Title

Unveiling the genes and pathways that are dysregulated in dopaminergic neurons during both familial and sporadic Parkinson's disease.

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Abbreviations

1. Dopaminergic	DA
2. Parkinson's Disease	PD
3. Substantia Nigra Par Compacta	SNpc
4. Induced Pluripotent Stem Cells	iPSCs
5. Common Differentially Expressed Genes	cDEGs
6. Gene Expression Omnibus	GEO
7. Protein-Protein Interaction	PPI
8. Gene Ontology	GO
9. Biological Processes	BP
10. Molecular Functions	MF
11. Cellular Components	CC
12. Molecular Complex Detection	MCODE
13. Database For Annotation, Visualization, And Integrated	

Discovery	DAVID
14. Transcription Factors	TFs
15. *Caenorhabditis* Genetics Center	CGC
16. The Nematode Growth Medium	NGM
17. First Larval Stage	L1
18. Basal Slowing Response	BSR
19. Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid	AMPA
20. Forkhead Box C1	FOXC1
21. Plant Homeodomain Finger 11	PHF11
22. Ras Association (Ralgds/AF-6) And Pleckstrin Homology Domains 1	RAPH1
23. Regulator Of G Protein Signaling 8	RGS8

Abstract

Identification of shared dysregulated genes and molecular mechanisms responsible for dopaminergic (DA) neuronal death in familial and sporadic forms of Parkinson's disease (PD) can offer the potential for common therapies in both types of PD. The gene expression profiles of DA neurons with sporadic and familial PD backgrounds and healthy DA neurons were obtained from the Gene Expression Omnibus database. Overlapping differentially expressed genes and hub genes were identified. These genes were subjected to Gene Ontology and Reactome pathway enrichment analyses. The roles of a select few differentially expressed genes in *C. elegans* DA neuron health were assessed. 40 genes, including 12 hub genes, were found dysregulated in DA neurons with sporadic and familial PD. Altering the expression of some of these genes in wild-type *Caenorhabditis elegans* promoted DA neuron degeneration and function. The study unveils shared molecular mechanisms in DA

neurons for familial and sporadic PD, potentially driving DA neuron degeneration. Understanding their roles may lead to targeted therapies, early detection, and intervention. *In vivo* experiments support our hypothesis, suggesting the implication of these genes in PD neurodegeneration and neuroprotection.

1. Introduction

Parkinson's disease (PD) is a complex neurodegenerative disorder that primarily involves the degeneration of midbrain dopaminergic (DA) neurons, thus reducing dopamine levels [1]. The accumulation of misfolded alpha-synuclein protein in the brain is the hallmark of the disease [1]. The prevalence of this disease increases with age, and most cases are diagnosed in people over the age of 60 [2]. PD can be broadly classified into two categories based on its etiology: familial PD and sporadic PD. Familial PD is relatively rare, accounting for 5-10% of all cases. It has a clear genetic basis, and several genes have been identified as responsible for its development, like *snca, lrrk2, park2, park6, pink1, and park7* [3]. These genes are critical for various cellular processes, including protein aggregation, mitochondrial function, and lysosomal degradation, which are essential for maintaining healthy neurons and other brain cells. Sporadic PD is the most common form of the disorder, making up approximately 90-95% of all cases [3]. Unlike familial PD, there is no clear genetic link in sporadic cases, and individuals affected by this form of the disease do not have a family history of PD. The exact cause of sporadic PD is not completely discerned but is believed to result from a combination of genetic susceptibility and environmental factors. Understanding the different forms of PD is crucial for advancing research and developing targeted therapies for affected individuals.

In both familial and sporadic PD, the progressive loss of DA neurons located in Substantia nigra par compacta (SNpc) is the defining feature of the disease.

Studying dysregulated genes in DA neurons is crucial for deepening our understanding of the molecular mechanisms underlying PD. Using powerful tools like high throughput RNA sequencing, attempts have been made to identify dysregulated genes in the disease state by comparing gene expression patterns in healthy versus PD-afflicted DA neurons [4-10]. Analyzing the postmortem brain tissue from PD patients provides a direct view of gene expression patterns in affected DA neurons [4-6]. Induced Pluripotent Stem Cells (iPSCs) derived from PD patients can be differentiated into DA neurons [7-10]. This approach offers a unique opportunity to study gene expression patterns in DA neurons specific to PD patients. CRISPR/Cas9 and other gene editing technologies have also enabled researchers to introduce or correct gene mutations associated with PD in cellular or animal models to investigate the direct impact of specific gene dysregulation on DA neuron function and survival [5].

Despite the differences in their genetic basis, some common pathways and mechanisms may contribute to the degeneration of DA neurons in both familial and sporadic PD. Identifying the shared dysregulated genes and pathways can help uncover the core cellular processes contributing to DA neuron degeneration in both PD forms. Understanding these fundamental mechanisms is crucial for developing targeted therapies that address the root causes of PD, potentially benefiting both familial and sporadic cases. Developing treatments for rare genetic subtypes of PD can be challenging due to the limited number of patients and diverse genetic mutations. By focusing on shared pathways and mechanisms, one can develop therapies that have the potential to be effective for a more significant proportion of PD patients, regardless of their genetic background. Understanding common pathways and mechanisms may also lead to identifying potential combinatorial

therapies. PD is a multifactorial disorder with various underlying causes. Simultaneous targeting of multiple pathways could offer a more comprehensive approach to potentially enhance therapeutic efficacy. Drug development is often a time-consuming and costly process. Drug discovery efforts can be streamlined by targeting pathways relevant to a broader population of PD patients by focusing on common genes dysregulated in familial and sporadic PD.

Utilizing the *Caenorhabditis elegans* model for neurodegeneration, especially in PD, offers significant benefits. Its short lifespan facilitates quick experimentation and observation of age-related neurodegenerative processes, while its well-understood genetics simplifies the study of specific genes implicated in PD [11]. The DA neurons *in C. elegans* have structural and functional similarities to human DA neurons, making it a relevant model for studying the degeneration of DA neurons in PD. The availability of transgenic *C. elegans* strains with specific fluorescent markers allows researchers to label and track specific cell types, such as DA neurons, in real-time [12].

In this study, by integrating different studies, we attempted to find common differentially expressed genes (cDEGs), hub genes, and metabolic pathways contributing to DA neuron degeneration in familial and sporadic PD. The gene expression datasets were obtained from the Gene Expression Omnibus (GEO) database, including gene expression profiles of DA neurons with different genetic backgrounds, representing both familial and sporadic PD. We identified 40 dysregulated genes in DA neurons common in familial and sporadic PD by analysing the gene expression datasets. Investigating the Protein-Protein Interaction (PPI) network of these genes identified the 12 hub genes. Gene ontology (GO) analysis was performed to understand the functional roles of the identified hub genes in various biological processes (BP), molecular functions (MF), and cellular

components (CC). Utilizing the Reactome databases, we identified enriched pathways in which the hub genes participate, shedding light on fundamental metabolic processes related to DA neuron degeneration in PD. Finally, we used *C. elegans* models to verify the implication of some of the cDEGs in DA neuronal health, and dopamine-associated behaviors. This *in vivo* approach added physiological relevance to our findings and provided insights into the impact of these genes on DA neurons. Our research findings are expected to provide a valuable understanding of the molecular mechanisms of DA neurodegeneration in both familial and sporadic PD. The identified cDEGs and hub genes may serve as potential candidates for further investigation in drug discovery approaches, potentially leading to the development of new therapeutics for PD.

2. Material and Methods

2.1. Data extraction

The data used in this study were obtained from the GEO (http://www.ncbi.nlm.nih.gov/geo/) database [13]. GEO database is a publicly accessible functional genomic data resource that includes microarrays, chips, and high-throughput gene expression data. The keyword "Parkinson's Disease" was used to search the GEO database, and the search results were filtered for *Homo sapiens*, returning 343 series in results (updated up to July 2023). The inclusion criteria for datasets used in this study were:

1. expression profiling is done via high-throughput sequencing
2. profiling on
    a) SNpc DA neurons from postmortem brains, or
    b) Human iPSC-derived DA neuron

3. samples included PD and healthy controls.

Based on the inclusion criteria, three gene datasets (GSE90469, GSE24378, and GSE125239) were used to identify DEGs[9,10,14].

2.2. Data processing and DEG screening

GEO2R is an online sequencing data analysis tool developed by the GEO database based on 2 R packages (https://www.r-project. org), GEO query, and Limma (Bioconductor, Roswell Park Comprehensive Cancer Center, NY, USA). The GEO query package is used to read the data, while the Limma package is used to calculate the multiples of differential expressions [15-17]. Gene expression profiles were analyzed by GEO2R to identify DEGs between PD and control DA neurons of the datasets independently. The $p \leq 0.05$ and $|\log2FC| \geq 0.5$ were set as thresholds for DEGs. The distribution of gene expression for the databases was visualized using a volcano plot and box plot. Overlapping DEGs from the three datasets were defined as cDEGs and were displayed with Venn diagrams, which were drawn by utilizing the online platform Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

2.3. PPI network analysis

A PPI network was predicted and constructed by the GeneMANIA plugin of Cytoscape software (version: 3.8.0; http://cyto-scape.org/) [18]. The minimum required interaction score for the PPI analysis was set at a combined score ≥ 0.4. The nodes represented genes, and the edges represented the interactive relations between the genes. The Molecular Complex Detection (MCODE) plugin in Cytoscape was used to perform gene network clustering analysis. This step aimed to identify key functional modules in the PPI network. The selection criteria used for clustering

were: K-core = 2, degree cutoff = 2, max depth = 100, and node score cutoff = 0.2. Significant modules obtained from the MCODE analysis were used to refine the hub candidate genes.

2.4. Functional enrichment analysis

The GO terms of hub genes were conducted with the online tool Database for Annotation, Visualization, and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) [19]. Significantly enriched function annotations were defined as GO terms and Reactome pathways with p-values of ≤ 0.05. The GO analysis, including BP, CC, and MF, provides a set of concepts for describing the molecular activity and the location where the genes execute their functions. The bubble plot of GO terms and Reactome pathways were visualized via the "ggplot2" package of RStudio software (version 1.3.959; https://rstudio.com/) [20].

2.5. Prediction of Transcription Factors (TFs)

TF-gene interactions with the hub genes point out the outcome of TF on functional pathways and expression levels of the genes. We employed the NetworkAnalyst platform (https://www.networkanalyst.ca/) to screen reliable TFs from the JASPAR database with hub genes to construct a TF-gene regulatory network [21,22].

2.6. Gene-Disease Association Analysis

With the intensive study of transcriptomics, gene regulation-related disease profiles are continuously identified, and different diseases can be linked by one or more similar genes. DisGeNET is a comprehensive database for the study of disease comorbidity and genetic features, with data integrated from multiple information sources, including the genome-wide association studies catalog and

literature collection sources, featuring different biomedical manifestations of the disease and highlighting new insights into human genetic disorders [23]. We studied gene-disease comorbidity through the NetworkAnalyst platform equipped with the DisGeNET database to identify diseases associated with hub genes.

2.7. Gene-Drug Association Analysis

Gene-Drug Association was assessed via the NetworkAnalyst platform (equipped with the DrugBank database to identify drugs associated with hub genes [24].

2.8. C. elegans Strains and Maintenance

The strain, BY250 (expressing GFP in DA neurons) was maintained on the nematode growth medium (NGM) plate seeded with *Escherichia. coli* OP50 as food at 20°C [25]. To isolate the eggs, adult worms were treated with a bleaching solution. The extracted eggs were age-synchronized at the first larval stage (L1) in M9 buffer at 20°C for 16-18 hours. The L1s were transferred to NGM RNAi plates for gene knock-down and imaging.

2.9. RNAi Induction

RNAi induction was performed by feeding worms with *E. coli* strain HT115 (DE3) expressing double-stranded RNA homologous of target genes as previously described [26]. The RNAi bacteria were grown overnight in Luria-Bertani broth containing 100 µg/ml ampicillin and 12.5 µg/ml tetracycline at 37°C. This was used to inoculate LB containing 100 µg/ml ampicillin in 1:100 ratio. The secondary cultures were grown at 37°C until an OD600 of 0.6. The cultures were centrifuged, the pellets were resuspended in 1X M9 buffer containing 1 mM IPTG and 100 µg/ml ampicillin and plated over the NGM plates already supplemented with 100 µg/ml Ampicillin and

2mM IPTG. RNAi-expressing plates were allowed to grow for approximately 24 hours at room temperature and stored at 4°C till further use. *C. elegans* were fed HT115 expressing double-stranded RNAi or empty vector at the L1 larval stage.

2.10. DA Neuron Imaging

The day three adult worms were collected from RNAi plates, washed with M9 buffer and transferred to a 2% agarose pad on a glass slide. Worms were anesthetized with 30 mM sodium azide and covered with a coverslip. Images of the head region were taken at 20X magnification using a fluorescence microscope (Carl Zeiss), and the fluorescence intensity of GFP in DA neurons was quantified with Image J software (NIH, Bethesda, MD) [27]. The assay was performed for at least 30 worms for each condition. Final datasets were presented as the mean of the value for each worm.

2.11. Pharyngeal Pumping Rate

DA neurons regulate the rate and coordination of pharyngeal pumping, and a reduction in pharyngeal pumping rate indicates DA neuron dysfunction, a hallmark of PD. Videos of Day 3 adult worms for at least 20 seconds was recorded using stereo-zoom microscope (Leica) [28]. The assay was performed for at least 30 worms for each condition. Final datasets were presented as the mean of the value for each worm.

2.12. Basal Slowing Response (BSR)

C. elegans adjust their locomotion speed based on food presence, moving slower when bacteria are present. A dopamine-driven neural circuit controls this response, which detects bacterial cues, potentially increasing their feeding time. BSR assays were performed as previously discussed [29]. Day 3 adult worms were

transferred to un-seeded NGM plates (off-feed). The number of body bends per 20 seconds for at least 10 worms was recorded for worms in off-feed and on-feed conditions using stereo-zoom microscope (Leica)[28]. The assay was performed for at least 30 worms for each condition. Final datasets were presented as the mean of the value for each worm.

2.13. 1-Nonanol Repulsion Assay

As variation from the normal dopamine levels affects worm response to volatile repellent 1-nonanol, the nonanol assay was also performed after knocking down the cDEGs in the nematodes [30]. The assay was performed in 6 cm NGM plates. During the assay, at least 50 day 3 adult worms in 10µl of M9 buffer were transferred to the centre of the plate, after which 1µl of 1-nonanol was added to two opposite quadrants of the plate. The worms were allowed to move around the plate for 30 min. Number of worms in each quadrant was determined and the repulsion index was calculated by:

$$\frac{\text{(Total no. of worms in quadrants with 1-nonanol - Total no. of worms in quadrants without 1-nonanol)}}{\text{Total no. of worms}}$$

The assay was performed in three biological repeats for each condition. Final datasets were presented as the mean of three repeats.

2.15. Statistics

The histograms of each assay were generated with GraphPad Prism v 8.0 (GraphPad Software Inc.). Statistical analysis of significance was carried out by unpaired student's t-test. A p-value ≤ 0.05 was considered to be statistically significant. In all figures, error bars represent SD; * $p<0.05$; ** $p<0.01$; *** $p<0.001$; and **** $p<0.0001$.

3. Results

3.1. Generation of cDEGs PPI Network and Functional Enrichment of Hub Genes

A total of 1,381 DEGs were identified through the gene expression profile data GSE90469 (Figure 1A). A total of 1,995 DEGs were identified through the gene expression profile data GSE24378 (Figure 1A). A total of 5,501 DEGs were identified through the gene expression profile data GSE125239 (Figure 1A). Gradient volcano plots are displayed in (Figure 1B). A Venn plot was used to identify the cDEGs in the three databases. 40 cDEGs, including 21 upregulated and 19 downregulated genes were screened (Figure 1(C-D)).

The PPI network of cDEGs, constructed using Cytoscape on the ground of the GeneMANIA database, selected 58 nodes and 326 edges in the network (Figure 2A). The top three significant modules were extracted through the MCODE plugin of Cytoscape, of which module 1 contained 17 nodes and 71 edges, module 2 contained four nodes and nine edges, and module 3 contained three nodes and six edges (Figure 2B). The nodes included 12 of the cDEGs (Table 1). Among these, increased levels and activity of PSMB8, Proteasome Subunit Beta 8, has been previously reported in postmortem human brains with PD [31]. Another gene, GRIA1, a subunit of AMPA receptors, has been observed to be significantly lowered in the SNpc of PD patients compared to normal samples [32].

GO enrichment analysis was performed to examine the biological characteristics of the 12 hub genes. The GO analysis of cDEGs in these modules identified chemical synaptic transmission, calcium ion transport, and neuron projection development as top 3 significant BP (Figure 2C). The postsynaptic density membrane, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

glutamate receptor complex, and excitatory synapse were top 3 CC (Figure 2D). Moreover, top 3 MF included voltage-gated calcium channel activity, neurotransmitter receptor activity involved in the regulation of postsynaptic cytosolic calcium ion concentration, and GTPase activity (Figure 2E). The Reactome Pathway analysis showed they were majorly involved in Neuronal System, Transmission across Chemical Synapses, and PPI at synapses (Figure 2F).

3.2. Exploring associations of hub genes

3.2.1. Gene-TF Regulatory Networks

Since, TFs are primarily responsible for regulating gene transcription; TFs can be master regulators of the expression of the hub genes. The TF-gene interaction network consisted of 58 nodes and 86 edges. The node consists of 12 hub genes and 46 TFs. The PHF11 was found to be regulated by the highest number of TFs, i.e., 14, and Forkhead box C1 (FOXC1) was the TF regulating a maximum number of hub genes, i.e. 9 (Figure 3A).

3.2.2. Gene-Disease Associations

The gene-disease correlations comprised 4 subnetworks with 84, 14, 11, and 3 nodes respectively and 83, 13, 10, and 2 edges, respectively. The node in subnetwork 1 had one hub gene, PSMB8, and 83 diseases (Figure 3B(i)). The subnetwork 2 associated GRIA1 with 13 diseases (Figure 3B(ii)). 10 diseases were linked to HCN4 according to subnetwork 3 (Figure 3B(iii)). The subnetwork 4 showed an association between PHF11 and two diseases (Figure 3B(iv)). Among all the diseases few are also associated with nervous system other than PD, like Mental Depression, Morphine Dependence, Schizophrenia, Substance Withdrawal Syndrome, Cocaine-Related Disorders, and Basal ganglia calcification.

3.2.3. Gene-Drug Associations

The gene-drug correlations consisted of 11 nodes and ten edges. The node consists of 1 hub gene, GRIA1, and ten drugs, namely, L-Glutamic Acid, Enflurane, Isoflurane, Ethanol, Methoxyflurane, Desflurane, Sevoflurane, Talampanel, CX717, and Perampanel (Figure 3C). Among these Talampanel, CX717, and Perampanel have previously been investigated in various neurological disorders. Talampanel is an AMPA receptor antagonist under study for treating brain tumors and other brain disorders, such as epilepsy and PD [33]. CX-717 is an ampakine compound previously investigated for the treatment of Attention-Deficit/Hyperactivity Disorder and AD [34]. Perampanel is a AMPA glutamate receptor antagonist used to treat seizures and epilepsy [35].

3.3. Raph1 and rgs8 RNAi degenerates DA neurons and deteriorates dopamine dependent behaviour in healthy C. elegans

The reduced expression of a set of cDEGs in PD-associated DA neurons suggests that these genes may have a detrimental role in DA neurons if their expression is below normal. This hypothesis was assessed by knocking down the *C. elegans* homologs of a few of these genes, *raph1* and *rgs8* in nematode strain BY250, which expresses GFP in DA neurons, making it possible to assess DA neuron health under a fluorescence microscope. At day 3 of adulthood, the GFP intensity in DA neurons was reduced when BY250 was grown on RNAi against homolog of *raph1* (F10E9.6) (15.20 ± 7.05) and *rgs8* (C29H12.3) (17.60 ± 6.36), as compared to control RNAi (23.92 ± 0. 6.83) (Figure 4(A-B)). It suggests that reduced expression of certain cDEGs like *raph1* and *rgs8* may have significant effects on DA

neurons health and that modulating the expression of these genes could be a potential avenue for therapeutic intervention in PD.

We also tested the effect of cDEGs knock-down on the DA neuron-linked behaviour like pharyngeal pumping rate, BSR, and 1-nonanol repulsion index. BY250 worms showed slower pharyngeal pumping per 20 seconds on F10E9.6 (106.33 ± 6.21) and C29H12.3 RNAi (110 ± 8.17) when compared with the control RNAi (140.53 ± 16.65) (Figure 4C). BY250 worms on all the RNAis showed similar locomotion rate on the off-food plates (control RNAi- 10.63 ± 2.53; F10E9.6 RNAi- 9.2 ± 2.23; C29H12.3 RNAi- 10.00 ± 2.16) (Figure 4D). However, on the on-food condition F10E9.6 and C29H12.3 RNAi displayed higher motility 5.8 ± 2.3, and 5.33 ± 1.58, respectively as compared to control RNAi (1.13 ± 0.77) (Figure 4D). This suggests that *raph1* and *rgs8* knock-down result in the disruption of the food-sensation capability in wild-type worms, induce the worms to move around and look for food even on seeded NGM-plates. BY250 worms displayed suppressed repulsion behaviour to 1-nonanol on F10E9.6 (-0.08 ± 0.02) and C29H12.3 RNAi (-0.13 ± 0.07) when compared with the control RNAi (-0.54 ± 0.14) (Figure 4E). These observations suggest that the expression levels of cDEGs like *raph1* and *rgs8* play a significant role in regulating the dopamine dependent behavior in the nematode, and altering the expression of these genes can lead to changes in behavior related to DA neurons.

4. Discussion

The progressive loss of DA neurons in the SNpc is a hallmark of PD and leads to characteristic motor symptoms [36]. Both familial and sporadic PD cases involve the degeneration of DA neurons [37]. Understanding the pathological alterations in these neurons is crucial for understanding the molecular mechanisms involved in the

disease development and progression. Identifying and studying shared target genes altered in DA neurons in both familial and sporadic cases of PD can help gain insights into common underlying pathways that contribute to the DA neuron degeneration and progression of the disease. This knowledge can help unravel the complex biology of PD and provide potential therapeutic targets. Identifying common target genes can aid in developing more accurate diagnostic markers for PD. This is crucial for early detection and intervention, potentially slowing disease progression and improving patient outcomes.

In the current study, we re-analyzed the GEO datasets GSE24378, GSE90469, and GSE125239, focusing on DA neurons representing familial and sporadic PD. Through a comprehensive comparison of gene expression profiles in these three datasets, we identified a set of 40 genes that were consistently differentially expressed across the three datasets. Notably, some of these cDEGs namely PPP1R1B, DEPTOR, DLGAP3, DNER, GJA1, GREM1, GRIA1, IL13RA1, LIPA, NECAB1, and PSMB8 have previously been reported to exhibit altered expression in cellular and mice model of PD as well as in human PD brain tissues [38-63]. This strengthens our findings and provides additional validation to the relevance of these genes in DA neurodegeneration in PD. Importantly, a significant proportion of the cDEGs are uncovered for the first time in our study, shedding new light on the shared molecular mechanisms underlying familial and sporadic PD. Using in vivo assays, we have reported the effect of some of these cDEGs on DA neuronal health and function (Figure 4). Further research is needed to understand the specific roles of these genes in DA neurodegeneration and how their dysregulation may contribute to PD development.

The 12 hub genes we screened out via PPI network analysis may be the core genes involved in the pathological process of DA neurodegeneration in PD. This hypothesis is substantiated by previous reports, particularly concerning PSMB8 and GRIA1. The other hub genes have been associated with DA neurons in context of PD for the first time in our study. For instance, PHF11 (Plant Homeodomain Finger 11), which is involved in chromatin remodeling and transcriptional regulation was found constantly upregulated PD-DA neuronsin our study [64]. While the exact role of PHF11 in PD remains to be fully understood, our findings mark the first step towards exploring its potential implications in this disorder. Perturbations in PHF11 expression or function may lead to dysregulation of genes involved in DA neuron survival, differentiation, or neurotransmitter production and release. This, in turn, could result in the dysfunction or degeneration of DA neurons, contributing to the pathogenesis of PD.

The GO analysis of hub genes identified postsynaptic neurotransmitter receptor diffusion trapping, neuron spine, and neurotransmitter receptor activity involved in regulation of postsynaptic cytosolic calcium ion concentration as the highly enriched BP, CC, and MF respectively. The Reactome Pathway analysis showed the hub genes were highly enriched in Presynaptic depolarization and calcium channel opening. Dysregulation of these mechanisms, along with altered neurotransmitter receptor activity, may disrupt calcium homeostasis and contribute to the degeneration of DA neurons and the developments of PD. Few reports addressing these processes have offered therapeutic strategies for managing PD-related neurodegeneration [65,66].

The TF-gene interaction network revealed that FOXC1 was the TF regulating largest proportion of hub genes. Another study has reported decreased FOXC1

levels in the SNpc of PD patients compared to matched controls [67]. This and our study suggest that certain genes, regulated by FOXC1 which is involved in developmental processes, may have later implications for neurodegenerative disorders like PD. Hence, FOXC1 may play a critical role in the progression and pathogenesis of PD.

Furthermore, using *C. elegans* model we have provided evidence for the impact of dysregulation of some of the cDEGs on DA neuronal health. As a Ras-associated protein, RAPH1 (Ras Association (RalGDS/AF-6) And Pleckstrin Homology Domains 1) interacts with Ras GTPases, which are known to be involved in cell proliferation, differentiation, and survival. Studies have suggested that RAPH1 may have a role in neuronal function and maintenance, making it a potential candidate for involvement in PD [68]. Dysregulation of RAPH1 expression or function could potentially disrupt cellular processes vital for the survival and function of DA neurons, leading to their degeneration and contributing to PD. RGS8 (Regulator Of G Protein Signaling 8) is involved in regulating G protein-coupled receptor (GPCR) signalling by acting as a GTPase-activating protein [69]. GPCRs are key components of dopamine signalling. If RGS8 expression is altered, it may result in abnormal dopamine signaling, affecting the release, uptake, or sensitivity of dopamine, and could potentially impact the function and survival of DA neurons. In the current study, we have shown that knocking down RAPH1 and RGS8 in BY250 resulted in DA neurodegeration, and impaired dopamine-linked behaviour. These findings indicate that RAPH1 and RGS8 are likely involved in the maintenance of DA neuron health their inhibition results in neurodegeneration.

A selection of pharmaceutical agents has garnered attention as potential candidates for repurposing in the treatment of PD. In fact, very recently Jun Ueda et

al. has shown that Perampanel, an antiepileptic drug, can impede the progression of α-synuclein pathology within mouse hippocampal neurons by preventing the uptake of α-synuclein preformed fibrils through the inhibition of macropinocytosis. Additionally, oral administration of perampanel effectively mitigated the development of α-synuclein pathology in wild-type mice that had been inoculated with α-synuclein preformed fibrils. Thus, Perampanel emerges as a novel candidate for PD [70].

5. Conclusions

In conclusion, our study provides valuable insights into the DA neuron-specific shared molecular mechanisms underlying familial and sporadic PD. Dysregulation of these processes could contribute to the degeneration of DA neurons seen in PD. The potential therapeutic relevance of these genes is further supported by their association with other neurodegenerative disorders and the identification of drugs like nimodipine, spironolactone, carvedilol, and others that could be repurposed for PD treatment. Understanding the role of these genes and their interactions in DA neuron degeneration can lead to the development of targeted therapies and diagnostic markers for early detection and intervention in PD. The hypothesis that these genes may have role in DA neuronal health in familial and sporadic PD was supported by the outcomes from *C. elegans* experiment performed in the study. Comprehensive investigations in the future focussed on identification of agonists/ antagonists of these genes, further supported by rigorous pre-clinical studies utilizing rodent models will help develop more effective and improved therapeutic strategies for PD.

Declaration of interest

The authors declare no conflict of interest.

Acknowledgements

PKP is supported by Senior Research Fellowship from CSIR. We thank Dr. Aamir Nazir for providing the fluorescence microscope facility. BY250 strain was provided by *Caenorhabditis* Genetics Center (CGC), University of Minnesota, MN, USA.

Author Contribution

PKP- Investigation; Data curation; Formal analysis; Validation; Visualization Interpretation, Manuscript writing - Review.

SV – Conceptualization; Investigation; Data curation; Formal analysis; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft; and Writing - review & editing.

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Tables

Table 1: The list of 12 hub genes identified from the modules.

ID	Gene Name
CELF3	CUGBP Elav-like family member 3
PHF11	PHD finger protein 11
RAB6B	RAB6B, member RAS oncogene family
ADGRB3	Adhesion G protein-coupled receptor B3
BTN3A2	Butyrophilin subfamily 3 member A2
GRIA1	Glutamate ionotropic receptor AMPA type subunit 1
HCN4	Hyperpolarization activated cyclic nucleotide gated potassium channel 4
KIF3C	Kinesin family member 3C
PSMB8	Proteasome 20S subunit beta 8
RGS8	Regulator of G protein signaling 8
SEPTIN3	Septin 3
SAMD14	Sterile alpha motif domain containing 14

Figures

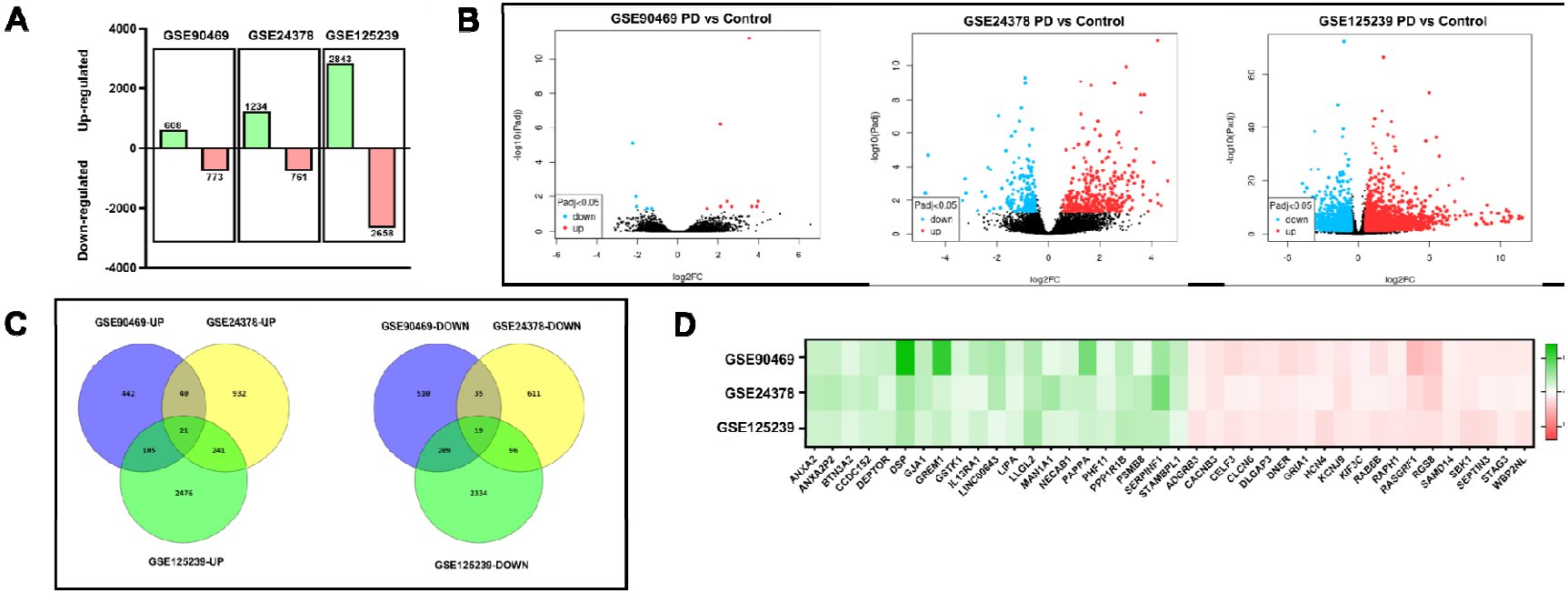

Figure 1. High throughput gene expression analysis of the three selected datasets using GEO2R. (A) Bar graph of up- and downregul genes in PD vs healthy DA neurons from each dataset. **(B)** Volcano plots to demonstrate gene expression differences between PD and co groups in the datasets. The criteria for selection of differentially expressed genes was set $p \leq 0.05$ and $|\log2FC| \geq 0.5$. **(C)** In the 3 datasets DEGs were upregulated, and 19 DEGs were downregulated, as depicted in the Venn diagram. **(D)** Heat map showing the cDEGs in PI Control DA neurons within the three datasets. The rows represent the GSE accession number of the datasets, and columns represent cDEGs.

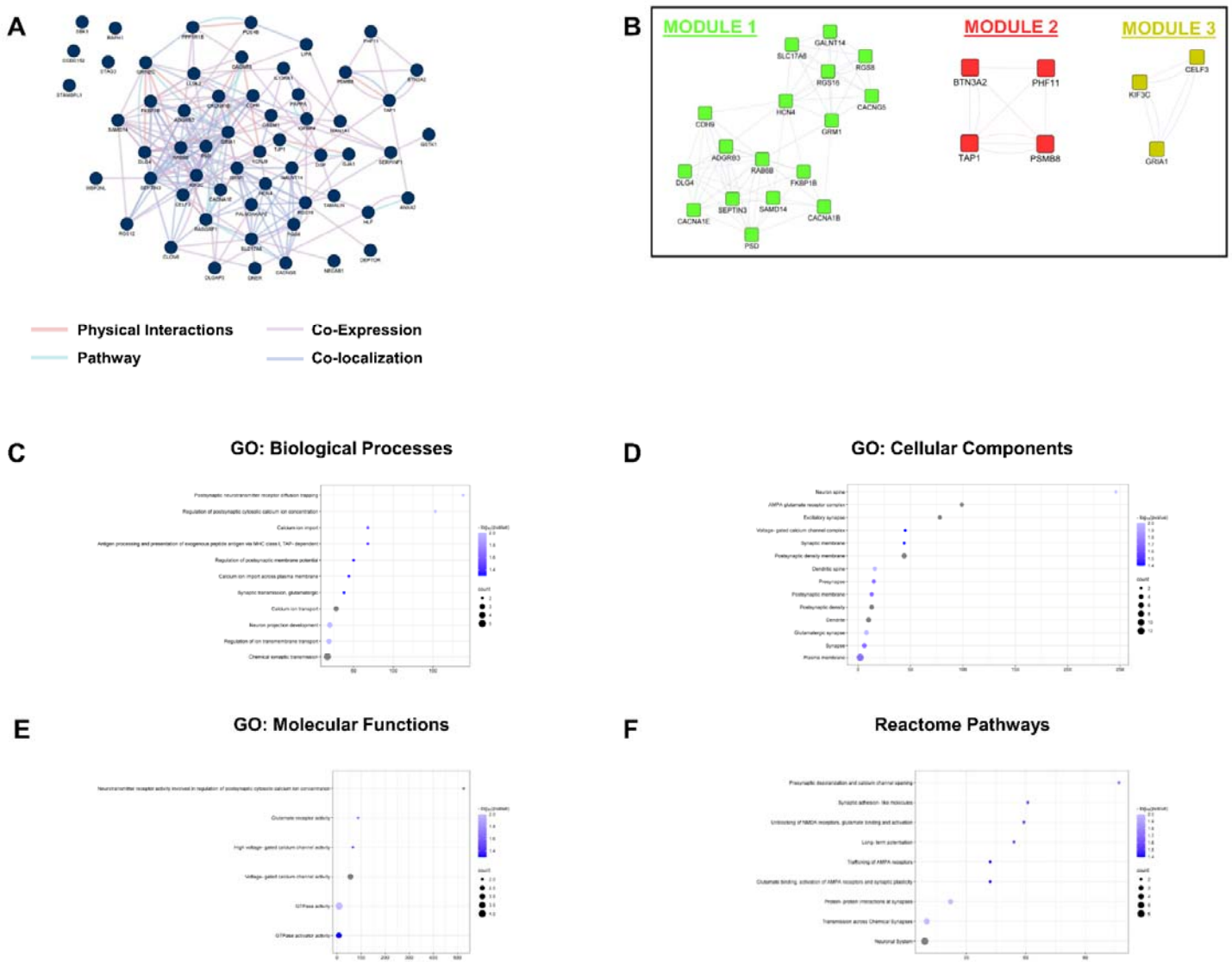

Figure 2: Identification of hub genes among the cDEGs. (A) The 40 cDEGs were filtered into the PPI network complex using GENEMA plugin in the Cytoscape software. The network nodes include proteins encoded by the cDEGs. The edges represent their functional associati

(B) The most significant modules were screened using the MCODE plugin in the Cytoscape software. Bubble plots showing the significant GO terms for **(C)** Biological Processes, **(D)** Cellular Components, and **(E)** Molecular Function ranked by fold enrichment following analysis of hub genes. **(F)** Reactome pathway analysis of the hub genes.

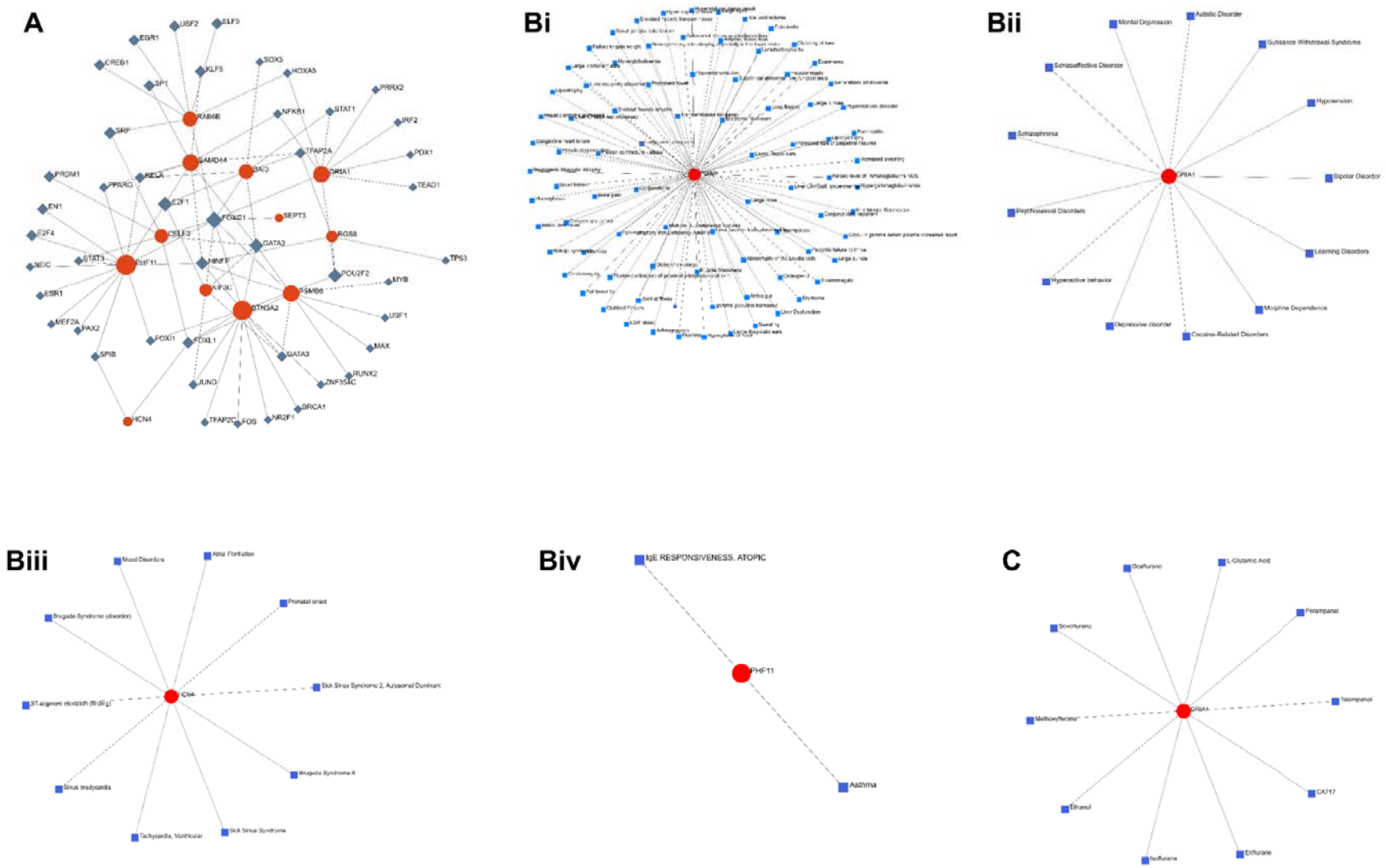

Figure 3. Biological networks of hub genes as created by Networkanalyst tool. (A) Gene-Transcription factor Interaction network. **(B** Gene-Disease Interaction network, and **(C)** Gene-Drug Interaction network.

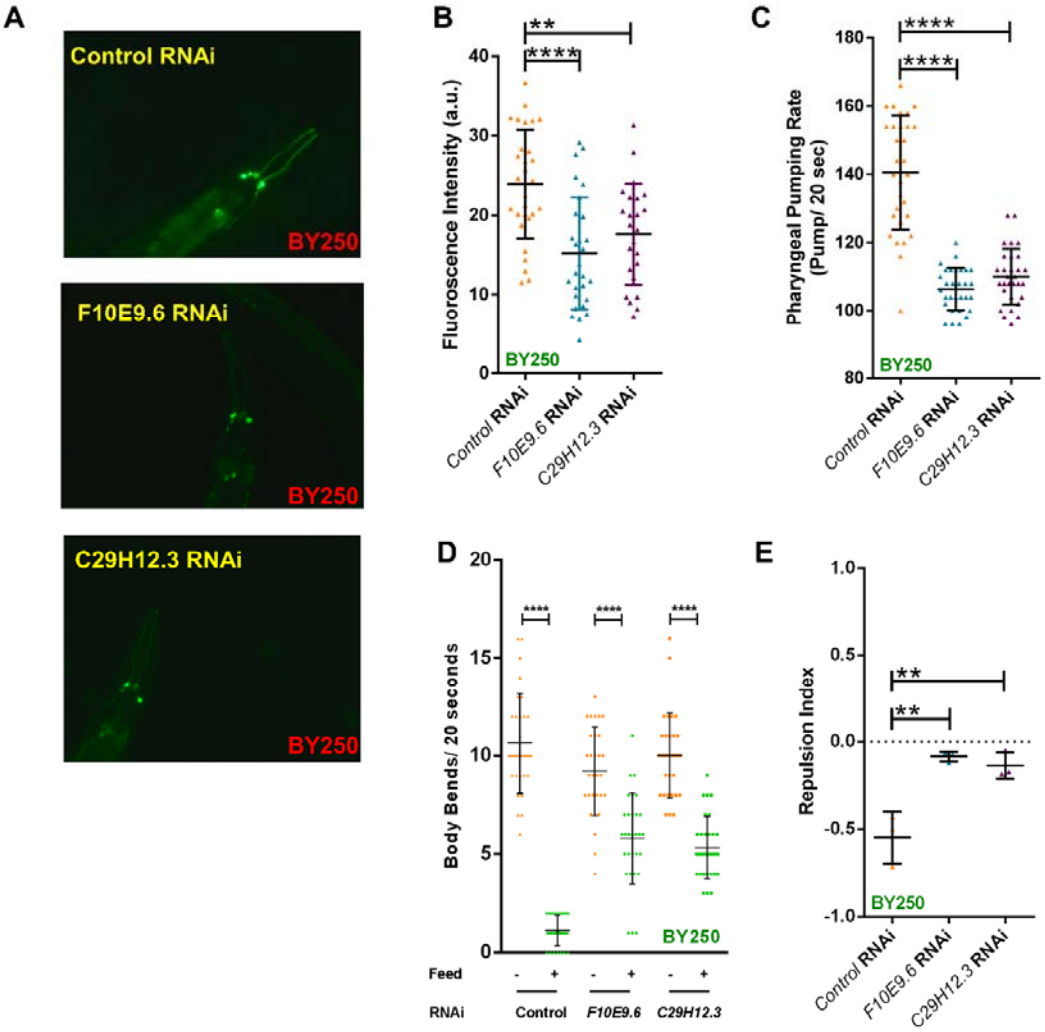

Figure 4. Downregulation of RAPH1 and RGS8 causes DA neuronal degeneration and induces deterioration in dopamine-associa behavior in the BY250 strain of *C. elegans*. (A) Knocking down homologs of RAPH1 (F10E9.6) and RGS8 (C29H12.3) in BY250 s

expressing GFP in DA neurons causes DA neurodegeneration and reduction in GFP expression in DA neurons. Images were taken at 20X magnification. **(B)** Graph showing fluorescence intensities of GFP expression in DA neurons as quantified by the ImageJ software. **(C)** Graph representing changes in pharyngeal pumping rate in BY250 when grown on the RNAis **(D)** Representative graphs for Basal slowing response of BY250 grown on the RNAis. The data is represented as mean ± S.D. n ≥ 30 in each RNAi. . ** $p<0.01$; **** $p<0.0001$; Unpaired t-test **(E)** Repulsion index of BY250 for 1-nonanol upon knock-down of RAPH1 (F10E9.6) and RGS8 (C29H12.3). The data is represented as the mean of three biological repeats ± S.D. n ≥ 30 in each RNAi per repeat. ** $p<0.01$; Unpaired t-test.