

# MiR-190a Potentially Ameliorates Postoperative Cognitive Dysfunction by Regulating Tiam1

**Qiang Liu**

Zhejiang University

**Aisheng Hou**

Chinese PLA General Hospital

**Yongyi Zhang**

Chinese PLA General Hospital

**Ying Guo**

Chinese PLA General Hospital

**Jingjing Li**

Zhejiang University

**Yinghao Yao**

Zhejiang University

**Kaimeng Niu**

Zhejiang University

**Hao Li**

Chinese PLA General Hospital

**Yunlong Ma**

Zhejiang University

**Jiangbei Cao** (✉ [cjb2000@sina.com](mailto:cjb2000@sina.com))

PLA general hospital of China <https://orcid.org/0000-0003-1218-4639>

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## Research article

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# Abstract

Postoperative cognitive dysfunction (POCD) affects a large number of post-surgery patients, especially for the elderly. However, the etiology of this neurocognitive disorder is largely unknown. Even if several studies have reported a small number of miRNAs as the essential modulatory factors in POCD, these findings are still rather limited. In this study, we used two miRNA microarray datasets to perform differential expression analyses of miRNAs in the hippocampus of POCD model mice. We found that nine miRNAs showed significant associations with POCD in both datasets. Among these miRNAs, mmu-miR-190a-3p was the most significant one. By performing weighted gene co-expression network analysis, we found 25 co-expression modules, of which mmu-miR-190a-3p was significantly anti-correlated with the red module. Moreover, in this red module, 314 genes were significantly enriched in four pathways such as axon guidance and calcium signaling pathway, which are well-documented to be associated with psychiatric disorders and brain development. Also, 169 of the 314 genes were highly correlated with mmu-miR-190a-3p, and four genes (*Sphkap*, *Arhgef25*, *Tiam1*, and *Ntrk3*) had putative binding sites at 3'-UTR of mmu-miR-190a-3p. Based on protein-protein network analysis, we detected that *Tiam1* was a central gene regulated by the mmu-miR-190a-3p. Taken together, we conclude that mmu-miR-190a-3p is involved in the etiology of POCD and may serve as a novel predictive indicator for POCD.

## Background

Postoperative cognitive dysfunction (POCD), a commonly-seen postoperative complication especially for elderly patients (age > 65-year-old), is characterized by cognitive impairments in patients underwent major surgery, and associated with high morbidity and mortality [1]. It covers a wide range of cognitive dysfunctions including short or long-term memory loss and attention deficit [2]. POCD was reported to occur in 25.8% of the patients seven days post-surgery, and in 12.7% of the patients 3 months post surgery [3-5]. This indicates that when the early postoperative neurocognitive disorders happen, the human body simultaneously initiates the self-repair mechanism for improving or restoring the cognitive function [6]. However, the mechanisms remain to be fully elucidated. Since POCD often leads to prolonged hospital stays, decreased quality of life, and increased social dependence as well as medical expenses [3], it is important to elucidate its underlying molecular mechanisms for the prevention, diagnosis, and treatment of POCD.

MicroRNAs (miRNAs), which are extensively distributed in eukaryotes, regulate expression of genes by 1) combining with 3-UTR of target gene to inhibit the translation or to degrade target mRNAs at the post-transcription level; and 2) interacting with other target non-coding RNAs, e.g. long non-coding RNA (lncRNA) or circular RNA (circRNA) [7, 8]. A great number of miRNAs enriched in human brain has been shown to express in a developmental stage-specific, tissue-specific, and cell-specific pattern [9] and play crucial roles in development of the neural system and in cognitive process such as learning and memorizing [10, 11]. Recently, multiple lines of evidence have demonstrated that miRNAs are involved in the pathogenesis of several neuropsychiatric diseases [11-15]. For example, based on genome-wide transcription sequencing, Liu et al. demonstrated that miRNAs and lncRNAs were important contributors

in the pathogenesis of schizophrenia [14]. Furthermore, multiple aberrantly expressed miRNAs were implicated in the development of Huntington's disease [13], neural tube defects [16], and Alzheimer's disease [17]. As for POCD, several miRNAs have been reported to be associated with it [6, 18-20], such as, Yu et al. [6] reported that miR-572 was implicated in the development and restoration of POCD and might serve as a biological marker in early diagnosis of POCD. However, the specific roles and underlying mechanism of miRNAs in POCD are still not yet clear.

Hence, in this study, by focusing on the biology of the dysregulated miRNAs in POCD, we conducted a comprehensive analysis of miRNAs expression profiles in hippocampus tissue of POCD model mice, and made further investigation of the miRNAs' potential interaction mechanisms with target genes. Our findings not only extend the understanding of miRNAs' role in the pathogenesis of POCD but also facilitate the identification of the miRNA biomarkers for improving the prognosis of POCD patients.

## Methods

### Animals

Healthy aged male C57BL/6 mice (n = 22, aged 18 months, weights from 40.2g to 46.6 g) were purchased from SiBeiFu Experimental Animal Science and Technology Co. Ltd (Beijing, China. Permit Number: SCXK (Jing) 2016-0002). The mice were individually housed in an air-conditioned room with a temperature of  $24\pm 4^{\circ}\text{C}$  and 55-65% humidity, under a standard 12h-12h light-dark cycle (lights on 6 AM to 6 PM), and had free access to standard food and water. The mice were acclimatized for 1 week before the experiment. The protocol of animal experiment was approved by the Animal Care Committee of the Chinese People's Liberation Army General Hospital (Beijing, China).

### Tissue Sampling

Mice were numbered by weight and randomly divided into two groups: Surgery and Sham, with 11 mice per group. The mice in the Surgery group were exposed to abdominal surgery under local bupivacaine anesthesia (according to the protocol from Xu *et al.* [21]), whereas those in the Sham group did not suffer from the anesthesia and surgery. At 24 hours after the surgery, three mice were randomly selected from each group and sacrificed. The hippocampus tissue was removed immediately and stored in the sterile tube (RNase Free) in liquid nitrogen.

### Morris Water Maze (MWM)

The MWM test, as a hippocampal-dependent test, was applied to evaluate the spatial learning, spatial memory and cognitive flexibility for the mice [22]. The cognitive function of remaining 8 mice in each group were assessed by the MWM experiment. The water maze was a black circular tank (120 cm in

diameter and 50cm in depth) and filled with water of  $22\pm 1^{\circ}\text{C}$  to a depth of 35 cm. Several visual objects were installed above the pool to help mice identify the direction. The maze was divided into four quadrants, an invisible platform (10 cm in diameter) was placed 1.5 cm below the water surface in the first quadrant (target quadrant). The whole experiment was performed under a dark and quiet environment.

During the experiment, mice were released into the water facing the wall of the tank from one of the four quadrants. The mice were trained to find the hidden platform and climb onto it within 60 seconds. The animals were allowed to stay on the platform for at least 10 seconds after each trial. If the mice were unable to find the platform in 60 seconds, it was then placed on the platform for 10 seconds. After that, the mice were put back to the cage and the second mouse was tested on trial 1. This rotation was repeated until all animals completed trial 1. Subsequently, the process was repeated for subsequent trials until 4 trials completed per day for 5 consecutive days. At 6th day, the platform was removed, and the mice were sent to evaluate their reference memory by being released from the third quadrant. The swimming speed, platform-site crossing numbers, dwelling time in the target quadrant and the escape latency were recorded.

## RNA Extraction

Total miRNAs were extracted from the hippocampal tissue ( $n = 3$  from each group) by the miRcute miRNA kit (TIANGEN, DP501). The nanodrop was utilized to determine the miRNA concentration of each sample according to the optical absorption at 260 nm and the gel electrophoresis was used to detect the miRNA integrity.

## Microarray and Statistical Analysis

MiRNA microarray (Affymetrix miRNA 4.0) was conducted by PREMEDICAL Co. Ltd (Beijing, China). The microarray was utilized to find the aberrant expression of miRNAs from the POCD model mice to normal mice. Fluorescent signals were transformed from picture signal to digital data based on the degree of fluorescent for each probe, and then the data were saved as .DAT files by AGCC software (Affymetrix Genechip Command Console Software). The differential expression analyses were performed by using Transcriptome Analysis Console (v 4.0) and using an FDR correction for multiple testing. Considering that none of the identified miRNAs reached the threshold of adjusted significance, we chose the threshold of a nominal P value of  $< 0.05$  for further replication.

The miRNA expression profile data of replication cohort were downloaded from the Gene Expression Omnibus (GEO) database (Accession number: GSE95070; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE95070>), which were deposited by Wei et al. [19] from the Chaoyang Hospital. With regard to this dataset, hippocampus tissues of 10 mice (5 per group) were dissected, and the different miRNA expression levels between two groups were detected by

Affymetrix miRNA 4.0 as well. Similarly, the differential expression analysis was performed by using the Transcriptome Analysis Console (v 4.0) with same parameter.

## Quantitative Real-time PCR

Expression level of the most significantly aberrant miRNA, mmu-miR-190a-3p, was validated by using real-time PCR assay. Reverse transcription reaction was performed with M-MLV Reverse Transcriptase kit (Takara Code: D2639A) based on the manufacturers' protocol. Real-time PCR was performed with SYBR Premix Ex Taq kit (Takara Code: DRR041A). The miRNA expression level was evaluated relative to the expression of U6 of the  $2^{-\Delta\Delta C_t}$ . The primers for miRNA mmu-miR-190a-3p are listed in Supplemental Table 1.

## Statistical Analysis

Data were analyzed using GraphPad PRISM (version 6; GraphPad Prism Software, Inc. San Diego, CA, USA). Measurements of dwelling time, number of grossing, escape latency, and speed in MWM test among preoperative and postoperative mice were analyzed by using Student's t-test. qRT-PCR data were also analyzed with Student's t-test. A P value of < 0.05 was considered statistically significant.

## WGCNA Analysis

The genes and miRNAs expression data of GSE73507 were acquired from GEO database (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73507>). The GSE73507 dataset was designed to gain insight into the relationship between the CAG repeat length and the Huntington disease [23]. By excluding the expression data from other brain regions and mutant mice, we only downloaded the mRNAs and miRNAs expression data from hippocampus tissue of wild type mice for our analysis (n = 24). Because of recent progress of alignment and mapping approaches, which are capable of detecting the transcriptome profiles in a more accurate and effective way compared with former tools [24]. The RNA and miRNA sequencing data were aligned and mapped to the GRCm38 version of mice genome using Hisat2 (v 2.1.0) and StringTie (v 1.3.4) [25], and the miRNA expression data by miRDeep2 (v 2.0.0.7) [26]. A total of 16,425 mRNAs and 1,057 miRNAs were correctly mapped onto the mouse genome. Threshold for filtering out genes expressed at low levels was set to greater than 1 of the average fpkm. After the filtering process, 13,241 mRNAs and 546 miRNAs were included for WGCNA analysis.

The R package of WGCNA was used to construct the network modules of highly correlated transcripts subsets [27]. This approach aims to find the gene pairs with similar expression patterns and highly topological overlap, and it represents a valuable tool for identifying promising target genes and understanding the pathology of complex disorders [28]. In order to provide a comprehensive expression pattern among the mRNAs and miRNAs and detect the interaction of the transcripts, we performed our co-

expression analysis by combined the mRNA and miRNA dataset together. First, we constructed a weighted network according to the gene pair correlations among all the mRNAs and miRNAs; second, by using the default parameters to assess the network interconnection, 25 specific modules were hierarchically clustered. These module sizes were from 50 to 17,500 genes. In the network we only showed a connection of the corresponding topological overlap is above a threshold of 0.05 with mmu-miR-190a-3p in the red module (n = 169). The visual gene-gene network plot was displayed by using the Cytoscape version 3.5.1 (<https://www.cytoscape.org/>) [ 29].

Concordance between the highly correlated mRNAs with mmu-miR-190a-3p and those not involved in the module genes were assayed by performing density plots. We compared the distribution of Pearson correlation coefficients of the 169 potential interaction targets of mmu-miR-190a-3p to a control distribution of non-predicted targets which consisted of all other mRNAs that we mapped. The mRNAs which were possibly modulated by mmu-miR-190a-3p displayed more significant negative correlation compared with the control.

## Pathway Analysis

ClueGO (v. 2.3.4), a plug-in software of Cytoscape, was used to decipher the pathways network and determine their biological functions for the candidate genes [30]. The potential biological functions of each gene set were annotated using the pathway profiles of Kyoto Encyclopedia of Genes and Genomes (KEGG) [31].

## Target Prediction

We conducted further analysis to screen the most potential regulated genes by mmu-miR-190a-3p. We first employed the miRWalk3.0 (<http://mirwalk.umm.uni-heidelberg.de/>) to predict the target genes regulated by mmu-miR-190a-3p [32]. By using a stringent standard to obtain reliable targets (Supplemental Table 2), we set the parameters for target prediction in the miRwalk3.0 as following: 1) Binding over than 0.9; 2) Energy less than -16; and 3) Accessibility less than 0.05. Second, using these predicted targets for mmu-miR-190a-3p to overlap with the WGCNA results of those highly correlated genes. Finally, we conducted a protein-protein interaction network analysis for those overlapped genes according to the STRING v 10.5 under default parameters (<https://string-db.org/cgi/input.pl>). By combining the gene-gene interaction result, prediction targets and the protein-protein interaction analysis, we attempted to find the high degree receivable genes regulated by mmu-miR-190a-3p.

## Conclusions

To sum up, we provided robust evidence supporting that mmu-miR-190a-3p has a strong positive correlation with the incidence of POCD. The pathway analysis displayed that these mmu-miR-190a-3p targeted genes were enriched in the psychiatry-related pathways and immune system-related pathways.

Among of these target genes, *Tiam1* was inhibited by mmu-miR-190a-3p in the hippocampus tissue of POCD model mice. These results demonstrated that mmu-miR190a-3p probably serve as an important regulator to inhibit target genes expression in the hippocampus tissue of POCD patients. More molecular-based studies are warranted to explore the underlying biological mechanisms of target genes by mmu-miR-190a-3p in the pathogenesis of POCD.

## Abbreviations

POCD: Postoperative cognitive dysfunction; PCR: Polymerase chain reaction; WGCNA: Weighted gene co-expression network analysis; miRNA: microRNA; lncRNA: long non-coding RNA; circRNA: circular RNA; MWM: Morris water maze; AGCC: Affymetrix Genechip Command Console; GEO: Gene expression omnibus; KEGG: Kyoto Encyclopedia of Genes and Genomes.

## Declarations

## Ethics approval and consent to participant

The protocol of animal experiment was approved by the Animal Care Committee of the Chinese People's Liberation Army General Hospital (Beijing, China).

## Consent to publish

Not applicable.

## Availability of data and materials

The datasets generated and/or analysed during the current investigation are available from the corresponding author until it becomes available from a public repository.

## Competing Interests

The authors declare no conflicts of interest.

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# Authors' contributions

QL, JL, YY, KN managed the literature searches and data analysis. AH, YZ, YG, HL performed laboratory experiments. QL and AH wrote the first draft of the manuscript. YM, and JG conceived the study and wrote the manuscript. All authors read and approved the final manuscript.

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## Tables

**Table 1. Results of Morris Water Maze test among preoperative and postoperative mice**

Sample ID	Dwelling Time	Number of Crossing	Escape Latency	Speed
CTRL1	33.1	1.0	36.3	0.2
CTRL2	16.9	2.0	36.0	0.2
CTRL3	26.3	1.0	60.0	0.2
CTRL4	23.5	2.0	60.0	0.2
CTRL5	36.3	3.0	47.9	0.2
CTRL6	16.2	3.0	31.9	2.1
CTRL7	25.9	2.0	46.9	0.2
CTRL8	26.3	2.0	43.1	0.2
CASE1	26.0	2.0	60.0	0.3
CASE2	15.4	2.0	55.0	0.2
CASE3	13.7	1.0	60.0	0.2
CASE4	4.8	0.0	60.0	1.3
CASE5	30.6	2.0	60.0	0.2
CASE6	12.3	1.0	60.0	0.2
CASE7	17.1	1.0	56.3	0.2
CASE8	18.0	1.0	53.6	0.2
<i>p</i> Value	0.044	0.0596	0.005	0.754

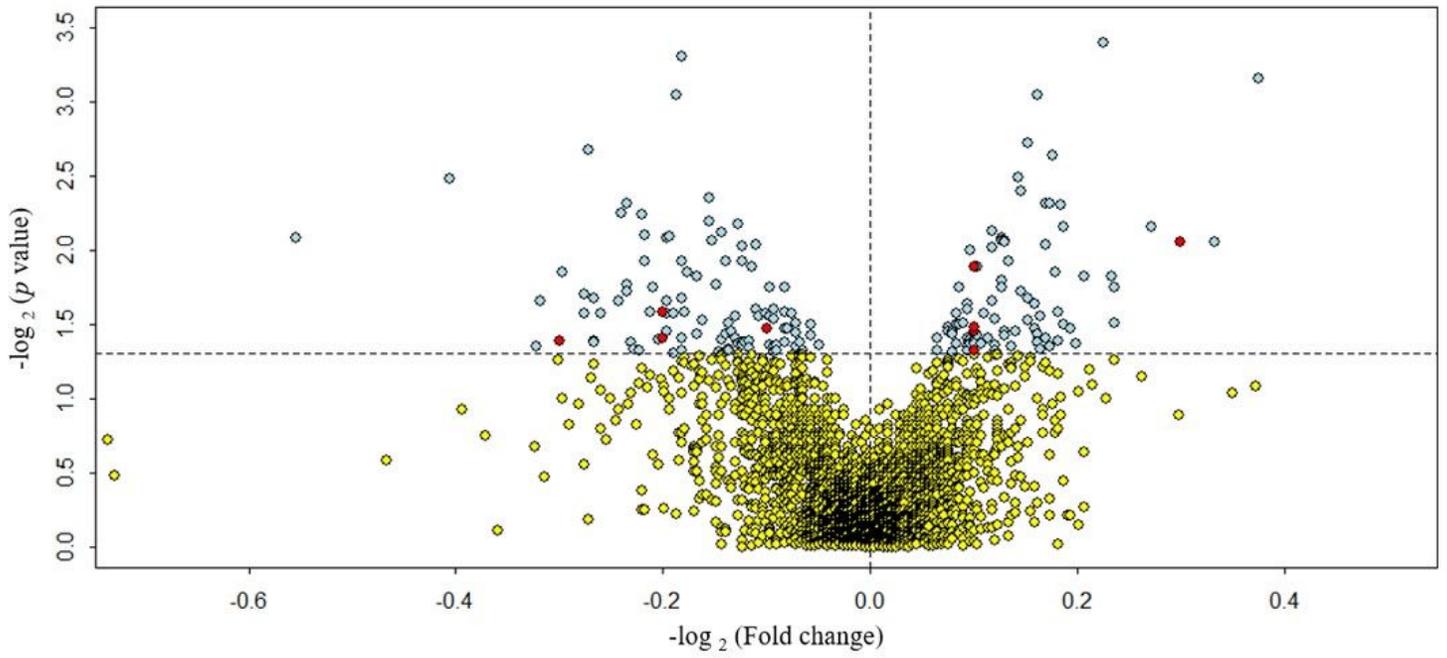
Note: CTRL represents preoperative mice, CASE represents postoperative mice.

**Table 2. The replicated differential expressed miRNAs in hippocampus tissues**

miRNA	Chr	Start	End	Discovery Cohort		Replication Cohort	
				Log2(FC)	<i>p</i> Value	Log2(FC)	<i>p</i> Value
mmu-miR-190a-3p	chr9	67236664	67236685	2.4	7.0E-04	1.9	2.4E-03
mmu-mir-7648	chr15	90224360	90224412	1.3	1.3E-02	1.1	2.3E-02
mmu-mir-1907	chr15	50889025	50889114	1.2	3.3E-02	1.4	2.1E-02
mmu-miR-184-5p	chr9	89802302	89802323	1.2	3.5E-02	1.2	3.9E-02
mmu-miR-6999-5p	chr2	91944908	91944930	1.2	4.8E-02	1.3	3.6E-02
mmu-miR-496a-3p	chr12	109739165	109739186	-1.5	2.6E-02	-1.1	3.3E-02
mmu-miR-592-3p	chr6	27936672	27936693	-1.9	4.1E-02	-2.3	6.6E-05
mmu-miR-6389	chr7	57581070	57581090	-1.5	3.9E-02	-1.2	5.0E-02
mmu-miR-6939-5p	chr12	112659327	112659347	-1.2	3.4E-02	-1.4	4.0E-02

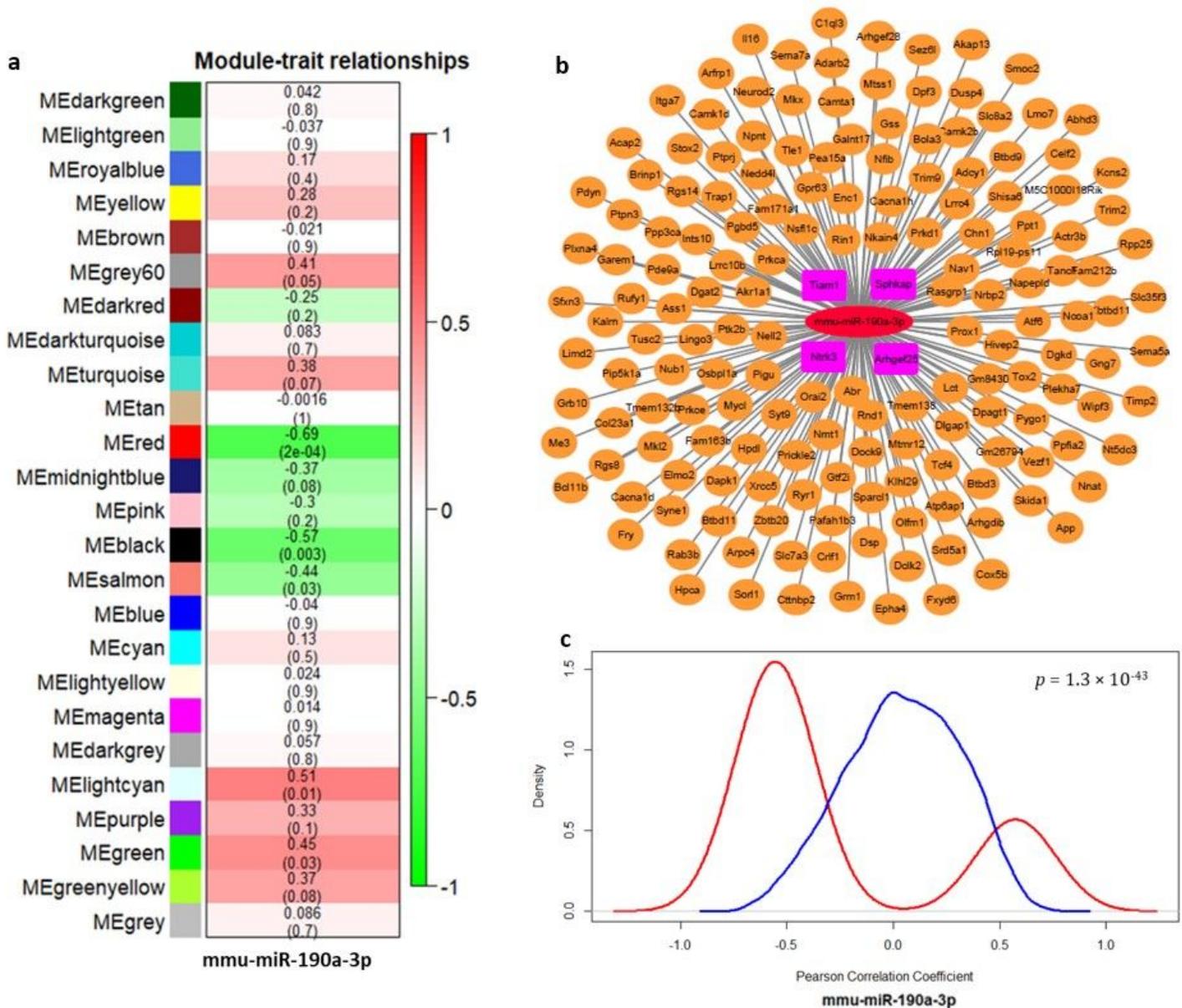
Notes: 1) Chr: Chromosome; 2) FC: Fold Change.

## Figures



**Figure 1**

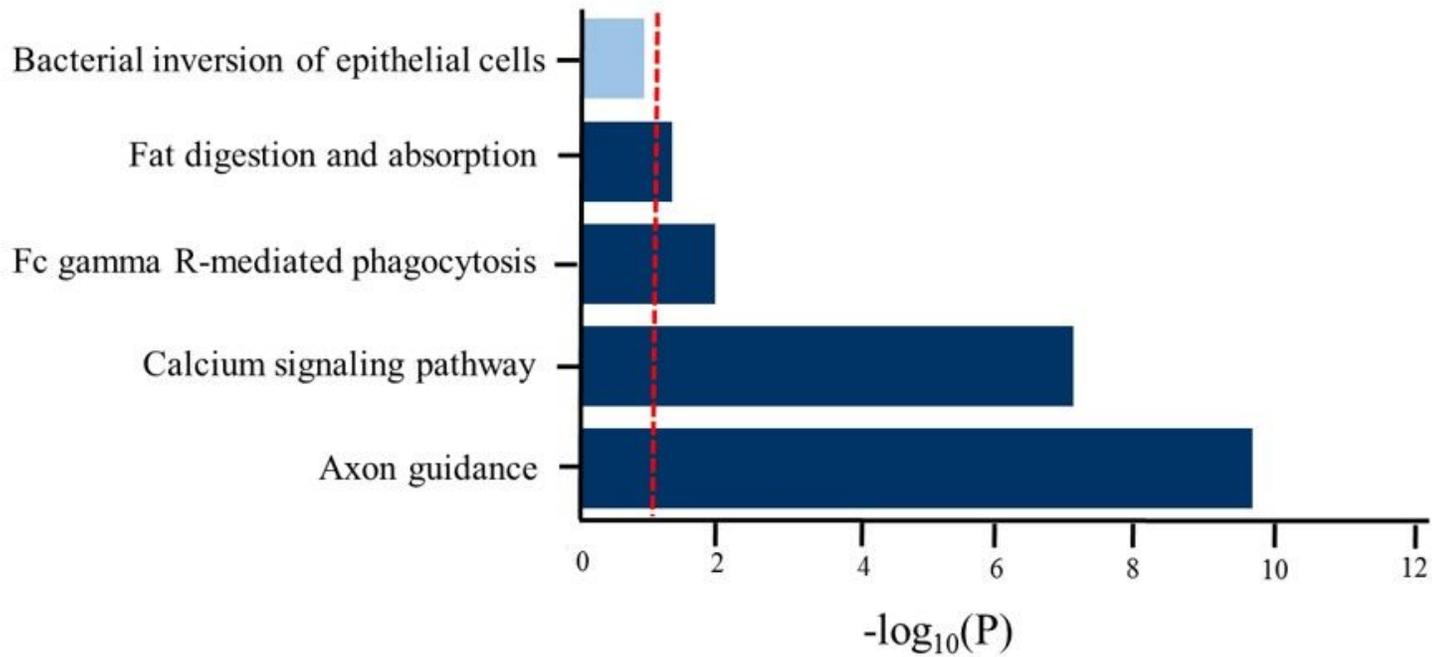
Volcano plot of miRNA expression in discovery cohort. Plotted along the x-axis is the mean of log2 fold-change, along the y-axis the negative logarithm of the log2 p values. Light blue demonstrates the miRNAs with significant p value ( $p < 0.05$ ), and red denotes these significant miRNAs validated by the replication cohort. The horizontal line represents the threshold of significant p value.



**Figure 2**

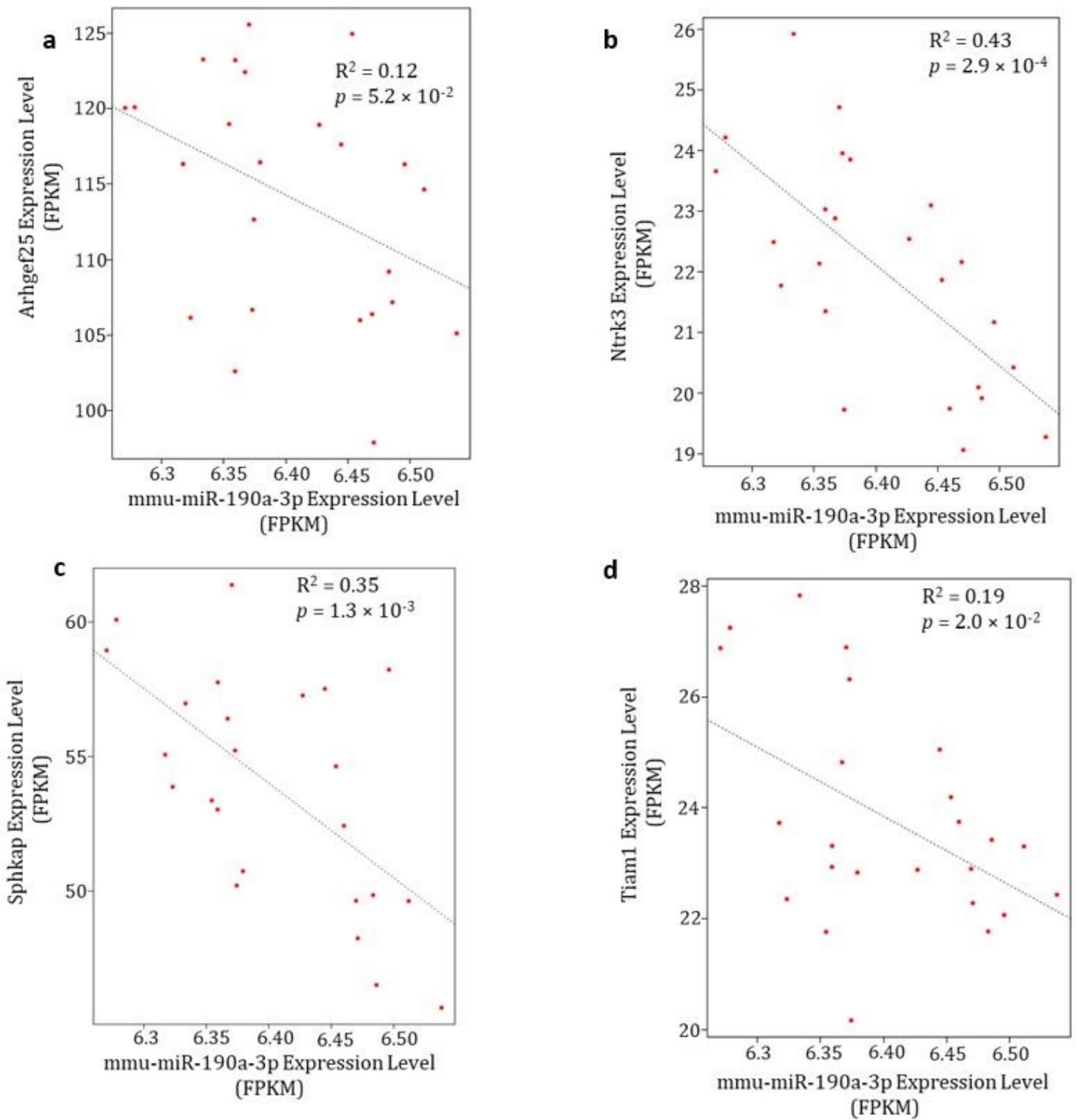
The mmu-miR-190a-3p involved module in the hippocampal tissue. (a) Clustering dendrograms of genes according to the topological overlap to distinguish the subgroup and used same color to assign highly correlated modules. As the result, 25 co-expression modules were constructed and painted with different colors. Pearson correlation analysis was used to determine whether co-expression modules are correlated with mmu-miR-190a-3p. Red color indicates the module was positively correlated with mmu-miR-190a-3p, and green demonstrates negative correlation. (b) Overview of candidate genes modified by the mmu-miR-190a-3p. Nodes are defined as the target genes modulated by the mmu-miR-190a-3p which was representing by red ellipse. The 4 pink rectangular represented the prediction results from miRwalk3.0 databases. (c) Distribution of Pearson correlation coefficients of predicted mmu-miR-190a-3p-mRNA pairs. We determined the correlation between the expression of that miRNA and the expression of a predicted mRNA targets in all samples by calculating a Pearson correlation coefficient. This process was repeated for all targets and the red line indicated the distribution of these coefficients as a density plot.

The blue line as the control curve represented the distribution of the coefficient of others unrelated mRNA with mmu-miR-190a-3p. A left shift of the red line comparing with the red line demonstrated that the potential targets for mmu-miR-190a-3p are preferentially more negative correlated with targets gene than random.



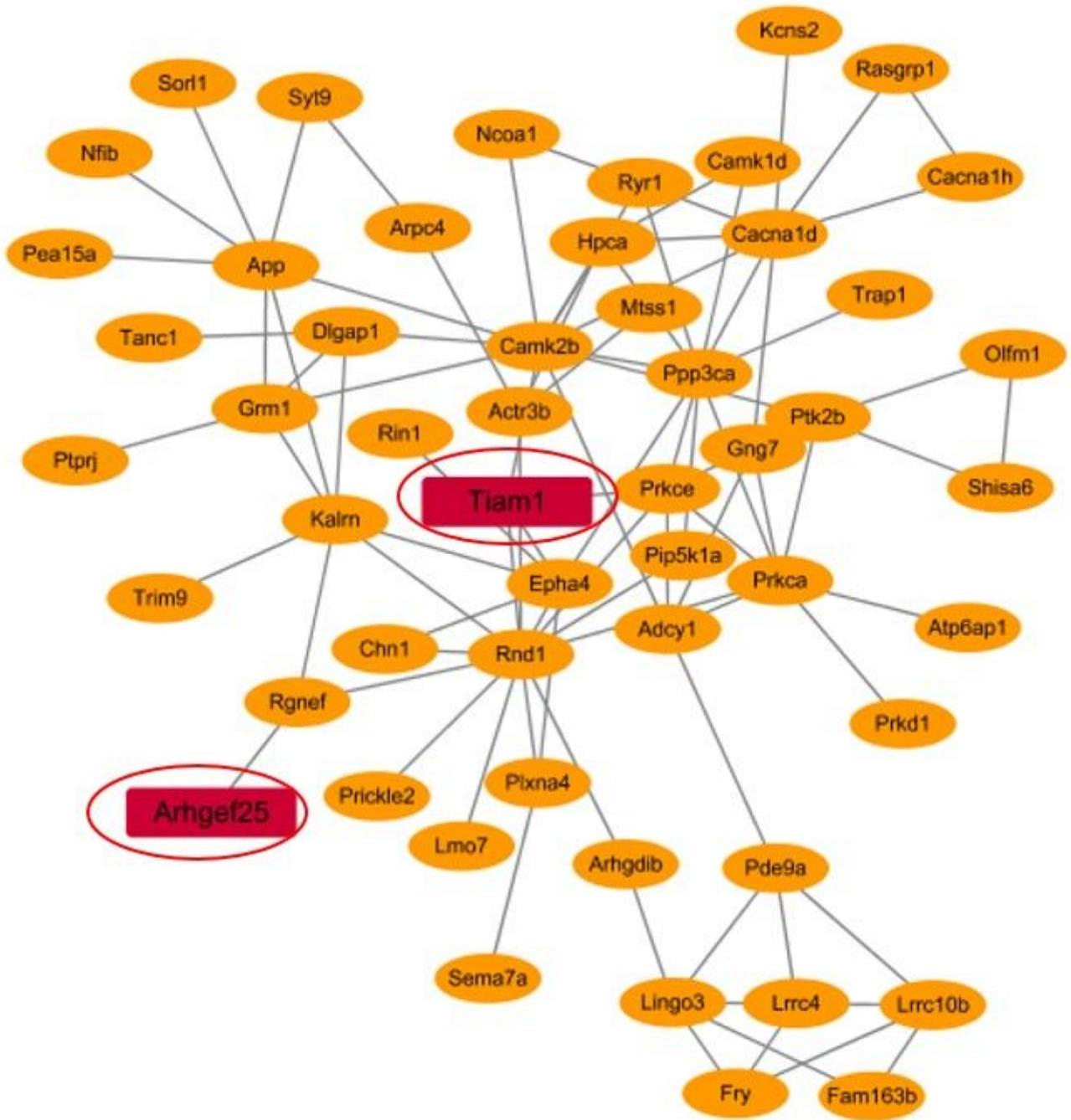
**Figure 3**

KEGG pathway enrichment analysis for genes in red module. The red vertical line indicates the threshold of the significant pathway enrichment p value after FDR correction. Dark blue color of horizontal bars represents the significantly enriched pathway, and the light blue color represents the non-significantly enriched pathway.



**Figure 4**

Prediction binding targets show anti-correlation with mmu-miR-190a-3p. The target mRNAs expression level (FPKM) of: a) Arhgef25; b) Ntrk3; c) Sphkap and d) Tiam1 are inversely correlated with the expression level of mmu-miR-190a-3p.



**Figure 5**

Protein-protein interaction network for mmu-miR-190a-3p potential regulated genes in mice hippocampus tissue. The genes overlapped with prediction targets by mmu-miR-190a-3p were highlighted as red rectangular.

## Supplementary Files

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

- [supplement1.pdf](#)