

# Differential Network Analysis Reveals Regulatory Patterns in Neural Stem Cell Fate Decision

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## Research article

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# Abstract

## Background

Neural stem cell (NSC) differentiation is one of many multi-stage lineage systems that require multiple cell fate decisions. Recent single-cell transcriptome datasets became available at individual differentiation, however, a systematic and integrative analysis of multiple datasets at multiple temporal points of NSC differentiation is lacking.

## Results

Here we investigate five NSC differentiation paths by analyzing and comparing four different single-cell transcriptome datasets. By constructing gene regulatory networks for each cell type, we delineate their regulatory patterns via analyses of differential topology and network diffusion. Among the five NSC differentiation paths, we find 12 common differentially expressed genes, with one common three-gene regulatory pattern shared by all paths. The identified regulatory pattern, partly supported by previous experimental evidence, is found to be essential to all differentiation paths, however, plays a different role in each path when regulating other genes.

## Conclusions

Together, our integrative analysis provides both common and specific regulatory mechanisms for each of the five NSC differentiation paths, and the approach can be applied to analyzing other complex multi-stage lineage systems.

# Background

Neural stem cell (NSC) differentiation is a complex biological process with many unresolved mysteries [1, 2]. Identifying the key regulators on NSC fate decision have potential of treating neurological diseases [3]. For the past ten years, researchers have developed multiple biological methods [4, 5] and computational methods [6–8] to explore the key regulators in the complex differentiation systems. However, differentiation is thought to require one or more discrete transitions from one intermediate state to another, each of which is determined by a set of genes that interact in a complex network, instead of a single perturbed gene [9]. Although these approaches have identified the key participants for lineage programming of each differentiation stage, the regulatory patterns (the set of genes that drive differentiation) are not yet fully understood and determined.

The bulk RNA-seq technology [10] was developed to describe the average level of gene expression in cell populations, but it is difficult to analyze heterogeneous systems such as brain development and cell differentiation [11]. Recently, the gradual rise of single-cell RNA-seq technology, which measures the distribution of gene expression at the single-cell level, allows for the study of new biological problems [12]. Based on single-cell RNA-seq profiles, researchers have been able to explore the matters of NSC

differentiation more comprehensively. For instance, multiple clusters of co-varying genes enriched in differentiation were identified by pseudotime trajectory analysis based on unsupervised clustering [8]. 34 key regulatory genes related to neural differentiation were predicted by a four-way stochastic gradient-boosting classification model [13]. New neuronal cell subtypes were identified based on hierarchical clustering and principal components analysis [14].

The above-mentioned researches at a single-cell level have furthered our understanding of NSC differentiation. However, they focused on single path or dataset of neural stem cell differentiation, and there is no regulatory pattern that drive multi-path differentiation has ever been found. Recently, it is surveyed that the same cell type family may share common regulatory programming driving differentiation, while the expression of some effector genes are lost or gained anew to effect cellular phenotypes [15]. Likewise, the state-of-the-art study of pan-cancer suggests that there may exist common and specific patterns in different cancers, which means that different cancers may be cured by the same pan-cancer genes [16]. However, in the NSC differentiation system, there is still no clear understanding of such mechanisms, so that identifying such common and specific patterns will open up a new perspective of NSC differentiation.

The traditional biological analysis, e.g. differential expression analysis, is hard to capture common and specific patterns at a system level [17]. Unlike differential expression analysis, network analysis, especially differential network analysis, provides critical novel biological insights by identifying important genes or modules implicated in complex life processes from both system biology and bioinformatics perspective [18, 19]. Many researches show that differential network analysis is potential of predicting and identifying essential modules in life processes transition, e.g. differential subnetwork identification [20], prioritizing driver genes [21], cell types classification [22], etc.

In this study, with the aim of exploring the common and specific patterns of neural stem cell lineage differentiation, we analyzed five NSC differentiation paths in four single-cell RNA-seq datasets. Firstly, differential expression analysis and function enrichment analysis were performed to identify the differentially expressed genes (DEGs). Secondly, state networks and differential networks integrated with single-cell transcriptome and proteome were constructed based on the identified DEGs of each differentiation paths. Thirdly, all differential networks were topological overlapped to explore the regulatory pattern (RP). Fourthly, network analyses, including differential network analyses and network diffusion analyses, were performed on the constructed networks to assess the importance of the RP. Finally, the RP was validated by literature and pathway enrichment analysis.

## Methods

### Single-cell RNA-seq datasets

In this study, four single-cell RNA-seq datasets of mice were used. These datasets were separately downloaded from PRJNA324289 [13], GSE76381 [23], GSE71585 [24] and GSE87544 [25]. PRJNA324289 contains NSC, neural progenitor cells (NPC) and astrocyte cells (AST). GSE76381 contains NPC and

radial glial cells (RGL). GSE71585 contains oligodendrocyte progenitor cells (OPC) and oligodendrocyte cells (OLI). GSE87544 contains OPC and myelinating oligodendrocyte cells (MO). The number of cells in each dataset is shown in Table 1.

Table 1  
The number of cells in four single-cell RNA-seq datasets of mice

	<b>NSC</b>	<b>NPC</b>	<b>AST</b>	<b>RGL</b>	<b>OPC</b>	<b>OLI</b>	<b>MO</b>
PRJNA324289	152	64	32	-	-	-	-
GSE76381	-	149	-	166	-	-	-
GSE71585	-	-	-	-	22	38	-
GSE87544	-	-	-	-	1792	-	3692

NSC lineage differentiation tree contains various differentiation paths (as is shown in Fig. 1). We are interested in the transition before and after each differentiation paths. As a result, five paths of neural stem cell differentiation were obtained. The name of each path was given by their cell types as follows: NSC\_NPC, NPC\_AST, NPC\_RGL, OPC\_OLI, OPC\_MO. Then cell and gene filtering were performed for each path. For cell filtering, the top 50 samples with total molecular in each path were selected. For gene filtering, one gene was selected if it was expressed by five cells over ten counts in each path, and two cells over four counts in each type of cells, the parameters used here is similar to the previous study [13].

#### Differential expression analysis

For each path, differential expression analysis based on statistical methods was performed. The DEGs in each path were detected by the Bioconductor package ‘edgeR’ [26], with p-value < 0.05 and

$$|\log_2 \text{fold change}(FC)| > 0.5 .$$

#### Network construction by integrating single-cell transcriptome and proteome

Network analysis requires a robust network skeleton. The choice of the network skeleton is important, which would allow the network-based approaches to achieve higher precision [27]. Therefore, network construction here integrated single-cell transcriptome and proteome, which was based on both correlation of each pair of gene expression and confidence score in a background network. The workflow of network construction was shown in Fig. 2. Each path will produce three networks, containing two state networks and one differential network. For each path, firstly, the identified DEGs were used to build a background network by STRING database (<http://string-db.org>). Next, the state-specific networks were produced by assigning each edge an initial weight corresponding to the Spearman correlation coefficients (SCC) of gene expression of the adjacent nodes in the respective single-cell RNA-seq dataset; edges with absolute

SCCs lower than 0.1 are removed (see Additional File 1 for more detail about the parameter selection). Finally, the differential network was produced by the differential SCCs across two correlation networks; edges with absolute differential SCCs lower than 0.1 were removed.

#### Network diffusion analysis for each differential network

Differential topology analysis fails to capture some important genes with subtle topological differences in networks, such as bridge genes. To investigate the direct and indirect influence of such genes on network, we employ the MC method [28], one of the diffusion network analysis methods based on perturbation, which enables us to quantify the potency of label propagation(gene signaling). The MC method uses a network topology structure and a list of node initial scores as input. After a perturbation test, a list of node influence scores is calculated. In this study, the topology of differential network and the average scores of differential topology analysis are used to perform diffusion network analysis. This method is implemented by the 'diffuStats' package [29], with all default parameters.

#### Differential topology analysis between two state networks

Differential topology analysis focuses on the topological differences between two state networks. However, different differential topology analysis methods rely on different network topology structures, which may not comprehensively balance the importance of genes between different biological states. In this study, five differential topology analysis methods were employed for each path, including differential degree centrality (DDC) [30], differential eigenvector centrality (DEC) [19], differential PageRank centrality (DPC) [31], differential betweenness centrality (DBC) and DiffRank [32], among which there is one local measurement method, three global measurement methods and one hybrid (local and global) measurement method. DDC takes the normalized differences between a node's degree in two networks. DEC/DPC/DBC take the different global centrality measurement methods to find changes between two networks. DiffRank linearly combines both differential connectivity and DBC into a single score to order the importance of genes. All methods above are implemented by 'igraph' package. In order to prioritize genes at the same level, the result of each differential topology analysis is standardized, ranging from 0 to 1, where 1 characterizes the most significant difference and 0 the least significant difference. Then, the average score of the results of all standardized differential topology analysis is regarded as the overall important score of genes in each path.

#### Enrichment analysis

To evaluate the functional relevance and biological processes of selected genes, enrichment analysis was performed by Bioconductor package 'ClusterProfiler' [33]. It contains gene ontology (GO) enrichment analysis, and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis. The significant enrichment terms were obtained by Benjamini-Hochberg with adjusted p-value ( $p.adjust < 0.05$ )

## Results

Differential expression analysis reveals the common differentially expressed genes among five NSC differentiation paths

Differential expression analysis was performed on the five preprocessed NSC differentiation paths (NSC\_NPC, NPC\_AST, NPC\_RGL, OPC\_OLI, OPC\_MO). It yielded a set of DEGs with different numbers (ranging from 566 to 2582) for each path. Interestingly, 12 common DEGs co-occurred in the five NSC differentiation paths (Fig. 3A), among which some genes presented over-expressed (represented by the red bar) and down-expressed (represented by the blue bar) across different NSC differentiation paths, while some genes consistently down-expressed across different NSC differentiation paths. Commonly, the alterations of gene expression reflect on the functional changes in biological processes. Therefore, as shown in Fig. 3A, it implies that the specific genes play different roles in different cell differentiation paths.

To evaluate the biological processes related to these common DEGs, we performed the GO enrichment analysis. The GO enrichment analysis (Fig. 3B) showed that these 12 common DEGs were significantly enriched in differentiation and development of neural system. For example, Cdk5, App, Gsk3b, Ednrb and Ptprz1 were enriched in negative regulation of neuron differentiation(adjusted p-value = 1.14e-7); Cdk5, Dclk1, Gsk3b, Ptprz1 were enriched in neuron migration(adjusted p-value = 1.64e-4); Cdk5, App, Dclk1, Gsk3b, Ptprz1 were enriched in axon development(adjusted p-value = 2.04e-4). These GO terms serve as further evidence to support that the identified common DEGs play important roles in NSC differentiation and development.

Although the result of differential expression analysis helped us narrow the candidate gene sets, it failed to assess the importance of these candidate genes and the association among them. In contrast to conventional differential expression analysis, network analysis, especially differential network analysis, allows us to study these complex life processes quantitatively and qualitatively from both bioinformatics and system biology perspectives [19]. To explore which genes, pathways or patterns are responsible for making the cell fate decision of NSC differentiation, comprehensive analyses of differential network were performed in the following sections.

Overlapping differential networks of five paths reveals the candidate regulatory pattern

In order to assess these common DEGs compared with all DEGs in a systematic perspective, three networks (two state networks and one differential network) were separately built for each path, which integrated single-cell transcriptome and proteome data (see Methods for detail). A state network characterizes the association of each pair of genes in a specific cell type, while a differential network characterizes the changed association of each pair of genes from one cell type to another. After overlapping these differential networks of each path, two topologically overlapped subnetworks were identified: subnetwork 1 (Slc1a3, Slc38a2) and subnetwork 2 (Gsk3b, App, Cdk5). Here we focused on the Gsk3b\_App\_Cdk5 as a candidate RP because there is significant evidence that GSK3 signaling has a critical role in the regulation of neurogenesis, neurodevelopment, and in neuroplasticity [34]. Therefore, the associated genes of Gsk3b in differential networks may play the similar role.

The weight of edges in a differential network measures the changed correlation of each pair of genes and indicates the alterations of regulation between genes. It can be observed that the differences of edges correlation in the RP present an inconsistency in each path (Fig. 4). In path NSC\_NPC, NPC\_RGL and OPC\_OLI, the correlations among Cdk5, Gsk3b and App were weakened, while in path NPC\_AST and OPC\_MO, the correlations among Cdk5, Gsk3b and App were strengthened, which reflects the specificity of the RP.

According to the recently published literatures relating to the RP Gsk3b\_App\_Cdk5, all genes in the RP were separately found to be related to NSC differentiation in some degree. For instance, neuronal and glial differentiations of human neural stem cells are regulated by amyloid precursor protein (APP) levels [35]. GSK-3b inhibits degradation of  $\beta$ -catenin and thereby potentiates its downstream signaling, significantly enhancing neuronal differentiation [36]. Fine-tuning of Synapsin III expression and phosphorylation by CDK5 activation through Sema3A activity are essential for proper neuronal migration and orientation [37]. In total, all these literatures have confirmed that, each gene in the RP separately participates in specific stage of NSC differentiation. However, it is not clear whether the RP is involved in the regulation of all differentiation stages of the NSC lineage.

#### Network diffusion analysis reveals an indirect role of the regulatory pattern

In order to explore those important genes that indirectly influence topological differences (gene signaling propagation), the MC method based on perturbation test was performed on the differential network of each path [28], which is capable of quantifying the indirect influence of perturbing one gene on network propagation and network stability.

The median score of the network diffusion in each path is around 0.75 (the white dots of Fig. 5A), while the score of the most of the 12 common DEGs is greater than the median score (Fig. 5B). The result of network diffusion suggests that the scores of these three genes (Gsk3b, App, Cdk5) in the RP are consistently high, and the result of gene hierarchical clustering based on their scores on network diffusion (Fig. 5B) shows that these three genes play an interactive role in complex life processes.

#### Differential network analysis of common DEGs shows inconsistent topology in each path

To comprehensively assess the differences of network topology, five kinds of differential network measurement analysis methods (DDC, DEC, DPC, DBC and DiffRank) were performed on each path, and the average score of the five methods is regarded as the overall network topological differences (See Methods for details).

The topological differences of 12 common DEGs in five paths were shown in Fig. 6. There were inconsistencies of network topology of these 12 DEGs during different differentiation paths. When compared to the analysis of network diffusion, most of the 12 DEGs show no significant network topology differences (the score smaller than 0.2), which implies that they play indirect regulatory roles in the five differentiation paths. However, it should be noted that more than one of the top three genes with

most significantly topology difference in each path were involved in the RP Gsk3b\_App\_Cdk5. Therefore, the inconsistent scores of topological differences and consistent high scores of network diffusion reveal that these 3 genes in the RP may alternate their direct or indirect regulatory roles in different differentiation paths in order to promote cell differentiation towards different trajectory development.

KEGG enrichment analysis reveals the common and specific pathways implicated in the regulatory pattern

In order to explore the pathways of the RP Gsk3b\_App\_Cdk5 and their interactive genes, we performed the KEGG enrichment analysis on the pattern and their first-order neighbors in each differential network. The top ten enriched pathways in each path are shown in (Fig. 7A). Different paths are enriched in pathways related to distinct chemical signaling, which suggests the heterogeneity of NSC lineage differentiation. After intersecting all enriched pathways in each path, four common pathways are identified (Fig. 7B), including axon guidance, Alzheimer's disease, prostate cancer and endocrine resistance. Importantly, one of the common pathways, axon guidance, refers to the process by which growing neural axons follow specific, predictable paths to reach their target locations, which may play an important role in cell differentiation and development [38]. In this manner, specific regulators are able to establish cell type identity through shaping molecular programs for axon guidance [39], while aberrant axon guidance regularly results in most neurodegenerative diseases, such as Alzheimer's disease [40]. The above analysis confirms that the identified pattern plays important roles in NSC lineage differentiation and development.

## Discussion And Conclusion

Investigating common and specific patterns across multiple NSC differentiation paths is essential for understanding the mechanisms of different cell-fate decision in stem cell differentiation. In this work, we obtained 12 common DEGs by investigating five NSC differentiation paths. Extensive differential network topology-overlap analysis of all DEGs in each differentiation path revealed a pattern, termed RP Gsk3b\_App\_Cdk5, which co-occurred in five differentiation paths. In addition, literature analyses of recently published researches validated that each gene of the RP was significantly related to stem/progenitor cell differentiation and proliferation.

In each path, we further measured the pattern contributing to network differences quantitatively, including one network diffusion analysis and five differential topology analysis methods. In the diffusion analysis, most of the 12 common DEGs presented the consistently high diffusion score among five NSC differentiations, which suggests that they play an indirect role in NSC differentiation. For the five differential topology analyses, more than one gene in the RP show the highest topological differences, which suggests that they play a direct role in NSC differentiation. All of the above analyses revealed that the identified RP simultaneously influence and contribute to NSC differentiation, while different differentiation paths present specific pattern, that is, how the same genes play different roles (indirect or direct) in different differentiation paths.

In summary, we explored the common and specific patterns through different neuronal cell differentiations. The RP Gsk3b\_App\_Cdk5 was considered responsible for the cell-fate decision pattern of neural stem cell lineage, while different regulatory rules (indirect or direct) would contribute to different cell differentiation trajectory. Although we have investigated the common and specific pattern across different cell differentiation from a system perspective, there are still some problems needed to be addressed. The differential networks and state networks we built were undirected networks, which could not support the directed association between each pair of genes. Limited to the technology and cost, the causal relationship among genes is still unknown [41]. To explain which genes are upstream or downstream of the cascade, more perturbation researches are needed (e.g. gene knockdown). To validate our result, further biological experiments of the pattern may be done to provide more concrete evidence.

## Abbreviations

NSC  
Neural Stem Cell  
DEG  
Differentially Expressed Gene  
RP  
Regulatory Pattern  
NPC  
Neural Progenitor Cell  
AST  
Astrocyte cell  
RGL  
Radial Glial cell  
OPC  
Oligodendrocyte Progenitor Cell  
OLI  
Oligodendrocyte cell  
MO  
Myelinating Oligodendrocyte cell  
FC  
Fold Change  
SCC  
Spearman Correlation Coefficient  
DEC  
Differential Eigenvector Centrality  
DPC  
Differential PageRank Centrality  
DBC

Differential Betweenness Centrality

DDC

Differential Degree Centrality

GO

Gene Ontology

KEGG

Kyoto Encyclopedia of Genes and Genomes

## References

1. Schwartz PH, Brick DJ, Stover AE, Loring JF, Muller FJ: **Differentiation of neural lineage cells from human pluripotent stem cells.** *Methods* 2008, **45**(2):142-158.
2. English D, Sanberg PR: **Neural specification of stem cell differentiation.** *Stem Cells Dev* 2006, **15**(2):139-140.
3. Qiao SP, Liu Y, Han FT, Guo M, Hou XL, Ye KR, Deng S, Shen YJ, Zhao YF, Wei HY *et al:* **An Intelligent Neural Stem Cell Delivery System for Neurodegenerative Diseases Treatment.** *Adv Healthc Mater* 2018, **7**(12).
4. Hashimshony T, Wagner F, Sher N, Yanai I: **CEL-Seq: Single-Cell RNA-Seq by Multiplexed Linear Amplification.** *Cell Reports* 2012, **2**(3):666-673.
5. Picelli S, Bjorklund AK, Faridani OR, Sagasser S, Winberg G, Sandberg R: **Smart-seq2 for sensitive full-length transcriptome profiling in single cells.** *Nature Methods* 2013, **10**(11):1096-1098.
6. Pina C, Teles J, Fugazza C, May G, Wang D, Guo Y, Soneji S, Brown J, Eden P, Ohlsson M *et al:* **Single-Cell Network Analysis Identifies DDIT3 as a Nodal Lineage Regulator in Hematopoiesis.** *Cell Rep* 2015, **11**(10):1503-1510.
7. Hu Y, Hase T, Li HP, Prabhakar S, Kitano H, Ng SK, Ghosh S, Wee LJ: **A machine learning approach for the identification of key markers involved in brain development from single-cell transcriptomic data.** *BMC Genomics* 2016, **17**(Suppl 13):1025.
8. Kee N, Volakakis N, Kirkeby A, Dahl L, Storvall H, Nolbrant S, Lahti L, Bjorklund AK, Gillberg L, Joodmardi E *et al:* **Single-Cell Analysis Reveals a Close Relationship between Differentiating Dopamine and Subthalamic Nucleus Neuronal Lineages.** *Cell Stem Cell* 2017, **20**(1):29-40.
9. MacLean AL, Hong T, Nie Q: **Exploring intermediate cell states through the lens of single cells.** *Current Opinion in Systems Biology* 2018, **9**:32-41.
10. Wang Z, Gerstein M, Snyder M: **RNA-Seq: a revolutionary tool for transcriptomics.** *Nature Reviews Genetics* 2009, **10**(1):57-63.
11. Rostom R, Svensson V, Teichmann SA, Kar G: **Computational approaches for interpreting scRNA-seq data.** *Febs Lett* 2017, **591**(15):2213-2225.
12. Tang FC, Barbacioru C, Wang YZ, Nordman E, Lee C, Xu NL, Wang XH, Bodeau J, Tuch BB, Siddiqui A *et al:* **mRNA-Seq whole-transcriptome analysis of a single cell.** *Nature Methods* 2009, **6**(5):377-U386.

13. Dulken BW, Leeman DS, Boutet SC, Hebestreit K, Brunet A: **Single-Cell Transcriptomic Analysis Defines Heterogeneity and Transcriptional Dynamics in the Adult Neural Stem Cell Lineage.** *Cell Rep* 2017, **18**(3):777-790.
14. Zurauskiene J, Yau C: **pcaReduce: hierarchical clustering of single cell transcriptional profiles.** *Bmc Bioinformatics* 2016, **17**.
15. Arendt D, Bertucci PY, Achim K, Musser JM: **Evolution of neuronal types and families.** *Curr Opin Neurobiol* 2019, **56**:144-152.
16. Yang X, Gao L, Zhang S: **Comparative pan-cancer DNA methylation analysis reveals cancer common and specific patterns.** *Brief Bioinform* 2017, **18**(5):761-773.
17. de la Fuente A: **From 'differential expression' to 'differential networking' - identification of dysfunctional regulatory networks in diseases.** *Trends Genet* 2010, **26**(7):326-333.
18. West J, Bianconi G, Severini S, Teschendorff AE: **Differential network entropy reveals cancer system hallmarks.** *Sci Rep* 2012, **2**:802.
19. Lichtblau Y, Zimmermann K, Haldemann B, Lenze D, Hummel M, Leser U: **Comparative assessment of differential network analysis methods.** *Brief Bioinform* 2017, **18**(5):837-850.
20. Xie J, Lu D, Li J, Wang J, Zhang Y, Li Y, Nie Q: **Kernel differential subgraph reveals dynamic changes in biomolecular networks.** *J Bioinform Comput Biol* 2018, **16**(1):1750027.
21. Dimitrakopoulos C, Hindupur SK, Hafliger L, Behr J, Montazeri H, Hall MN, Beerenwinkel N: **Network-based integration of multi-omics data for prioritizing cancer genes.** *Bioinformatics* 2018, **34**(14):2441-2448.
22. Ji J, He D, Feng Y, He Y, Xue F, Xie L: **JDINAC: joint density-based non-parametric differential interaction network analysis and classification using high-dimensional sparse omics data.** *Bioinformatics* 2017, **33**(19):3080-3087.
23. La Manno G, Gyllborg D, Codeluppi S, Nishimura K, Salto C, Zeisel A, Borm LE, Stott SRW, Toledo EM, Villaescusa JC *et al:* **Molecular Diversity of Midbrain Development in Mouse, Human, and Stem Cells.** *Cell* 2016, **167**(2):566-580 e519.
24. Tasic B, Menon V, Nguyen TN, Kim TK, Jarsky T, Yao Z, Levi B, Gray LT, Sorensen SA, Dolbeare T *et al:* **Adult mouse cortical cell taxonomy revealed by single cell transcriptomics.** *Nat Neurosci* 2016, **19**(2):335-346.
25. Chen R, Wu X, Jiang L, Zhang Y: **Single-Cell RNA-Seq Reveals Hypothalamic Cell Diversity.** *Cell Rep* 2017, **18**(13):3227-3241.
26. Robinson MD, McCarthy DJ, Smyth GK: **edgeR: a Bioconductor package for differential expression analysis of digital gene expression data.** *Bioinformatics* 2010, **26**(1):139-140.
27. Chasman D, Fotuhi Siahpirani A, Roy S: **Network-based approaches for analysis of complex biological systems.** *Curr Opin Biotechnol* 2016, **39**:157-166.
28. Bersanelli M, Mosca E, Remondini D, Castellani G, Milanesi L: **Network diffusion-based analysis of high-throughput data for the detection of differentially enriched modules.** *Sci Rep-Uk* 2016, **6**.

29. Picart-Armada S, Thompson WK, Buil A, Perera-Lluna A: **diffuStats: an R package to compute diffusion-based scores on biological networks.** *Bioinformatics* 2018, **34**(3):533-534.
30. Wang H, Li M, Wang J, Pan Y: **A New Method for Identifying Essential Proteins Based on Edge Clustering Coefficient.** In: *2011; Berlin, Heidelberg*. Springer Berlin Heidelberg: 87-98.
31. Koschützki D, Schreiber F: **Centrality analysis methods for biological networks and their application to gene regulatory networks.** *Gene Regulation and Systems Biology* 2008.
32. Odibat O, Reddy CK: **Ranking Differential Hubs in Gene Co-Expression Networks.** *J Bioinf Comput Biol* 2012, **10**(1).
33. Yu G, Wang LG, Han Y, He QY: **clusterProfiler: an R package for comparing biological themes among gene clusters.** *OMICS* 2012, **16**(5):284-287.
34. Hur EM, Zhou FQ: **GSK3 signalling in neural development.** *Nat Rev Neurosci* 2010, **11**(8):539-551.
35. Coronel R, Lachgar M, Bernabeu-Zornoza A, Palmer C, Domínguez-Alvaro M, Revilla A, Ocaña I, Fernández A, Martínez-Serrano A, Cano E et al: **Neuronal and Glial Differentiation of Human Neural Stem Cells Is Regulated by Amyloid Precursor Protein (APP) Levels.** 2019, **56**(2):1248-1261.
36. Rammensee S, Kang MS, Georgiou K, Kumar S, Schaffer DV: **Dynamics of Mechanosensitive Neural Stem Cell Differentiation (vol 35, pg 497, 2017).** *Stem Cells* 2018, **36**(3):467-469.
37. Perlini LE, Szczurkowska J, Ballif BA, Piccini A, Sacchetti S, Giovedi S, Benfenati F, Cancedda L: **Synapsin III Acts Downstream of Semaphorin 3A/CDK5 Signaling to Regulate Radial Migration and Orientation of Pyramidal Neurons In Vivo.** *Cell Reports* 2015, **11**(2):234-248.
38. Inestrosa NC, Varela-Nallar L: **Wnt signalling in neuronal differentiation and development.** *Cell Tissue Res* 2015, **359**(1):215-223.
39. Kuwajima T, Soares CA, Sitko AA, Lefebvre V, Mason C: **SoxC Transcription Factors Promote Contralateral Retinal Ganglion Cell Differentiation and Axon Guidance in the Mouse Visual System.** *Neuron* 2017, **93**(5):1110-+.
40. Frei JA, Stoeckli ET: **SynCAMs - From axon guidance to neurodevelopmental disorders.** *Mol Cell Neurosci* 2017, **81**:41-48.
41. Fuellen G: **Evolution of gene regulation—on the road towards computational inferences.** *Brief Bioinform* 2011, **12**(2):122-131.

## Additional Files

Additional file 1. The parameter selection of Spearman Correlation Coefficient (SCC) used in our network construction. We explain the parameter selection by the plot of the features of distribution of SCCs in the different network in five paths of neural stem cells differentiation.

## Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are available in <https://github.com/FuzhangYang/RP>.

Competing interests

The authors declare that they have no competing interests.

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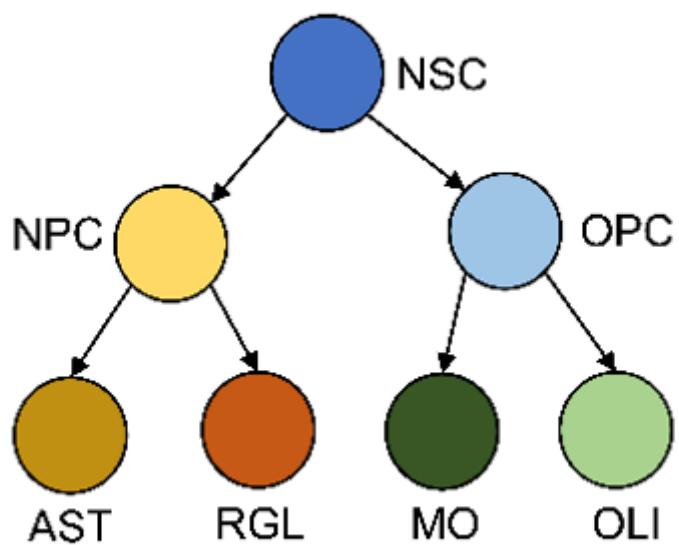
Author's contributions

FY conceived the study. JX, FY, JS, JW improved the study based on the original model. FY implemented the methods corresponding to the study. JX and JW supervised the study. FY wrote the manuscript of the study. All authors reviewed and improved the manuscript.

Acknowledgements

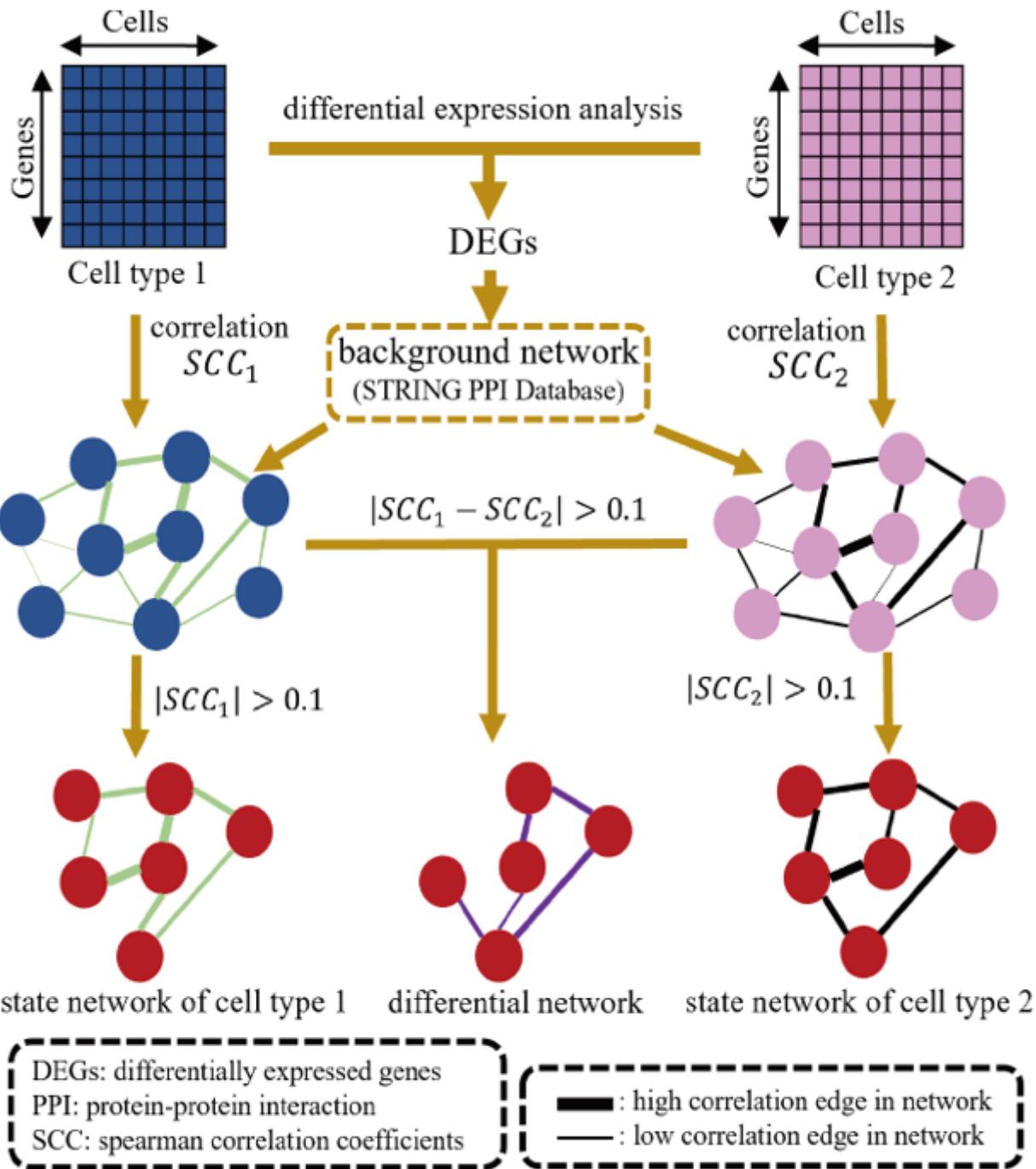
Not applicable

## Figures



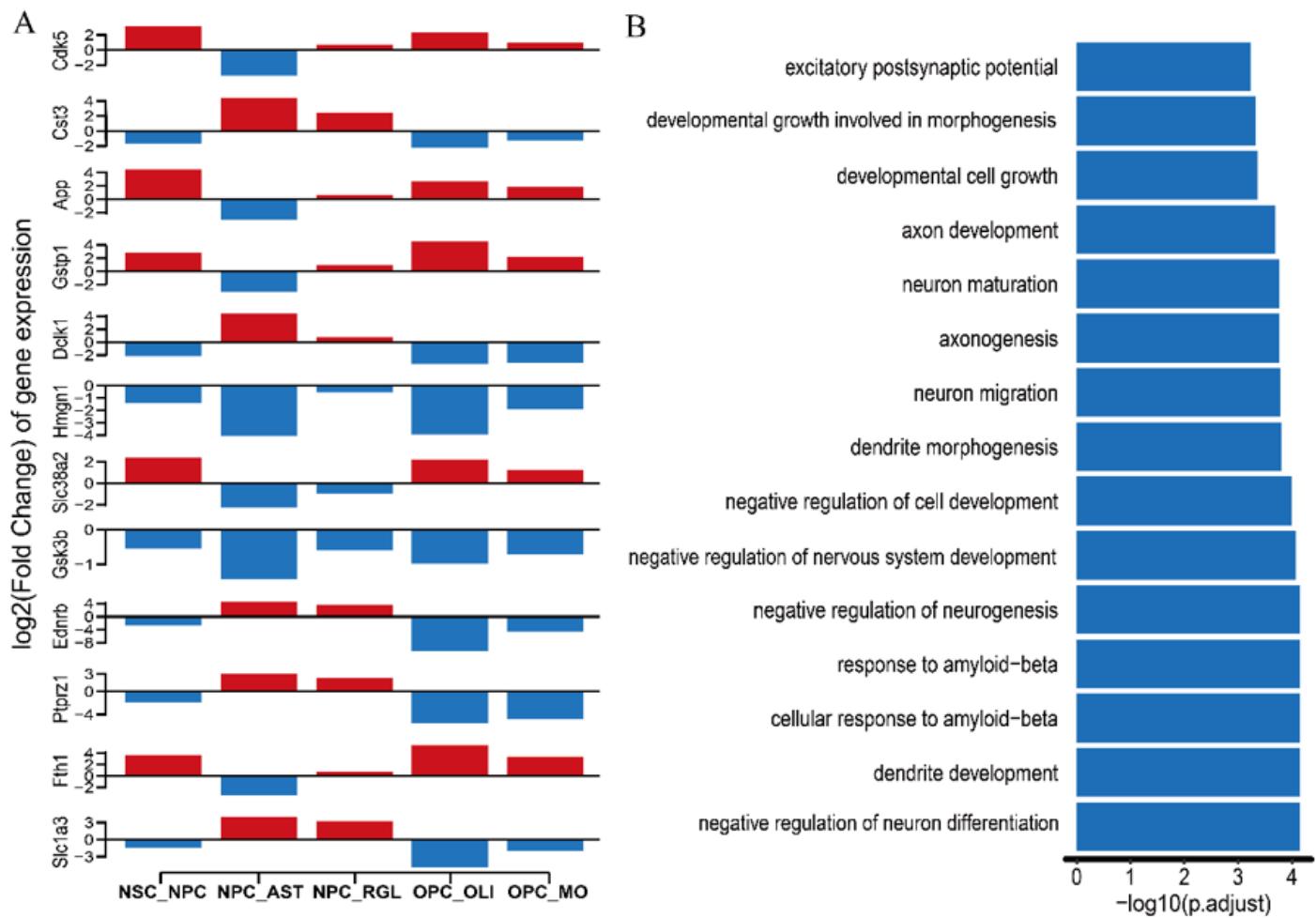
**Figure 1**

Neural stem cell lineage differentiation tree. Each arrow corresponds to a cell differentiation path in our datasets, excluding one arrow from NSC to OPC.



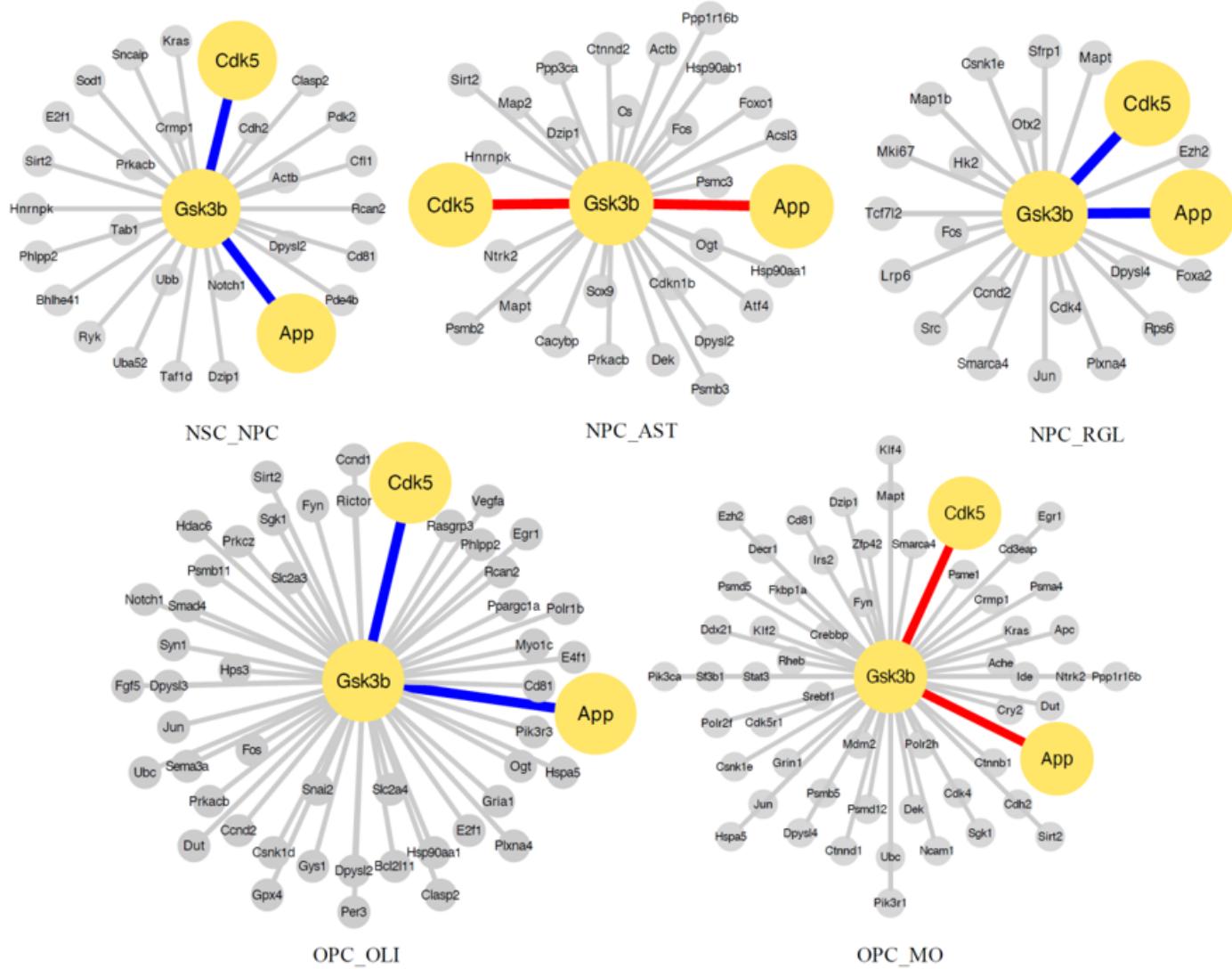
**Figure 2**

The workflow of network construction. Two state networks are constructed by the correlation of gene expression and the topology of protein-protein interaction network. A differential network is constructed by the differential correlation of the two state networks.



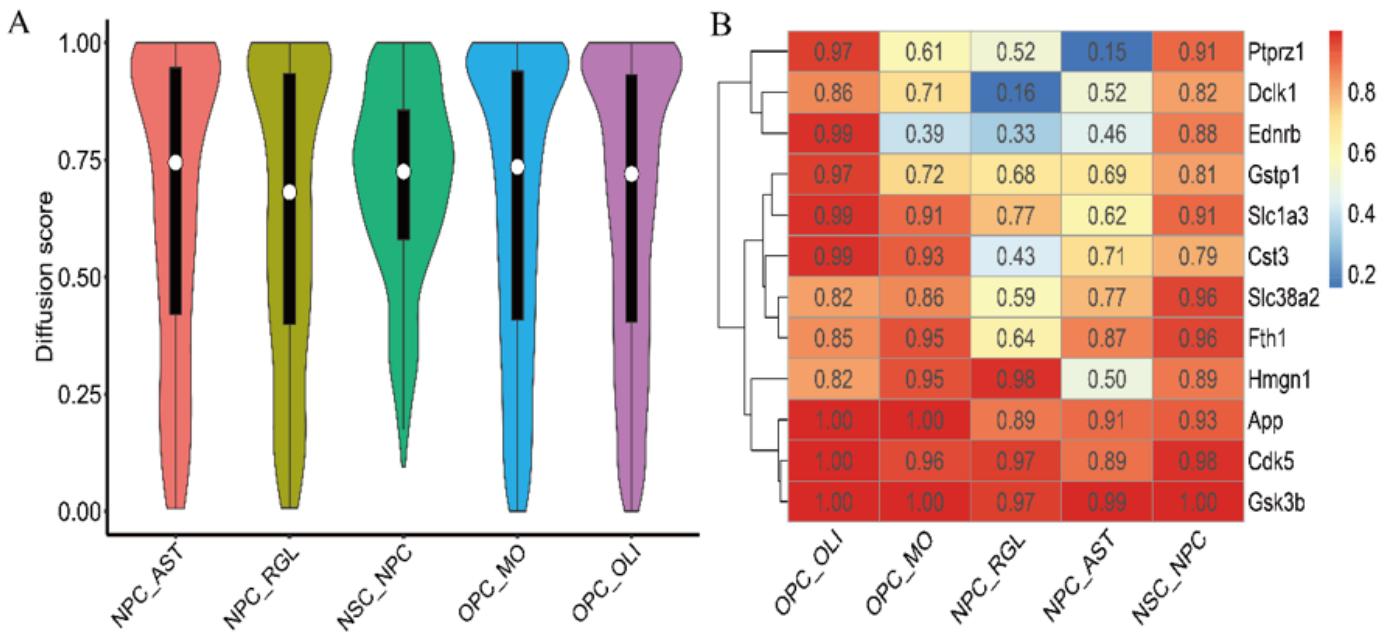
**Figure 3**

Differential expression analysis reveals 12 common differentially expressed genes among five NSC differentiation paths. (A) The bar plot of the fold change of 12 common differentially expressed genes, where blue color bars represent down-regulated function and red color bars mean up-regulated function. (B) The bar plot represents GO enrichment result of 12 common differentially expressed genes. For visualization, we chose the top 15 enriched terms in GO enrichment result.



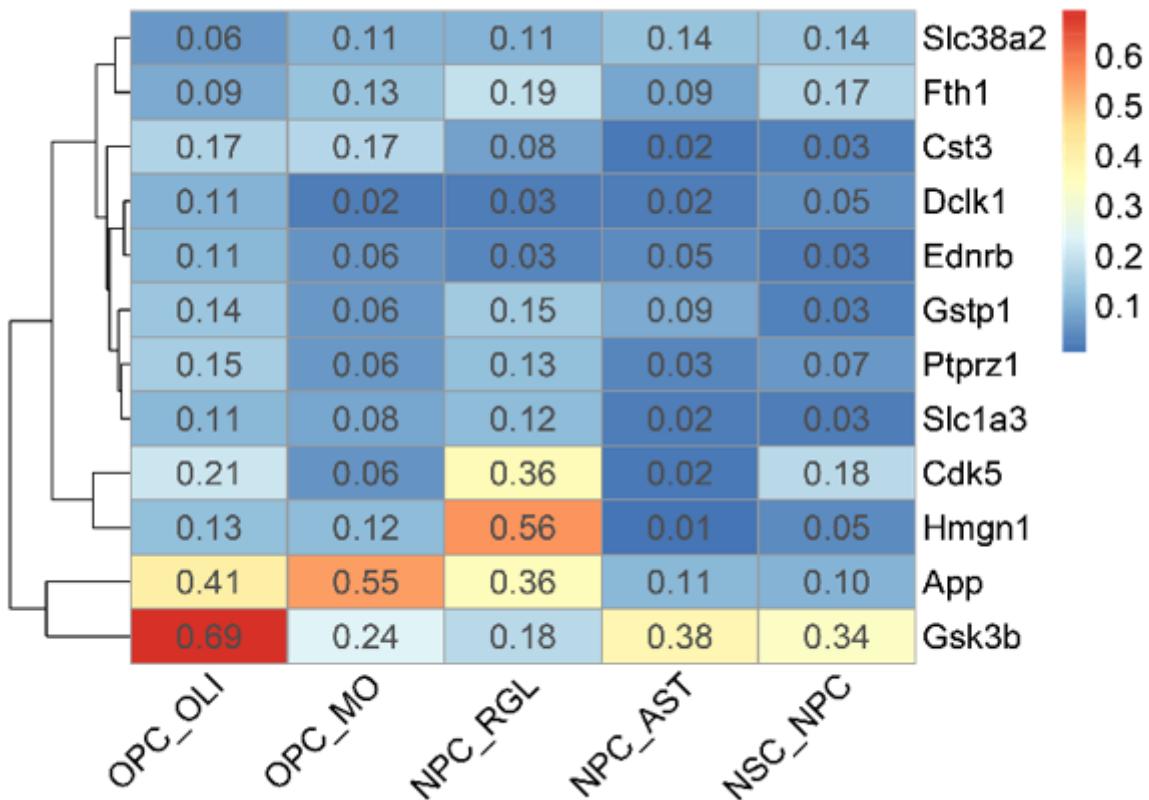
**Figure 4**

The network topology of the candidate RP among five NSC differentiation paths. The subnetworks are extracted by selecting the first-order neighbors of Gsk3b from differential network in different paths. The color of edge means the correlation difference; red represents correlation gain while blue represents correlation lost.



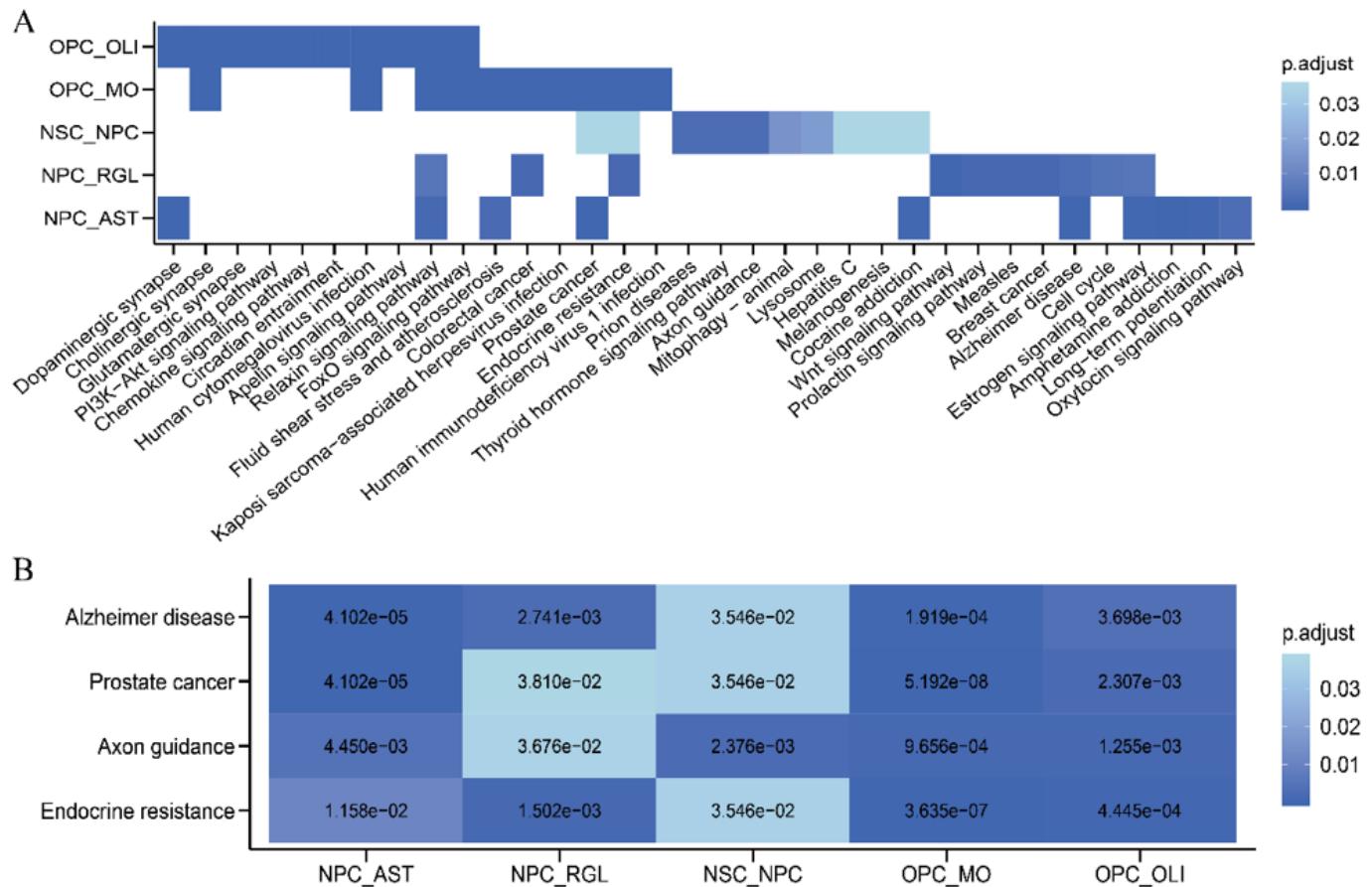
**Figure 5**

The score of network diffusion reveals the indirect roles of the 12 common DEGs. (A) The violin plot and box plot of diffusion represent scores of all genes in each path. (B) The heatmap of 12 common DEGs in each path, and the colors of heatmap describe the diffusion scores.



**Figure 6**

The heatmap of average score of 5 differential topology analyses reveals the direct role in the RP. The different columns describe different paths. The row describes the 12 common differentially expressed genes among 5 paths. The scores and colors of heatmap describe the difference of network topology of a specific gene in a specific path.



**Figure 7**

KEGG enrichment analysis shows the common and specific pathways of the RP. (A) Top 10 enriched pathways in each NSC differentiation path. (B) The intersection of all enriched pathways in each NSC differentiation path.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx