.49
R07E5.7	-1.18	*T02H6.6*	1.49
Y41E3.7	-1.17	*acs-17*	1.49
Y48C3A.8	-1.17	*F36H1.6*	1.49
D2089.1a	-1.17	*zig-1*	1.49
cul-2	-1.17	*Y54E10A.16b*	1.49
efa-6	-1.17	*cyp-33C5*	1.49
npp-16	-1.17	*bre-1*	1.49
ZK1127.6	-1.17	*C43H6.1*	1.49
F38A5.13	-1.17	*nhr-97*	1.49
ZK1127.12	-1.16	*C14A4.7*	1.49
guk-1	-1.16	*tag-163*	1.49
Y57A10A.7	-1.16	*ceh-18*	1.49
pqe-1	-1.16	*K03E6.7*	1.49
wsp-1	-1.16	*C43H6.1*	1.49
jmjd-1.2	-1.16	*F56F12.1*	1.49
C16A3.8	-1.16	*ifb-1*	1.48
F55C12.5	-1.16	*acs-5*	1.48
D34290	-1.15	*mec-1*	1.48
C08F8.2	-1.15	*fbxa-219*	1.48
symk-1	-1.15	*str-7*	1.48
F44E2.10	-1.15	*F28A10.3*	1.48
bpl-1	-1.15	*T19C3.4*	1.48

NP016715	-1.15	*lbp-3*	1.48
Y73B3A.9	-1.15	*spon-1*	1.48
hrp-1	-1.15	*sto-5*	1.48
M01E5.3	-1.14	*R05H5.7*	1.48
Y41E3.7	-1.14	*cyp-25A3*	1.48
rad-26	-1.14	*F54C8.4*	1.48
Y59A8A.1	-1.14	*aagr-2*	1.48
tcer-1	-1.14	*C43H6.1*	1.48
H06I04.1	-1.14	*ZC334.12*	1.48
npp-10	-1.13	*apl-1*	1.48
Y87G2A.10b	-1.13	*T08A9.13*	1.48
lst-3	-1.12	*fbl-1*	1.48
rga-1	-1.12	*acs-17*	1.48
Y116A8C.34.2	-1.12	*lurp-1*	1.47
spe-5	-1.12	*nmy-1*	1.47
Y41E3.7	-1.12	*nhr-146*	1.47
F21F3.6	-1.12	*ugt-5*	1.47
dcr-1	-1.11	*nhr-105*	1.47
C05G5.3	-1.11	*nhr-142*	1.47
T05A12.3	-1.11	*T08A9.13*	1.47
R12C12.8	-1.10	*pbo-5*	1.47
noca-1	-1.10	*aagr-2*	1.47
F55A11.3	-1.09	*acs-5*	1.47
cyk-1	-1.09	*gst-33*	1.47
ddx-23	-1.05	*nipi-3*	1.47
		ucr-2.1	1.47
		elk-2	1.47
		cal-2	1.47
		ttr-48	1.47
		cTel55X.1	1.47
		dhs-2	1.47
		C07B5.4	1.47
		F59E11.7	1.47

	nhr-32	1.47
	AF319615	1.47
	nhr-143	1.46
	Y39B6A.7	1.46
	ugt-33	1.46
	klp-11	1.46
	M03A8.3	1.46
	acs-17	1.46
	col-187	1.46
	ZC376.3	1.46
	rcn-1	1.46
	Y51H7BR.4	1.46
	F48G7.9	1.46
	ceh-18	1.46
	nkat-1	1.46
	ZC334.12	1.46
	C50B8.6	1.46
	F25H5.4.4	1.46
	NP395157	1.46
	prx-3	1.45
	C06A8.8b	1.45
	F35B12.9	1.45
	T19C4.5	1.45
	T23G5.6	1.45
	mtrr-1	1.45
	F01G4.6	1.45
	F47F2.1	1.45
	srd-74	1.45
	R12E2.4a	1.45
	ZK593.1	1.44
	cars-1	1.44
	kqt-2	1.44
	mtrr-1	1.44

	nhr-104	1.44
	TC189707	1.44
	deb-1	1.44
	NP435080	1.44
	F59B1.2	1.44
	cars-1	1.44
	M60.6	1.43
	F34H10.3	1.43
	nhr-94	1.43
	R09H10.5	1.43
	nmr-2	1.43
	ceh-18	1.43
	ZK593.1	1.43
	eat-16	1.43
	Y48G1BR.1	1.43
	pept-3	1.43
	T26E4.9	1.43
	T01G6.8	1.43
	Y37F4.8	1.42
	eat-16	1.42
	F31E3.2	1.42
	F31E3.2	1.42
	ztf-16	1.42
	R11G1.6	1.42
	kgb-2	1.42
	R11G10.3	1.42
	R11G1.6	1.42
	TC200062	1.42
	dct-10	1.42
	T28C6.8	1.42
	C01B10.6	1.42
	M60.6	1.41
	ZK1193.4	1.41

	eat-16	1.41
	F49E2.1	1.41
	ain-1	1.41
	npr-16	1.41
	srh-70	1.41
	mab-31	1.41
	T01A4.3	1.41
	F49E2.1	1.41
	T21G5.2	1.41
	odd-1	1.41
	tag-163	1.41
	pcp-5	1.41
	unc-62	1.41
	F08F3.10	1.41
	F53H4.4	1.40
	mtrr-1	1.40
	F26F2.8	1.40
	F22A3.2	1.40
	F53H4.4	1.40
	elt-7	1.40
	C35B1.7	1.40
	R11G1.6	1.40
	egl-9	1.40
	glb-17	1.40
	C56G2.3	1.40
	aat-2	1.40
	C01B10.6	1.40
	T02B11.4	1.40
	B0280.8	1.40
	Y69A2AR.7	1.40
	C54D2.2	1.40
	mup-2	1.40
	C07B5.4	1.40

	C46E1.3	1.39
	F49E2.1	1.39
	alh-4	1.39
	Y53F4B.42	1.39
	F14B6.6	1.39
	pkg-1	1.39
	acs-17	1.39
	Y66D12A.13	1.39
	cdka-1	1.39
	vha-12	1.39
	Y47H10A.4	1.39
	ceh-37	1.39
	Y51H7BR.3	1.39
	C54F6.6	1.39
	clec-56	1.39
	W10G11.17	1.39
	T25F10.6	1.39
	C24A3.4	1.39
	F16H6.10	1.39
	T04A11.14	1.39
	Y18H1A.12	1.39
	gpa-11	1.39
	Y48B6A.6	1.39
	unc-62	1.39
	ldb-1	1.39
	npr-12	1.38
	nhr-19	1.38
	C39B10.1	1.38
	cyp-34A6	1.38
	crh-1	1.38
	prx-5	1.38
	eat-16	1.38
	nas-31	1.38

	F18G5.6	1.38
	tag-163	1.38
	F59E11.7	1.38
	alh-12	1.38
	T16G12.1	1.38
	Y54G9A.9.1	1.38
	R09F10.5	1.38
	F42E11.1	1.38
	T23F11.6	1.38
	K08F8.1	1.38
	C06G8.3a	1.38
	gba-3	1.38
	islo-1	1.37
	ins-36	1.37
	egl-9	1.37
	Y97E10AR.2	1.37
	R57.1	1.37
	M04F3.4	1.37
	nas-31	1.37
	eat-16	1.37
	glt-7	1.37
	pms-2	1.37
	dhs-18	1.37
	C28G1.6	1.37
	K06A9.2	1.37
	R12E2.11	1.37
	F53H4.4	1.37
	C24A3.4	1.37
	K08F8.1	1.37
	ins-36	1.37
	sptf-1	1.37
	C06H5.6	1.36
	R05G6.10	1.36

	C71236	1.36
	T23G5.6	1.36
	Y46H3A.4	1.36
	glb-13	1.36
	catp-5	1.36
	F49E2.1	1.36
	egl-9	1.36
	egl-44	1.36
	C33A11.1	1.36
	K03H6.1	1.36
	nhr-116	1.36
	R05G6.10	1.36
	C44B7.10.3	1.36
	nas-31	1.36
	C34D10.1	1.36
	TC206236	1.35
	dhs-21	1.35
	F40G9.5	1.35
	dop-3	1.35
	egl-9	1.35
	vha-16	1.35
	T02D1.8	1.35
	oat-1	1.35
	C35E7.4	1.35
	R151.2	1.34
	Y59C2A.1	1.34
	T21H8.5	1.34
	ZC190.2	1.34
	cnb-1	1.34
	vha-13	1.34
	TC189707	1.34
	M04F3.4	1.34
	oat-1	1.34

	vha-8	1.34
	K09E2.3	1.34
	sulp-4	1.34
	M04F3.4	1.34
	rhr-2	1.34
	cex-2	1.34
	Y18H1A.12	1.34
	T01C1.4	1.34
	Y51B9A.6	1.34
	nuo-4	1.34
	nhr-184	1.34
	ztf-16	1.34
	asm-1	1.34
	snb-2	1.33
	F02C12.1	1.33
	Y69H2.15	1.33
	oat-1	1.33
	sptf-1	1.33
	acl-8	1.33
	F18G5.6	1.33
	F13E6.4	1.33
	cyp-33D3	1.33
	H03A11.1	1.33
	tat-4	1.33
	mtp-18	1.33
	R05F9.6	1.33
	C17E7.10	1.33
	T13H5.1	1.33
	nhr-12	1.33
	K08F8.1	1.33
	unc-44	1.33
	Y66H1B.3.1	1.32
	fkb-1	1.32

	R07E3.7	1.32
	H32K21.1	1.32
	F10G8.5	1.32
	R07D5.2	1.32
	prx-13	1.32
	H32K21.1	1.32
	fkb-1	1.32
	npr-31	1.32
	Y56A3A.36	1.32
	M04F3.4	1.31
	D2021.4	1.31
	nhr-184	1.31
	unc-69	1.31
	TC181997	1.31
	gob-1	1.31
	nas-31	1.31
	ztf-16	1.31
	M176.5	1.31
	H32K21.1	1.31
	H32K21.1	1.31
	F17H10.1	1.31
	H32K21.1	1.30
	ZK180.2	1.30
	ztf-16	1.30
	pes-22	1.30
	K02G10.1	1.30
	C24A3.4	1.30
	C33A11.1	1.30
	Y57G11C.41	1.30
	unc-44	1.30
	nlp-17	1.30
	K10B2.2	1.30
	F53H4.4	1.30

	Y87G2A.4	1.30
	oig-5	1.30
	frpr-17	1.30
	F53H4.4	1.30
	btb-14	1.30
	F13E6.4	1.30
	F48E3.8	1.30
	vps-45	1.30
	C06H5.6	1.30
	D1046.5	1.30
	C53A3.1	1.29
	T11B7.4b	1.29
	F41G4.7	1.29
	C06H5.6	1.29
	egl-9	1.29
	K12B6.9	1.29
	C06H5.6	1.29
	M01H9.4	1.29
	mps-4	1.29
	flp-10	1.29
	lgc-47	1.29
	F48E3.8	1.29
	cpr-6	1.29
	vha-16	1.29
	C05C12.4	1.29
	F41C3.8	1.28
	T19E7.6	1.28
	rab-19	1.28
	Y119D3B.17	1.28
	egl-44	1.28
	C12D12.1	1.28
	F32B6.2.2	1.28
	ags-3	1.28

	T01G1.4	1.28
	F55A11.6	1.28
	nhr-184	1.28
	vha-14	1.27
	Y38C1AA.11	1.27
	prx-11	1.27
	T02B11.8	1.27
	gpc-1	1.27
	C09E8.1	1.27
	rab-19	1.27
	nhr-87	1.27
	B0563.4	1.27
	Y73B6BL.31	1.27
	gbb-2	1.27
	zig-7	1.27
	Y56A3A.36	1.27
	F13E6.4	1.27
	acp-1	1.27
	T02B11.8	1.27
	R10E8.8	1.27
	U68260	1.27
	pdcd-2	1.26
	Y77E11A.16	1.26
	F52C9.3	1.26
	F40A3.6	1.26
	sft-4	1.26
	flp-10	1.26
	nhr-135	1.26
	unc-98	1.26
	pqn-42	1.26
	T02B11.8	1.26
	Y102E9.5	1.26
	ZK742.6	1.26

	K12H4.7	1.26
	nhr-205	1.26
	C44B7.10.3	1.25
	ZK180.2	1.25
	shk-1	1.25
	glb-23	1.25
	unc-44	1.25
	egl-9	1.25
	T23B12.8	1.25
	C08B6.10	1.25
	F35C8.5	1.25
	F49H12.3	1.25
	shk-1	1.25
	T02B11.8	1.25
	C18B12.4	1.25
	T04B2.5	1.24
	T13H5.1	1.24
	nhr-34	1.24
	tag-89	1.24
	clec-173	1.24
	T23B12.8a	1.24
	dhs-30	1.24
	atgp-2	1.24
	oig-2	1.24
	C08G9.1	1.24
	dmsr-1	1.24
	ZK813.4	1.24
	Y38C1AA.11	1.24
	T20F5.3	1.24
	unc-44	1.24
	sfxn-1.5	1.24
	D2096.9	1.23
	Y63D3A.7.1	1.23

	flr-1	1.23
	C46G7.2	1.23
	K04C1.4	1.23
	afd-1	1.23
	Y63D3A.7.1	1.23
	shk-1	1.23
	C44H4.4	1.23
	R57.1	1.23
	gsr-1	1.22
	F33H2.6	1.22
	frpr-3	1.22
	TC204028	1.22
	C36A4.11	1.22
	pes-9	1.22
	ZK688.3	1.22
	pxl-1	1.22
	F48E8.4	1.22
	F38B2.1c.4	1.22
	shk-1	1.22
	alh-8	1.21
	C36A4.11	1.21
	F38B2.1c.4	1.21
	ZK1073.1	1.21
	B0303.3	1.21
	C24G6.2	1.21
	Y63D3A.7.1	1.21
	K07F5.15	1.21
	skr-19	1.21
	K04C2.7	1.21
	Y102E9.5	1.21
	T19D2.3	1.20
	skr-19	1.20
	C46731	1.20

	ZK180.2	1.20
	K02D10.1	1.20
	C29824	1.20
	atgp-2	1.20
	aco-2	1.20
	T25G12.6	1.20
	Y63D3A.7.1	1.20
	C11G10.2	1.19
	sft-4	1.19
	trxr-1	1.19
	spp-15	1.19
	nhr-40	1.19
	C26F1.9.1	1.19
	ZK1073.1	1.19
	Y73B6BL.31	1.19
	ubh-1	1.19
	F57E7.1	1.19
	nhr-142	1.19
	Y71H10B.1	1.19
	bca-1	1.18
	C45E1.4	1.18
	lim-9	1.17
	clec-51	1.17
	R186.8	1.17
	K07B1.2	1.17
	C51E3.6	1.17
	F01G4.6	1.16
	lron-10	1.16
	best-4	1.16
	mrpl-12	1.16
	F32H5.4	1.16
	aco-2	1.15
	C01H6.4	1.15

	F20D1.9	1.14
	ugt-20	1.14
	F09F7.4	1.14
	rhi-1	1.14
	syx-3	1.14
	F26F4.10c	1.14
	sar-1	1.13
	lron-10	1.13
	T08A11.1	1.13
	rab-18	1.13
	mrpl-2	1.13
	CB400149	1.12
	F42A10.9	1.12
	ZC434.7	1.11
	Y54E10BR.4	1.11
	rab-18	1.11
	ndx-6	1.11
	F55A12.2	1.11
	ZK512.4	1.11
	T23F11.1	1.09
	let-721	1.09
	C26E6.11	1.07
	glrx-22	1.05

Appendix A.2 Genes differentially expressed in the hlh-17 (ns204); hlh-31(ns217); hlh-32(ns223) microarray analysis

Gene (down-regulated)	Fold Change	Gene (up-regulated)	Fold Change
T09B4.5	-32.17	*Y57A10C.9*	2.00
F53B3.5	-22.39	*col-151*	2.00
sma-1	-20.49	*sru-27*	2.01
sma-1	-20.45	*srbc-82*	2.01
erm-1	-19.29	*B0034.2*	2.01

tag-279	-18.70	*F53A2.1*	2.01
inf-1	-18.36	*lgc-52*	2.01
F53B3.5	-17.12	*srw-55*	2.02
igcm-3	-16.99	*R04B3.1*	2.02
inx-3	-16.02	*add-1*	2.02
gsp-1	-14.81	*sre-38*	2.02
tag-60	-14.42	*lgc-42*	2.02
hmp-1	-13.94	*E01G4.5*	2.02
sma-9	-13.69	*F07C6.2*	2.02
hbl-1	-13.63	*B0348.5*	2.02
rig-1	-13.38	*str-217*	2.02
ptp-3	-13.20	*Y75B8A.39*	2.03
pos-1	-13.17	*clec-127*	2.03
mep-1	-13.05	*K04F1.6*	2.03
atg-18	-12.91	*F55A11.11*	2.03
F23F12.9	-12.73	*vit-4*	2.03
K10C3.4	-12.43	*K01D12.5*	2.03
sdc-2	-12.32	*ugt-18*	2.03
dlg-1	-11.85	*F57A10.2*	2.03
chitinase	-11.68	*Y116F11B.9*	2.04
rab-5	-11.41	*C36C9.5*	2.04
xpo-1	-11.33	*C36C5.14*	2.04
hmr-1	-11.32	*F54D12.8*	2.04
ajm-1	-11.24	*tra-1*	2.04
hmg-1.2	-11.17	*F53G2.8*	2.04
mtm-3	-10.97	*nhr-36*	2.04
cey-1	-10.94	*C44H9.6*	2.04
cyb-1	-10.82	*C32H11.3*	2.04
noah-1	-10.75	*M6.4*	2.04
F26B1.2	-10.69	*F28C6.2*	2.04
sca-1	-10.64	*C07A12.2*	2.05
pha-4	-10.37	*T10C6.10*	2.05
C53A3.2	-10.25	*anc-1*	2.05

rab-11.1	-10.19	*WBGene00017553*	2.05
ajm-1	-10.18	*sre-39*	2.05
Y75B8A.24	-10.17	*F02D8.1*	2.05
T24G10.2	-10.16	*ZK858.2*	2.05
rab-1	-10.12	*ZK899.7*	2.05
sel-10	-10.10	*gcy-22*	2.05
C10G11.7	-10.09	*Y40H7A.4*	2.05
tag-192	-9.97	*AC3.5*	2.05
C18E3.2	-9.93	*Y47G6A.5*	2.05
hsr-9	-9.84	*Y105E8A.1*	2.06
C10C6.6	-9.82	*D1054.11*	2.06
F09G2.9	-9.71	*T06E4.7*	2.06
ZK858.6	-9.70	*fli-1*	2.06
T28B8.1	-9.66	*unc-7*	2.06
C18A3.5	-9.61	*F54C8.6*	2.06
set-1	-9.50	*C42C1.6*	2.06
Y46G5A.4	-9.49	*stdh-2*	2.06
lsy-2	-9.48	*T22E7.2*	2.06
tag-179	-9.46	*pqn-62*	2.06
mcm-7	-9.31	*Y51B9A.9*	2.06
arx-2	-9.30	*W09G12.9*	2.07
eel-1	-9.08	*unc-79*	2.07
spc-1	-9.06	*grd-1*	2.07
F18C5.10	-8.95	*F46A8.5*	2.07
mrck-1	-8.94	*oac-45*	2.07
clec-87	-8.94	*R106.1*	2.07
mrp-5	-8.89	*F47F6.9*	2.07
spectrin	-8.85	*Y54G11A.1*	2.07
T20F5.7	-8.82	*sri-69*	2.07
ceh-32	-8.78	*Y116A8C.29*	2.08
daf-19	-8.78	*K10C9.4*	2.08
let-92	-8.68	*C15F1.5*	2.08
tag-310	-8.57	*W10G11.2*	2.08

F35D2.3	-8.56	*nhr-250*	2.08
Y57G11C.15	-8.48	*R102.2*	2.08
rbr-2	-8.45	*C06E1.7*	2.10
Y59A8B.10	-8.42	*Y75B8A.33*	2.10
vps-26	-8.41	*W01C9.2*	2.10
xbp-1	-8.41	*tre-3*	2.10
swan-2	-8.35	*str-255*	2.10
Y113G7B.17	-8.31	*Y39A1A.17*	2.10
unc-43	-8.28	*srx-39*	2.10
ain-1	-8.21	*par-2*	2.10
cdh-4	-8.10	*egg-1*	2.10
pab-2	-8.07	*F07H5.7*	2.10
man-9	-8.02	*msp-33*	2.10
tsp-9	-8.00	*str-264*	2.10
paa-1	-7.98	*srw-82*	2.10
F08C6.2	-7.98	*F35C11.2*	2.11
skn-1	-7.97	*F54D12.2*	2.11
ccf-1	-7.95	*F07G6.3*	2.11
asd-1	-7.95	*Y39G10AR.18*	2.11
arx-1	-7.89	*K02B2.6*	2.11
nhr-34	-7.85	*fhod-2*	2.11
lin-25	-7.76	*fbxa-133*	2.11
pak-1	-7.73	*F22B3.4*	2.11
abts-1	-7.72	*lipase*	2.11
spas-1	-7.66	*Y47G6A.25*	2.11
gei-4	-7.63	*WBGene00011257*	2.11
C30A5.3	-7.61	*srx-128*	2.12
ttyh-1	-7.58	*F20C5.3*	2.12
WBGene00020683	-7.53	*xbx-4*	2.12
tag-123	-7.45	*F57A8.6*	2.12
F18A1.7	-7.44	*WBGene00019261*	2.12
let-805	-7.42	*kin-24*	2.12
E01A2.6	-7.40	*WBGene00020897*	2.12

R05D3.2	-7.37	*Y32B12C.3*	2.13
unc-32	-7.37	*R03H10.5 /// WBGene00019857*	2.13
gap-2	-7.35	*mop-25.2*	2.13
cam-1	-7.33	*gcy-8*	2.13
atf-7	-7.32	*F55G1.7*	2.13
ubc-3	-7.32	*W03D8.8*	2.13
F22D6.2	-7.30	*srz-100*	2.13
bath-43	-7.29	*clec-223*	2.13
sax-3	-7.29	*F37A4.5*	2.13
alg-2	-7.28	*ifc-2*	2.13
cht-1	-7.26	*fbxa-18*	2.14
egl-27	-7.22	*Y53G8AR.7*	2.14
his-24	-7.19	*K03A11.4*	2.14
apg-1	-7.18	*col-78*	2.14
F20D1.1	-7.15	*lim-6*	2.14
prp-8	-7.14	*F41G4.7*	2.14
WBGene00016620	-7.14	*ZK813.2*	2.14
fzr-1	-7.11	*F13H10.1*	2.15
cdh-6	-7.10	*srw-107*	2.15
ret-1	-7.09	*lge-1*	2.15
mrg-1	-7.06	*tag-243*	2.16
pup-2	-7.05	*nhr-204*	2.16
mop-25.2	-7.05	*ZC504.1*	2.16
rig-5	-7.04	*R06B9.4*	2.16
tag-172	-7.01	*nlp-23*	2.16
nhr-2	-7.01	*Y39B6A.27*	2.16
trap-1	-6.99	*Y37E11B.10*	2.17
pabp-2	-6.96	*F54D10.8*	2.17
D2013.6	-6.95	*csp-3*	2.17
anc-1	-6.95	*dylt-2*	2.17
tag-169	-6.94	*W05F2.4*	2.17
M163.1	-6.94	*R13A5.7*	2.17
cyb-2.1	-6.93	*gnrr-8*	2.17

gdi-1	-6.91	*E04A4.3*	2.17
acn-1	-6.83	*C16D9.3*	2.17
dpl-1	-6.81	*F28H1.4*	2.18
egl-46	-6.79	*C30F12.4*	2.18
rsp-6	-6.76	*dao-4*	2.18
clec-88	-6.76	*nhr-78*	2.18
knl-1	-6.73	*Y18H1A.1*	2.18
tag-333	-6.69	*C28D4.8*	2.18
cfz-2	-6.69	*srw-130*	2.18
noah-2	-6.67	*M151.3*	2.19
gei-4	-6.67	*F55C10.4*	2.19
dab-1	-6.63	*E02H9.5*	2.19
bra-2	-6.61	*Y23B4A.2*	2.19
ddx-19	-6.59	*R10F2.5*	2.19
T08B6.5	-6.52	*clec-131*	2.19
plk-1	-6.51	*C04G2.11*	2.20
let-2	-6.48	*sru-25*	2.20
pqn-53	-6.46	*R08C7.5*	2.20
set-2	-6.45	*F16H6.3*	2.20
Y57G11C.3	-6.44	*F28F8.7*	2.20
cdk-1	-6.43	*Y69A2AR.9*	2.20
frm-1	-6.39	*srd-74*	2.20
B0001.6	-6.38	*B0207.1*	2.20
isw-1	-6.34	*F42G2.5*	2.20
C48A7.2	-6.33	*F35E12.2*	2.20
rsp-3	-6.30	*R160.4*	2.21
lst-1	-6.25	*cutl-1*	2.21
cyl-1	-6.24	*Y105C5A.9*	2.21
T05H10.4	-6.23	*F58D5.6*	2.21
lam-3	-6.23	*Y92H12BL.4*	2.21
pqn-95	-6.21	*fbxa-214*	2.21
plp-1	-6.18	*T26C11.2*	2.22
F47H4.1	-6.17	*T06E8.2*	2.22

cyb-2.2	-6.17	*C04B4.1*	2.22
pqn-13	-6.16	*F31D5.1*	2.22
smo-1	-6.16	*F12A10.7*	2.22
D1007.5	-6.16	*C18H2.3*	2.22
ceh-20	-6.15	*M04D8.7*	2.23
F40F4.7	-6.15	*srh-133*	2.23
hum-2	-6.15	*srh-142*	2.23
pat-3	-6.14	*tag-19*	2.23
F46F6.2	-6.13	*K06H6.6*	2.23
F31E8.4	-6.12	*tbb-6*	2.23
pbrm-1	-6.11	*F56B6.6*	2.23
mbk-2	-6.09	*F42G2.2*	2.23
frm-4	-6.08	*srbc-14*	2.23
glit-1	-6.07	*sri-42*	2.23
R02D5.1	-6.01	*grl-22*	2.23
Y40D12A.1	-6.00	*F35E12.6*	2.23
T05B9.1	-6.00	*K06A4.6*	2.23
ZC262.3	-5.99	*H09F14.1*	2.23
wdr-23	-5.97	*T07A9.1*	2.23
daf-18	-5.96	*str-78*	2.23
tsp-12	-5.94	*Y6B3B.1*	2.23
imp-2	-5.92	*Y40B10A.5*	2.23
unc-37	-5.91	*F35A5.2*	2.24
fbn-1	-5.90	*fat-7*	2.24
lgg-2	-5.90	*K04G11.1*	2.24
lrp-2	-5.89	*fbxa-72*	2.24
K08D10.1	-5.89	*srbc-42*	2.25
R07E5.3	-5.87	*R03H10.6*	2.25
lmp-1	-5.85	*srz-53*	2.25
Y55F3BR.1	-5.84	*F54E7.6*	2.25
C01B12.2	-5.84	*fbxa-38*	2.25
fzy-1	-5.82	*Y37E11AR.7*	2.25
ZK484.3	-5.82	*odr-1*	2.25

F40E10.6	-5.81	*T07H6.4*	2.25
lmn-1	-5.81	*lgc-19*	2.25
cap-2	-5.79	*F07B7.2*	2.25
C25A1.4	-5.79	*sqv-6*	2.26
dnj-13	-5.77	*nas-16*	2.26
spk-1	-5.77	*ugt-10*	2.26
patr-1	-5.77	*F26F4.2*	2.26
ani-1	-5.75	*kqt-3*	2.26
F13C5.2	-5.75	*K09F5.4*	2.26
C27A12.7	-5.73	*nhr-51*	2.26
fbp-1	-5.71	*C29F4.2*	2.26
K10D3.4	-5.70	*C43F9.7*	2.27
F56C9.10	-5.70	*Y67A10A.11*	2.27
inf-1	-5.68	*R10E4.11*	2.27
unc-84	-5.68	*T15D6.5*	2.27
lag-1	-5.68	*H28O16.2*	2.27
F47B7.2	-5.66	*T09E11.6*	2.27
C06B8.7	-5.65	*Y57G7A.5*	2.27
C33H5.18	-5.65	*F34H10.1*	2.28
F22D3.2	-5.63	*C37A5.1*	2.28
vha-12	-5.62	*F07G11.3*	2.28
sip-1	-5.62	*C16H3.1*	2.28
ubc-9	-5.60	*Y49G5A.1*	2.28
zip-2	-5.56	*cyp-35B3*	2.28
jmjd-2	-5.55	*F38B2.3*	2.28
unc-62	-5.54	*F47F2.3*	2.28
emb-9	-5.52	*C30G4.5*	2.28
R10E4.1	-5.50	*str-222*	2.28
C06G3.6	-5.46	*F36D3.4*	2.28
ppn-1	-5.46	*F56A11.6*	2.28
gpdh-2	-5.45	*Y17G9B.6*	2.29
Y71G12B.8	-5.43	*C02E7.7*	2.29
F48E3.3	-5.41	*Y38E10A.13*	2.29

C56G2.7	-5.41	*mac-1*	2.29
R151.2	-5.40	*str-60*	2.29
F56D2.6	-5.40	*Y38H6C.9*	2.29
prp-17	-5.40	*C41G6.13*	2.29
sex-1	-5.39	*str-87*	2.29
C29H12.2	-5.37	*F26F2.4*	2.30
lit-1	-5.37	*Y39G10AR.16*	2.30
eif-3.B	-5.36	*C35A5.4*	2.30
aqp-2	-5.35	*chitinase*	2.30
F54A5.1	-5.34	*F49E11.7*	2.30
nurf-1	-5.33	*ZK1290.7*	2.30
mtm-3	-5.33	*T22H9.4*	2.30
mig-13	-5.30	*F55C9.3*	2.30
spr-4	-5.29	*sri-38*	2.30
hrp-1	-5.29	*C07D8.2*	2.30
lin-40	-5.28	*Y39G8B.7*	2.30
rsd-2	-5.28	*T06D8.2*	2.30
tpi-1	-5.25	*nhr-275*	2.30
cyk-4	-5.25	*C44E4.2*	2.30
tbc-3	-5.25	*nlp-29*	2.31
kin-20	-5.24	*T13F3.6*	2.31
ccdc-47	-5.23	*Y53F4B.17*	2.31
tam-1	-5.21	*ZK783.3*	2.31
Y71F9AL.9	-5.21	*R07A4.3*	2.31
csn-4	-5.21	*Y57G7A.1*	2.31
F01G4.6	-5.21	*srh-130*	2.32
lpr-3	-5.20	*Y110A2AL.5*	2.32
calu-1	-5.18	*clec-45*	2.32
ile-1	-5.18	*acr-3*	2.32
F19B6.1	-5.18	*ZC190.8*	2.32
xbx-6	-5.16	*F35E8.9*	2.32
F47B10.1	-5.16	*F40G12.11*	2.33
tag-147	-5.15	*syn-4*	2.33

ptr-4	-5.14	*Y48A6B.7*	2.33
M05D6.2	-5.14	*M04G7.1*	2.33
ZK938.2	-5.13	*dsc-1*	2.33
C02B10.4	-5.13	*str-27*	2.34
sym-5	-5.12	*Y105E8B.11*	2.34
dgn-1	-5.09	*ZC239.15*	2.34
sec-6	-5.09	*str-23*	2.34
unc-68	-5.07	*F28B1.1*	2.34
dsh-2	-5.06	*C28A5.5*	2.34
cids-2	-5.05	*srj-27*	2.34
hsp-60	-5.04	*Y38H6C.21*	2.34
hmg-1.1	-5.04	*Y53F4B.1*	2.35
gex-2	-5.04	*T25D3.3*	2.35
vha-13	-5.04	*C03F11.4*	2.35
let-418	-5.03	*K02B9.3*	2.35
C49H3.9	-5.02	*spg-7*	2.35
snr-2	-5.02	*gsto-1*	2.35
dpy-30	-5.01	*K08C9.6*	2.35
F55C5.8	-4.99	*B0205.10*	2.35
T09A5.11	-4.97	*WBGene00019349*	2.35
sgt-1	-4.97	*col-120*	2.36
ZC247.1	-4.96	*arf-1.1*	2.36
dcn-1	-4.95	*cyp-33C7*	2.36
T08A11.2	-4.95	*ZK418.2*	2.36
ZK507.6	-4.94	*dgk-1*	2.36
dmd-6	-4.94	*srh-122*	2.36
aph-2	-4.90	*srh-105*	2.36
R166.2	-4.89	*F35C5.3*	2.36
F58E10.3	-4.89	*C17F3.1*	2.36
ftt-2	-4.89	*C49G7.5*	2.36
rab-11.1 4	-4.85	*F56G4.4*	2.36
trap-3	-4.84	*Y34D9A.7*	2.36
F26F12.3	-4.83	*F59A7.7*	2.36

nhr-46	-4.82	*F48G7.10*	2.37
unc-14	-4.82	*C04C3.6*	2.37
emb-5	-4.82	*M02F4.2*	2.37
ufd-2	-4.82	*F58F9.6*	2.37
C35A5.8	-4.81	*1-Oct*	2.37
F14E5.2	-4.79	*K08D12.5*	2.37
arf-3	-4.79	*WBGene00017026*	2.38
vha-2	-4.79	*Y41G9A.3*	2.38
Y102A11A.8	-4.78	*Y51A2D.1*	2.38
mlt-11	-4.77	*F36F12.3*	2.38
M05B5.2	-4.75	*F58F6.3*	2.38
knl-3	-4.75	*Y54G2A.21*	2.38
F52G3.1	-4.73	*C18H9.3*	2.38
cyb-2.2	-4.72	*nhr-276*	2.39
ife-3	-4.72	*srj-21*	2.40
B0303.3	-4.72	*tag-293*	2.40
sca-1	-4.70	*let-756*	2.40
ZK353.1	-4.70	*K01A6.6*	2.40
rbc-1	-4.70	*srh-109*	2.40
C34D4.4	-4.69	*T10C6.7*	2.40
dif-1	-4.69	*F13E9.9*	2.40
F52A8.1	-4.69	*srw-10*	2.40
rgs-5	-4.68	*F31E8.5*	2.41
cls-2	-4.68	*cdr-1*	2.41
C05C8.7	-4.67	*F21D9.3*	2.41
phosphofructokinase	-4.67	*C18B12.2*	2.41
tbc-12	-4.67	*cyp-32A1*	2.41
rsd-3	-4.66	*F38E9.4*	2.41
F59E12.9	-4.65	*cyp-13A8*	2.41
yop-1	-4.65	*clec-134*	2.41
C56E6.3	-4.64	*Y69A2AR.20*	2.41
tbp-1	-4.64	*WBGene00007472*	2.41
mdf-2	-4.63	*C04H5.1*	2.41

WBGene00019295	-4.63	*ZK380.4*	2.42
F25B4.2	-4.61	*F53B2.5*	2.42
dgk-2	-4.60	*C33D9.3*	2.42
hexokinase	-4.60	*nhr-37*	2.42
hil-3	-4.60	*nhr-38*	2.42
uba-1	-4.59	*F44F4.10*	2.42
npp-19	-4.57	*Y39E4B.10*	2.42
R11A8.7	-4.57	*C27D6.4*	2.42
K04C2.3	-4.56	*srx-59*	2.42
inx-11	-4.55	*grl-29*	2.42
apl-1	-4.55	*C27H5.6*	2.42
T05H4.10	-4.55	*srt-58*	2.42
lpr-4	-4.54	*Y111B2A.24*	2.43
F54D5.5	-4.53	*F38B2.2*	2.43
set-3	-4.53	*nhr-255*	2.43
T26A5.6	-4.52	*C05C10.1*	2.43
rpn-7	-4.49	*egl-2*	2.43
vha-8	-4.48	*Y53G8AM.2*	2.43
C46C2.2	-4.47	*dyf-14*	2.43
C03C10.5	-4.46	*C15C7.6*	2.43
F48F7.6	-4.45	*flp-14*	2.44
C17E4.6	-4.45	*nspd-4*	2.44
T06D10.1	-4.45	*C40H1.2*	2.44
W01C8.5	-4.45	*srbc-65*	2.44
rpt-2	-4.44	*Y113G7A.13*	2.44
cul-4	-4.44	*WBGene00008142*	2.44
F08G12.2	-4.43	*clec-112*	2.44
F52E1.13	-4.43	*srx-112*	2.44
cul-1	-4.42	*Y39B6A.16*	2.45
F31E3.4	-4.42	*C47F8.6*	2.45
hel-1	-4.42	*F07G6.8*	2.45
pph-4.1	-4.41	*Y55H10A.2*	2.45
mcm-7	-4.41	*ceeh-2*	2.45

T05H4.6	-4.41	*C14C6.3*	2.45
R11H6.2	-4.40	*nhr-115*	2.46
sms-1	-4.40	*T07C12.11*	2.46
T19D12.1	-4.39	*F10C2.3*	2.46
K02F2.2	-4.39	*C01B12.5*	2.46
WBGene00009552	-4.37	*C28C12.4*	2.46
WBGene00013919	-4.36	*sre-26*	2.46
C40A11.6	-4.36	*fbxa-126*	2.46
farl-11	-4.36	*F46A8.4*	2.46
C44E4.4	-4.35	*ugt-16*	2.46
ergo-1	-4.35	*str-260*	2.47
tsp-14	-4.34	*Y46E12A.3*	2.47
cav-1	-4.34	*acc-1*	2.47
F59B8.2	-4.33	*ZK856.5*	2.47
vab-10	-4.32	*dac-1*	2.47
arx-5	-4.32	*F47G3.1*	2.47
pqn-55	-4.31	*srbc-12*	2.47
dnj-27	-4.31	*col-116*	2.47
K05F1.6	-4.30	*D1046.5*	2.47
flap-1	-4.30	*F47G6.2*	2.48
hmr-1	-4.30	*ZK218.11*	2.48
D1044.2	-4.30	*srj-54*	2.48
F49E12.6	-4.30	*clec-45*	2.48
rig-4	-4.30	*T01G5.6*	2.48
rpn-1	-4.28	*C25D7.12*	2.48
F56A8.3	-4.26	*C12D5.4*	2.48
wrt-10	-4.26	*M151.4*	2.48
gale-1	-4.23	*F36D1.7*	2.48
Y39G10AR.10	-4.22	*F54D10.7*	2.48
unc-44	-4.22	*srw-113*	2.49
YER141W	-4.22	*Y116F11A.3*	2.49
rsp-1	-4.22	*B0379.7*	2.49
unc-84	-4.21	*Y39G8C.2*	2.49

wwp-1	-4.20	*ace-2*	2.49
tbc-9	-4.20	*cyp-33C2*	2.50
pdi-2	-4.18	*str-4*	2.50
act-1	-4.18	*C46A5.2*	2.50
F33D11.9	-4.18	*frm-5*	2.50
rnp-5	-4.17	*F41G4.1*	2.50
ubh-4	-4.15	*ZK353.4*	2.50
T19B10.2	-4.15	*ncx-7 /*	2.50
dnj-5	-4.15	*T13G4.7*	2.50
Y37D8A.10	-4.15	*W02B3.4*	2.51
F11G11.5	-4.14	*F27C1.10*	2.51
E04A4.5	-4.14	*fbxa-209*	2.51
rbx-1	-4.14	*srh-169*	2.51
ham-1	-4.14	*C52E2.2*	2.51
sec-23	-4.13	*ZC53.2*	2.51
unc-1	-4.12	*WBGene00021553*	2.51
rap-2	-4.11	*srg-24*	2.51
H19N07.4	-4.11	*sre-40*	2.51
tag-125	-4.11	*C24A11.1*	2.52
efk-1	-4.10	*F09E5.10*	2.52
ile-2	-4.10	*srsx-40*	2.52
lfi-1	-4.09	*str-180*	2.52
unc-15	-4.08	*F46B6.10*	2.52
ceh-21	-4.08	*srab-7*	2.52
K09G1.1	-4.07	*T05D4.5*	2.53
ape-1	-4.07	*kin-26*	2.54
Y47G6A.18	-4.06	*pqn-88*	2.54
nid-1	-4.06	*K04F1.1*	2.54
4-NITROPHENYLPHOSPHATASE	-4.05	*F02D10.4*	2.55
chd-3	-4.05	*C10E2.2*	2.55
siah-1	-4.05	*twk-34*	2.55
ugt-57	-4.05	*K02F6.3*	2.55
T12E12.1	-4.04	*Y40B1A.3*	2.55

T21C9.4	-4.03	*T24B8.7*	2.55
nhr-48	-4.02	*T21D11.1*	2.56
car-1	-4.01	*fbxa-12*	2.56
imp-1	-4.00	*xtr-1*	2.56
kle-2	-4.00	*F57F4.2*	2.56
C10E2.6	-4.00	*W02H5.4*	2.56
smp-1	-3.99	*sre-47*	2.56
skr-1	-3.99	*srd-48*	2.57
C35E7.1	-3.99	*F29G9.7*	2.57
tag-253	-3.98	*nkcc-1*	2.57
rnr-1	-3.97	*T25B9.6*	2.57
pqn-65	-3.97	*xbx-1*	2.57
D1007.15	-3.96	*H28G03.4*	2.57
top-2	-3.95	*bath-33*	2.57
kin-25	-3.94	*fbxa-112*	2.57
snb-1	-3.94	*haf-8*	2.57
vha-1	-3.93	*sru-44*	2.57
npp-10	-3.93	*F54B8.4*	2.58
C05D10.4	-3.93	*B0554.2*	2.58
flh-1	-3.93	*sre-29*	2.58
phb-2	-3.92	*F26F12.2*	2.58
F57A8.2	-3.91	*E03H12.9*	2.58
npp-12	-3.91	*srx-65*	2.58
sta-1	-3.91	*sri-57*	2.59
mel-11	-3.90	*C25G6.4*	2.59
pqe-1	-3.90	*C03B1.5*	2.59
T20D3.11	-3.90	*ssu-1*	2.59
Y55F3AM.3	-3.89	*Y48G1A.1*	2.59
pccb-1	-3.89	*srab-16*	2.59
D1081.7	-3.89	*gei-3*	2.60
bath-9	-3.89	*R11G11.3*	2.60
mlc-4	-3.88	*F41D3.8*	2.60
bec-1	-3.88	*C18D4.4*	2.60

H17B01.1	-3.88	*T15D6.8*	2.60
M01B12.4	-3.87	*Y116A8C.4*	2.61
akt-1	-3.87	*F38C2.1*	2.61
apa-2	-3.86	*C45E1.4*	2.61
toca-1	-3.86	*T07D3.5*	2.61
F22B5.10	-3.86	*VC27A7L.1*	2.61
C32D5.11	-3.86	*srd-35*	2.61
F15A4.2	-3.85	*F26D2.3*	2.62
hda-1	-3.85	*srh-79*	2.62
K01G5.5	-3.84	*W06H3.3*	2.62
mel-32	-3.84	*ZK856.4*	2.62
T26A5.5	-3.82	*T22G5.1*	2.62
F46H5.2	-3.82	*scl-21*	2.62
set-16	-3.82	*C05D2.8*	2.62
him-17	-3.81	*oac-27*	2.62
ent-1	-3.80	*Y119D3B.13*	2.63
Y69A2AR.16	-3.80	*K01A2.10*	2.63
hke-4.2	-3.80	*srd-27*	2.63
cTel55X.1	-3.79	*ZC204.13*	2.63
npp-1	-3.79	*scl-10*	2.63
gcs-1	-3.79	*R12G8.1*	2.63
ldb-1	-3.79	*WBGene00011166*	2.63
Y57A10A.23	-3.79	*R07E5.6*	2.64
mcm-2	-3.78	*F10F2.2*	2.64
clec-91	-3.78	*W06D4.3*	2.64
nuo-3	-3.77	*F28F5.4*	2.64
Y48G10A.1	-3.77	*Y22D7AL.11*	2.64
dim-1	-3.77	*Y48E1B.7*	2.64
crb-1	-3.77	*Y55F3BR.7*	2.64
T06D8.3	-3.77	*Y51A2D.8*	2.64
T10C6.5	-3.76	*nas-18*	2.65
vha-4	-3.76	*srd-17*	2.65
Y44A6D.2	-3.76	*nhr-287*	2.65

ldb-1	-3.75	*Y53F4A.2*	2.65
F36G3.1	-3.75	*msp-74*	2.65
rab-6.2	-3.75	*F58F12.3*	2.65
cey-4	-3.75	*nhr-210*	2.65
M106.4	-3.74	*Y105E8A.13*	2.66
his-72	-3.74	*Y37E11B.10*	2.66
W07G4.4	-3.74	*Y50D4A.4*	2.66
let-711	-3.74	*Y53F4B.11*	2.66
ebp-1	-3.74	*C26B2.2*	2.66
Y32H12A.5	-3.74	*Y40B1A.1*	2.67
dnj-13	-3.73	*srbc-66*	2.67
prp-4	-3.73	*C49D10.7*	2.67
nuo-4	-3.72	*Y110A2AL.9*	2.67
dlc-1	-3.72	*col-180*	2.68
frs-1	-3.72	*F12E12.12*	2.68
cdc-48.1	-3.72	*F20B4.3*	2.68
tag-320	-3.71	*srt-11*	2.68
ZK616.4	-3.71	*B0524.3*	2.68
F30A10.10	-3.71	*sri-47*	2.68
T20B12.7	-3.70	*unc-33*	2.68
F25G6.8	-3.70	*Y39F10A.3*	2.69
hyl-1	-3.69	*dop-1*	2.69
K04F10.7	-3.69	*sre-52*	2.69
aly-3	-3.69	*F58E1.7*	2.69
F55C5.4	-3.69	*F31D5.4*	2.69
T12D8.6	-3.69	*K08D9.6*	2.69
kpc-1	-3.68	*C31B8.4*	2.69
WBGene00008506	-3.68	*nhr-5*	2.70
vab-1	-3.68	*Y48E1B.8*	2.70
unc-71	-3.67	*lact-6*	2.70
F59A3.4	-3.67	*soc-2*	2.70
nduf-7	-3.67	*K08C9.1*	2.70
ama-1	-3.66	*F52C9.1*	2.70

mat-1	-3.66	*nlp-22*	2.71
ZC13.1	-3.66	*Y66A7A.4*	2.72
Y105E8A.3	-3.65	*clec-34*	2.72
sec-24.1	-3.65	*K01A6.4*	2.72
tol-1	-3.65	*C09B9.3*	2.72
hil-2	-3.64	*T28F2.1*	2.72
R05F9.9	-3.64	*clec-245*	2.73
lin-17	-3.63	*Y116A8C.23*	2.73
WBGene00007904	-3.63	*srw-1*	2.73
dpy-10	-3.62	*K06A9.3*	2.73
WBGene00019477	-3.62	*fbxa-43*	2.73
tba-2	-3.62	*Y119C1B.6*	2.73
F44E2.7	-3.62	*cyp-35B2*	2.74
C09G4.2	-3.61	*F31F7.2*	2.74
F53B1.2	-3.61	*srab-25*	2.74
F28H6.4	-3.60	*dct-7*	2.74
C37H5.6	-3.60	*Y47H9B.2*	2.74
sym-2	-3.59	*clec-107*	2.74
C28H8.4	-3.58	*fbxb-79*	2.74
spd-1	-3.58	*clec-238*	2.75
klc-2	-3.58	*F43C11.3*	2.75
ZK418.9	-3.58	*F43C11.12*	2.75
F11E6.7	-3.58	*vit-4*	2.75
pbs-7	-3.57	*srw-108*	2.76
dpy-18	-3.57	*srw-61*	2.76
F56A8.8	-3.57	*gcy-29*	2.76
dao-3	-3.56	*C36C5.5*	2.76
somi-1	-3.56	*B0218.7*	2.77
R148.5	-3.56	*srv-21*	2.77
K11G12.5	-3.56	*str-103*	2.77
tag-175	-3.56	*B0285.6*	2.77
ncx-2	-3.55	*W06H8.6*	2.77
F16D3.4	-3.55	*C44B9.1*	2.77

cas-1	-3.55	*WBGene00016862*	2.78
B0334.5	-3.55	*gst-17*	2.78
M106.8	-3.55	*WBGene00017550*	2.78
K08F4.1	-3.55	*srd-60*	2.78
W02F12.5	-3.55	*Y48B6A.5*	2.78
C43H6.4	-3.54	*C28D4.4*	2.78
lin-26	-3.54	*C14C6.12*	2.78
elo-6	-3.54	*T11A5.1*	2.78
pas-5	-3.54	*F14H12.2*	2.79
T02E9.5	-3.53	*Y70G10A.2*	2.79
irs-1	-3.53	*Y50E8A.2*	2.79
Y57G7A.10	-3.52	*Y17G7B.8*	2.79
T05E11.3	-3.52	*srj-37*	2.79
atn-1	-3.52	*F20B4.4*	2.79
cutl-2	-3.51	*ZK1248.7*	2.79
tag-335	-3.51	*F15D3.5*	2.79
ZK484.1	-3.51	*rol-1*	2.80
tir-1	-3.51	*daf-2*	2.80
K04B12.2	-3.51	*srd-66*	2.80
dcr-1	-3.51	*C26E1.3*	2.80
erv-46	-3.51	*ZC239.2*	2.80
F38E9.5	-3.51	*F08D12.7*	2.80
pbs-3	-3.50	*cyp-13A11*	2.80
T04B8.5	-3.50	*msp-3*	2.81
egl-4	-3.50	*C07G1.6*	2.81
ZK686.3	-3.50	*W05H5.1*	2.81
rpc-1	-3.49	*aat-4*	2.81
T19A6.1	-3.48	*srw-84*	2.81
snap-1	-3.48	*H10E21.2*	2.81
pdi-1	-3.48	*srw-88*	2.81
F44F1.6	-3.47	*WBGene00013479*	2.82
T19C4.1	-3.47	*F20A1.6*	2.82
Y113G7B.17	-3.47	*ugt-32*	2.83

C37E2.1	-3.47	*T25B9.4*	2.83
cdc-14	-3.46	*ZK593.9*	2.83
mcm-4	-3.46	*grd-4*	2.83
gip-1	-3.44	*Y66D12A.11*	2.83
elt-1	-3.44	*WBGene00009345*	2.84
F40F9.7	-3.44	*nhr-173*	2.84
eat-20	-3.43	*Y60A9.1*	2.84
M01A10.3	-3.43	*T17A3.10*	2.84
WBGene00019819	-3.42	*W04G5.9*	2.84
C01G6.4	-3.41	*R06B9.1*	2.84
T22H6.2	-3.41	*M03E7.1*	2.85
npp-17	-3.41	*T24D5.1*	2.85
dpy-11	-3.41	*W03G1.8*	2.85
pqn-18	-3.40	*C29F7.6*	2.86
trap-4	-3.40	*clec-115*	2.86
rab-21	-3.39	*F23B2.3*	2.86
C08B11.9	-3.39	*Y82E9BL.12*	2.86
ZK829.4	-3.39	*H28G03.4*	2.86
F25B4.5	-3.39	*Y54E2A.7*	2.87
T13C2.6	-3.38	*Y49F6C.2*	2.87
W08E3.2	-3.38	*bath-23*	2.87
ran-1	-3.38	*R02F2.5*	2.87
F57G12.1	-3.37	*srh-131*	2.87
cam-1	-3.37	*F46F5.1*	2.87
T03F1.8	-3.37	*K02F6.6*	2.88
D2096.8	-3.36	*C15H11.1*	2.88
rpn-3	-3.36	*oac-22*	2.88
inx-5	-3.36	*F01F1.14*	2.88
M28.5	-3.35	*R155.2*	2.88
hsp-3	-3.35	*amt-3*	2.89
eft-4	-3.35	*C16A11.3*	2.89
F25H5.3	-3.35	*Y111B2A.4*	2.89
R07H5.8	-3.35	*Y71H2AR.2*	2.90

eya-1	-3.34	*F56H6.2*	2.90
efn-2	-3.34	*str-205*	2.91
C09F9.2	-3.34	*F38E1.10*	2.92
oma-2	-3.33	*srd-46*	2.92
ZC376.7	-3.33	*R07E4.1*	2.92
F33G12.5	-3.32	*C32H11.7*	2.92
ceh-41	-3.31	*C38C3.6*	2.92
vhp-1	-3.31	*oac-10*	2.92
tbb-2	-3.31	*col-152*	2.93
ntl-2	-3.31	*WBGene00017453*	2.93
bra-1	-3.31	*T05A8.7*	2.93
unc-83	-3.31	*C29F5.5*	2.93
C06A8.3	-3.31	*clec-260*	2.94
pqn-14	-3.30	*cnc-2*	2.94
T19A5.1	-3.30	*C54F6.5*	2.94
Y60A3A.9	-3.30	*sru-16*	2.95
sop-2	-3.30	*sri-60*	2.95
Y47D3A.29	-3.29	*egl-10*	2.96
ran-5	-3.29	*F48B9.3*	2.96
lin-12	-3.28	*F35C8.8*	2.96
C50B8.1	-3.28	*F53E10.3*	2.96
ife-2	-3.28	*fbxa-47*	2.96
gei-12	-3.28	*C18A3.7*	2.97
C02E11.1	-3.28	*C41H7.1*	2.97
D1046.3	-3.27	*oac-4*	2.97
fib-1	-3.27	*F36H9.4*	2.97
pat-6	-3.27	*cwp-5*	2.98
atp-3	-3.27	*srw-68*	2.98
T05F1.1	-3.25	*gcy-19*	2.98
casy-1	-3.25	*F56B3.3*	2.98
F46E10.2	-3.25	*R12E2.6*	2.98
let-19	-3.24	*str-37*	2.99
aco-1	-3.24	*F47G4.5*	2.99

cap-1	-3.24	*Y62H9A.14*	2.99
C54G7.2	-3.24	*gld-2*	2.99
lin-28	-3.24	*unc-63*	2.99
C35E7.5	-3.23	*F35E8.6*	2.99
T07C4.3	-3.23	*C56E10.3*	3.00
tag-72	-3.23	*T15D6.9*	3.00
T04F8.2	-3.23	*K02D10.3*	3.00
act-2	-3.22	*C06C6.7*	3.00
H42K12.3	-3.21	*Y105C5A.24*	3.00
sur-2	-3.21	*wht-4*	3.01
bus-17	-3.20	*clec-263*	3.01
srs-2	-3.19	*sru-29*	3.01
qdpr-1	-3.18	*W05F2.7*	3.01
inx-7	-3.18	*B0507.6*	3.01
daf-5	-3.18	*Y6D1A.1*	3.02
nsf-1	-3.17	*srh-123*	3.02
gbh-2	-3.17	*K04C1.5*	3.02
K01A2.3	-3.17	*srh-32*	3.02
his-32	-3.16	*K01A11.1*	3.02
C18E9.10	-3.16	*C50A2.2*	3.02
frm-5	-3.16	*F22B3.7*	3.02
pdk-1	-3.15	*zig-2*	3.02
Y71G12B.11	-3.15	*F46F5.5*	3.03
npp-4	-3.15	*Y110A2AL.2*	3.03
C32D5.10	-3.15	*C34D10.2*	3.04
oga-1	-3.14	*C30B5.5*	3.04
R10E11.3	-3.14	*sra-27*	3.05
sli-1	-3.14	*F47G9.4*	3.05
bre-3	-3.14	*tag-336*	3.06
ptr-10	-3.13	*ZK218.5*	3.06
WBGene00011089	-3.13	*W02G9.3*	3.07
F40F9.6	-3.13	*try-8*	3.07
inx-2	-3.13	*C50E3.11*	3.08

npp-3	-3.12	*sra-14*	3.08
B0395.3	-3.12	*K09C4.2*	3.08
dnj-25	-3.12	*F54H5.3*	3.09
tag-349	-3.12	*F11D11.3*	3.09
baf-1	-3.12	*C29F5.3*	3.09
cgt-3	-3.12	*tag-80*	3.09
syd-9	-3.11	*str-257*	3.09
F53C3.13	-3.11	*T04C9.2*	3.09
tre-1	-3.11	*ZK1290.10*	3.10
cct-5	-3.11	*Y71H2B.1*	3.10
gln-6	-3.11	*ZK402.2*	3.10
pac-1	-3.11	*K02F6.2*	3.10
ZK682.2	-3.10	*acr-23*	3.10
F57C7.1	-3.10	*F16B12.1*	3.11
M05D6.6	-3.10	*rnp-6*	3.11
ZK512.1	-3.10	*C25H3.10*	3.11
ttm-1	-3.10	*sru-12*	3.11
ZK673.2	-3.10	*Y55D5A.4*	3.11
C29H12.6	-3.10	*srbc-40*	3.11
spt-5	-3.10	*T28A11.18*	3.12
F52E1.9	-3.10	*F56A4.11*	3.12
flh-2	-3.09	*sre-33*	3.12
Y47D3B.6	-3.09	*cpt-3*	3.12
icp-1	-3.08	*H25K10.4*	3.13
ftn-2	-3.08	*fbxa-81*	3.13
F52D1.1	-3.08	*F31F4.11*	3.13
F38A5.1	-3.08	*srh-271*	3.13
cap-1	-3.07	*clec-106*	3.14
ZK856.10	-3.07	*sru-11*	3.14
elb-1	-3.07	*sri-11*	3.14
F54D7.2	-3.07	*grd-16*	3.14
F19B6.1	-3.06	*srh-186*	3.14
acdh-3	-3.06	*K08H2.5*	3.15

T21H8.1	-3.06	*clh-4*	3.15
fmo-2	-3.06	*F20B6.6*	3.16
aly-1	-3.05	*pqn-11*	3.17
rfc-2	-3.05	*M117.6*	3.17
srgp-1	-3.05	*F23D12.1*	3.17
R02F2.1	-3.05	*jip-1*	3.17
C11E4.1	-3.05	*srx-4*	3.18
sym-5	-3.04	*clec-116*	3.18
hbl-1	-3.04	*C26B9.2*	3.18
lin-24	-3.04	*srb-18*	3.19
R05F9.1	-3.04	*srh-272*	3.19
hrp-2	-3.04	*tag-312*	3.20
dpy-2	-3.04	*ZK1240.2*	3.20
ubq-1	-3.04	*R01H2.1*	3.20
eat-6	-3.03	*srh-295*	3.20
C28H8.9	-3.03	*Y69A2AR.14*	3.21
C17E4.10	-3.03	*F49H12.2*	3.21
rpa-2	-3.03	*srg-2*	3.21
gob-1	-3.02	*hil-8*	3.22
T01E8.4	-3.02	*hlh-26*	3.24
wht-2	-3.02	*snt-2*	3.24
rpb-6	-3.02	*T20B12.5*	3.25
ZK632.10	-3.02	*T07D10.3*	3.25
nhr-3	-3.02	*Y43F8C.11*	3.25
T24F1.2	-3.01	*Y60C6A.1*	3.25
B0041.5	-3.01	*M04G7.2*	3.26
C18D11.1	-3.01	*acr-21*	3.26
gei-13	-3.01	*ZK355.3*	3.26
F32A7.5	-3.01	*cyp-14A4*	3.27
mlt-8	-3.01	*F59D6.4*	3.28
cdc-25.2	-3.01	*T26C11.3*	3.29
C49F8.3	-3.00	*F21F3.2*	3.29
coh-1	-3.00	*F49E2.5*	3.29

gdi-1	-3.00	*C10C6.3*	3.30
aqp-4	-3.00	*clec-130*	3.30
ubxn-3	-3.00	*vab-19*	3.30
cyn-8	-3.00	*F33E2.6*	3.30
trap-3	-3.00	*Y49F6B.6*	3.31
egl-45	-3.00	*Y47H9B.2*	3.31
F54B3.3	-3.00	*F21E9.6*	3.31
vbh-1	-2.99	*egl-38*	3.33
K08E7.1	-2.99	*srh-231*	3.33
T12G3.4	-2.99	*unc-10*	3.33
par-5	-2.99	*Y71G12B.2*	3.34
R02F2.1	-2.98	*ZC404.10*	3.35
M176.3	-2.98	*C49G7.12*	3.35
ftt-2	-2.98	*F56H6.13*	3.37
SR-famC	-2.98	*nkat-1*	3.37
D1044.2	-2.98	*C08A9.3*	3.37
Y22D7AL.14	-2.98	*Y40B1A.2*	3.38
Y25C1A.5	-2.97	*Y57G7A.5*	3.38
Y54E5A.7	-2.97	*srh-220*	3.39
WBGene00015232	-2.96	*bath-21*	3.39
Y73B6BL.33	-2.96	*Y17D7B.2*	3.40
eat-20	-2.96	*F02H6.1*	3.41
C31C9.2	-2.96	*ZK355.4*	3.41
F17C11.9	-2.95	*F19H6.5*	3.41
fce-1	-2.95	*ugt-30*	3.42
F08G12.1	-2.95	*sra-39*	3.42
tbg-1	-2.95	*C35B1.4*	3.42
psa-4	-2.94	*F36D1.4*	3.43
his-72	-2.94	*fat-5*	3.44
hke-4.2	-2.94	*ZC404.1*	3.44
trs-1	-2.94	*F44D12.8*	3.44
tbx-2	-2.94	*K03D3.2*	3.44
atg-4.1	-2.93	*cyp-35A3*	3.45

vha-17	-2.93	*oac-38*	3.45
pdi-3	-2.93	*btb-18*	3.45
F46H5.3	-2.92	*fbxb-52*	3.45
gfi-1	-2.92	*col-148*	3.45
ace-3	-2.92	*T10E9.6*	3.46
F16B12.6	-2.92	*cnc-7*	3.46
Y71G12B.23	-2.92	*F36D1.5*	3.46
pqn-32	-2.91	*srx-24*	3.47
Y17G7B.10	-2.91	*F36D1.6*	3.48
ubc-7	-2.91	*R13D11.10*	3.48
Y4C6B.1	-2.90	*nlp-42*	3.49
srp-7	-2.90	*F18G5.5*	3.49
tba-2	-2.90	*F13A2.4*	3.49
K08D12.3	-2.90	*srx-28*	3.51
emb-27	-2.90	*irk-1*	3.52
K05C4.2	-2.89	*C46H11.1*	3.52
erp72	-2.89	*egl-20*	3.52
ZK154.4	-2.89	*srxa-9*	3.52
C06H5.7	-2.89	*T23D5.3*	3.53
K08E3.5	-2.89	*srw-85*	3.54
T20B12.7	-2.89	*srab-17*	3.54
rha-1	-2.89	*srw-2*	3.55
fmo-4	-2.89	*srg-31*	3.56
F38E9.5	-2.88	*sri-54*	3.59
exos-3	-2.87	*K09F6.7*	3.59
snf-1	-2.86	*M01G12.2*	3.61
rpb-7	-2.86	*drh-1*	3.62
uaf-1	-2.86	*R11G10.2*	3.62
F15B9.8	-2.85	*F36G9.13*	3.62
rgr-1	-2.85	*F33E2.10*	3.65
rab-7	-2.85	*F49E2.5*	3.66
rnp-3	-2.85	*str-256*	3.66
nit-1	-2.84	*Y53H1B.4*	3.67

Y48G9A.3	-2.84	*C50B6.1*	3.67
egl-9	-2.84	*WY54G2A.20*	3.68
F10C5.2	-2.84	*K04H4.5*	3.70
rnp-6	-2.84	*fbxa-40*	3.73
H19N07.4	-2.84	*fbxa-84*	3.74
cdc-25.1	-2.83	*cdh-12*	3.75
C47D12.2	-2.83	*Y47D3B.4*	3.75
fib-1	-2.82	*T01B6.4*	3.79
mig-5	-2.82	*C50H2.5*	3.81
mex-6	-2.82	*F56H6.4*	3.83
Y46G5A.8	-2.81	*Y19D10A.4*	3.89
H24K24.3	-2.81	*F44B9.9*	3.90
T23B12.2	-2.81	*clec-150*	3.90
F40F8.11	-2.80	*srz-13*	3.91
pbs-2	-2.80	*ZC239.14*	3.94
apb-1	-2.80	*srd-43*	3.95
F43D9.1	-2.80	*F44D12.6*	3.99
Y23H5A.3	-2.80	*cyp-14A2*	4.04
cfz-2	-2.80	*T19H12.3*	4.11
C56G2.4	-2.79	*fip-2*	4.13
C05G5.4	-2.79	*C07D8.3*	4.23
rad-26	-2.79	*F35F10.6*	4.25
Y48A6B.3	-2.79	*F43C11.4*	4.40
C02B10.5	-2.78	*C52E4.8*	4.42
sna-2	-2.78	*ins-24*	4.42
dpy-26	-2.78	*ZK813.1*	4.45
dhhc-1	-2.78	*C14E2.1*	4.49
cyn-5	-2.78	*Y22D7AR.1*	4.49
sqrd-1	-2.78	*clec-208*	5.07
T01G9.2	-2.78	*F49E2.5*	5.14
F43G6.4	-2.78	*cnc-7*	5.77
act-1	-2.77		
atp-5	-2.77		

F43G9.2	-2.76
C36E8.1	-2.76
pha-4	-2.76
lin-1	-2.76
dpy-11	-2.75
mtx-1	-2.75
H28G03.1	-2.75
pmr-1	-2.74
C14F11.6	-2.73
F37B12.3	-2.73
F32E10.6	-2.73
C26B9.3	-2.72
him-4	-2.71
lev-11	-2.71
atf-7	-2.71
WBGene00019298	-2.71
Y69A2AR.31	-2.71
Y57G11C.9	-2.70
F07A11.4	-2.70
Y67A10A.7	-2.70
col-76	-2.70
ost-1	-2.70
hil-5	-2.70
nuo-1	-2.70
unc-2	-2.69
C13B9.3	-2.68
F30A10.6	-2.68
F47A4.5	-2.68
sup-17	-2.68
cdk-7	-2.68
T14G10.5	-2.67
cpt-2	-2.67
tag-131	-2.67

ZK899.2	-2.67
K12H4.4	-2.66
D1043.1	-2.66
C15C6.1	-2.66
T23F2.5	-2.66
pct-1	-2.66
set-10	-2.66
gas-1	-2.66
fbxb-37	-2.65
Y39A1A.8	-2.65
WBGene00019624	-2.65
ung-1	-2.65
elt-6	-2.65
lam-2	-2.65
K11H12.8	-2.65
daf-3	-2.65
F13C5.2	-2.65
Y57E12AL.6	-2.65
ztf-12	-2.64
K04H4.2	-2.64
F26E4.12	-2.64
dhs-29	-2.64
nhr-237	-2.64
mps-2	-2.63
ben-1	-2.63
C53D5.2	-2.63
sop-2	-2.63
rgs-8.1	-2.63
H03E18.1	-2.62
hcf-1	-2.62
WBGene00019620	-2.62
bub-1	-2.61
ZK353.9	-2.61

atg-7	-2.61
pst-1	-2.61
C06E7.4	-2.61
hch-1	-2.61
M01E5.6	-2.60
C45G3.5	-2.60
gei-17	-2.60
Y37A1B.17	-2.60
M18.6	-2.60
T09E8.3	-2.59
Y45G5AM.6	-2.59
npp-5	-2.59
F53E10.1	-2.59
H17B01.4	-2.59
unc-37	-2.58
C28A5.1	-2.58
fbxb-107	-2.58
F56C9.3	-2.58
phy-2	-2.58
egl-27	-2.58
syn-3	-2.58
cnb-1	-2.58
T25E4.1	-2.57
pmt-2	-2.57
Y44A6C.1	-2.57
W02B12.10	-2.57
asp-1	-2.57
ZK370.4	-2.57
unc-112	-2.57
alh-9	-2.57
F18H3.4	-2.56
lec-1	-2.56
ain-1	-2.56

Y54E10BR.5	-2.56
dgn-1	-2.56
skr-9	-2.55
F25B5.2	-2.55
vab-1	-2.55
Y60A3A.14	-2.54
dcn-1	-2.54
gpc-2	-2.54
nurf-1	-2.54
mps-2	-2.54
atf-5	-2.53
Y47D9A.1	-2.53
patr-1	-2.53
vps-39	-2.53
gfi-4	-2.52
snt-4	-2.52
F43E2.7	-2.52
mbk-1	-2.51
ZK809.3	-2.51
mlp-1	-2.51
R08E5.3	-2.50
ham-2	-2.50
rpb-8	-2.50
Y57G11C.33	-2.50
F30B5.4	-2.50
fbxb-24	-2.50
K10H10.1	-2.50
exc-9	-2.50
snr-4	-2.49
Y73B3A.18	-2.49
scd-1	-2.49
C02D5.2	-2.49
mak-2	-2.49

C24A3.2	-2.49
R11.4	-2.49
nsy-1	-2.48
C34F6.8	-2.48
vha-19	-2.48
fum-1	-2.47
rpb-2	-2.47
nhr-4	-2.47
aly-2	-2.47
B0495.8	-2.47
smc-4	-2.47
abl-1	-2.46
F38H4.10	-2.46
cdc-14	-2.46
F22D3.2	-2.46
T16H12.4	-2.45
tgt-2	-2.45
isp-1	-2.45
cct-4	-2.45
tag-135	-2.45
C27F2.8	-2.45
F32D1.5	-2.45
lim-9	-2.45
his-71	-2.44
F41C3.4	-2.44
Y25C1A.7	-2.44
Y22D7AL.10	-2.44
F57B1.6	-2.44
his-17	-2.43
F10D11.2	-2.43
zer-1	-2.43
cpt-1	-2.43
pfd-5	-2.43

C14C11.7	-2.43
pbs-5	-2.43
lgg-1	-2.42
K09E2.3	-2.42
F41D9.2	-2.42
lpr-3	-2.42
Y82E9BR.17	-2.42
C17G10.9	-2.41
tag-294	-2.41
R03E1.2	-2.41
C49H3.9	-2.40
clic-1	-2.40
ZK154.1	-2.40
rgs-7	-2.40
Y5F2A.4	-2.40
fkb-2	-2.40
C47C12.4	-2.40
WBGene00010704	-2.39
C27H2.2	-2.39
C30B5.2	-2.39
mog-1	-2.39
R151.6	-2.39
W02D3.4	-2.39
F13E6.1	-2.39
hmp-2	-2.38
hcp-4	-2.38
gpdh-2	-2.38
R08C7.2	-2.38
unc-112	-2.38
cgp-1	-2.38
R53.4	-2.38
F41G3.6	-2.37
Y64G10A.7	-2.37

sdhb-1	-2.37
idi-1	-2.37
C06A6.2	-2.37
rsa-2	-2.37
T22E7.1	-2.37
haf-4	-2.36
dpy-7	-2.36
skr-10	-2.36
C05D11.7	-2.36
unc-97	-2.36
F21F3.6	-2.35
daf-1	-2.35
pnk-1	-2.35
T09B4.1	-2.35
asp-4	-2.35
vha-9	-2.35
B0336.3	-2.35
ubxn-2	-2.35
psa-4	-2.35
K10C8.3	-2.34
mlt-11	-2.34
hel-308	-2.34
pqn-74	-2.34
F02E9.10	-2.34
prkl-1	-2.33
Y38F2AR.9	-2.33
C24H10.2	-2.33
rpn-9	-2.33
ldh-1	-2.32
tag-192	-2.32
bath-38	-2.31
tag-170	-2.31
K02E10.4	-2.31

T05D4.1	-2.31
WBGene00008971	-2.31
C34C12.8	-2.31
spd-5	-2.31
hid-1	-2.30
pqn-51	-2.30
C08B11.3	-2.30
T01E8.5	-2.30
ZC8.6	-2.30
pqn-85	-2.29
gad-1	-2.29
npl-4.1	-2.29
acs-16	-2.29
dct-18	-2.29
R05G6.7	-2.29
dnj-19	-2.29
F23H11.3	-2.29
ttll-4	-2.28
stau-1	-2.28
pir-1	-2.28
phosphofructokinase	-2.28
F31C3.3	-2.28
Y105C5B.12	-2.27
ash-2	-2.27
sel-9	-2.27
wrt-2	-2.27
sec-3	-2.26
1-Jun	-2.26
fkb-6	-2.26
F53A2.3	-2.25
sphk-1	-2.25
F11A10.2	-2.25
K08D12.3	-2.25

pqn-46	-2.25
ZK675.4	-2.24
R06C7.1	-2.24
xbx-6	-2.24
ncx-3	-2.24
acy-1	-2.24
npp-5	-2.24
T23G11.6	-2.24
F14B8.2	-2.24
C07E3.6	-2.24
pat-3	-2.24
F02E9.5	-2.24
F20D1.1	-2.23
hpk-1	-2.23
npp-13	-2.23
fbxb-42	-2.23
dsh-2	-2.23
C54E4.2	-2.23
ubxn-4	-2.23
cpl-1	-2.23
ZC308.4	-2.22
ula-1	-2.22
pqn-39	-2.22
T20H4.5	-2.22
F52H2.7	-2.22
acn-1	-2.22
tag-232	-2.22
ttll-5	-2.22
ndg-4	-2.21
hrd-1	-2.21
nuo-2	-2.21
pis-1	-2.20
tag-173	-2.20

C17C3.1	-2.20
R107.5	-2.20
scm-1	-2.20
ugt-50	-2.19
B0303.14	-2.19
unc-60	-2.19
mdl-1	-2.19
npl-4.2	-2.19
F22G12.4	-2.19
Y45G5AM.9	-2.19
R10E12.2	-2.18
cco-2	-2.18
tag-297	-2.18
mec-7	-2.18
col-121	-2.17
alx-1	-2.17
knl-1	-2.17
Y111B2A.3	-2.17
F36D4.5	-2.17
WBGene00001562	-2.16
phg-1	-2.16
WBGene00008986	-2.16
C48B6.10	-2.16
Y17G9B.5	-2.16
apl-1	-2.16
ify-1	-2.15
K02C4.3	-2.15
F54B11.5	-2.15
somi-1	-2.15
tat-4	-2.15
C05D11.10	-2.14
ntl-2	-2.14
C37E2.1	-2.14

T12G3.2	-2.14
crs-1	-2.14
mev-1	-2.14
tab-1	-2.14
T28D9.1	-2.13
ceh-6	-2.13
rbg-1	-2.13
rps-2	-2.13
sec-24.1	-2.13
C47B2.2	-2.13
C14F11.1	-2.13
lin-49	-2.12
lin-66	-2.12
hlh-2	-2.12
dsl-3	-2.12
WBGene00010759	-2.12
fbxc-10	-2.12
wapl-1	-2.11
bli-4	-2.11
R04E5.9	-2.11
iff-2	-2.11
F13H8.7	-2.11
cki-1	-2.11
clh-5	-2.11
rsp-1	-2.11
hgo-1	-2.11
dnj-14	-2.11
mtss-1	-2.10
F09F7.4	-2.10
nhr-23	-2.09
ptr-2	-2.09
add-2	-2.09
ubq-1	-2.09

W05H12.2	-2.09
unc-119	-2.09
K10C2.1	-2.09
eft-1	-2.09
Y57A10A.26	-2.08
hil-7	-2.08
B0511.12	-2.08
dif-1	-2.08
hsr-9	-2.08
sup-17	-2.08
T19A6.3	-2.08
T07F10.3	-2.08
rps-17	-2.08
mex-3	-2.07
bus-8	-2.07
F45E4.3	-2.07
tmbi-4	-2.07
unc-9	-2.06
inft-2	-2.06
nuc-1	-2.06
Y53C12B.2	-2.05
T24H10.4	-2.05
F53F10.3	-2.05
his-6	-2.05
F02E9.10	-2.05
Y39A1A.7	-2.05
rps-0	-2.05
gfl-1	-2.05
rpt-3	-2.04
D2096.6	-2.04
arf-1.2	-2.03
K09E9.3	-2.03
pfd-2	-2.03

rpl-5	-2.03
egg-4	-2.03
chk-1	-2.02
gpr-2	-2.02
Y71G12B.10	-2.02
tag-197	-2.02
F45H11.3	-2.02
sqt-3	-2.02
gei-8	-2.02
F55F3.3	-2.02
ubxn-6	-2.02
Y48E1B.3	-2.02
Y45G12B.3	-2.02
pqn-13	-2.02
aos-1	-2.01
C26H9A.2	-2.01
C18C4.5	-2.01
C34G6.1	-2.01
psa-1	-2.01
aminoacylase-1 /// WBGene00007509	-2.01
R148.3	-2.00
pqn-52	-2.00
C36E6.1	-2.00