Co-expression network analysis identifies specific hub genes in association with developmental neuronal remodeling in *Drosophila melanogaster*

Yunze Liu [1], **Xiaojie Sun** [1], **Aijun Qu** [Corresp. 1]

[1] Shandong Agricultural University, Tai'an, China, China

Corresponding Author: Aijun Qu
Email address: ajqu1965@163.com

As an evolutionarily conserved mechanism, developmental neuronal remodeling is needed for the proper wiring of the nervous system and is critical for understanding the neurodevelopment mechanisms. Previous studies have shown that during metamorphosis lots of *Drosophila melanogaster* mushroom body neurons experience stereotypic remodeling. However, the related regulators and downstream executors of pathways are yet unclear, especially studies of transcriptional gene co-expression analysis of nervous systems remain insufficient. In this study, we develop a weighted gene co-expression network (WGCNA) to classify gene modules associated with neuronal remodeling. Moreover, functional and pathway enrichment analysis with protein-protein network construction is applied to detect high informative hub genes in the targeted gene module. Thus, we select a total of five hub genes that play critical roles in neuronal remodeling and identify them with functional enrichment analysis and protein-protein interaction network. Overall, this study provides insight into the underlying molecular mechanism of developmental neuronal remodeling in *Drosophila melanogaster*.

1

Co-expression network analysis identifies specific hub genes in association with developmental neuronal remodeling in *Drosophila melanogaster*

5   Yunze Liu, Xiaojie Sun, Aijun Qu*

6
7   College of Plant Protection, Shandong Agricultural University, Tai'an, China
8
9   *Authors for correspondence:
10   Aijun Qu*
11   Daizong Road No.61, Tai'an, Shandong, 271018, China
12   Email address: ajqu1965@163.com
13

Abstract

As an evolutionarily conserved mechanism, developmental neuronal remodeling is needed for the proper wiring of the nervous system and is critical for understanding the neurodevelopment mechanisms. Previous studies have shown that during metamorphosis lots of *Drosophila melanogaster* mushroom body neurons experience stereotypic remodeling. However, the related regulators and downstream executors of pathways are yet unclear, especially studies of transcriptional gene co-expression analysis of nervous systems remain insufficient. In this study, we develop a weighted gene co-expression network (WGCNA) to classify gene modules associated with neuronal remodeling. Moreover, functional and pathway enrichment analysis with protein-protein network construction is applied to detect high informative hub genes in the targeted gene module. Thus, we select a total of five hub genes that play critical roles in neuronal remodeling and identify them with functional enrichment analysis and protein-protein interaction network. Overall, this study provides insight into the underlying molecular mechanism of developmental neuronal remodeling in *Drosophila melanogaster*.

Keywords

Neuronal remodeling, Weighted Gene Co-expression Network Analysis, Significant modules, Hub genes

Introduction

32   Both in invertebrates and vertebrates, neuronal remodeling is a necessary process to establish a developed and

33   functional nervous system [1-3]. Remodeling has been studied and characterized in a broad variety of organisms

34   including worms, insects, human and m[3-5]. In the remodeling process, degenerative phenomena, such as neurite

35   pruning may be accompanied by regeneration to form different connections. Studies over the past decades have

36   demonstrated deregulated neuronal remodeling leads to various neuropsychiatric diseases such as

37   schizophrenia[6,7]. Consequently, a general perception of the mechanisms that manage developmental neuronal

38   remodeling might elucidate some neurotic degradation and disorders.

39   *Drosophila melanogaster* is a well-known model organism to investigate the structural and functional reorganization

40   of the nervous system [8,9]. Besides, throughout the processes of metamorphosis, the elimination of larval neurons

41   via programmed cell death and the birth of adult neurons continues. The neurons regularly undertake individual

42   remodeling neurites such as pruning of the larva and outgrowth of the adult. Among the neuronal remodeling

43   examples, the mushroom body (MB) is well-characterized, involving three neuronal sub-populations (γ, α'/β' and

44   α/β), wherein only neurons undergo spatially stereotypic and temporal remodeling manner [10,11]. Remodeling

45   manner of MB neurons is interfered partly by the regulated transcriptional factor (TF) in neuronal development. For

46   instance, the activation of MB γ neuron pruning demands the initiation of nuclear ecdysone receptor (EcR); EcR

47   also performs a critical role in the thoracic ventral (TV) neurons and remodeling of the olfactory projection neurons

48   (PNs) [12,13]. Recently, a well-studied target of E75 in EcR is reported to involve in axon regrowth after

49   pruning[14]. Another acknowledged EcR target, the transcription factor Sox14, regulates dendrite pruning of the

50   sensory da neurons [15].

51   For years past, the improvement of gene expression profiling such as microarray and RNA sequencing has been

52   utilized to classify genes relevant to neuronal remodeling of MB γ neurons. Most studies mainly concentrate on the

53   screening of differentially expressed genes and the pattern of gene expression, while the functional interaction

54   among genes is neglected [16](Tavazoie et al. 1999). We attempt to unveil the implied molecular mechanisms of

55   MB γ neuronal remodeling with system biology strategy. We applied the method of weighted gene co-expression

56   network analysis (WGCNA) and established a neuronal remodeling network on Drosophila γ neuron gene

57   expression data. At last, we identified five hub genes that may be potentially crucial in developmental neuronal

58   remodeling.

Materials & Methods

Data collection

Normalized raw data of gene expression was retrieved from the NCBI Gene Expression Omnibus database (http://www.ncbi.nlm.nih. gov/geo/) [17,18]. In our study, the dataset GSE101946 was used as a training set to compose an expression network and characterize hub genes [19]. The dataset GSE101946 was sequenced on the Illumina NextSeq 500 platform and comprised two independent RNA-sequencing data. One was gene expression result of various cell types and developmental stages from sample GSM2719262 to GSM2719325. The other contained three transcriptional genotype profiling of Drosophila γ neurons when perturbed by EcR-DN, E75 RNAi or Sox14 RNAi, including 43 samples from GSM2719326 to GSM2719368 which were the main focus of the current study.

Data preprocessing and DEG screening

After GSE101946 downloaded, quality control was applied to remove those genes without expression values in over half of 43 samples. The dataset then went through the $\log_2$ transformation. For computational restriction, the network analysis was restrained to a subset of genes[20]. Genes with high median absolute deviation (MAD) value were selected for subsequent analysis. Depending on the differential expression genes among three key factor and control groups, the flashClust R package [21](Langfelder and Horvath 2012) was adopted to implement the cluster analysis to detect and remove the outliers.

Co-expression network construction

A scale-free network for the gene expression dataset[22](Horvath and Dong 2008). The "WGCNA" package in R (version 1.64, https://cran.r-project.org/web/packages/WGCNA/index.html) was performed for genes in 43 samples by median absolute deviation (MAD) algorithm with high expression variance[23,24](Carlson et al. 2006; Leys et al. 2013). Firstly, we computed the Pearson's correlation matrices for each pair of genes and determined the correlation coefficient between gene x and gene y as $Smn = \left| cor(x,y) \right|$. Next, we constructed a weighted network using a power function $a_{xy} = \left| c_{xy} \right|^{\beta}$ (c_{xy} = Pearson's correlation between gene x and gene y; a_{xy} = adjacency

83   between gene x and gene y). This step could calculate a soft-thresholding parameter that emphasizes high

84   correlations and reduced the influences of low correlations on an exponential scale. Besides, we reconstructed the

85   weighted network with topological overlap (TO), which could weigh the network connectivity of a gene across the

86   weighted network as a total of adjacency with other genes [25](Yip and Horvath 2007). Based on the TO-based

87   dissimilarity measure, classification of gene co-expression modules with similar patterns of expression was

88   conducted by average linkage hierarchical clustering.

89   After identifying the modules, the principal component analysis for each gene module was used to describe the

90   module eigengenes (MEs). Gene significance (GS) was calculated as the log10 conversion of the P value, and the

91   average GS of whole genes in a module was represented as the module significance (MS). Generally, the module

92   with the highest absolute MS is regarded as the one correlated with neuronal remodeling. Besides, the functional

93   annotation of the modules was also conducted to distinguish the most relevant module. We used clusterProfiler R

94   package (version 3.8.1, https://guangchuangyu.github.io/software/clusterProfiler) to test each module for enrichment

95   in genes with particular Gene Ontology (GO) analysis[26]. GO analysis contains three parts: cellular component

96   (CC), biological process (BP) and molecular function (MF), revealing functions of gene products[27]. The Kyoto

97   Encyclopedia of Genes and Genomes (KEGG) database comprises pathway functions of molecules or

98   genes[28](Kanehisa 2000). The result p-value < 0.05 also q-value < 0.05 after correction was used as the cut-off

99   threshold.

Hub genes identification

101   Hub genes involved intense connection among nodes which were functionally significant within a

102   module[29](Albert 2005). In one module, hub genes might obtain much importance than other genes in the entire

103   network. In this study, genes with module membership (MM) value over 0.8 were collected as hub genes

104   [30](Presson et al. 2008). Predicted related genes in the similar complex or pathway with hub genes were also

105   considered to build a functional association network with GeneMANIA[31](Warde-Farley et al. 2010). Moreover, to

106   narrow down our research targets, we visualized the protein-protein interaction (PPI) network of interest genes by

107   Cytoscape software (Version 3.7.0) and calculated the intramodular connectivity[32]. In the PPI network, a gene

108   with over ten connectivity degree was considered as intramodular hub genes for further analyses. We also selected

109   high informative hub genes to detect expression level in the brain which might give us some clue for further study.

Results

DEGs screening

112   The normalized gene expression matrix of 16026 genes was obtained from the 43 samples. After low-count filtering

113   and $\log_2$ transforming, 7807 genes remained for the next selection. With the MAD and WGCNA algorithm, we

114   selected 3,300 highly informative genes for further weighted gene co-expression network construction. The result of

115   flashClust analysis on the 43 samples was shown in (Fig. 1).

Construction of co-expression module

117   Working with the R WGCNA package, we grouped the genes with similar expression patterns into modules by the

118   average linkage hierarchical clustering. As a result, a total of 13 modules (Fig. 2A) were identified. Two approaches

119   were performed to examine the relevance between the critical neurodevelopment factors and every module. At first,

120   the ME in several modules revealed an association with certain neurotic development factors (p-value < 0.05), then

121   all modules were ordered in consonance with the significant p-value (Table 1).

122   Nevertheless, most of the correlations had lower R squared value ($R^2 < 0.5$), and only one between the tan module

123   and EcR was strong (p-value = 1 x 10^{-19}, $R^2 = 0.93$) (Fig. 2B). Afterward, the tan module also had the highest MS

124   value among all selected modules (Fig. 2C). Hence, the tan module with EcR factor was recognized as the

125   neurodevelopmental module, which was selected for further analysis.

Enrichment analysis

127   Enrichment analysis of GO and KEGG was conducted in the tan module. Notable GO enrichment was located in a

128   biological process (Fig. 3A) and cellular components (Fig. 3B). All GO and KEGG terms with significant p-value

129   and q-value were listed (Table 2).

Identification and characterization of hub genes in the tan module

Hub genes with a strong connection in a specific module are fundamental in biological processes. Accordingly, the top 28 genes shared the high connectivity with weighted correlation coefficients over 0.8 and were preferred as potential hub genes, including transient receptor potential cation channel γ (*Trpgamma*), SIFamide receptor (*SIFaR*), Hairy/E(spl)-related with YRPW motif (*Hey*), Heparan sulfate 6-O-sulfotransferase (*Hs6st*), Centaurin gamma 1A (*CenG1A*), supernumerary limbs (*slmb*) and Swiprosin-1 (*Swip-1*). Subsequently, a network included hub genes and related genes was also built, in which nodes represented genes and links represented networks (pink link indicated co-expression network, orange link indicated physical interactions network and blue link indicated co-localization network) (Fig. 4).

Furthermore, according to the DroID database[33], a network of protein-protein interaction (PPI) was created for the genes in the tan module (Fig. 5), and 8 hub genes (*Swip-1, CenG1A, Rdx, e(r), Tnks, Cul4, slim* and *Trpgamma*) in the PPI network were also found in the co-expression network.

Gene expression mining using Fly Atlas Database[34] found that 5 of 8 hub genes had a significantly high expression level in the brain while one gene revealed significant low level (Table 3).

Discussion and Conclusion

Our analysis revealed 28 highly connected hub genes in the tan module, such as *Trpgamma, SIFaR, Hey, Hs6st, CenG1A, slmb, and Swip-1*. Functional and PPI network intersection analysis further selected eight genes (*Swip-1, CenG1A, Rdx, e(r), Tnks, Cul4, slim* and *Trpgamma*) in which five genes (*Swip-1*, *Tnks*, *CenG1A*, *slim*, *Rdx*) showed significant higher expressions in the brain.

Pruning and remodeling of exuberant neuronal connections is a broadly accepted mechanism in metazoan development for performing the mature pattern of neural connectivity[3,35]. Cells will either be extravagantly activated or become silent without this regulation. As a major model species, *Drosophila* is well suited for studying neuronal remodeling mechanisms. Several studies have found that transcriptional changes during developmental neuronal remodeling resulting from several significant TFs perturbations[36-38]. Each of these TFs has some potential targets. Thus a network-driven integrative analysis is needed to distinguish hub genes in neuronal remodeling. Idan Alyagor et al. [19](Alyagor et al. 2018) investigated their dataset using WGCNA and constructed a

156   co-expression network of three key TFs, in which both DEGs and their intercommunications were studied.

157   Hierarchical clustering analysis was implemented to assort genes with co-expression into 13 modules. The most

158   significant tan module was then recognized by both module significance and the feature vector. In the tan module,

159   genes were mainly enriched in protein regulated functions, hinting that genes in the tan module might be crucial in

160   protein regulation during neuronal remodeling.

161   *Tnks* (Tankyrase) exists in the poly (ADP-ribose) polymerase (PARP) superfamily, which is defined by a similar

162   PARP catalytic domain that transfers ADP-ribose from NAD^+ onto substrate proteins successively. Also, it

163   interferes a novel type of post-translational adjustment through energy homeostasis and vesicle traffic[39,40](Moore

164   et al. 2007; Kobayashi and Fukuda 2012)ing in Drosophila [41](Nagashima et al. 2011). *CenG1A* is a GTPase that

165   may have a role in regulating the second to third instar larval transition, which is ecdysone signaling-dependent. It

166   also takes parts in both presynaptic and postsynaptic cells [42](Homma et al. 2014). As a member of Centaurin

167   family, CenG1A (Centaurin gamma 1A) plays an essential role in neurite outgrowth and dendritic differentiation in

168   neural cells (Moore et al., 2007; Kobayashi & Fukuda, 2012). *Slim* (scruin like at the midline) activates the

169   Apoptosis signal-regulating kinase1 (ASK1) by the suppression of protein phosphatase 5 (PP5) and thereby to

170   contribute to oxidative stress-induced cell death [43](Sekine et al. 2012). *Rdx* (Roadkill) functions as a substrate

171   identification component of Cul3-based E3 ubiquitin ligase complexes. It promotes the degradation of the

172   transcriptional factor ci to regulate the Hedgehog signaling pathway [44](Seong et al. 2010).

173   In summary, a total of 3300 high informative genes were selected from 16026 gene matrix and 13 modules

174   established. Module tan was regarded as the essential module in the developmental neuronal remodeling. Moreover,

175   the analysis revealed several hub genes including *Swip-1*, *Tnks*, *CenG1A, slim* and *Rdx* that all play essential roles in

176   neuronal functions and may serve as potential biomarkers of developmental neuronal remodeling. Nevertheless, due

177   to the limitation of sample size, further studies are needed to understand this complicated process fully.

178   ## Acknowledgements

180

181

182

183

Conflict of interests

The authors declare no conflict of interests.

References

Albert R (2005) Scale-free networks in cell biology. Journal of Cell Science 118:4947–4957. doi: 10.1242/jcs.02714

Alyagor I, Berkun V, Keren-Shaul H, et al (2018) Combining Developmental and Perturbation-Seq Uncovers Transcriptional Modules Orchestrating Neuronal Remodeling. Developmental Cell 47:38–52.e6. doi: 10.1016/j.devcel.2018.09.013

Awasaki T, Lee T (2011) Orphan nuclear receptors control neuronal remodeling during fly metamorphosis. Nature Publishing Group 14:6–7. doi: 10.1038/nn0111-6

Barrett T, Troup DB, Wilhite SE, et al (2007) NCBI GEO: mining tens of millions of expression profiles--database and tools update. Nucleic Acids Research 35:D760–D765. doi: 10.1093/nar/gkl887

Carlson MRJ, Zhang B, Fang Z, et al (2006) Gene connectivity, function, and sequence conservation: predictions from modular yeast co-expression networks. BMC Genomics 2015 16:1 7:40. doi: 10.1186/1471-2164-7-40

Ding M, Chao D, Wang G, Shen K (2007) Spatial Regulation of an E3 Ubiquitin Ligase Directs Selective Synapse Elimination. Science 317:947–951. doi: 10.1126/science.1145727

Dixit A, Parnas O, Li B, et al (2016) Perturb-Seq: Dissecting Molecular Circuits with Scalable Single-Cell RNA Profiling of Pooled Genetic Screens. Cell 167:1853–1857.e17. doi: 10.1016/j.cell.2016.11.038

Edgar R, Domrachev M, Lash AE (2002) Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Research 30:207–210.

Faludi G, Mirnics K (2011) Synaptic changes in the brain of subjects with schizophrenia. International Journal of Developmental Neuroscience 29:305–309. doi: 10.1016/j.ijdevneu.2011.02.013

Gogtay N, Giedd JN, Lusk L, et al (2004) Dynamic mapping of human cortical development during childhood through early adulthood. PNAS 101:8174–8179. doi: 10.1073/pnas.0402680101

Homma M, Nagashima S, Fukuda T, et al (2014) Downregulation of Centaurin gamma1A increases synaptic transmission at Drosophilalarval neuromuscular junctions. Eur J Neurosci 40:3158–3170. doi: 10.1111/ejn.12681

Hoopfer ED, Penton A, Watts RJ, Luo L (2008) Genomic Analysis of Drosophila Neuronal Remodeling: A Role for the RNA-Binding Protein Boule as a Negative Regulator of Axon Pruning. Journal of Neuroscience 28:6092–6103. doi: 10.1523/JNEUROSCI.0677-08.2008

Horvath S, Dong J (2008) Geometric interpretation of gene coexpression network analysis. PLoS Comput Biol 4:e1000117. doi: 10.1371/journal.pcbi.1000117

Jaitin DA, Weiner A, Yofe I, et al (2016) Dissecting Immune Circuits by Linking CRISPR- Pooled Screens with Single-Cell RNA-Seq. Cell 167:1883–1888.e15. doi: 10.1016/j.cell.2016.11.039

231   Kanehisa M (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Research 28:27–30. doi:
232   10.1093/nar/28.1.27

234   Kirilly D, Gu Y, Huang Y, et al (2009) A genetic pathway composed of Sox14 and Mical governs severing of
235   dendrites during pruning. Nat Neurosci 12:1497–1505. doi: 10.1038/nn.2415

237   Kobayashi H, Fukuda M (2012) Rab35 regulates Arf6 activity through centaurin-β2 (ACAP2) during neurite
238   outgrowth. Journal of Cell Science 125:2235–2243. doi: 10.1242/jcs.098657

240   Langfelder P, Horvath S (2012) Fast R Functions for Robust Correlations and Hierarchical Clustering. J Stat Softw.
241   doi: 10.1038/nm0113-3

243   Leader DP, Krause SA, Pandit A, et al (2018) FlyAtlas 2: a new version of the Drosophila melanogaster expression
244   atlas with RNA-Seq, miRNA-Seq and sex-specific data. Nucleic Acids Research 46:D809–D815. doi:
245   10.1093/nar/gkx976

247   Lee T, Marticke S, Sung C, et al (2000) Cell-autonomous requirement of the USP/EcR-B ecdysone receptor for
248   mushroom body neuronal remodeling in Drosophila. Neuron 28:807–818.

250   Leys C, Ley C, Klein O, et al (2013) Detecting outliers: Do not use standard deviation around the mean, use
251   absolute deviation around the median. Journal of Experimental Social Psychology 49:764–766. doi:
252   10.1016/j.jesp.2013.03.013

254   Luo L, O'Leary DDM (2005) Axon retraction and degeneration in development and disease. Annu Rev Neurosci
255   28:127–156. doi: 10.1146/annurev.neuro.28.061604.135632

257   Marin EC (2005) Developmentally programmed remodeling of the Drosophila olfactory circuit. Development
258   132:725–737. doi: 10.1242/dev.01614

260   Moore CD, Thacker EE, Larimore J, et al (2007) The neuronal Arf GAP centaurin 1 modulates dendritic
261   differentiation. Journal of Cell Science 120:2683–2693. doi: 10.1242/jcs.006346

263   Nagashima S, Fukuda T, Kubota Y, et al (2011) CRMP5-associated GTPase (CRAG) protein protects neuronal cells
264   against cytotoxicity of expanded polyglutamine protein partially via c-Fos-dependent activator protein-1 activation.
265   J Biol Chem 286:33879–33889. doi: 10.1074/jbc.M111.234997

267   Presson AP, Sobel EM, Papp JC, et al (2008) Integrated Weighted Gene Co-expression Network Analysis with an
268   Application to Chronic Fatigue Syndrome. BMC Systems Biology 2:95. doi: 10.1186/1752-0509-2-95

270   Rabinovich D, Yaniv SP, Alyagor I, Schuldiner O (2016) Nitric Oxide as a Switching Mechanism between Axon
271   Degeneration and Regrowth during Developmental Remodeling. Cell 164:170–182. doi: 10.1016/j.cell.2015.11.047

273   Schuldiner O, Yaron A (2014) Mechanisms of developmental neurite pruning. Cellular and Molecular Life Sciences
274   72:101–119. doi: 10.1007/s00018-014-1729-6

276   Sekar A, Bialas AR, de Rivera H, et al (2016) Schizophrenia risk from complex variation of complement component
277   4. Nature 530:177–183. doi: 10.1038/nature16549
278

279 Sekine Y, Hatanaka R, Watanabe T, et al (2012) The Kelch Repeat Protein KLHDC10 Regulates Oxidative Stress-
280 Induced ASK1 Activation by Suppressing PP5. Molecular Cell 48:692–704. doi: 10.1016/j.molcel.2012.09.018
281
282 Seong K-H, Akimaru H, Dai P, et al (2010) Inhibition of the Nuclear Import of Cubitus Interruptus by Roadkill in
283 the Presence of Strong Hedgehog Signal. PLoS ONE 5:e15365–12. doi: 10.1371/journal.pone.0015365
284
285 Shannon P, Markiel A, Ozier O, et al (2003) Cytoscape: a software environment for integrated models of
286 biomolecular interaction networks. Genome Res 13:2498–2504. doi: 10.1101/gr.1239303
287
288 Tavazoie S, Hughes JD, Campbell MJ, et al (1999) Systematic determination of genetic network architecture. Nat
289 Genet 22:281–285. doi: 10.1038/10343
290
291 Tweedie S, Ashburner M, Falls K, et al (2009) FlyBase: enhancing Drosophila Gene Ontology annotations. Nucleic
292 Acids Research 37:D555–D559. doi: 10.1093/nar/gkn788
293
294 Warde-Farley D, Donaldson SL, Comes O, et al (2010) The GeneMANIA prediction server: biological network
295 integration for gene prioritization and predicting gene function. Nucleic Acids Research 38:W214–W220. doi:
296 10.1093/nar/gkq537
297
298 Weeks JC (2003) Thinking globally, acting locally: steroid hormone regulation of the dendritic architecture,
299 synaptic connectivity and death of an individual neuron. Progress in Neurobiology 70:421–442. doi: 10.1016/S0301-
300 0082(03)00102-3
301
302 Williams DW, Truman JW (2005) Remodeling dendrites during insect metamorphosis. J Neurobiol 64:24–33. doi:
303 10.1002/neu.20151
304
305 Yaniv SP, Schuldiner O (2016) A fly's view of neuronal remodeling. WIREs Dev Biol 5:618–635. doi:
306 10.1002/wdev.241
307
308 Yip AM, Horvath S (2007) Gene network interconnectedness and the generalized topological overlap measure.
309 BMC Bioinformatics 8:22. doi: 10.1186/1471-2105-8-22
310
311 Yu G, Wang L-G, Han Y, He Q-Y (2012) clusterProfiler: an R package for comparing biological themes among
312 gene clusters. OMICS 16:284–287. doi: 10.1089/omi.2011.0118
313
314 Yu J, Pacifico S, Liu G, Finley RL Jr (2008) DroID: the Drosophila Interactions Database, a comprehensive
315 resource for annotated gene and protein interactions. BMC Genomics 2015 16:1 9:461–9. doi: 10.1186/1471-2164-
316 9-461
317
318 Yu XM, Gutman I, Mosca TJ, et al (2013) Plum, an Immunoglobulin Superfamily Protein, Regulates Axon Pruning
319 by Facilitating TGF-β Signaling. Neuron 78:456–468. doi: 10.1016/j.neuron.2013.03.004
320
321 Zhang B, Horvath S (2005) A general framework for weighted gene co-expression network analysis. Stat Appl
322 Genet Mol Biol 4:Article17. doi: 10.2202/1544-6115.1128
323
324 Zheng X, Wang J, Haerry TE, et al (2003) TGF-β Signaling Activates Steroid Hormone Receptor Expression during
325 Neuronal Remodeling in the Drosophila Brain. Cell 112:303–315. doi: 10.1016/s0092-8674(03)00072-2
326

Figure 1(on next page)
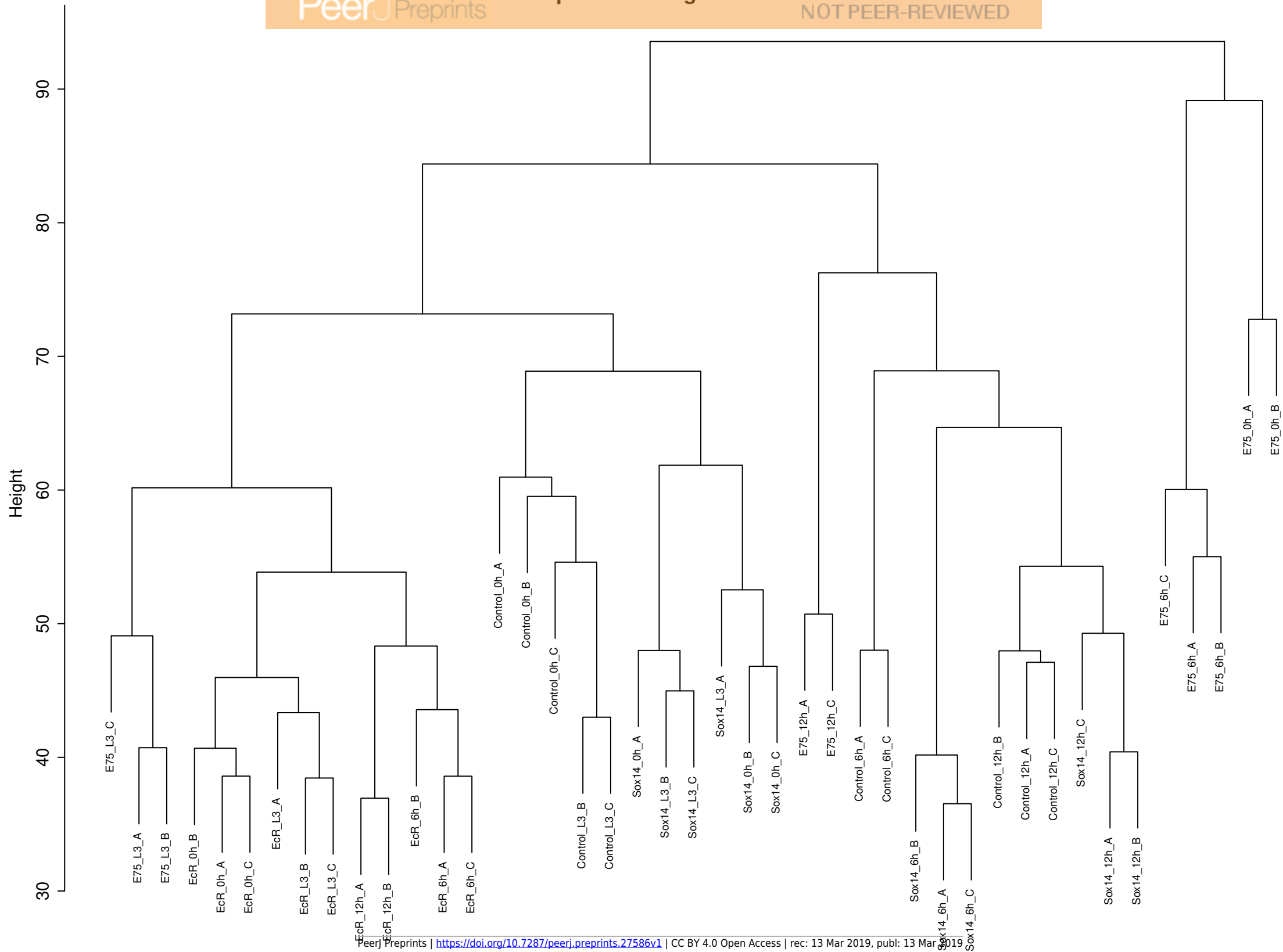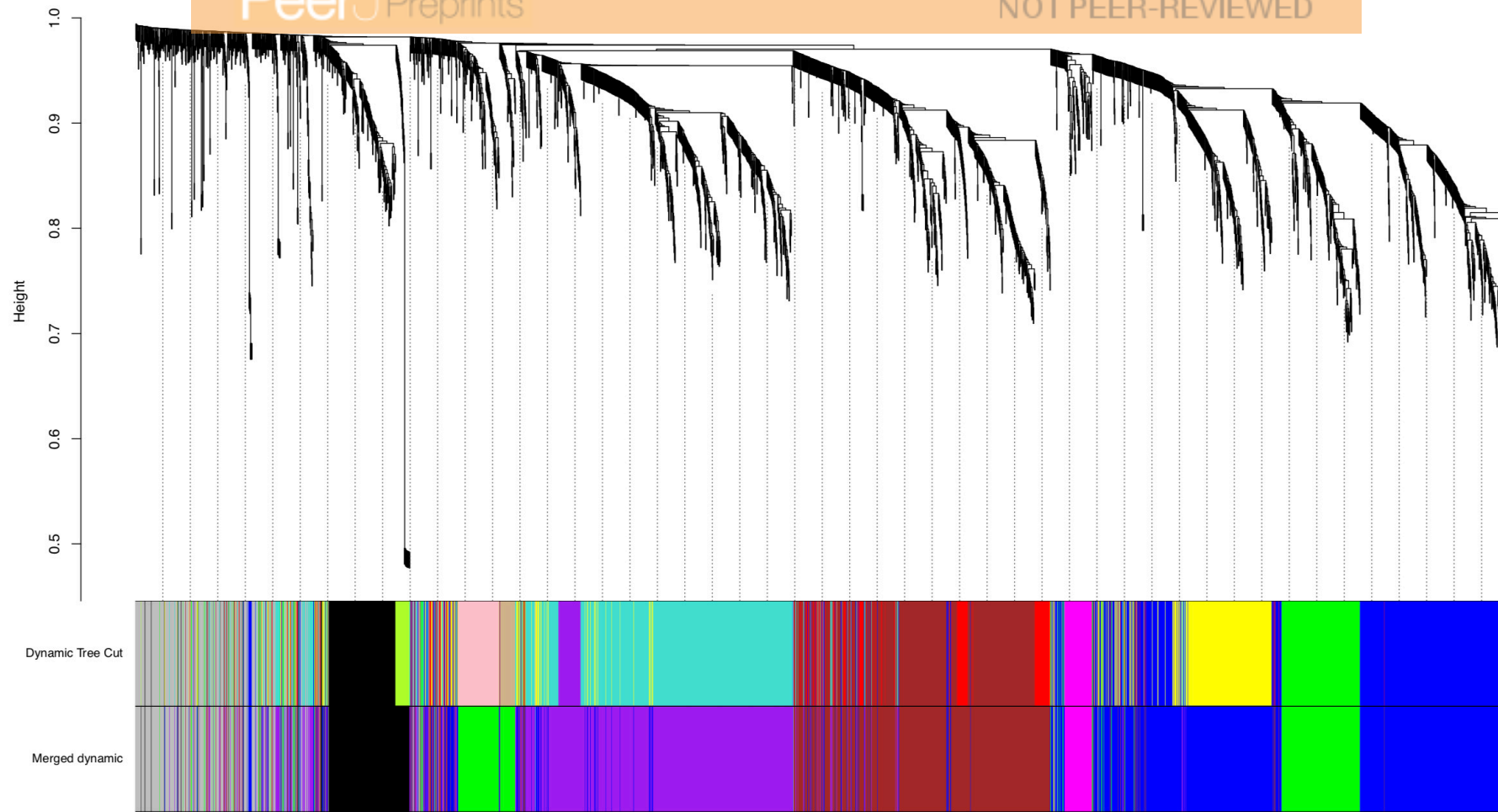
Figure 1: Clustering dendrogram of 43 samples.

Figure 2(on next page)

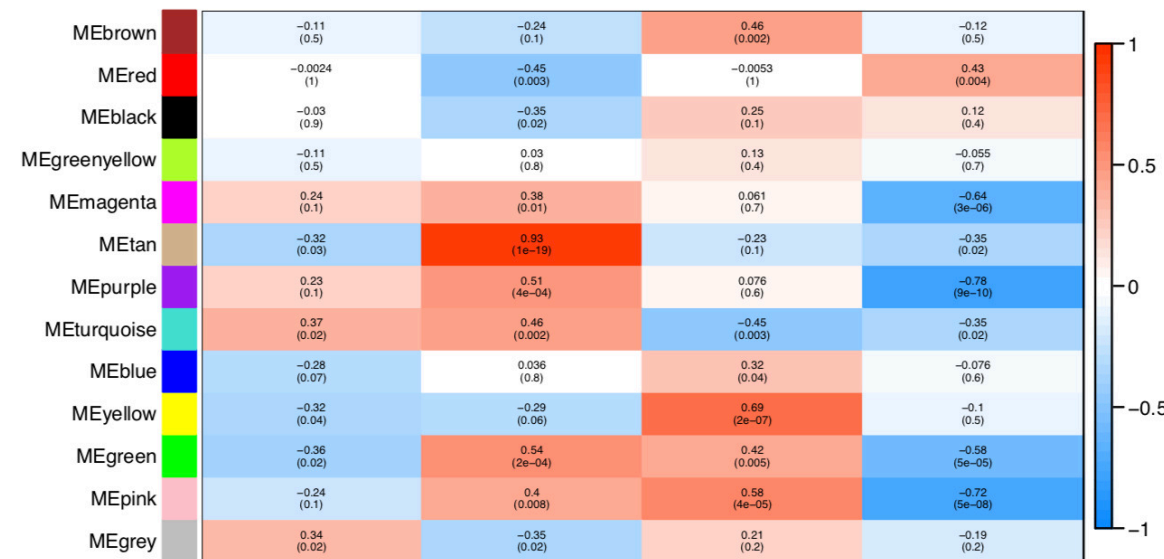*Figure 2: Identification of modules associated with developmental neuronal remodeling*

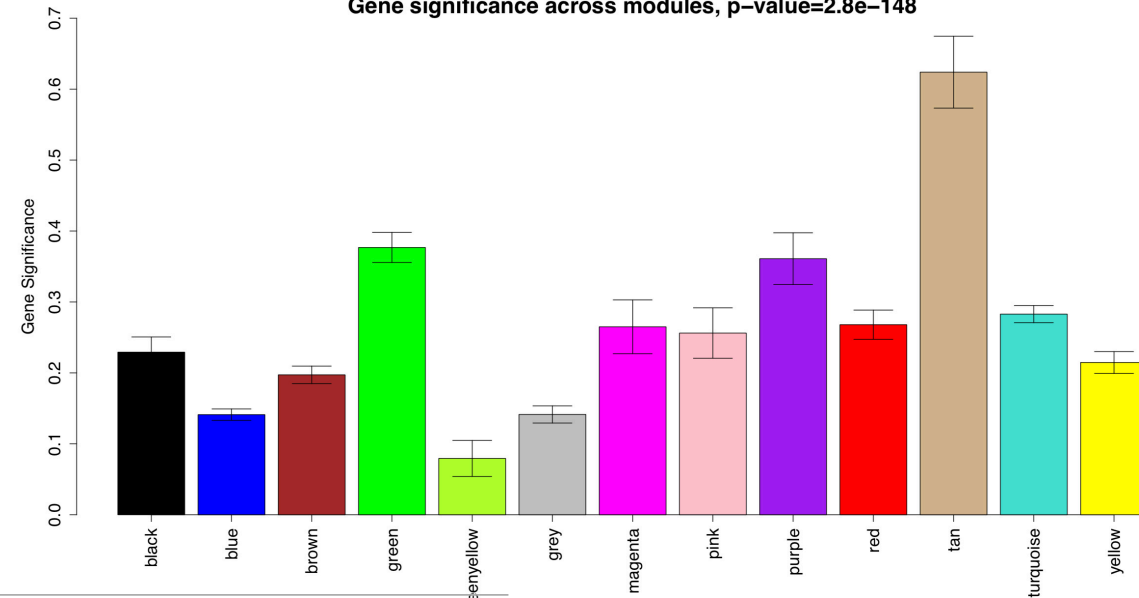**(A)** Clustering dendrogram of co-expression modules presented in diverse colors of drosophila

Figure 3(on next page)

Fig 3: The top 5 GO terms enriched in the tan module.
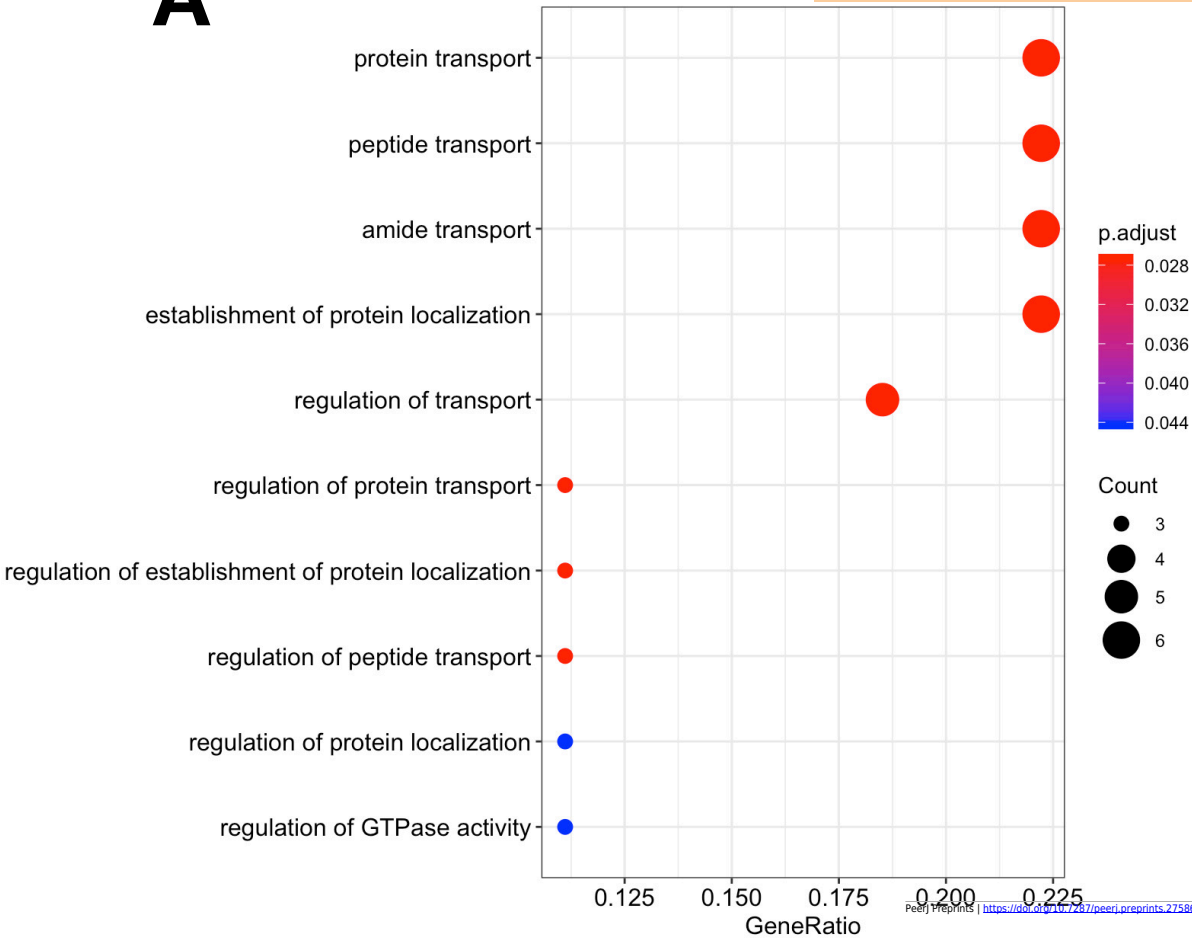
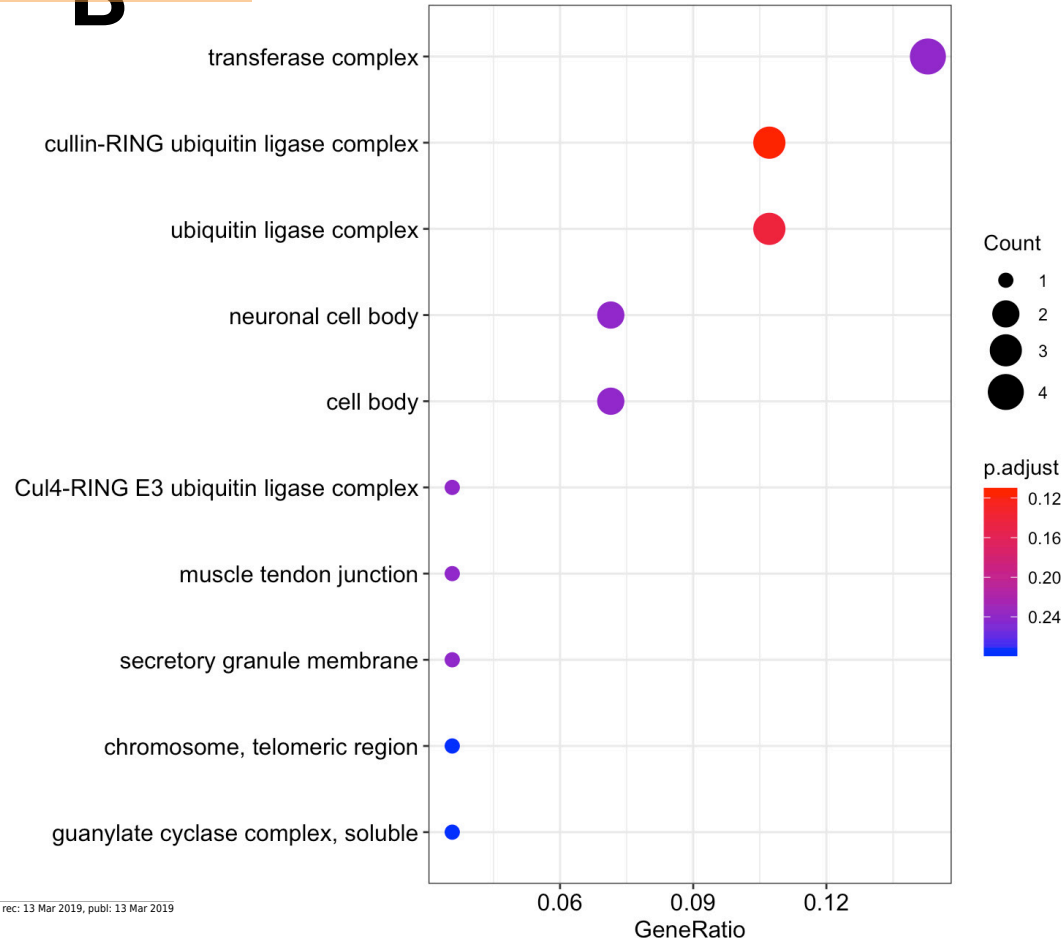*(A) Biological process; (B) Cellular components*

Figure 4(on next page)

Fig 4: Functional network in the tan module.

The network of top 28 genes in the tan module. (Inner ring indicated hub genes in the tan module, outer ring indicated functionally related genes. Node color: Blue indicated genes with a similar function of regulation of intracellular signal transduction).
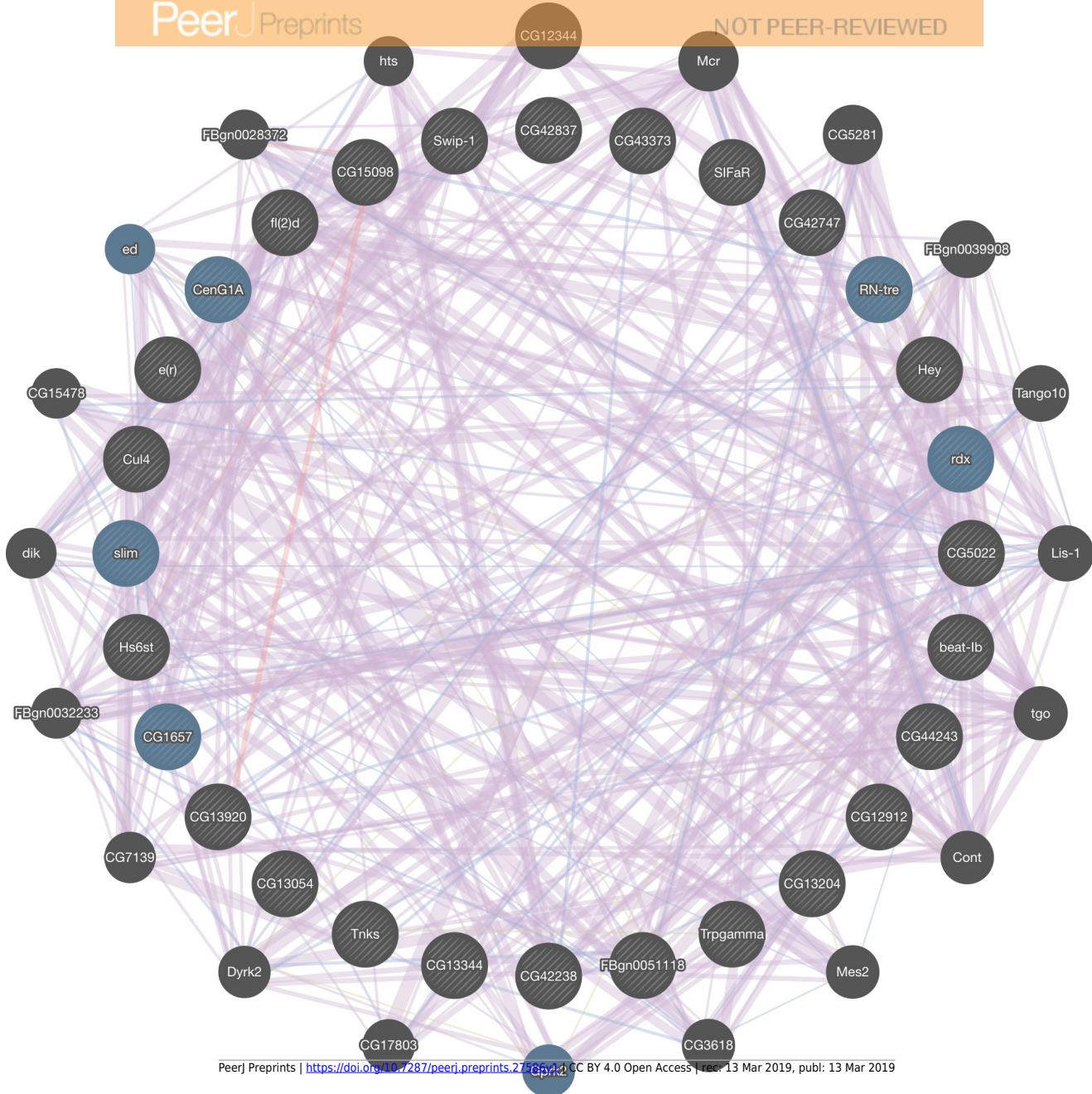
Figure 5(on next page)

Fig 5: Protein-protein interaction (PPI) network of genes in the tan module.

The light yellow circle represented similar GO term annotation. The central nodes of every circle were hub genes in the PPI network. The color of nodes was based on gene eccentricity from high (red) to low (yellow).
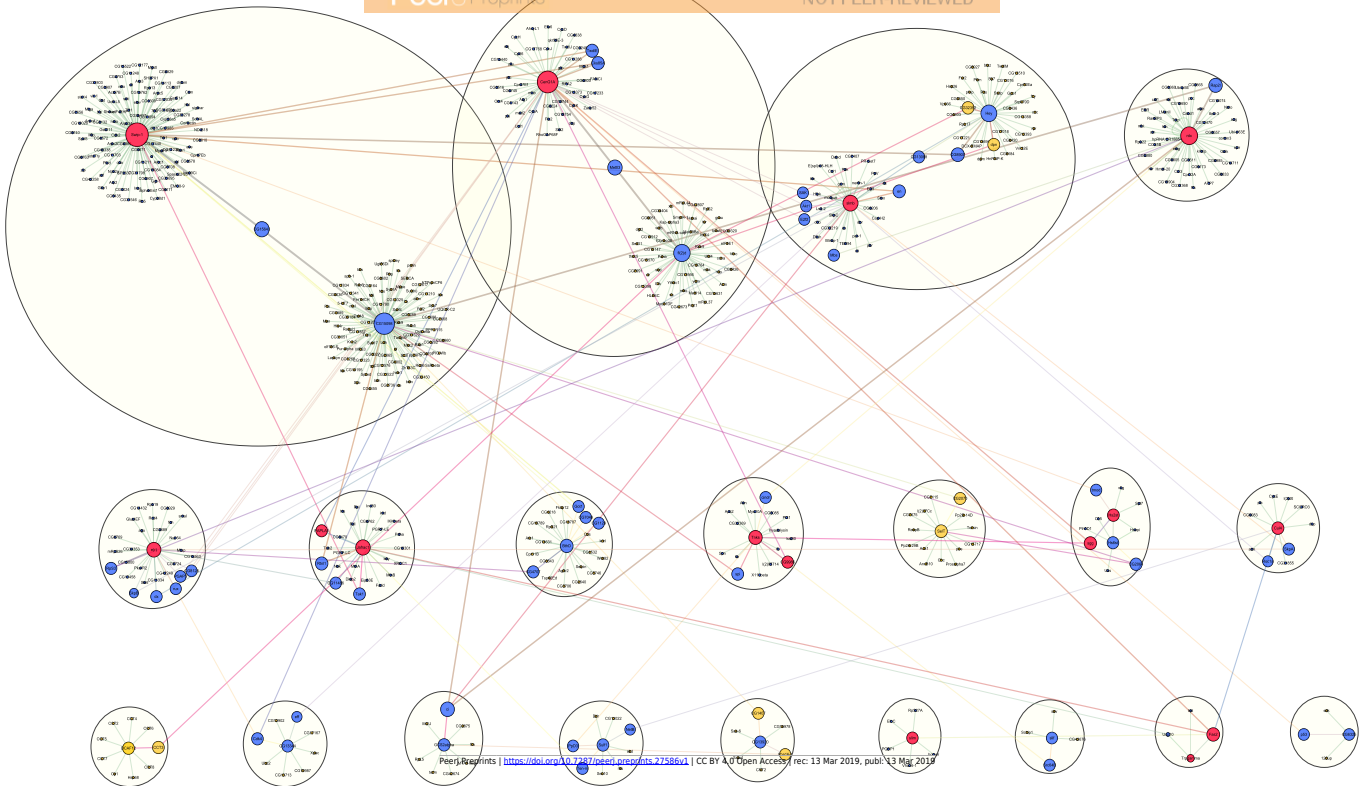
Table 1(on next page)

Table 1. Correlation between modules and developmental neuronal remodeling

1 **Table 1. Correlation between modules and developmental neuronal remodeling**

2

MODULE	Correlation	*P*-value
Tan	0.93	1.00E-19
Green	0.54	1.68E-04
Purple	0.51	4.39E-04
Turquoise	0.46	1.86E-03
Pink	0.40	7.59E-03
Magenta	0.38	1.13E-02
Blue	0.04	8.20E-01
Greenyellow	0.03	8.47E-01
Brown	-0.24	1.17E-01
Yellow	-0.29	6.20E-02
Grey	-0.35	2.07E-02
Black	-0.35	1.99E-02
Red	-0.45	2.51E-03

3
4
5
6

Table 2(on next page)

Table 2. GO and KEGG pathway enrichment analysis of tan module

KEGG: Kyoto Encyclopedia of Genes and Genomes; GO_BP: Gene ontology term of the biological process; GO_CC: Gene ontology term of the cellular component

1
2
3

Table 2. GO and KEGG pathway enrichment analysis of tan module

Category	Term	Count	*P*-value
KEGG	dme00534~ glycosaminoglycan biosynthesis	1	4.41E-02
GO_BP	GO:0051049~regulation of transport	5	1.88E-04
GO_BP	GO:0051223~regulation of protein transport	3	2.01E-04
GO_BP	GO:0070201~regulation of establishment of protein localization	3	2.24E-04
GO_BP	GO:0090087~regulation of peptide transport	3	2.87E-04
GO_BP	GO:0015031~protein transport	6	3.42E-04
GO_BP	GO:0015833~peptide transport	6	3.88E-04
GO_BP	GO:0042886~amide transport	6	4.01E-04
GO_BP	GO:0045184~establishment of protein localization	6	4.28E-04
GO_BP	GO:0032880~regulation of protein localization	3	8.30E-04
GO_BP	GO:0043087~regulation of GTPase activity	3	9.14E-04
GO_BP	GO:0046822~regulation of nucleocytoplasmic transport	2	9.55E-04
GO_BP	GO:0006886~intracellular protein transport	5	1.12E-03
GO_BP	GO:0032874~positive regulation of stress-activated MAPK cascade	2	1.24E-03
GO_BP	GO:0070304~positive regulation of stress-activated protein kinase signaling cascade	2	1.24E-03
GO_BP	GO:0033157~regulation of intracellular protein transport	2	1.44E-03
GO_BP	GO:0032879~regulation of localization	5	1.47E-03
GO_CC	GO:0031461~cullin-RING ubiquitin ligase complex	3	2.23E-03
GO_CC	GO:0000151~ubiquitin ligase complex	3	5.58E-03
GO_CC	GO:0080008~Cul4-RING E3 ubiquitin ligase complex	1	2.56E-02
GO_CC	GO:1990234~transferase complex	4	2.60E-02
GO_CC	GO:0005927~muscle tendon junction	1	3.24E-02
GO_CC	GO:0043025~neuronal cell body	2	3.36E-02
GO_CC	GO:0030667~secretory granule membrane	1	3.70E-02
GO_CC	GO:0044297~cell body	2	3.77E-02

4

5   KEGG: Kyoto Encyclopedia of Genes and Genomes; GO_BP: Gene ontology term of the biological process;

6   GO_CC: Gene ontology term of the cellular component

7
8

Table 3(on next page)

Table 3. Hub gene expression in brain tissue

1
2 **Table 3. Hub gene expression in brain tissue**
3

Gene Symbols	Genes Secondary Identifier	Gene Name	Fly Atlas Enrichment	Fly Atlas Affy Call	Fly Atlas mRNA Signal
Rdx	CG12537	roadkill	1.3	Up	246.4
slim	CG5186	scruin like at the midline	2	Up	403.2
CenG1A	CG31811	Centaurin gamma 1A	2.1	Up	352.9
Tnks	CG4719	tankyrase	2.5	Up	217.0
Swip-1	CG10641	Swiprosin-1	2.7	Up	503.4
Cul4	CG8711	Cullin 4	0.7	Down	153.2

4
5