Interplay of neuronal and non-neuronal genes regulates intestinal DAF-16-mediated immune response during *Fusarium* infection of *Caenorhabditis elegans*

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Although precisely controlled innate immune response is governed by conserved cellular events in phylogenetically diverse hosts, the underlying molecular mechanisms by which this process is regulated against a multi-host pathogen remain unknown. *Fusarium oxysporum* is a model multi-host pathogen, known to be associated with neuronal stress in humans and vascular wilt in plants. The interaction between innate immune and neuronal pathways is the basis of many diverse biological responses. How these processes are coordinated in response to fungal disease is not well understood. Here, we show that *F. oxysporum* f. sp. *ciceri* causes neuronal stress and intestinal disintegration, ultimately leading to the death of *Caenorhabditis elegans*. To explore the regulatory framework of *Fusarium*-associated disease, we analysed the gene expression during infection, integrated temporal gene expression, and network analysis with genetic inactivation data in *Caenorhabditis elegans*. We identified 1024 genes showing significant changes in expression (corrected P-values < 0.05) in response to *Fusarium* infection. Co-expression network analysis of our data identified prognostic genes related to disease progression. These genes were dynamically expressed in various neuronal and non-neuronal tissues exhibiting diverse biological functions, including cellular homeostasis, organ patterning, stress response, and lipid metabolism. The RNA-seq analysis further identified shared and unique signalling pathways regulated by DAF-16/FOXO and SIR-2.1 linking neuronal stress, which facilitates negative regulation of intestinal innate immunity. Genetic analysis revealed that GCY-5 in ASE functions upstream of DAF-16, whereas ASI-specific SRD-1 regulates behavioural immunity. Overall, our results indicate that a ubiquitous response occurs during *Fusarium* infection mediated by highly conserved regulatory components and pathways, which can be exploited further for the identification of disease-responsive genes conserved among animals and plants. Finally, this study provided a novel insight into cross-species immune signalling and may facilitate the discovery of cellular therapeutic targets for *Fusarium*-associated disease.

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**INTRODUCTION**

Morbidity and mortality associated with fungal infections and emergence of resistant fungal strains necessitate the study of fungal pathogenesis and host innate immunity. Evidences suggest that a common virulence mechanism exists for a wide array of pathogenic fungi with broad host ranges. Several pathogens, including *Fusarium*, have the ability to infect both animals and plants. It has emerged as the second most frequent mould causing invasive fungal infection in humans and exhibits a broad resistance to antifungal drugs. *Fusarium*-induced neuronal stress and mycotoxicosis are considered as potential risk factors in humans and rats. In plants, it causes vascular wilt, head blight, root rot, seedling blight, and foot rot diseases, while *Fusarium*-mediated killing of *Caenorhabditis elegans* has recently been described. Previous, cross-kingdom pathogenicity of *F. oxysporum* f. sp. *lycopersici* was investigated in mice to unravel the disease mechanism in plants and mammals. Growing evidence indicates that Rho1, a signal component is indispensable for the virulence of *F. oxysporum* in plants, but not in mammalian hosts. In these reports, *Fusarium* pathogenicity in different host systems has been shown; however, which pathways might serve as the functional basis of *Fusarium*-associated disease or an immune state remains to be explored.

Mechanistic frameworks of defense and disease state accommodate certain common and contrasting themes that regulate host-specific pathogen surveillance system in eukaryotes. Innate immunity cannot be considered as autonomous. Increasing evidence suggests that actuation of the immune system is coordinated with the nervous system to regulate defense responses, as it perceives and responds to various pathogens in mammals. Their complexity led us to study how these systems influence each other at the molecular and cellular level in a well-characterised model organism *Caenorhabditis elegans*. Characterisation of nematode immunity is largely based on nosocomial bacterial pathogens. However, immune response directed towards a medically and agronomically important fungal pathogen, such as *F. oxysporum* has not been defined till date. Recent studies indicate that sensory and dopaminergic neurons regulate innate immune pathways in *C. elegans*. Furthermore, the regulation of DAF-16-mediated innate immunity in bacteria is well
explored in worms. Epidermal DAF-16 is known to be involved in immunity against *Drechmeria coniospora*, however, the role of intestinal DAF-16 in fungal infection is yet unknown.

Although these studies provide targeted information associated with immune pathways, a global overview of gene expression and function in a spatiotemporal manner defining organ specificity and pathway conservation across kingdoms due to fungal invasion is lacking. Studies on transcriptional variations have been widely used to analyse inter-kingdom differences and dissect changes in regulatory sequences and expression divergence among them. In addition, signal transduction defines functional homology, and genetic screens offer the detection of candidate genes involved in immune system programming.

Here, we employed integrated transcriptomic, genetic analysis, and a system-level approach to understand molecular parsimony associated with neuro-immune pathways. Using RNA-seq analysis, we created a transcriptional landscape of *C. elegans* invaded with *Fusarium* that exhibits an interconnected cascade of DAF-16- and SIR-2.1-regulated genes linking neuronal stress and immunity. We then constructed a correlation network and assessed the biological significance of modules focussing on disease/immunity-related genes. Organ-based network shows a distinct disease/immune signatures for specific organs. Using a genetic screen, we observed that intestinal DAF-16 is mainly responsive to *F. oxysporum* infection. Altogether, our study demonstrates the ability of a fungus to induce neuronal stress and trigger a non-canonical pathway, which regulates pathogen-induced immune response through avoidance and the activation of several novel immune-responsive genes. In addition, the approach would be applicable to identify the analogous pathways of defense response and its regulation across kingdoms.

**RESULTS**

*F. oxysporum* infection leads to intestinal disintegration in *C. elegans*

To establish potential *C. elegans–F. oxysporum* pathosystem, we screened *F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f. sp. *methioli*, and *F. oxysporum* f. sp. *lycopersici* for worm survivability. Worms showed a high susceptibility to *F. oxysporum* f. sp. *ciceri*, as compared to *F. oxysporum* f. sp. *methioli*. However, survivability of the worms grown on *F. oxysporum* f. sp. *lycopersici* was comparable to control worms grown on *Escherichia coli* OP50 (Figure 1a). Although *F. oxysporum* is known to infect plants in a host-specific manner, we found that both wild-type N2 and BA15 (rf-3) exhibited a susceptibility to *F. oxysporum* f. sp. *ciceri* under non-avoidance conditions (Figure 1b). In contrast, under avoidance conditions, Fusarium-infected *C. elegans* survived longer, exhibiting avoidance behaviour (Supplemental Figure 1). Microscopic studies revealed that infected worms ingest fungal spores in the absence of a food source, such as *E. coli* (Figure 1c). Histopathological analysis depicted the signs of fungal pathogenesis, including intestinal colonisation of germinating hyphae, resulting in their gross disintegration leading to death (Figure 1d). Of note, Fusarium pathogenesis exhibits similarities among diverse kingdoms. In plants, it causes clogging of vascular bundles and hypoxia, and in humans, the infection leads to fusariosis and haematological malignancies associated with inflammation and hypoxia.
Global transcriptional reprogramming in response to Fusarium
Next, to understand the complexity of disease mechanism and the
associated molecular parsimony, we performed RNA-seq analysis of
patho-stressed worms (Supplemental Figure 2; also detailed in
Supplemental Information). Comprehensive analysis of a tran-
scriptome by plotting the alignments of reads matched along the
exons of *C. elegans* chromosomes revealed an extensive
transcriptional activity in the genome (Supplemental Figure 3).
As expected, RNA-seq reads matched multiple locations in the
genome. Differential expression analysis led to the identification of
1312 dysregulated transcripts representing 1024 genes with the
Gene Ontology (GO), common and specific
that the most variable transcripts during infection were pre-
signatures. We performed hierarchical clustering, in which all
clusters, as early and late reprogrammers have distinct gene
signatures. We performed hierarchical clustering, in which all
analysed regions/areas were followed across time, showing stress-
regulated clustering of transcripts. Increased correlations between
some of the transcripts indicate that transcriptional differences are
pronounced during invasion (Figure 2a). Further analysis revealed
that the most variable transcripts during infection were pre-
dominantly protein coding, for example, gcy-4, gcy-5, srd-1, ktp-1,
ches-57, nhr-17, hsp-12.6, hsp-16.41, and hsp-70; whereas relatively
stable transcripts were dominated by non-coding RNAs. Using Gene Ontology (GO), common and specific themes across the
disease state were determined that included a response to stimuli,
membrane-bound receptors, signal transducer, and enzymatic
activity (Supplemental Figure 4c). To validate the results, we
investigated expression levels of 10 DEGs with highest-reads
abundance by qRT-PCR and obtained a positive correlation for 6
DEGs (a true positive rate between 50 and 60%) (Supplemental
Figure 5).

Disease network reveals organ-specific deregulated cellular
programmes as major drivers of pathogenesis
For delineating the global architecture of disease networks,
subsequently, we developed a three-step methodology to
construct biological modules associated with pathogenesis. First,
we identified differentially regulated disease pathways using
literature search. We then built a co-expression network of 245
nodes and 17 857 edges using WGCNA that identified prognostic
genes with dense interconnections (Figure 2b). Finally, network
modules were examined for disease gene signatures that allowed
the identification of novel targets to combat pathogenicity,
particularly in worms and other host systems. We classified these
modules into five functional categories, namely module 1
(homeostasis and co-signal regulatory control) mapped to 47
transcripts involved in signal transduction and transcriptional
regulation, including tkr-3, ttk-2, ac7-3, bath-20, oct-16, gpa-18,
ils-5, msp-52, and md-4. A closer scrutiny of the module revealed
the activation of signalling pathways densely linked to
G-protein signalling, serpentine receptor, and chemosensory
regulation. Deregulated molecular machines and organ
pattern-related module 2 with 41 DEGs was functionally
distinguishable in modular organisation. It is hypothesised that
multiple but relatively independent regulatory programmes might
govern organ-specific disease-associated factors during invasion.
Also, a group of stress-related genes associated with a common
activity. Interestingly, 126 DEGs found in this study are known to
be regulated by SIR-2.1, and a few of them have human orthologues such as *C. elegans*, particularly the ins/IGF-1-signalling path-
way, age-related disorders, and inflammations in humans.20-21 we
focused on 78 DAF-16-regulated DEGs that might be associated with *Fusarium* pathogenesis in worms (Figures 4a and d). To
tackle their relevance in response, we constructed a targeted gene expression
network, encompassing asp-12 (human orthologue NAPSA),
cle-2, hsp-16.41, ils-5, cyp-25A1, bath-20, R13G10.4, R13H9.5,
and nhr-6 having a distinct functionality during disease condition.
Further, it is known that SIR-2.1 in worms modulates DAF-16
activity.22 Interestingly, 126 DEGs found in this study are known to
be regulated by SIR-2.1, and a few of them have human orthologues such as *C. elegans*, particularly the ins/IGF-1-signalling path-
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and nhr-6 having a distinct functionality during disease condition.

Subnetwork analyses of DAF-16 and SIR-2.1-regulated genes
A disease network detected several genes known to be regulated
by DAF-16, SIR-2.1, or both. Given that DAF-16 controls innate
immunity in *C. elegans*, particularly the ins/IGF-1-signalling path-
way, age-related disorders, and inflammations in humans,20-21 we
focused on 78 DAF-16-regulated DEGs that might be associated with
*Fusarium* pathogenesis in worms (Figures 4a and d). To
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cle-2, hsp-16.41, ils-5, cyp-25A1, bath-20, R13G10.4, R13H9.5,
and nhr-6 having a distinct functionality during disease condition.

Both SIR-2.1 and DAF-16 co-regulate diverse biological pro-
cesses, including stress response, UPR, and longevity.23 As gene
expression may vary both as a cause and a consequence of the
disease, we investigated a set of common genes co-regulated by

These genes may exert a fine-tuned control of cellular processes and
prioritise as potential candidates for drug discovery to treat
fusariosis in humans and vascular wilt in plants. Further, we
interrogated module 4 (46 DEGs) encompassing development
regulators and immune-related factors associated with morpho-
genesis, organogenesis, development, and response to stimuli.
A coordinated interplay of DEGs in this module indicates the role
of genome plasticity during patho-stress. Finally, module 5 (38
DEGs) represents a molecular signature associated with lipid
metabolism and stress. Of these, asah-1, lip-3, C11D2.3, lip-1, and
lips-5 are the core element of lipid biogenesis. Whereas, ant-1,2,
C01B4.2, str-76, T22F3.11, C10G11.1, clec-3, fbxb-28, his-10, and
mth-11 are known to be involved in stress-associated processes
linking metabolism to immune response.

We took a step further to explore the crosstalk among different
organisms (neuron, pharynx, muscle, intestine, and hypodermis)
deriving fungal invasion (Figure 3). A total of 227 DEGs enriched in
neurons were deregulated, including 37 daf-16-regulated genes,
suggesting that *F. oxysporum* infection induces neuronal stress
in worms. Further, human orthologues of 4 genes enriched in
neurons, namely ktr-3 (orthologue GPBAR1), ugt-8 (orthologue
UGT3A2), R05G6.5.1 (orthologue NMES), and svn-85 (orthologue
GPR142) might act as potential candidates to unravel the link
between neuronal stress and fusariosis. In addition, the enrich-
ment of 139 intestinal genes associated with disease response
detected human orthologues in worms such as ant-1,2 (ortholo-
gue adenine nucleotide translocase ANT genes), C01B4.2 (orthol-
ologue progranulin), and ZK813.6 (orthologue SPINK5). Molecular
changes were also evidenced in hypodermis (14 DEGs), pharynx
(24 DEGs), and muscle (35 DEGs). Organ-specific global disease
network analyses suggest that the nervous system distinctly
perceives stress signals, while pharynx, intestine, muscle, and
hypodermis present a consistent tendency and functionality to
respond to a fungal pathogen.

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both DAF-16 and SIR-2.1 to construct a targeted gene expression network, including *asp-12*, *cyp-25A1*, *bath-20*, *ceh-57*, *clec-2*, and *nhr-6* for dysregulated transcriptional programmes associated with *Fusarium* disease (Figure 4c). In our analysis, regulatory relationships were found to be common among DEGs enriched in diverse organs. We detected neuronal genes of diverse categories such as *srd-1*, *gcy-4*, *gcy-5*, and *ceh-57* regulated by an interplay of the above-mentioned genes. Interestingly, of the 74 intestinal

Figure 2. Transcriptional dynamics during *F. oxysporum* infection. (a) Unsupervised hierarchical clustering of DEGs in the samples. The heatmap indicates Pearson’s correlation between pairwise sample comparisons, and the dendrogram indicates the average linkage distance between the samples. (b) Co-expression network representing five functional modules in *C. elegans* during *F. oxysporum* infection. The node colour denotes network modules, as determined using Cytoscape and the edge width represents a sign of association.
DEGs, 32 were regulated by SIR-2.1 and 21 were regulated by DAF-16. Many genes in the network were related to mitochondria (MT) and endoplasmic reticulum (ER) stress, including hsp-16.41, hsp-70, cyp-25A1, and bath-20. In addition, UPR-related genes were also regulated by DAF-16 and SIR-2.1. Therefore, we hypothesised that *F. oxysporum* infection in *C. elegans* activate intestinal and neuronal immunity mediated by DAF-16 and SIR-2.1 signal transduction pathway. This analysis prioritises functional recapitulation of a transcriptional complex involved in disease response, including asp-12, ceh-57, clec-2, and nhr-6. Further, a targeted gene expression network detected regulatory 'hotspots' for a disease or immunity with implications in *Fusarium*-associated aetiology.

Figure 3. Distribution of tissue-specific gene expression. Tissue-specific genes identified in RNA-Seq data and their distribution in (a) neuron, (b) pharynx, (c) muscle, (d) intestine, and (e) hypodermis.
Genetic interaction reveals DAF-16-mediated regulation of TGF-β pathway during infection

Global gene expression and disease network indicated that F. oxysporum invasion induces daf-16-mediated response in C. elegans. As the canonical immune-signalling pathways are known to respond to various bacterial and fungal infections in C. elegans,\textsuperscript{15} we carried out survivability assays of different pathway mutants to understand whether DAF-16 influences other
immune pathways or acts independently. Worms fed on daf-16: RNAI died significantly earlier than the control worms fed on vector control (P < 0.0001). We observed that silencing of daf-16 by RNAI had no effect on the survival of ERK and TOLL pathway mutants (P > 0.05; Figures 5a and b). Thus, we assume that ERK and TOLL pathways operate independently of Ins/IGF-1. We further demonstrate that daf-16 was epistatic to p38MAPK pathway mutants except sek-1 and ncy-1, which exhibited enhanced resistance compared to daf-16 RNAI worms (P = 0.0024 and 0.0016, respectively (Figure 5c). Hence, from our screen, we conclude that the p38MAPK cascade might be involved during the initial stages of infection when the worms come in contact with the fungal pathogen, F. oxysporum and triggers a signalling cascade similar to the response during Pseudomonas infection in C. elegans.25 Similarly, daf-16 was epistatic to TGF-β and DBL-1 pathway mutants (P > 0.05, Figure 5d). It has been shown that the ASI neuron expressing TGF-β influences longevity through daf-16.26 Thus, Ins/IGF-1 is mainly responsive to F. oxysporum infection, while DBL-1, TGF-β, TOLL, and ERK-1 are involved in behavioural response through pathogen avoidance.

ASE and ASI neurons are important for the regulation of innate immunity through DAF-16

An organ-based network and transcriptional profiling depicted that subsets of DEGs were enriched in the intestine and neurons. Among deregulated genes in a chemosensory neuron, gcy-5 and gcy-4 expressed in ASER27 showed downregulation at 6 hpi. In contrast, CEH-57, a homoeodomain box transcription factor was upregulated, supporting that it acts in concert with GCY-5 to regulate downstream genes. Also, srd-1 expressed in ASI28 exhibited downregulation at 6 hpi followed by upregulation till 48 hpi, suggesting that chemosensory neurons function in fungal perception. Furthermore, we postulated that F. oxysporum infection causes neuronal stress that directly or indirectly affects the expression of DAF-16- and SIR-2.1-regulated genes. To understand the interplay between innate immune systems, neuronal, and intestinal pathways, we compared a set of DAF-16- and SIR-2.1-regulated genes with DEGs identified in response to other bacterial and fungal pathogens.15 Few DEGs show a commonality, possibly due to the conservation of innate immune regulators in C. elegans during patho-stress (Supplemental Figure 8). Genetic analysis displayed that daf-16, sir-2.1, and sir-2.1;daf-16 RNAI worms exhibited a comparable survivability during infection (Figure 6a). To explore the influence of DAF-16 on GCY-5 and SIR-1, we performed F. oxysporum-mediated killing of gcy-5, srd-1;gcy5;daf-16 RNAI, srd1;daf-16 RNAI, and daf-16-silenced worms. gcy-5 worms died earlier than control worms, whereas no difference was observed for gcy-5;daf-16 RNAI worms (Figure 6a). In contrast, srd-1 worms, known to be influenced by DAF-1624 and TGF-β pathways26 showed enhanced resistance than gcy-5 worms. Survivability of srd-1;daf-16 worms was intermediate of single mutants, implying that SIR-1 contributes to immunity indirectly through DAF-16 and might be involved in avoidance. To further explain the avoidance behaviour, we performed an aversive olfactory-learning assay on srd-1, gcy-5, and wild-type worms (Figure 6b). The choice index (CI) of the wild-type worms showed that it can naturally avoid F. oxysporum; however, previous exposure of the wild-type worms (training) to a fungus altered their behaviour (Figure 6c). Further, a learning index (LI) showed that both srd-1 and daf-16-silenced worms avoid a fungus better than wild-type worms. Interestingly, daf-16-silenced srd-1 worms exhibited negative LI, reiterating that DAF-16 influences SIR-1 for avoidance (Figure 6d). In contrast, a...
The gcy-5 mutant had better CI under naive conditions, but was defective in learning to avoid a fungus (Figure 6e), as shown previously by Stein and Murphy.29 These observations indicate that both gcy-5 and srd-1 cause F. oxysporum avoidance at an early stage, while during later stages, it may lead to attraction and ingestion of spores. Altogether, our results pointed that srd-1 governs short- and long-term memory, whereas gcy-5 might have a role in memory accusation. Finally, DAF-16 controls SRD-1 and regulates behavioural response and innate immunity in worms during Fusarium infection.

DAF-16 activity is tightly controlled in varied subcellular localisation and by post-translational modifications. Nuclear localisation of DAF-16 in the intestine is required for developmental decisions.20 Besides, daf-16 isoforms have tissue specificity and functional patterns.21 Conversely, epidermal DAF-16 controls innate immunity against bacterial pathogens.19 Surprisingly, in our study, no expression change was observed for DAF-16. To understand how DAF-16 exerts its function in the signalling cascade, we examined transgenic worms expressing DAF-16::GFP reporter and found a nuclear translocation of DAF-16 in the intestine at 24–48 hpi (Figure 7a). Next, we analysed intestinal daf-16 RNAi to understand tissue-specific response. We found that VP303 worms30 fed on daf-16 RNAi died significantly earlier than control worms (P < 0.0001, Figure 7b). Thus, we show that non-neuronal daf-16 regulates the avoidance and immune response in C. elegans. Conclusively, nuclear localisation of intestinal DAF-16 activates signal transduction to ASI, leading to upregulation of srd-1. We conclude that gcy-5 downregulation affects the expression of other stress-related genes that might translocate DAF-16 to the nucleus directly or indirectly through SIR-2.1. During

Figure 6. Influence of DAF-16 on neuronal and non-neuronal genes and aversion assay for studying pathogen avoidance in C. elegans. (a) Wild-type daf-16 RNAi, gcy-5, srd-1, sir-2.1 mutants, gcy-5; daf-16 RNAi, srd-1; daf-16 RNAi, and sir-2.1; daf-16 RNAi were infected with F. oxysporum under non-avoidance conditions. N = 50 adult animals for each strain. Error bars represent S.E. from three independent experiments. *P < 0.05, **P < 0.01, and ***P < 0.001 by one-way ANOVA and Tukey’s post hoc test. P-values are relative to N2; daf-16 (RNAi) worms. (b) A schematic representation of the assays developed for understanding the behavioural response to F. oxysporum in C. elegans. (c) Choice index of wild-type srd-1, gcy-5, daf-16, and gcy-5;daf-16 worms. The white bar represents the choice index for E. coli OP50 and the coloured bar represents the choice index for F. oxysporum. (d) Learning index of wild-type srd-1, gcy-5, daf-16, and gcy-5;daf-16 worms. (e) Normalised choice index of wild-type srd-1 and gcy-5 worms. N = 30 adult animals for each strain. Error bars represent S.E. from three independent experiments. *P < 0.05, t test with Bonferroni correction.
the initial phase, ASE works upstream to intestinal \textit{daf-16}; later on, nuclear \textit{daf-16} and \textit{srd-1} in ASI primes invasion.

**DISCUSSION**

Here, we studied the complex regulatory network and molecular mechanism of \textit{Fusarium} pathogenesis in \textit{C. elegans}. This study indicates the crosstalk of \textit{Ins/IGF-1} signalling pathway and neuronal stress response, facilitating the negative regulation of intestinal innate immunity in a patho-stressed worm (Figure 8). RNA perturbation during invasion represents a global signature of infection, including lipid metabolism and neural development, which is known to be regulated by NHR transcription factors and \textit{Fusarium} toxin, fusimosin. \textsuperscript{31} A lipolytic enzyme, ASAH-1 is essential for neuronal development\textsuperscript{32} and GLF-1 plays a crucial role in the synthesis of a surface coat.\textsuperscript{33} Moreover, stress signals from MT and ER lead to altered lipid metabolism and unfolded protein response (UPR). UPR\textsuperscript{MT} is known to be stimulated by enhanced SIR-2.1 (Sirtuin orthologue in \textit{C. elegans}) and a lower level of mitochondrial ribosomal proteins.\textsuperscript{23} Thus, the imbalance of neuronal mitonuclear proteins results in intestinal UPR\textsuperscript{MT}.\textsuperscript{34} SIR-2.1, an epigenetic regulator involved in stress and longevity exhibits a neuroprotective role through the activation of multiple targets, indicating the conservation of neuronal signalling across kingdoms.\textsuperscript{24} It also controls mitochondrial function through deacetylation of DAF-16/FOXO and plays a role in aging and disease.\textsuperscript{35} CEP-1 (mammalian homologue p53) acts as a regulatory component for stress signals. However, its role in innate immunity is less explored. Focussed RNA-seq analysis used in this study demonstrated that lipid biosynthetic genes, namely \textit{asah-1} and \textit{glf-1} were downregulated, suggesting the deposition of ceramides in tissues and decline in sphingolipids synthesis. \textit{acdh-1}, a mitochondrial enzyme and \textit{ant-1.2}, a \textit{C. elegans} orthologue of adenine nucleotide translocase were upregulated, whereas \textit{nduo-3} was downregulated (Supplemental Table 4), reflecting an altered respiration state of the cell. Interestingly, six DEGs involved in lipid biosynthesis had human orthologues such as \textit{asah-1} (orthologues of N-acylsphingosine amidohydrolase), \textit{C18H9.5} (orthologues of SLC), \textit{mboa-4} (orthologues of MBOAT1), \textit{lipl-3} (orthologues of lipase), \textit{hsd-3} (orthologues of SDR43E2), and \textit{ZK896.1} (orthologues of SDR43E2).
of EphX1) that might be potential targets to link metabolism and immunity. In addition, a nuclear hormone receptor (nhr-17), C-type lectins (clec-192 and clec-193), and 1,5-5 were upregulated at 48 hpi. An altered expression of 109 DEGs (~9.25%) regulated by Sir-2, 1, 29 CEP-1-regulated DEGs, and three by both CEP-1 and Sir-2.1 might be related to Fusarium-induced UPR response and points towards the possible interplay of CEP-1, DAF-16, and Sir-2.1. Further, UPR leads to chaperone activation and its role in innate immunity has recently been recognised.36 This is consistent with our results, as ER chaperones such as hsp-12.6, hsp-16.4, and hsp-70 were upregulated during invasion (Supplemental Table 4). Interestingly, Notch receptors had non-developmental roles in nervous systems of adult mammals, Drosophila, and C. elegans.37 DOS-3 is a transmembrane protein and is predicted to function as a bipartite ligand activating C. elegans Notch receptors. Downregulation of dos-3 in our study may affect the susceptibility of worms to Fusarium. Thus, regulatory pathways affect cellular homeostasis and provided a link between metabolism and fungal pathogenesis in worms.

FOXO orthologue of DAF-16 induces the expression of antimicrobial peptides in Drosophila and humans. Further, a transcriptional target of DAF-16, srz-57 belongs to a serpentine receptor present in plants and regulates various cellular processes associated with extracellular stimuli. It is also known that targets of mir-59, M02G9.2, and 21UR-14847, and H04M03.6 present in our data set are influenced by DAF-16, (http://www.wormbase.org/) suggesting that non-coding RNAs might control the immune response through DAF-16. Our findings were in concordance with the previous data36 reporting non-coding RNA-mediated regulation of PMK-1 and DAF-2/DAF-16 deactivation of innate immune system. The identification of upstream genetic regulators of these pathways in worms might offer opportunities for understanding disease aetiology. It has been well documented that the interplay of a neuro-immune system in the context of non-neuronal tissues forms the basis of crosstalk among different organs; however, the underlying mechanisms by which these processes are coordinated in response to fungal disease remain to be poorly understood. Of these, the intestine and nervous system occupy a central role in environmental adaptation. Elegant work has shown that non-autonomous signals from different neurons have the potential to regulate non-neuronal tissues39 and immunity.42,43 However, a mechanistic crosstalk between the intestine and nervous system that influences innate immunity through forkhead transcription factor DAF-16 remains to be less explored. Differential expression of Gcy-4 and Gcy-5, expressed in ASE and SRD-1, and expressed in ASI highlights the role of ASE and ASI neurons in sensing the fungus. This hypothesis is further supported by genetic analysis, depicting that daf-16, sir-2, and sir-2;daf-16 RNAi worms exhibited a comparable survivability. Further, gcy-5 worms died earlier than control worms; whereas srd-1 worms showed enhanced resistance. In addition, CEH-57 and KQT-1 (an orthologue of human cardiac KvLQT-1 channel) were also differentially expressed. Our finding indicates hypoxia as a major cause and concern in the case of Fusarium pathogenesis in C. elegans similar to its functional homology in plants and humans. Concomitantly, we identified an oxygen sensor (BAG neuron) such as R05G6.5. DEGs related to pharyngeal neurons (W03F8.2) and musculature (C17F4.7, C24D10.5; daf-16, M02H5.8, and Y45G12C.10) in pathogen-stressed C. elegans. Additionally, pan-neuronal G-protein-coupled receptor was predominantly dysregulated in ASH and ASI during Pseudomonas aeruginosa infection in C. elegans.17 Ligand-gated ion channel LGC-8, the gap junction protein; INX-18,30 and GLR-3, an extracellular-glutamate-gated ion channel41 expressed in neurons, coelomocytes, and excretory cells, respectively, was downregulated (Supplementary Table 4). Also, WRT-6, a hedgehog-like protein expressed in hypodermis, neuronal sheath cells, and socket cells was upregulated at 48 hpi, suggesting that intercellular signalling is enhanced during infection. Thus, these DEGs might provide potential targets to unravel the relation between neuronal synopsis and innate immunity across a kingdom. In addition, our data identified diverse regulatory hubs displaying synergistic or antagonistic associations of disease or immune regulators and signal components.

In this study, we showed for the first time how a functional interplay of neuronal genes, intestinal DAF-16 regulatory components, and innate immune system regulates fungal pathogenesis. Potential mechanisms include the dysregulated expression of neuronal genes, translocation and combinatorial expression of DAF-16- and Sir-2.1-regulated DEGs, and perturbation of upstream-and downstream-signalling cascades. C. elegans – Fusarium oxysporum pathosystem might be instrumental in testing disease orthologues in plants and humans. Our study identifies novel molecular cassettes that perceive and respond to fungal virulence factors and provides mechanistic and diagnostic implications. Finally, we detected compendia of C. elegans orthologues of human genes in various innate immune pathways, including asp-12 (orthologue NAPSA), R08F11.4 (orthologue Williams–Beuren syndrome), and C18H7.4 (orthologue FES proocogene). These orthologues might possess translational potential to understand fusiariosis in humans.

MATERIALS AND METHODS

Strain maintenance

C. elegans strains were maintained on nematode growth medium (NGM) agar plates seeded with OP50 E. coli at 25 °C, as described earlier, except for BA15 (rrf-3).42 The strains used in this study were obtained from the C. elegans Genetics Center (University of Minnesota, MN, USA) and are listed in Supplemental Table 5.

E. coli strain OP50 was grown in Luria-Bertani (LB) broth at 37 °C, while strain HT115 was grown in LB supplemented with 25 μg/ml tetracycline.43 The fungal strains used in this study are Fusarium oxysporum f. sp. ciceri,8 Fusarium oxysporum f. sp. meliolii,44 and Fusarium oxysporum f. sp. lycopersici,11 which are known to infect Cicer arietinum, Arabidopsis thaliana, and Solanum lycopersicum, respectively. These stains were maintained on potato dextrose agar at 28 °C.

C. elegans killing assay

F. oxysporum was grown on PDB medium for 3–5 days, the culture was filtered, and the spores were washed twice with NGM broth. The spores were resuspended at a concentration of 10^7/ml of NGM liquid medium for the plate assay. The avoidance assay was performed, as described earlier and small lawns were prepared by spreading spores covering a small area on a 60-mm plate. A non-avoidance assay was performed in a 12-well plate with the agar fully covered with spores (60 μl/well). The plates were dried at room temperature (RT) for 2 h. Synchronised L4 worms were raised as described and further washed with a medium containing 100 μg/ml kanamycin. The worms were plated in NGM broth containing 100 μg/ml kanamycin and 1 mg/ml 5-fluoro-2'-deoxyuridine (Sigma), except when BA15 was used and incubated at 25 °C for 2 h for starvation so as not to hamper the growth of F. oxysporum.45 The worms were examined for viability using either Nikon 80i DIC microscopic or SXZ7 Olympus stereozoom microscope and scored at the times indicated, and were considered dead when they did not show pharyngeal pumping. All the experiments were conducted in two to three biological replicates.

Statistical analysis of experimental data

Statistical significance was calculated using PRISM v.6 and R software environment. Longrank Mantel–Heanszel test was used to compare the survival curves. Survival curves were considered to be different than the corresponding control, as indicated in Supplemental Table 5 when the P-value was < 0.05.

Localisation study

Synchronised worms were exposed to F. oxysporum till 48 h at 25 °C and mounted on 2% agarose with 30 mM NaCl in M9 medium for DAF-16 localisation study. The worms were visualised using Nikon 80i DIC.
microscope using the FITC filter. At least two independent biological replicates were screened for GFP expression.

Cellular integrity assay

The integrity of intestinal cells was visualised using propidium iodide staining, as described previously. Briefly, wild-type worms were transferred to plates seeded with either *F. oxysporum* under non-avoidance conditions or OP50 at 24 and 48 h. Worms were stained with 20 mg/ml propidium iodide for 30 min, washed twice with M9 buffer to remove the excess dye, and visualised under Nikon 80i DIC microscope using the TRITC filter.

RNA extraction, library preparation, and sequencing

Worms were grown in NGM broth, collected, and the total RNA was isolated using TRIzol reagent-based extraction method (Invitrogen, Carlsbad, CA, USA). RNA was further purified using RNAeasy kit (Qiagen, Hilden, Germany) and the quality was assessed on an Agilent 2100 bioanalyzer. High-quality RNA samples were used for subsequent studies. RNA-seq experiments were performed using RNA libraries generated from synchronised L4 worms representing three distinct time points. Altogether, seven samples were collected at 6, 24, and 48 h with and without infection and at 0 h as control (Supplemental Table 6). Libraries were sequenced on an Illumina HiSeq 2000 using paired-end chemistry and 101-bp cycles. qRT-PCR was performed to validate the expression of differentially expressed transcripts. To attain a higher depth, libraries were prepared from two replicates.

Raw reads mapping and assembly

Libraries were analysed with quality control tools in CLC Genomics Workbench (version 7.0.4) and FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We proceeded with an adapter sequence that removed high-quality reads for the RNA-Seq analysis using the workflow, as depicted in Supplemental Figure 2. Our replicates were significantly correlated. Therefore, we merged the replicates together to improve statistical power and further analyses were performed using the merged data. The TopHat-Cufflinks pipeline (version 2.0.9) was used to align the sequences, with the default parameters allowing two mismatches. The expected fragment length and the ‘small-anchor-fraction’ were set to default, 200 bp and 0.09, respectively, with at least 9 bp on each side of an exon junction for the 101-bp RNA-seq data. The sequence-aligned files generated by Tophat were subjected to Cuf exon junction for the 101-bp RNA-seq data. The sequence-aligned default, 200 bp and 0.09, respectively, with at least 9 bp on each side of an expected fragment length and the sequences, with the default parameters allowing two mismatches. The existing gene annotations. Isoform prediction of genes was classiﬁed as intestine and neuron associated. In order to compare between different releases of the Wormbase, we used the software ‘WormBase Converter’.

Gene co-expression network construction

The differentially expressed genes identiﬁed in time-lapse analyses were used to construct the weighted gene co-expression network using the R package WGCNA (version 1.51). A total of 327 transcripts were used to construct the network of all 7 samples (C0, C6, I6, C24, I24, C48, and I48). In the WGCNA algorithm, the elements in the co-expression matrix are deﬁned as the weighted value of the correlation coefﬁcient. The absolute values of Pearson’s correlation coefﬁcients were calculated for all possible gene pairs and the correlation matrix was transformed into a weighted adjacency matrix using a β-power of 6, so that the final correlation matrix followed an approximate scale-free topology. The connection strength between each set of gene pairs varies with the expression proﬁle and is used to calculate the topological overlap measure (TOM). Genes were clustered using an average linkage with their TOM distances. Co-expression modules were deﬁned as branches of the resulting clustering tree by specifying the branch height to cut, as well as the minimal number of genes to be included into a module. WGCNA cut-tree hybrid algorithm was used optimising the minimum module size to 30 and a tree-cut height of 0.25 in order to merge the neighbouring network modules with similar expression trends in different samples. Subnetworks were extracted for DAF-16 and SIR-2.1-regulated genes and genes expressed in specific organs. Co-expression networks were visualised in Cytoscape (http://www.cytoscape.org/) with topological overlap values as the edge weight. The CPM values were log2 transformed, converted into RGB colour codes, and used to display the relative expression levels in different networks.

miRNA and piRNA target prediction

miRNA-binding sites were obtained from Miranda with an mirSVR score (a measure of the likelihood that an miRNA targets a certain sequence) less than -0.3. Targets for piRNA were identiﬁed based on Bagijn et al.

Quantitative RT-PCR analysis

The total RNA was used to generate double-stranded cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qRT-PCR was done using the Power SYBER green PCR master mix (Applied Biosystems) on an Applied Biosystems 7500 real-time PCR machine in a 96-well-plate format. Actin (act-1) was used as the endogenous control and relative fold changes were calculated using the comparative \( \Delta \Delta C_T \) method. Primer sequences are listed in Supplemental Table 8.

RNAi experiments

RNAi phenotypes were generated by feeding worms with *E. coli* strain HT115 (DE3) expressing double-stranded RNA that is homologous to a target gene, as described earlier. HT115 (DE3) expressing the appropriate protein-coding genes. The identified novel protein-coding contigs were then filtered and the final processing was performed on the remaining unannotated contigs using BLASTX (E-value \( \leq 1e^{-10} \)) against the NON-CODE database and BLASTn against Rfam database to curate a confident set of novel non-coding transcripts. To predict splice junctions, RNA-seq data sets were subjected to SpliceMap using default parameters.

Differential gene expression and gene ontology analyses

We used EdgeR to normalise tag distribution per library and determined the transcript abundance by mapping reads against the reference genome (WS240). The Benjamini and Hochberg’s approach was used to adjust the resulting P-values for controlling the false-discovery rate. The transcripts having a false-discovery rate of < 0.05 were considered as differentially expressed. GO analysis was performed using Blast2GO.

Heatmap and tissue-enrichment analyses

K-means clustering was performed on differentially expressed genes with the K-means/K-median support module and heatmaps were generated using MeV v4.9.0 (MultiExperiment Viewer) (http://www.tm4.org/). Tissue-enrichment study was done using Wormmine tool (http://www.wormbase.org/tools/wormmine) based on previous studies and genes were classified as intestine and neuron associated. In order to compare between different releases of the Wormbase, we used the software ‘WormBase Converter’.

Identification of unannotated coding, non-coding RNAs, and novel splice junctions

Trinity and Cufflinks that generated fasta files were analysed further (Supplemental Figure 2) to identify regions of active transcription that do not overlap to the existing gene annotations using BLASTX (E-value \( \leq 1e^{-10} \)) against the following databases: (1) *C. elegans* genome (WS 240) and (2) *C. elegans* ESTs. Further, we searched for any possible match against *F. oxysporum* using Fusarium comparative database from Broad Institute (https://www.broadinstitute.org/annotation/genome/fusarium_group/Mul.tiHome.html). The annotated contigs and the contigs having a match with *Fusarium* genome were filtered and the contigs that did not have a significant BLASTX hit to *C. elegans* genome, ESTs, and *Fusarium* were processed using BLASTX (E-value \( \leq 1e^{-10} \)) against NCBI nr, PfamA, and PfamB, and UniProtKB/Swiss-Prot databases to identify potential targets of novel non-coding transcripts. To predict splice junctions, RNA-seq data sets were subjected to SpliceMap using default parameters.
vector was grown on LB agar plates containing 50 μg/ml ampicillin and 25 μg/ml tetracycline. For seeding, a single colony was inoculated in LB broth containing 100 μg/ml ampicillin at 37 °C overnight and plated onto NGM plates containing 100 μg/ml carbenicillin and 3 mM isopropyl β-D thiogalactoside. Synchronised L1 worms were placed on RNAi or vector control plates for 24 h at 25 °C and L4 worms were used for subsequent infection assays.

Aversive olfactory-learning assay

The aversive olfactory-training assays were performed, as described previously with modifications. Briefly, worms were synchronised by bleaching and L4-stage larvae were used for further studies on training and naive plates. One group was grown on a control NGM plate with ~ 300 μl of an overnight culture of E. coli OP50 (untrained or naive plate) and the other group was grown on a NGM plate with ~ 200 μl of spore culture F. oxysporum on one side and ~ 50 μl of an E. coli OP50 culture on the other side (training plate). After 48 h of training at 25 °C, the worms from both trained and untrained plates were placed on assay plates and counting was done after 1 h. Assay plates were prepared on 35-mm NGM plates with 20 μl of F. oxysporum and OP was placed on each end of the plate.

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AUTHOR CONTRIBUTIONS

SC conceived the project. SC, PN, and PA designed the study. PN and PA performed the wet-lab experiments. RT and NM performed RNA-seq analysis. SG and KN performed network and statistical analysis. PN, PA, KN, RT, SG, NC, and SC contributed to data interpretation. SC supervised the project. SC, KN, PN, and PA wrote the manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

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REFERENCES

1 Dickman MB, Figueiredo PD. Comparative pathology of fungal pathogen of plants and animals. PLoS Pathog 2011; 7: e1002224.
2 Staskawicz J, Mudgett MB, Dangl JL, Galan JE. Common and contrasting themes of plant and animal diseases. Science 2001; 292: 2285–2289.
3 Boutati EI, Anaissie EJ. Fusarium, a significant emerging pathogen in patients with hematologic malignancy: ten years’ experience at a cancer center and implications for management. J Gen Intern Med 2014; 29: 3384–3387.
4 Yu S, Avery L, Baude E, Garbers DL. Guanylyl cyclase expression in specific neurons functions as a regulator of epidermal innate immunity. Proc Natl Acad Sci USA 2005; 102: 16536–16541.
5 Novelli JF, Chaudhary K, Canovas J, Bennett JS, Madinger CL, Kelly P et al. Characterization of the Caenorhabditis elegans UDP-galactopyranosemutase homolog with aversive olfactory-training assays. Cell Death Discovery (2017) 17073 Of 8 Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
glf-1 reveals an essential role for galactofuranose metabolism in nematode surface coat synthesis. Dev Biol 2009; 335: 340–355.

34 Taylor RC, Berendzen KM, Dillin A. Systemic stress signalling: understanding the cell nonautonomous control of prostatectasis. Nat Rev 2014; 15: 211–217.

35 Houkooper RH, Pinrin E, Xuwier J. Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol 2012; 13: 225–238.

36 Richardson CE, Kooistra T, Kim DH. An essential role for XBP-1 in host protection against immune activation in C. elegans. Nature 2010; 463: 1092–1095.

37 Singh K, Choy MY, Somers GA, Komatsu H, Corkins ME, Larkins-Ford J et al. C. elegans notch signaling regulates adult chemosensory response and larval molting quiescence. Curr Biol 2011; 21: 825–834.

38 Kudlow BA, Zhang L, Han M. Systematic analysis of tissue-restricted microRNAs reveals a broad role for microRNAs in suppressing basal activation of the C. elegans pathogen response. Mol Cell 2012; 46: 530–541.

39 Mak HY, Nelson LS, Basson M, Johnson CD, Ruvkun G. Polygenic control of fat storage. Nature 2006; 38: 363–368.

40 Altun ZF, Chen B, Wang Z-W, Hall DH. High Resolution Map of Caenorhabditis elegans gap junction proteins. Dev Dyn 2009; 238: 1936–1950.

41 Brockie PJ, Madsen DM, Zheng Y, Mellem J, Maricq AV. Differential expression of glutamate receptor subunits in the nervous system of Caenorhabditis elegans and their regulation by the homeodomain protein UNC-42. J Neurosci 2001; 21: 1510–1522.

42 Miyata S, Begun J, Troemel ER, Ausubel FM. DAF-16-dependent suppression of immunity during reproduction in Caenorhabditis elegans. Genetics 2008; 178: 903–918.

43 Kamath RS, Ahringer J. Genome-wide RNAi screening in C. elegans. Methods 2003; 30: 313–321.

44 Diener AC, Ausubel FM. Resistance to Fusarium oxysporum, a dominant Arabidopsis disease-resistance gene, is not race specific. Genetics 2005; 171: 305–321.

45 Sternegle T. Maintenance of C. elegans. In: WormBook (ed). The C. elegans Research Community, WormBook: Pasadena, CA, USA, 2006, pp 1–11.

46 Shivers KP, Kooistra T, Chu SW, Pagano DJ, Kim DH. Tissue-specific activities of an immune signaling module regulate physiological responses to pathogenic and nutritional bacteria in C. elegans. Cell Host Microbe 2009; 6: 320–327.

47 Estes KA, Szumowski SC, Troemel ER. Non-Lytic, actin-based exit of intracellular parasites from C. elegans intestinal cells. PLoS Pathog 2011; 7: e1002227.

48 Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 2009; 8: 1105–1111.

49 Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010; 28: 511–515.

50 Kakumanu A, Ambavaram MM, Klumas C, Krishnan A, Batlang U, Myers E et al. Effects of drought on gene expression in maize reproductive and leaf meistem tissue revealed by RNA-Seq. Plant Physiol 2012; 160: 846–867.

51 R Core Team. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing: Vienna, Austria, 2005.

52 Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 2011; 29: 644–652.

53 Finn RD, Bateman A, Clements J, Coghill P, Eberhardt RY, Eddy SR et al. Pfam: the protein families database, Nucleic Acids Res 2014; 42: 222–230.

54 The UniProt Consortium. The Universal Protein Resource (UniProt), Nucleic Acids Res 2008; 36: 190–195.

Immunity against multi-host pathogen Fusarium
P Nag et al

55 Bu D, Yu K, Sun S, Xie C, Skogerbo G, Miao R et al. NONCODE v30: integrative annotation of long noncoding RNAs. Nucleic Acids Res 2012; 40: 210–215.

56 Gardner PP, Daub J, Tate JG, Nawrocki EP, Kolbe DL, Lindgreen S et al. Rfam updates to the RNA families database. Nucleic Acids Res 2008; 37: 136–140.

57 Au KF, Jiang H, Lin L, Xing Y, Wong WY. Detection of splice junctions from paired-end RNA-seq data by SpliceMap. Nucleic Acids Res 2010; 38: 4570–4578.

58 Robinson MD, McCarthy DJ, Smyth GK. ‘edgeR: A Bioconductor package for differential expression analysis of digital gene expression data’. Bioinformatics 2010: 26: 1.

59 Conesa S, Götz S. Blasts2GO: A comprehensive suite for functional analysis in plant genomics. Int J Plant Genomics 2008; 2008: 619832.

60 Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N et al. TMA: a free, open-source system for microarray data management and analysis. Biotechniques 2003; 34: 374–378.

61 Von Stetina SE, Watson JD, Fox RM, Olszewski KL, Spencer WC, Roy PJ et al. Cell-specific microarray profiling experiments reveal a comprehensive picture of gene expression in the C. elegans nervous system. Genome Biol 2007; 8: R135.

62 Spencer WC, Zeller G, Watson JD, Henz SR, Watkins KL, McWhirter RD et al. A spatial and temporal map of C. elegans gene expression. Genome Res 2011; 21: 325–341.

63 Rizki G, Iwata TN, Li J, Riedel CG, Picard CL, Jan M et al. The evolutionarily conserved longevity determinants HCF-1 and SIR-21/SIRT1 collaborate to regulate DAF-16/FOXO. PLoS Genet 2011; 7: e1002235.

64 Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J et al. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 2003; 424: 277–284.

65 Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 2008; 9: 559.

66 Ho R, Sances S, Gowrig V, Amoroso MW, O'Rourke JG, Sahabian A et al. ALS disrupts spinal motor neuron maturation and aging pathways within gene co-expression networks. Nat Neurosci 2016; 19: 1256–1267.

67 Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498–2504.

68 Bagijn MP, Goldstein LD, Sapetschnig A, Weick EM, Bouasker S, Lehrbach NJ et al. Function, targets, and evolution of Caenorhabditis elegans piRNAs. Science 2012; 337: 574–578.

69 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2ΔΔCt method. Methods 2001; 25: 402–408.

70 Zhang Y, Lu H, Bargmann CI. Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. Nature 2005; 438: 179–184.