# Upregulation of Dicer and MicroRNA Expression in the Dorsolateral Prefrontal Cortex Brodmann's Area 46 in Schizophrenia

Danielle M. Santarelli[1,2,3], Natalie J. Beveridge[1,2,3], Paul A. Tooney[1,2,3], Murray J. Cairns*[1,2,3]

**Affiliations:** [1]Schizophrenia Research Institute, Sydney, NSW 2006, Australia. [2]School of Biomedical Sciences and Pharmacy, Faculty of Health, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia. [3]Hunter Medical Research Institute, Lookout Road, New Lambton, NSW Australia.

*** Corresponding Author:** Dr Murray J Cairns, School of Biomedical Sciences, The University of Newcastle, University Drive, Callaghan, NSW 2308, Australia.

**Phone**: 61-2-4921-8670 **Fax**: 61-2-4921-7903

**Email**: murray.cairns@newcastle.edu.au

**Key Words:** Schizophrenia, microRNA, neuropathology, Dicer, gene silencing, BA46

**Word count:** **Abstract:** 225 **Text:** 3917

**Figures:** 2 **Tables:** 2 **Supplementary material:** 4

**Background:** MicroRNA (miRNA) are capable of regulating multitudes of target genes, and are essential factors in mediating healthy neurodevelopment. We hypothesize that abnormal miRNA levels contribute to the complex global changes in gene expression that underlie the pathophysiology of schizophrenia.

**Methods:** Using a commercial bead array platform, we investigated miRNA expression in 74 samples of postmortem dorsolateral prefrontal cortex (Brodmann's Area 46) (n=37 matched pairs schizophrenia/schizoaffective disorder and controls). A subset of differentially expressed miRNA and genes in the miRNA biogenesis pathway was also analyzed using quantitative RT-PCR (Q-PCR). Gene targets of miRNAs demonstrating significantly altered expression were predicted and pathways analysis performed.

**Results:** After correction for multiple testing, microarray analysis identified differential expression of 28 miRNA in the schizophrenia group. Significantly, 89% of these molecules were elevated in accordance with earlier work in other brain regions that showed a broad increase in miRNA expression in schizophrenia. These observations were supported by Q-PCR, for miR-328, miR-17-5p, miR-134, miR-652, miR-382 and miR-107, and were consistent with a schizophrenia-associated increase in miRNA processing through elevated Dicer expression. Target and pathways analysis provided insight into the potential cellular effects, with particular enrichment of miRNA targets in axon guidance and long term potentiation.

**Conclusions:** These results suggest that schizophrenia is associated with altered miRNA biogenesis and expression, which may have important implications in the complex pathophysiology of the disorder.

## Introduction

Schizophrenia is a complex neuropsychiatric disorder thought to arise during development as a consequence of multiple genetic and environmental risk factors (1, 2). While the precise genetic changes that give rise to the pathology are not clear, numerous genetic loci have been associated with the disorder, and dozens of schizophrenia candidate genes have been reported (3-5). Unfortunately, the effect size of these genes is often small, and alone, are not sufficient to support the heritability of schizophrenia. Genome-wide investigation of gene activity in postmortem brain tissue has identified changes in gene expression in postmortem brain of schizophrenia (6-10). These analyzes provide a biological dimension to the study of genes in schizophrenia that sometimes intersects with the genetics. In many more cases though, a functional relationship remains elusive. One trend seen often amongst many gene expression studies has been the bias toward downregulation of gene activity (8, 9, 11). These observations give rise to the possibility of an abnormality in gene regulation in schizophrenia. In this event, over representation of gene repression would implicate a loss of function in mechanisms that promote or elevate gene expression, or a gain of function in mechanisms capable of driving a systematic reduction of gene expression. In this study we explored this latter possibility by examining gene silencing and the influence of miRNA biogenesis in schizophrenia.

The discovery of endogenous regulatory non-coding RNA, known as microRNA (miRNA), has lead to wide spread interest and implication in several human diseases, including schizophrenia (12-14). MiRNA are short (~22nt) RNA sequences with the capacity to target hundreds of genes via sequence complementarity to the 3' untranslated region (UTR) of the target mRNA (15, 16). The consequence of this is predominantly a silencing of gene and/or protein expression. MiRNA have already been identified as essential factors in mediating healthy neurodevelopment (17). It is hence plausible to suggest that abnormalities at the miRNA levels may well contribute to the complex

differences in gene expression and disruptions in neurodevelopmental processes that are apparent in the pathophysiology of schizophrenia.

In the investigation presented here, miRNA expression profiling was performed on postmortem brain tissue of the dorsolateral prefrontal cortex (DLPFC) Brodmann's area 46 (BA46), from a cohort of 37 matched pairs of schizophrenia and non-psychiatric controls, using a recently developed commercial miRNA microarray platform (Illumina). BA46 is a region of the DLPFC adjacent to BA9, BA10 and the mid-ventrolateral region. Disturbances to BA46 result in a reduced ability to analyse information and make choices based on short-term memory; a cognitive function that is particularly known to be impaired in schizophrenia termed 'monitoring of working memory' (reviewed in 18). Over-activation of BA46 during working memory tasks has been reported in first-degree relatives of schizophrenia (19), further highlighting the anatomical specificity of this region in the disorder. To our knowledge, this is the first miRNA study to be performed in BA46 for schizophrenia, and the largest cohort to be used for any miRNA expression profiling across all previous brain regions studied in schizophrenia (12-14).

## Methods and Materials

### Tissue sample cohort

Postmortem brain tissue samples were collected by the NSW Tissue Resource Centre (University of Sydney). Use of this tissue was approved by the University of Newcastle Human Research Ethics Committee and consent was obtained from the next of kin. All cases had been diagnosed with schizophrenia or schizoaffective disorder according to the DSM-IV criteria, and were confirmed by medical file review using the Item Group Checklist of the Schedules for Clinical Assessment in Neuropsychiatry. The class 'schizoaffective' refers to patients that experience a combination of schizophrenia and affective disorder, particularly depressive forms of psychosis (20).

Subjects with a significant history of drug or alcohol abuse, neurological disorder or medical illness that might have influenced agonal state, any abnormality on neuropathological examination or head injury were excluded. Control subjects were excluded if there was a history of alcoholism or suicide. All subjects were of Caucasian descent. The cohort was comprised of 37 matched pairs of postmortem brain tissue from BA46 of the DLPFC on the middle frontal gyrus, from subjects with schizophrenia or schizoaffective disorder, and non-psychiatric controls. Samples were matched according to donor age, sex, brain pH, brain hemisphere, duration of illness, among other characteristics. Full cohort description, matching and analysis is detailed in (21) and summarized in Supplement 1. Demographic variables age ($p=0.95$), pH ($p=0.49$), PMI ($p=0.23$) and RIN ($p=0.81$) do dot differ between schizophrenia and control cohorts (two-tailed Student's t-test).

**RNA Extraction**

Cortical grey matter tissue was carefully dissected from postmortem brain slices of the crown of the middle frontal gyrus, anterior to the genu of the corpus callosum using a dental drill (22). The tissue was immediately homogenized and total RNA extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA concentration and integrity was determined using a bioanalyzer (Agilent). Mean RNA integrity number (RIN) was 7.3.

**miRNA microarray**

Profiling of miRNA expression was achieved using the commercial miRNA microarray platform developed by Illumina. Each array matrix on the Illumina microarray platform holds 96 sample arrays, and each array houses 1,536 unique oligonucleotide sequence probes for 470 annotated miRNA sequences as well as 265 recently identified miRNA sequences corresponding to miRBase version 9.1(23). Total RNA (1µg) was amplified and labeled within a 96-well plate format for hybridization to the miRNA beadarray matrix according to the manufacturer's instructions (Illumina).

**Microarray data normalization & analysis**

In this study, schizophrenia-associated miRNA expression was investigated in the DLPFC (BA46) using a commercial bead based microarray platform (Illumina) (23). Expression data was background subtracted and normalized to the geometric mean of U66 and U49 snoRNA expression using BeadStudio software (Illumina, version 3.0) as these had the most stable expression across the control cohort according to geNorm analysis (24). MiRNA were considered to be expressed if fluorescence intensity was at least twice the level of background fluorescence. Differential expression analysis was performed on normalized data using the Significance Analysis of Microarrays (SAM) statistical analysis program (full academic version 2.23) (25 - http://www-stat.stanford.edu/~tibs/SAM/). This program reports the validity of genes that it identifies as being significantly differentially expressed according to a q-value; an adaptation of the p-value that is appropriate for multiple hypothesis testing, and denotes the lowest possible false discovery rate that may occur before a reported gene is not to be considered significantly differentially expressed (26). SAM differential expression analysis was performed using a two-class unpaired Wilcoxon test of unlogged data and 5000 permutations. Significantly different miRNA were identified as those with a q-value < 5 (FDR < 5%).

**Quantitative real time reverse transcription PCR**

Validation of differentially expressed miRNA was performed by quantitative real time RT-PCR (Q-PCR) on the entire cohort, similarly to that described previously (12). Briefly, 500ng of sample RNA was treated with DNAse-I (Invitrogen) and multiplex reverse transcription performed with Superscript II reverse transcriptase (Invitrogen), a 3nM mix of miRNA sequence specific primers and primers for U6 snRNA and U49 snoRNA. For mRNA Q-PCR, random-primed reverse transcription was performed. For Q-PCR, triplicate reactions were set up in a 96-well format using the epMotion

5070 automated pipetting system (Eppendorf) and carried out using the Applied Biosystems 7500 real-time PCR machine. Serial dilutions of DLPFC cDNA were used as standards and data was analyzed using the relative quantitation method with efficiency correction. Relative microRNA expression was calculated as the ratio of the microRNA and the geometric mean of controls U6 and U49 (the most stable of the 3 controls as determined by geNorm). Relative mRNA (biogenesis gene) expression was calculated as the ratio of the gene and the geometric mean of controls hydroxymethylbilane synthase (HMBS) and beta-glucuronidase (GUSB). Outliers were identified as values ± 3SD outside of the mean and were removed. Statistical significance of differential miRNA and mRNA expression between schizophrenia and control samples was assessed by multivariate analysis of variance (ANOVA). To examine the influence of demographic variables (age, PMI and pH) on miRNA and mRNA expression, data was tested for normality and Spearman Product Moment correlations were performed on the entire cohort. If the expression of any miRNA or gene significantly correlated with any demographic variable, an analysis of covariance (ANCOVA) was performed to reassess its significance. The expression of the miRNA biogenesis genes were also examined in a pair-wise fashion. The expression for each schizophrenia sample was compared to the corresponding matched control sample and expressed as a ratio (schizophrenia:control); e.g. SZ#1 is matched to CTR#1. Pearson correlations (two-tailed) were also performed on expression data of validated miRNA and the biogenesis genes to identify any similarities in expression.

**Target gene and pathway analysis**

For those miRNA identified as being significantly differentially expressed by SAM analysis, gene target predictions were performed using the miRGen web-based prediction algorithm (version 4.0) (26 - http://www.diana.pcbi.upenn.edu/miRGen.html). MiRGen was directed to retrieve human miRNA targets from lists formulated by the union of prediction algorithms DIANA-microT, miRanda (microRNA.org), miRanda (miRBase), PicTar (4-way), Pictar (5-way), TargetScanS and miRanda.

Additional target prediction was performed using miRanda (www.microRNA.org) (27). The miRanda algorithm is capable of identifying numerous miRNA binding sites within the one 3'UTR. If a miRNA has more putative binding sites within the one gene, it increases its regulatory potential. The complete, unfiltered list of target genes (from validated miRNA) encompassed 4993 unique genes. This was reduced to 1792 target genes, when only genes with 3 or more "hits" were included. The 3 hits may arise from one miRNA having 3 binding sites within this 3'UTR from one individual miRNA, or, this gene had one putative site from 3 different validated miRNAs. Pathway analysis of these lists was achieved using the functional annotation tools on the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/) (28, 29); particular focus was paid to pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and biological processes from the Gene Ontology (GO) resource.

## Results

### Upregulation of miRNA expression in BA46 in schizophrenia

High throughput miRNA expression analysis of 470 annotated miRNA (from miRBase 9.1) revealed that 281 miRNAs (60%) were expressed in BA46. A scatter plot of the average fold change in expression of each expressed miRNA against its $\log_2$-transformed fluorescence intensity indicated that a large upregulation of miRNA in BA46 (Figure 1A), following the trend for global miRNA upregulation observed in BA22 and BA9 tissue. Differential expression analysis by SAM revealed 25 miRNA to be significantly upregulated in schizophrenia and 3 miRNA to be significantly downregulated (FDR < 5%) (Figure 1B). Of the upregulated miRNA, 10 have also been shown to be significantly increased in STG and 2 in BA9. Unsupervised clustering of the expression data for these miRNA reveals a moderate split between predominately schizophrenia samples with high miRNA expression (Figure 1B – right) and controls with lower miRNA expression (Figure 1B – left).

Validation of 10 differentially expressed miRNA was performed by Q-PCR similarly to that described previously (12). Although not reaching the threshold for significance by array, miR-107 was also included in the Q-PCR analysis as it has been previously shown to be differentially expressed in BA9 and BA22 (14). Relative miRNA expression was determined with respect to the geometric mean of U6 and U49 snoRNA expression. Significant changes in expression confirmed for miR-328 (1.32-fold increase, p=0.005 covaried for age), miR-17-5p (1.18-fold increase, p=0.029), miR-134 (1.16 fold-increase, p=0.037), miR-652 (1.15-fold increase, p=0.032), miR-382 (1.26-fold increase, p=0.042) and miR-107 (1.34-fold increase, p=0.042) (Figure 1C).

**Upregulation of miRNA biogenesis in BA46**

The microprocessor component genes DGCR8 and Drosha, and the type III ribonuclease responsible for cleavage of the pre-miRNA hairpin structure to form the mature miRNA, Dicer, have been shown to have increased expression in BA9 in schizophrenia (14). Alterations in microprocessor component genes may also be influential in the elevation of miRNA expression observed in this microarray analysis of the BA46 cohort. To this end, we examined the expression of these three miRNA biogenesis genes in the context of schizophrenia using Q-PCR. ANCOVA was used to assess the significance of gene expression changes in the miRNA biogenesis genes. Dicer displayed a 1.23-fold increase in expression in schizophrenia (p=0.025; covaried for PMI). DGCR8 and Drosha both displayed a robust 1.5-fold increase in the schizophrenia cohort though fell below the threshold of statistical significance due to greater variance within the samples (Drosha p=0.06 covaried for age; DGCR8 p=0.16 covaried for age, pH and RIN). These data support previous observations of increased miRNA biogenesis in schizophrenia. When exploring the pair-wise expression of these genes individually, DGCR8 was considered significantly increased (p=0.049), while Drosha (p=0.096) Dicer (p=0.087) fell below the threshold for significance. Interestingly, when examining the group as a

whole, it was revealed that 33 of the 37 matched pairs (89%) display schizophrenia-related upregulation in at least one of these miRNA biogenesis genes (Figure 2B - D).

Furthermore, correlation analysis indicated a significant relationship between the expression of DICER with miR-17-5p (p=0.002) and miR-382 (p=0.014); and also between the expression of DGCR8 with miR-382 (p=0.009). There were also numerous significant correlations between the expression of various miRNA, and a significant correlation between DICER and DROSHA expression (p=0.04) (Refer to Supplement 2 for the complete correlation matrix).

**Functional significance of upregulated miRNA**

To infer into the possible implications of upregulated miRNA in schizophrenia brain, target gene predictions using the miRanda prediction algorithm were conducted for the validated miRNA. Target predictions revealed a combined list of 4993 unique gene targets. Interestingly, several schizophrenia candidate genes as predicted target genes of the upregulated miRNA; including 9 glutamate receptors, 5 serotonin receptors, 2 GABA receptors, neuregulin 1 and 2, and brain-derived neurotrophic factor. Pathway analysis was performed on list of predicted target genes using the functional annotation tools on DAVID. Most attention was paid to the KEGG cellular pathway terms and biological processes from the Gene Ontology (GO) resource. The probability of pathway enrichment - EASE scores (modified Fisher's exact test p-value) was obtained following methods published by Huang *et al*. (29). From the target gene list, numerous KEGG pathways were identified as likely to be enriched with an EASE score < 0.05 (Table 1). Two highly enriched significant pathways of particular interest to this study were identified: axon guidance (EASE=0.017) and long-term potentiation (LTP) (EASE=0.039). Maps of these KEGG pathways were obtained via DAVID allowing visualization of the involvement of target genes within these pathways (Supplement 3). Gene ontology analysis revealed that numerous enriched biological processes with high relevance to the

pathophysiology of schizophrenia. Those biological processes with a p-value $\leq 0.05$ are listed as a percentage of total input genes in Table 2. To observe an extra level of stringency in the pathway analysis, the target gene list was subsequently filtered to only include genes which were identified with 3 or more conserved binding sites for the validated miRNA; effectively reducing the list of putative targets to 1792 genes. Target genes hosting a multiplicity of miRNA binding sites for differentially expressed miRNA, have a higher probability of being post-transcriptionally regulated and to a greater extent because of the cooperative or synergistic interactions between multiple RISCs on a single targets 3´UTR (30). Significantly, many of the pathways enriched in this multi-hit target gene analysis retained relevance in the context of schizophrenia and included the axon guidance pathway and LTP (Supplement 4).

## Discussion

We investigated miRNA expression in Brodmann's Area 46 of the DLPFC using commercial bead array technology. After background subtraction, normalization and correction for multiple testing using SAM, we were able to identify 28 miRNA that were differentially expressed in schizophrenia. Significantly, 25 of these miRNA were upregulated with respect to the controls, 10 of these in a manner consistent with previous investigation of the STG (BA22) and 2 consistent with investigations in BA9 (14). Many of these altered miRNA, such as miR-17-5p, miR-134, miR-148b, miR-150, miR-222, miR-328, miR-382 and miR-425-5p have been shown to be brain enriched, particularly in the cortex (31), and many also display neuron specific expression (32, 33). Consistent with the array and displaying significant upregulation in schizophrenia across the entire cohort, were miR-328, miR-17-5p, miR-134, miR-652 and miR-382. Also analysed by Q-PCR and shown to be significantly upregulated was miR-107, a miRNA displaying consistent elevation in studies of BA9 and STG. This support for an elevation of miRNA expression in schizophrenia and similarity to previous work led us

to again consider the influence of the miRNA biogenesis pathway. In this regard we observed a significant increase in Dicer mRNA expression in BA46 tissue in schizophrenia compared to controls. This increase in Dicer expression in BA46 was consistent with a previously reported increase in Dicer in BA9 (14). Interestingly, while the apparent increase in Drosha and DGCR8 expression is supportive of a trend increase with respect to the entire cohort, collectively, at least one biogenesis gene was found to be upregulated in an overwhelming majority of matched pairs (33 out of 37; 89%). Each of these genes has the potential to influence the level of cortical miRNA maturation in schizophrenia. Dicer in particular has been shown to play an important role in normal central nervous system development and function. In a Zebrafish knockout model Dicer deficiency was shown to induce failure of brain morphogenesis (17). In a Purkinje cell model system, Dicer inactivation results in cell degeneration and eventually neurodegeneration, due to a significant loss of neuron-specific miRNA (34). Moreover, Dicer has been shown to be enriched in the post-synaptic densities of neurons and activated by calpain in response to synaptic excitation (35). Increased Dicer expression is also associated with differentiation and maintenance of mature neural lineages during development (36, 37). In cortical neurons, Dicer has also been shown to localise in the dendrites (36). Upregulation of Dicer and consequential upregulation of global miRNA has also been reported in various tumor cells, such as adenocarcinomas (38). Dicer elevation in the DLPFC in schizophrenia could have important implications for structural and functional plasticity of the synapse and may hence contribute to the pathophysiology of the disorder.

The phenotypic implications of elevated miRNA biogenesis in the context of schizophrenia are very broad and difficult to predict even when considering individual miRNA. In the case of miR-328, upregulation has been observed in response to prion-induced neurodegeneration in mouse brain, leading to speculation that this may involve the targeting of the transcriptional co-repressor methyl CpG-binding protein 2 (MeCP2) (39). Mutations in MeCP2 are known to be linked to the

neurodevelopmental disorder Rett syndrome. MeCP2 has also been shown to modulate brain-derived neurotrophic factor (BDNF), a schizophrenia candidate gene involved in the coordination of dendrite/axon development in neurons, thus promoting dendritic spine growth (40, 41). On the other hand, miR-134 is a brain specific miRNA localised within dendrites that has been well characterised as a negative regulator of dendrite development and synaptic maturation, particularly by inhibition of *Limk1* mRNA, however, this inhibition may be relieved by BDNF (33, 42). More recently, miR-134 elevation in response to stress has been linked to alternative splicing of acetylcholinesterase (AChE), which is known to have downstream neurodegenerative effects (43). Also stress-responsive are miR-382 and miR-17-5p (43). Our group have previously shown expression of the miR-17 family, especially miR-17-5p, to be a feature of undifferentiated SH-SY5Y neuronal cells (44). In this study, miR-17-5p was found to inhibit the expression of differentiated neuronal cell markers B-cell CLL/lymphoma 2 (BCL2) and myocyte enhancer factor 2D (MEF2D), as well as the mitogen-activated protein kinase 12 (MAP3K12); MAPKs playing important roles in neurite outgrowth. Interestingly, miR-107 was also found to be significantly upregulated by Q-PCR, having previously shown to be significantly upregulated in the STG and DLPFC (BA9) of schizophrenia brain (14). Positive results were reported for a reporter gene assay investigating the interaction of miR-107 and a predicted target gene Reelin (RELN). RELN is a notable schizophrenia candidate gene with roles in regulating synaptic activity in the adult brain, and once again, dendritic spine growth (45). Numerous studies have reported reductions in RELN in schizophrenia and other neurological disorders (46-48), supporting the notion that increases in miRNA expression may be the cause of changes in neurological gene expression relevant to the pathophysiology of schizophrenia.

Prediction of potential target genes of the altered miRNA collectively revealed 299 schizophrenia candidate genes. Present within this list were 9 glutamate receptors, including GRIN1, GRIN2 and GRIN3 *N*-methyl-D-aspartate receptors (NMDARs), all potential targets of miR-328, and of which impaired transmission of has been linked to observations of decreased dendrite length and

density in cortical neurons in schizophrenia (49, reviewed in 50 2010, 51, 52). Also on the target list was the early growth response transcription factor EGR3, a schizophrenia candidate gene upregulated at times of high neuronal activity that is critical in numerous synaptic plasticity processes (53), and also reported to be downregulated in schizophrenia (54). According to our target predictions, all of the increased miRNA validated by Q-PCR in this study may potentially target EGR3. Furthermore, EGR3 regulates various schizophrenia candidate genes, as well miRNA genes that target additional schizophrenia candidate genes. According to Guo *et al.* (55), this may very well be an important, intricate feedback loop of which disturbances to would be highly relevant to schizophrenia.

Pathways analysis of the target list further highlighted the relevance of these upregulated miRNA to the pathophysiology of schizophrenia, with processes involved in nervous system development and synaptic transmission, among numerous other relevant processes, being highly enriched with target genes. Many of the differentially expressed miRNA share potential target genes, and may affect the same biological pathways/processes. Hence, various combinations of miRNA changes of small effect, especially those with roles in schizophrenia-related biological processes, may very well produce a similar outcome in terms of biological abnormalities, and ultimately schizophrenia symptoms.

Many of the miRNA found here to be upregulated in BA46 are either now known or thought to play a part in directing dendritic development. From the collection of information about these miRNA, it is possible that miR-17-5p, miR-134, miR-107 and possibly miR-328 may be involved in intricate modulation of neuron differentiation regulatory processes. Furthermore, target predictions found numerous neurotransmitter receptors to be potential miRNA targets. Pathway analysis of potential target genes further highlighted processes involved in synaptic transmission as major effect points of these upregulated miRNA. These observations are all consistent with the dysconnection hypothesis, which suggests that schizophrenia is a phenomenon of abnormal synaptic plasticity as a consequence of

inefficient or inappropriate wiring of neural networks (56). This hypothesis has also been supported by neuropathology with reports of dendrite length and density reductions in schizophrenia cortex (49, 57).

This study provides further support for a role of altered miRNA expression in the neuropathology of schizophrenia, and suggests that disturbances to miRNA biogenesis could be the underlying mechanism. Ultimately, the consequence of this change in regulatory environment is the alteration of gene expression and biological processes that may play a significant role in the pathogenesis and pathophysiology of schizophrenia.

*This study was supported by the Schizophrenia Research Institute, utilizing funding from NSW Health and an M.C. Ainsworth Research Fellowship in Epigenetics (MC); a NARSAD Young Investigator Award; a Hunter Medical Research Institute project grant; an NHMRC project grant 631057, the Neurobehavioral Genetics Unit and NSW Department of Health. Tissue was dissected and extracted in the Schizophrenia Research Laboratory at the Prince of Wales Medical Research Institute under the supervision of Professor Cynthia Shannon Weickert on behalf of the Australian Brain Donor Program's NSW Tissue Resource Centre (TRC). The TRC is supported by The University of Sydney, National Health and Medical Research Council of Australia, Schizophrenia Research Institute, National Institute of Alcohol Abuse and Alcoholism. The authors reported no biomedical financial interests or potential conflicts of interest.*

## References


1. Harrison PJ (1997): Schizophrenia: a disorder of neurodevelopment? *Curr Opin Neurobiol*. 7:285-289.
2. Roth TL, Lubin FD, Sodhi M, Kleinman JE (2009): Epigenetic mechanisms in schizophrenia. *Biochim Biophys Acta*. 1790:869-877.
3. O'Donovan MC, Williams NM, Owen MJ (2003): Recent advances in the genetics of schizophrenia. *Hum Mol Genet*. 12 Spec No 2:R125-133.
4. Tsuang M (2000): Schizophrenia: genes and environment. *Biol Psychiatry*. 47:210-220.
5. Waddington JL, Corvin AP, Donohoe G, O'Tuathaigh CM, Mitchell KJ, Gill M (2007): Functional genomics and schizophrenia: endophenotypes and mutant models. *Psychiatr Clin North Am*. 30:365-399.
6. Dean B, Keriakous D, Scarr E, Thomas EA (2007): Gene expression profiling in Brodmann's area 46 from subjects with schizophrenia. *Aust N Z J Psychiatry*. 41:308-320.
7. Mirnics K, Middleton FA, Lewis DA, Levitt P (2001): Analysis of complex brain disorders with gene expression microarrays: schizophrenia as a disease of the synapse. *Trends Neurosci*. 24:479-486.
8. Weidenhofer J, Bowden NA, Scott RJ, Tooney PA (2006): Altered gene expression in the amygdala in schizophrenia: up-regulation of genes located in the cytomatrix active zone. *Mol Cell Neurosci*. 31:243-250.
9. Bowden NA, Scott RJ, Tooney PA (2008): Altered gene expression in the superior temporal gyrus in schizophrenia. *BMC Genomics*. 9:199.
10. Kim S, Webster MJ Correlation analysis between genome-wide expression profiles and cytoarchitectural abnormalities in the prefrontal cortex of psychiatric disorders. *Mol Psychiatry*. 15:326-336.
11. Katsel P, Davis KL, Gorman JM, Haroutunian V (2005): Variations in differential gene expression patterns across multiple brain regions in schizophrenia. *Schizophr Res*. 77:241-252.
12. Beveridge NJ, Tooney PA, Carroll AP, Gardiner E, Bowden N, Scott RJ, et al. (2008): Dysregulation of miRNA 181b in the temporal cortex in schizophrenia. *Hum Mol Genet*. 17:1156-1168.
13. Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K, Newman MA, et al. (2007): microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. *Genome Biol*. 8:R27.
14. Beveridge NJ, Gardiner E, Carroll AP, Tooney PA, Cairns MJ (2009): Schizophrenia is associated with an increase in cortical microRNA biogenesis. *Mol Psychiatry*.
15. Lau NC, Lim LP, Weinstein EG, Bartel DP (2001): An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. *Science*. 294:858-862.
16. Murchison EP, Hannon GJ (2004): miRNAs on the move: miRNA biogenesis and the RNAi machinery. *Curr Opin Cell Biol*. 16:223-229.
17. Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, et al. (2005): MicroRNAs regulate brain morphogenesis in zebrafish. *Science*. 308:833-838.
18. Petrides M (2005): Lateral prefrontal cortex: architectonic and functional organization. *Philos Trans R Soc Lond B Biol Sci*. 360:781-795.
19. Seidman LJ, Thermenos HW, Poldrack RA, Peace NK, Koch JK, Faraone SV, et al. (2006): Altered brain activation in dorsolateral prefrontal cortex in adolescents and young adults at genetic risk for schizophrenia: an fMRI study of working memory. *Schizophr Res*. 85:58-72.
20. Tsuang MT, Simpson JC (1984): Schizoaffective disorder: concept and reality. *Schizophr Bull*. 10:14-25.
21. Weickert CS, Sheedy D, Rothmond DA, Dedova I, Fung S, Garrick T, et al. Selection of reference gene expression in a schizophrenia brain cohort. *Aust N Z J Psychiatry*. 44:59-70.

22. Weickert CS, Sheedy D, Rothmond DA, Dedova I, Fung S, Garrick T, et al. (2010): Selection of reference gene expression in a schizophrenia brain cohort. *Aust N Z J Psychiatry*. 44:59-70.
23. Chen J, Lozach J, Garcia EW, Barnes B, Luo S, Mikoulitch I, et al. (2008): Highly sensitive and specific microRNA expression profiling using BeadArray technology. *Nucleic Acids Res*.
24. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. (2002): Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. 3:RESEARCH0034.
25. Tusher VG, Tibshirani R, Chu G (2001): Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci U S A*. 98:5116-5121.
26. Storey JD (2002): A direct approach to false discovery rates. *J R Statist Soc B*. 64:479–498.
27. John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS (2004): Human MicroRNA targets. *PLoS Biol*. 2:e363.
28. Dennis G, Jr., Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. (2003): DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol*. 4:P3.
29. Huang da W, Sherman BT, Lempicki RA (2009): Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 4:44-57.
30. Hon LS, Zhang Z (2007): The roles of binding site arrangement and combinatorial targeting in microRNA repression of gene expression. *Genome Biol*. 8:R166.
31. Baskerville S, Bartel DP (2005): Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. *Rna*. 11:241-247.
32. Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, et al. (2004): Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc Natl Acad Sci U S A*. 101:360-365.
33. Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, et al. (2006): A brain-specific microRNA regulates dendritic spine development. *Nature*. 439:283-289.
34. Schaefer A, O'Carroll D, Tan CL, Hillman D, Sugimori M, Llinas R, et al. (2007): Cerebellar neurodegeneration in the absence of microRNAs. *J Exp Med*. 204:1553-1558.
35. Lugli G, Larson J, Martone ME, Jones Y, Smalheiser NR (2005): Dicer and eIF2c are enriched at postsynaptic densities in adult mouse brain and are modified by neuronal activity in a calpain-dependent manner. *J Neurochem*. 94:896-905.
36. Barbato C, Ciotti MT, Serafino A, Calissano P, Cogoni C (2007): Dicer expression and localization in post-mitotic neurons. *Brain Res*. 1175:17-27.
37. Potenza N, Papa U, Russo A (2009): Differential expression of Dicer and Argonaute genes during the differentiation of human neuroblastoma cells. *Cell Biol Int*. 33:734-738.
38. Chiosea S, Jelezcova E, Chandran U, Acquafondata M, McHale T, Sobol RW, et al. (2006): Up-regulation of dicer, a component of the MicroRNA machinery, in prostate adenocarcinoma. *Am J Pathol*. 169:1812-1820.
39. Saba R, Goodman CD, Huzarewich RL, Robertson C, Booth SA (2008): A miRNA signature of prion induced neurodegeneration. *PLoS One*. 3:e3652.
40. Manadas B, Santos AR, Szabadfi K, Gomes JR, Garbis SD, Fountoulakis M, et al. (2009): BDNF-induced changes in the expression of the translation machinery in hippocampal neurons: protein levels and dendritic mRNA. *J Proteome Res*. 8:4536-4552.
41. Cohen-Cory S, Fraser SE (1995): Effects of brain-derived neurotrophic factor on optic axon branching and remodelling in vivo. *Nature*. 378:192-196.
42. Ji Y, Pang PT, Feng L, Lu B (2005): Cyclic AMP controls BDNF-induced TrkB phosphorylation and dendritic spine formation in mature hippocampal neurons. *Nat Neurosci*. 8:164-172.
43. Meerson A, Cacheaux L, Goosens KA, Sapolsky RM, Soreq H, Kaufer D (2010): Changes in brain MicroRNAs contribute to cholinergic stress reactions. *J Mol Neurosci*. 40:47-55.

44. Beveridge NJ, Tooney PA, Carroll AP, Tran N, Cairns MJ (2009): Down-regulation of miR-17 family expression in response to retinoic acid induced neuronal differentiation. *Cell Signal*. 21:1837-1845.
45. Fatemi SH (2005): Reelin glycoprotein: structure, biology and roles in health and disease. *Mol Psychiatry*. 10:251-257.
46. Costa E, Davis J, Grayson DR, Guidotti A, Pappas GD, Pesold C (2001): Dendritic spine hypoplasticity and downregulation of reelin and GABAergic tone in schizophrenia vulnerability. *Neurobiol Dis*. 8:723-742.
47. Fatemi SH, Earle JA, McMenomy T (2000): Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. *Mol Psychiatry*. 5:654-663, 571.
48. Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, et al. (2000): Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch Gen Psychiatry*. 57:1061-1069.
49. Glantz LA, Lewis DA (2000): Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry*. 57:65-73.
50. Goto Y, Yang CR, Otani S (2010): Functional and dysfunctional synaptic plasticity in prefrontal cortex: roles in psychiatric disorders. *Biol Psychiatry*. 67:199-207.
51. Rajan I, Cline HT (1998): Glutamate receptor activity is required for normal development of tectal cell dendrites in vivo. *J Neurosci*. 18:7836-7846.
52. Sin WC, Haas K, Ruthazer ES, Cline HT (2002): Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. *Nature*. 419:475-480.
53. Li L, Yun SH, Keblesh J, Trommer BL, Xiong H, Radulovic J, et al. (2007): Egr3, a synaptic activity regulated transcription factor that is essential for learning and memory. *Mol Cell Neurosci*. 35:76-88.
54. Yamada K, Gerber DJ, Iwayama Y, Ohnishi T, Ohba H, Toyota T, et al. (2007): Genetic analysis of the calcineurin pathway identifies members of the EGR gene family, specifically EGR3, as potential susceptibility candidates in schizophrenia. *Proc Natl Acad Sci U S A*. 104:2815-2820.
55. Guo AY, Sun J, Jia P, Zhao Z A Novel microRNA and transcription factor mediated regulatory network in schizophrenia. *BMC Syst Biol*. 4:10.
56. Friston KJ (1998): The disconnection hypothesis. *Schizophr Res*. 30:115-125.
57. Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, et al. (1998): Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J Neurol Neurosurg Psychiatry*. 65:446-453.
58. Saldanha AJ (2004): Java Treeview--extensible visualization of microarray data. *Bioinformatics*. 20:3246-3248.

## Tables/Figures:

**Table 1.** Enriched KEGG pathways involving target genes of miRNA significantly upregulated in BA46 in schizophrenia.

| KEGG ID | Term | N | EASE |
|---|---|---|---|
| hsa04916 | Melanogenesis | 12 | 0.0063 |
| hsa04010 | MAPK signaling pathway | 23 | 0.0067 |
| hsa04660 | T cell receptor signaling pathway | 12 | 0.012 |
| hsa04360 | **Axon guidance** | 13 | 0.017 |
| hsa04020 | Calcium signaling pathway | 15 | 0.036 |
| hsa04720 | **Long-term potentiation** | 8 | 0.039 |
| hsa05410 | Hypertrophic cardiomyopathy (HCM) | 9 | 0.045 |

Enriched KEGG pathways identified with the DAVID functional annotation table tool. N, number of input genes in pathway; EASE, modified Fisher's exact test p-value (probability of enrichment); bold terms, terms of particular interest to the pathophysiology of schizophrenia. Some degree of over prediction of target genes is likely as multiple prediction algorithms were used in miRecords to ascertain miRNA targets.

**Table 2.** Enriched GO terms involving target genes with significant hits (≥3) by miRNA significantly upregulated in BA46 in schizophrenia.

| GO ID | Term | N | EASE | FDR |
|---|---|---|---|---|
| GO:0007399 | Nervous system development | 198 | 4.30E-18 | 7.92E-15 |
| GO:0032502 | Developmental process | 422 | 1.35E-12 | 2.49E-09 |
| GO:0022008 | Neurogenesis | 111 | 1.38E-10 | 2.54E-07 |
| GO:0048699 | Generation of neurons | 101 | 3.52E-09 | 6.49E-06 |
| GO:0030182 | Neuron differentiation | 80 | 1.11E-07 | 2.05E-04 |
| GO:0048666 | Neuron development | 64 | 7.65E-07 | 0.001410 |
| GO:0007417 | Central nervous system development | 75 | 1.16E-06 | 0.002140 |
| GO:0048812 | Neuron projection morphogenesis | 44 | 5.05E-06 | 0.009295 |
| GO:0007420 | Brain development | 54 | 8.47E-06 | 0.015597 |
| GO:0031175 | Neuron projection development | 49 | 1.21E-05 | 0.022363 |
| GO:0007409 | Axonogenesis | 39 | 3.15E-05 | 0.058061 |
| GO:0016337 | Cell-cell adhesion | 50 | 4.40E-05 | 0.080955 |
| GO:0050793 | Regulation of developmental process | 99 | 7.35E-05 | 0.135319 |
| GO:0030900 | Forebrain development | 32 | 8.28E-05 | 0.152382 |
| GO:0019226 | Transmission of nerve impulse | 58 | 1.33E-04 | 0.243955 |
| GO:0045664 | Regulation of neuron differentiation | 28 | 2.51E-04 | 0.461107 |
| GO:0048041 | Focal adhesion formation | 8 | 4.90E-04 | 0.898106 |
| GO:0050767 | Regulation of neurogenesis | 31 | 9.30E-04 | 1.698497 |
| GO:0030031 | Cell projection assembly | 19 | 0.001164 | 2.123024 |
| GO:0051960 | Regulation of nervous system development | 34 | 0.001300 | 2.366951 |
| GO:0009790 | Embryonic development | 80 | 0.001360 | 2.476111 |
| GO:0007268 | Synaptic transmission | 47 | 0.001779 | 3.226482 |
| GO:0030099 | Myeloid cell differentiation | 20 | 0.001818 | 3.297068 |
| GO:0050773 | Regulation of dendrite development | 7 | 0.002197 | 3.971032 |
| GO:0007155 | Cell adhesion | 94 | 0.002296 | 4.145431 |
| GO:0009966 | Regulation of signal transduction | 114 | 0.002393 | 4.317135 |

Enriched GO terms identified with the DAVID functional annotation table tool. N, number of input genes in the cellular process; EASE, modified Fisher's exact test p-value (probability of enrichment); table shows significant GO terms of particular interest to the pathophysiology of schizophrenia.

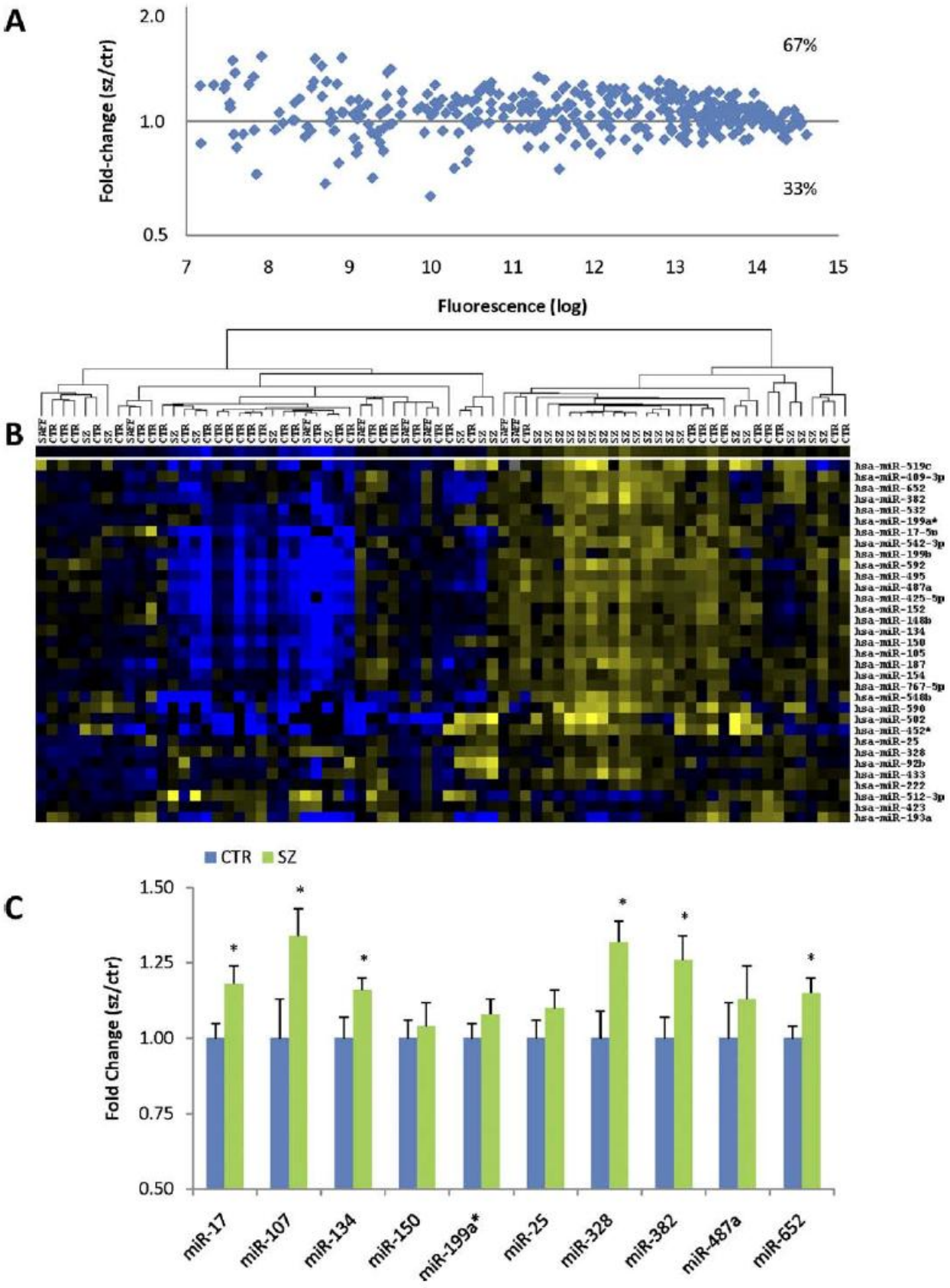

A
2.0
1.0
0.5
Fold-change (sz/ctr)
67%
33%
7
8
9
10
11
12
13
14
15
Fluorescence (log)
B
hsa-miR-519c
hsa-miR-409-3p
hsa-miR-652
hsa-miR-382
hsa-miR-532
hsa-miR-199a*
hsa-miR-17-5p
hsa-miR-542-3p
hsa-miR-199b
hsa-miR-592
hsa-miR-495
hsa-miR-487a
hsa-miR-425-5p
hsa-miR-152
hsa-miR-148b
hsa-miR-134
hsa-miR-150
hsa-miR-105
hsa-miR-187
hsa-miR-154
hsa-miR-767-5p
hsa-miR-518b
hsa-miR-590
hsa-miR-502
hsa-miR-452*
hsa-miR-25
hsa-miR-328
hsa-miR-92b
hsa-miR-433
hsa-miR-222
hsa-miR-512-3p
hsa-miR-423
hsa-miR-193a
C
CTR
SZ
1.50
1.25
1.00
0.75
0.50
Fold Change (sz/ctr)
miR-17
miR-107
miR-134
miR-150
miR-199a*
miR-25
miR-328
miR-382
miR-487a
miR-652

**Figure 1.** Schizophrenia-associated miRNA expression in the DLPFC (BA46). **(A)** Scatter plot of the average fold change in expression of each miRNA and $\log_2$ transformed fluorescence intensity. **(B)** Hierarchical clustering of significant upregulated miRNA microarray expression data (uncentered correlation, average linkage; Cluster 3.0). Blue, low expression; yellow, high expression. Produced with Java Treeview 1.1.1 (58 - http://jtreeview.sourceforge.net). **(C)** Significantly increased miRNA in schizophrenia (BA46) validated by Q-PCR. Bars represent mean fold change in expression (schizophrenia versus controls; n=37 matched pairs) + SEM; * p-value < 0.05 (ANOVA/ANCOVA).

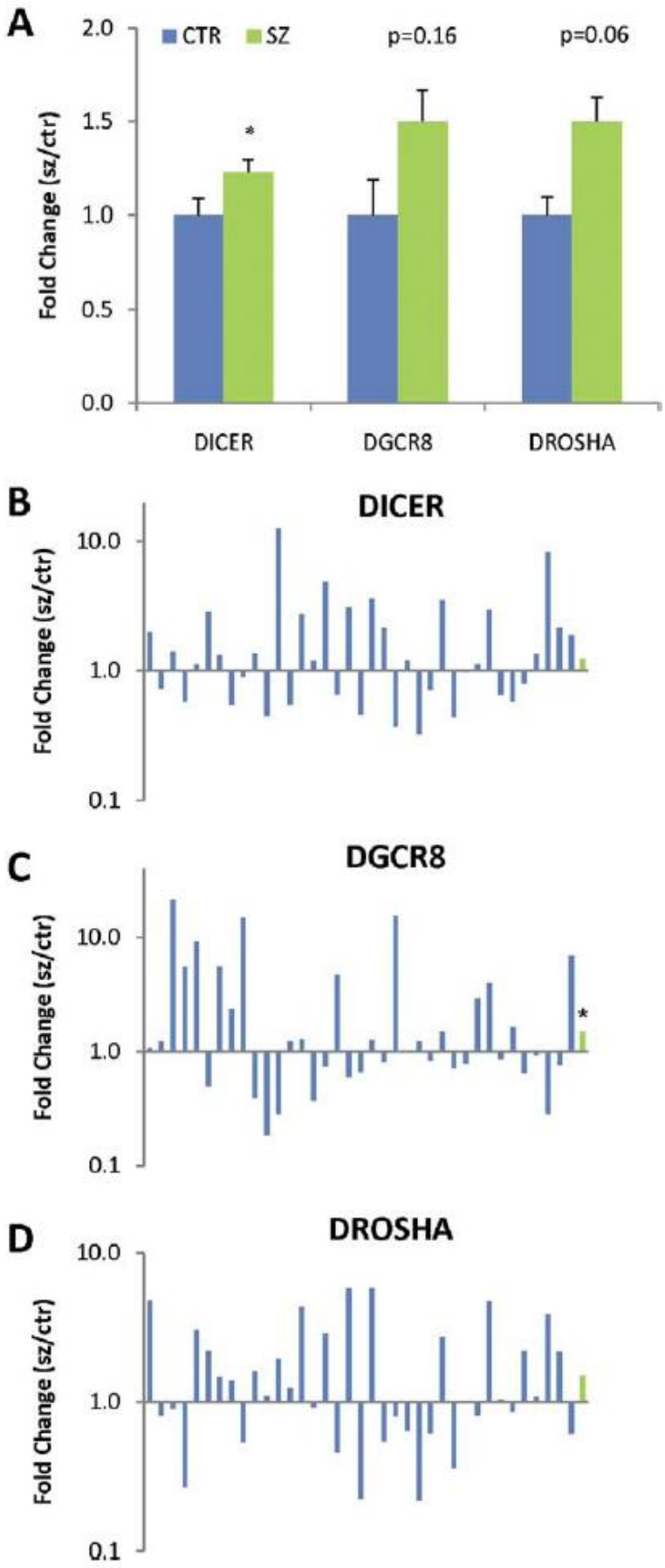

A
CTR
SZ
p=0.16
p=0.06
Fold Change (sz/ctr)
2.0
1.5
1.0
0.5
0.0
*
DICER
DGCR8
DROSHA
B
DICER
Fold Change (sz/ctr)
10.0
1.0
0.1
C
DGCR8
Fold Change (sz/ctr)
10.0
1.0
0.1
*
D
DROSHA
Fold Change (sz/ctr)
10.0
1.0
0.1

**Figure 2.** Upregulation of miRNA biogenesis genes in schizophrenia (BA46). **(A)** Q-PCR analysis of miRNA biogenesis genes in schizophrenia versus control samples (n=37 matched pairs). Bars represent fold change in expression + SEM. **(B-D)** Pair-wise expression if miRNA biogenesis genes. (B) Dicer expression was upregulated in 21 of the 37 matched pairs. The green bar furthest to the right is indicative of the overall fold increase. **(C)** DGCR8 expression was upregulated in 19 of the 37 matched pairs. **(D)** Drosha expression was upregulated in 20 of the 37 matched pairs.

## Supplementary Material:

**Supplement 1.** BA46 schizophrenia and non-psychiatric control cohort demographics and tissue characterisation.

| Pair | Diag | Sex | Age | pH | PMI | Hemi | Cause of death | Toxicology | APD | CPE | AO | DOI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | CRS | F | 51 | 5.7 | 12 | R | Li toxicity & congestive cardiac failure | Li 20mg/L (fatal), Midazolam 0.02mg/L | T | 570 | 35 | 16 |
| **2** | CDS | M | 56 | 6.2 | 15 | L | Chronic airways disease | NAD | TO | 450 | 21 | 35 |
| **3** | CUS | M | 52 | 6.4 | 46 | R | Cardiomegaly (cardiomyopathy) | N/A | A | 500 | 19 | 33 |
| **4** | CRS | M | 51 | 6.5 | 21 | L | Ischaemic heart disease | Thioridazine 2.2mg/L (fatal), Mesoridazine 2.4mg/L (fatal) | TO | - | 27 | 24 |
| **5** | CPS | M | 54 | 6.2 | 27.5 | R | Coronary artery thrombosis | Chlorpromazine: 0.7mg/L-Diazepam: <0.1mg/L, Nordiazepam: 0.1mg/L, Insulin: 2 uU/mL | T | 1560 | 19 | 35 |
| **6** | CPS | F | 58 | 6.3 | 19 | R | Sepsis & chronic renal failure | Morphine: 0.06 mg/L, Codeine: 0.05 mg/L, Carbamazepine: 7 mg/L, Pethidine: 0.1 mg/L, Paracetamol: 6mg/L, Metoclopramide 0.1mg/L, Diazepam: <0.1 mg/L | T | 450 | 19 | 39 |
| **7** | CPS | F | 66 | 6.3 | 12.5 | R | Faecaloid peritonitis | N/A | T | - | 19 | 47 |
| **8** | CUS | F | 55 | 6.3 | 33.5 | L | AMS toxicity & coronary artery disease | Amisulpride 18mg/L, Clozapine 1.4mg/L | T | 1300 | 17 | 38 |
| **9** | CPS | M | 55 | 6.4 | 72 | R | Ischaemic heart disease | Metoprolol: 0.2mg/L | TO | - | 30 | 26 |
| **10** | CPS | F | 54 | 6.4 | 29 | R | Asthma | Citalopram 0.6 mg/L | T | 700 | 19 | 35 |
| **11** | CDS | F | 67 | 6.4 | 27 | L | Empyema | Benztropine 0.2mg/L, Mesoridazine 0.9mg/L, Thioridazine 0.6mg/L, Paracetamol < 3.0mg/L | TO | 350 | 21 | 46 |
| **12** | CPS | M | 75 | 6.6 | 36 | L | Ischaemic heart disease | Olanzapine - 0.2 mg/L, Fluvoxamine - 0.7 mg/L | T | 500 | <33 | 44 |
| **13** | CPS | M | 40 | 6.5 | 21.5 | L | Dihydrocodeine toxicity & sleep apnoea | Valproic acid 20mg/L, Dihydrocodeine 0.7mg/L, Quetiapine 0.3mg/l, Sertraline 0.3mg/L | T | 986 | 17 | 23 |
| **14** | CUS | M | 44 | 6.5 | 27-43.5 | L | Hanging | Urine THC detected | T | 500 | 27 | 17 |
| **15** | CUS | M | 27 | 6.6 | 10 | L | Clozapine toxicity | Clozapine 8.6mg/L (fatal) | T | 300 | 18 | 11 |
| **16** | CPS | M | 51 | 6.7 | 18 | L | Ischaemic heart disease | N/A | T | 150 | 21 | 30 |
| **17** | CPS | M | 52 | 6.7 | 8.35 | R | Ischaemic heart disease | Temazepam <0.1mg/L | T | 680 | 21 | 31 |
| **18** | CPS | M | 33 | 6.8 | 48 | L | Hanging | Doxylamine: 0.9mg/L, Olanzapine: 0.2mg/L, Paracetamol: 3mg/L | A | 250 | 22 | 12 |
| **19** | CPS | M | 30 | 6.8 | 24 | L | CO poisoning - suicide | CO 74% saturation, Clozapine 0.7mg/L, HIV - | A/T | 300 | 26 | 3.5 |
| **20** | CDS | F | 56 | 6.8 | 34 | L | Pulmonary thrombo-embolism | Paracetamol 4.5mg/L | TO | 760 | 17 | 40 |
| **21** | CUS | M | 27 | 6.8 | 38.5 | L | Myocarditis | Clozapine 0.9mg/L | A | 200 | 23 | 4 |
| **22** | CPS | M | 57 | 7 | 33-38 | L | Cardiac arrythmia | Thioridazine 0.6mg/L, Seraline <0.1mg/L | T | 700 | 30 | 26 |
| **23** | CPS | M | 57 | 6.7 | 48 | R | Atherosclerotic cardiovascular disease | Carbamazepine 10 mg/L, Citalopram 0.2 mg/L, Quetiapine <0.1 mg/L | T | 618 | 40 | 17 |
| **24** | CPS | M | 27 | 6.8 | 33 | R | Hanging | Negative | T | - | 19 | 9 |
| **25** | CDS | M | 59 | 6.9 | 26.5 | R | Unknown | N/A | T | 750 | 21 | 39 |
| **26** | CUS | M | 67 | 6.8 | 5 | L | Ischaemic heart disease | N/A | TO | 1340 | 26 | 41 |
| **27** | CPS | F | 61 | 6.9 | 42 | R | Ischaemic heart disease | Clozapine 1.1 mg/L, Diazepam 0.2 mg/L, Audanosine 0.4 mg/L, Nordiazepame 0.4 mg/L, Olanzapine 0.2 mg/L | TO | 1200 | 19 | 42 |
| **28** | CDS | M | 32 | 7 | 26 | L | Hanging | Negative | T | 190 | 19 | 13 |
| **29** | CPS | F | 56 | 7.1 | 39 | R | Undetermined (obesity / hepatic fatty changes) | Thioridazine (1mg/L) & Mesoridazine (0.6mg/L) | T | 580 | 24 | 32 |
| **30** | CUS | F | 68 | 6.2 | 32 | L | Acute pancreatitis | EtOH 0.055 g/100ml, Paracetamol 3 mg/L | T | 190 | 23 | 46 |
| **31** | BPT | M | 34 | 7 | 26 | R | Hanging | Carbamazepine (1mg/L) | A | 250 | 27 | 8 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **32** | CDPS | F | 33 | 6.9 | 50 | R | Hanging | Negative | AO | 95 | 14 | 19 |
| **33** | BPT | M | 57 | 6.4 | 28 | R | Chronic obstructive airway disease | Paracetamol 3 mg/L, Li 4.6 mg/L | T | 415 | 30 | 27 |
| **34** | BPT | F | 61 | 6.4 | 17 | R | Myocarditis | Clozapine 0.4mg/L, Li: 0.1mg/L, nil EtOH | T | 100 | 31 | 30 |
| **35** | CDPS | M | 30 | 7 | 26 | L | Hanging | Venlafaxine 0.9mg/l | A | 285 | 27 | 4 |
| **36** | CDPS | F | 73 | 6.9 | 17-19 | L | Right ventricular dysplasia | Doxepin 0.1 mg/L, Chlorpheniramine 01, Trifluoperazine 01, Codeine 0.05, Pseudoephedrine 0.1, Paracetamol 10 mg/L | TO | 300 | 36 | 37 |
| **37** | CDPS | M | 73 | 6.8 | 14 | L | Asphyxia | Fluoxetine - 0.2 mg/l, 7-Amino nitrazepam - 0.1 mg/l, Diazepan - <0.1 mg/l, Paracetamol - <3 mg/l | T | 380 | 21 | 50 |
| **1** | CTR | M | 46 | 5.8 | 29 | L | Acute myocardial infarction | N/A | | | | |
| **2** | CTR | M | 60 | 6 | 25 | R | Bacterial peritonitis, ascites, carcinomatosis | Negative | | | | |
| **3** | CTR | M | 37 | 6.2 | 11 | R | Pulmonary embolism | Negative | | | | |
| **4** | CTR | M | 56 | 6.6 | 24 | R | Coronary artery atheroma | N/A | | | | |
| **5** | CTR | M | 61 | 6.3 | 27.5 | R | Unknown | N/A | | | | |
| **6** | CTR | M | 74 | 6.3 | 10 | L | Respiratory arrest | N/A | | | | |
| **7** | CTR | F | 78 | 6.4 | 11 | R | Pulmonary fibrosis | N/A | | | | |
| **8** | CTR | F | 56 | 6.5 | 23 | R | Massive pulmonary thrombo-embolus | N/A | | | | |
| **9** | CTR | F | 60 | 6.5 | 21 | L | Ischaemic heart disease | EtOH: 0.251g per 100mL, Paracetamol <3mgL | | | | |
| **10** | CTR | M | 60 | 6.6 | 13 | L | Acute myocardial infarction | Negative | | | | |
| **11** | CTR | M | 58 | 6.6 | 12 | L | Ischaemic heart disease | N/A | | | | |
| **12** | CTR | M | 73 | 6.6 | 48 | R | Ischaemic heart disease | N/A | | | | |
| **13** | CTR | M | 46 | 6.7 | 25 | R | Mitral valve prolapse | Negative | | | | |
| **14** | CTR | F | 49 | 6.6 | 15 | R | Arrhytmogenic right venticular dysplasia | Chloride ion 118 mmol/L | | | | |
| **15** | CTR | M | 34 | 6.5 | 20.5 | R | Acute exacerbation of asthma | N/A | | | | |
| **16** | CTR | M | 44 | 6.7 | 50 | L | Ischaemic heart disease | Negative | | | | |
| **17** | CTR | M | 50 | 6.6 | 19 | L | Ischaemic heart disease | NAD | | | | |
| **18** | CTR | M | 43 | 6.7 | 13 | R | Thrombotic coronary artery occlusion | Negative | | | | |
| **19** | CTR | M | 38 | 6.7 | 13.5 | L | Atherosclerotic cardiovascular disease | N/A | | | | |
| **20** | CTR | M | 54 | 6.8 | 29 | R | Coronary artery atheroma | Negative | | | | |
| **21** | CTR | M | 18 | 6.8 | 33 | L | Probable hypertrophic cardiomyopathy | Paracetamol (24mg/L) & Lignocaine (1mg/L) | | | | |
| **22** | CTR | F | 51 | 7.2 | 37.5 | R | Acute myocardial infarction | N/A | | | | |
| **23** | CTR | M | 53 | 6.7 | 27 | R | Acute myocardial infarct | N/A | | | | |
| **24** | CTR | F | 33 | 6.9 | 24 | L | Cardiac arrythmia; myocardial fibrosis | EtOH not detected | | | | |
| **25** | CTR | M | 59 | 7 | 20 | R | Coronary thrombosis | N/A | | | | |
| **26** | CTR | M | 56 | 7 | 37 | R | Hypertension & cardiomegaly | N/A | | | | |
| **27** | CTR | M | 57 | 6.9 | 18 | L | Ischaemic heart disease | HIV negative | | | | |
| **28** | CTR | M | 37 | 6.9 | 24 | R | Electrocution | Codeine <0.5 mg/L, paracetamol 3 mg/L, lignocaine <0.5 mg/L | | | | |
| **29** | CTR | M | 55 | 7.2 | 20 | L | Cardiac arrest | N/A | | | | |
| **30** | CTR | M | 78 | 6.3 | 6.5 | L | Dehydration, adenocarcinoma | N/A | | | | |
| **31** | CTR | M | 37 | 6.8 | 21 | L | Ischaemic heart disease | N/A | | | | |
| **32** | CTR | F | 21 | 6.8 | 39.5 | R | Primary cardiac arrhythmia | N/A | | | | |
| **33** | CTR | M | 62 | 6.6 | 37.5 | R | Acute myocardial infarction | Negative | | | | |
| **34** | CTR | M | 50 | 6.7 | 29 | R | Ischaemic heart disease | Negative | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **35** | CTR | M | 24 | 7 | 43 | R | Idiopathic cardiac arrhythmia | Negative |
| **36** | CTR | M | 64 | 7 | 39.5 | R | Coronary artery thrombosis | Negative |
| **37** | CTR | M | 60 | 7 | 21.5 | R | Ischaemic heart disease | N/A |

Diag, diagnosis; CRS, chronic residual schizophrenia; CDS, chronic differential schizophrenia; CUS, chronic undifferentiated schizophrenia; CPS, chronic paranoid schizophrenia; BPT, bipolar type; CDPS, chronic depressive schizophrenia; CTR, control; PMI, post mortem interval (hours); Hemi, brain hemisphere; APD, antipsychotic drug class (A, predominantly atypical; T, predominantly typicals; TO, typicals only; A/T, equal); CPE, chloropromazine equivalent (mg/day); AO, age of onset; DOI, duration of illness (years);

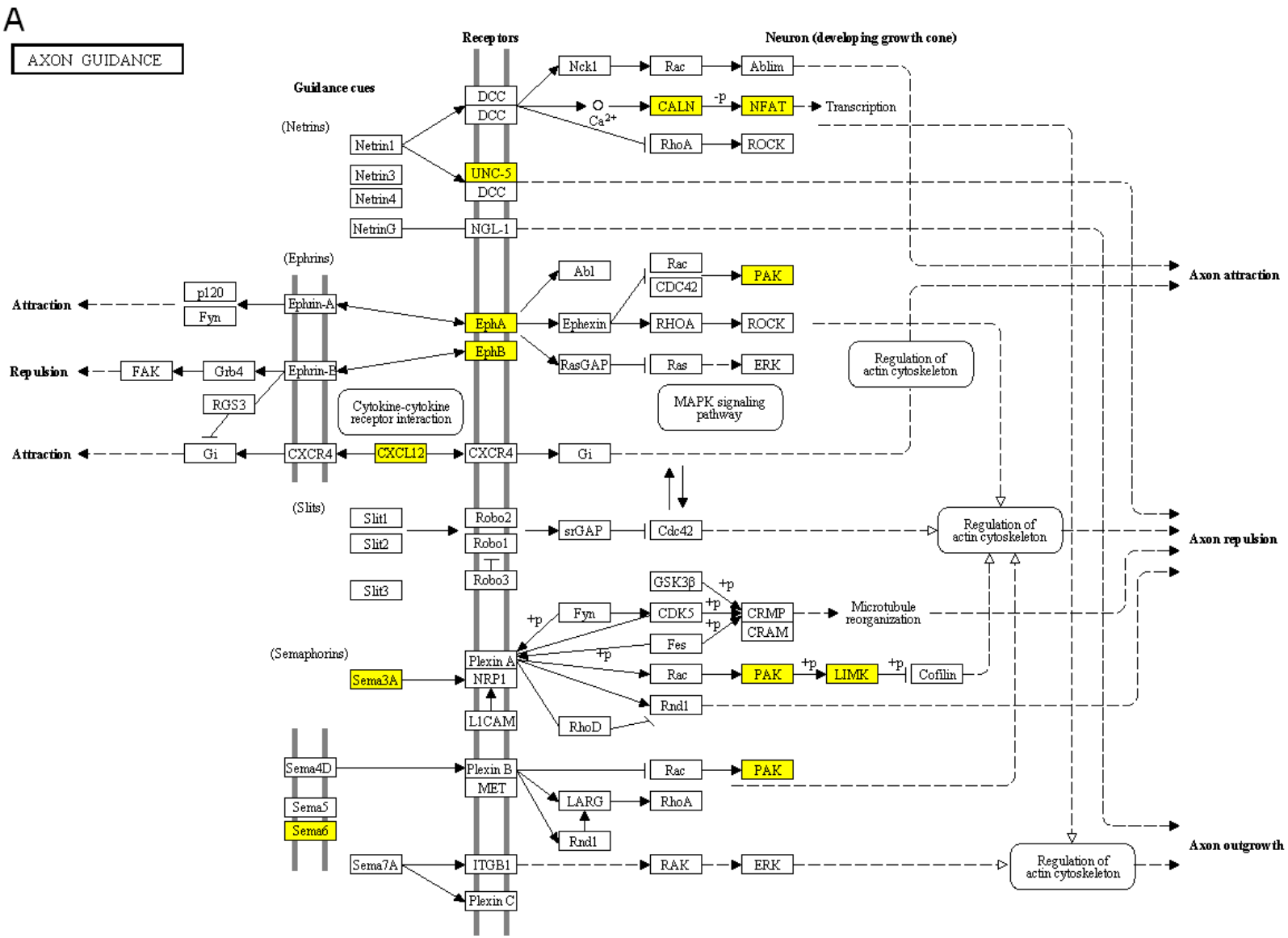
**Supplement 2.** MiRNA and biogenesis gene expression correlation matrix.

**Correlations**

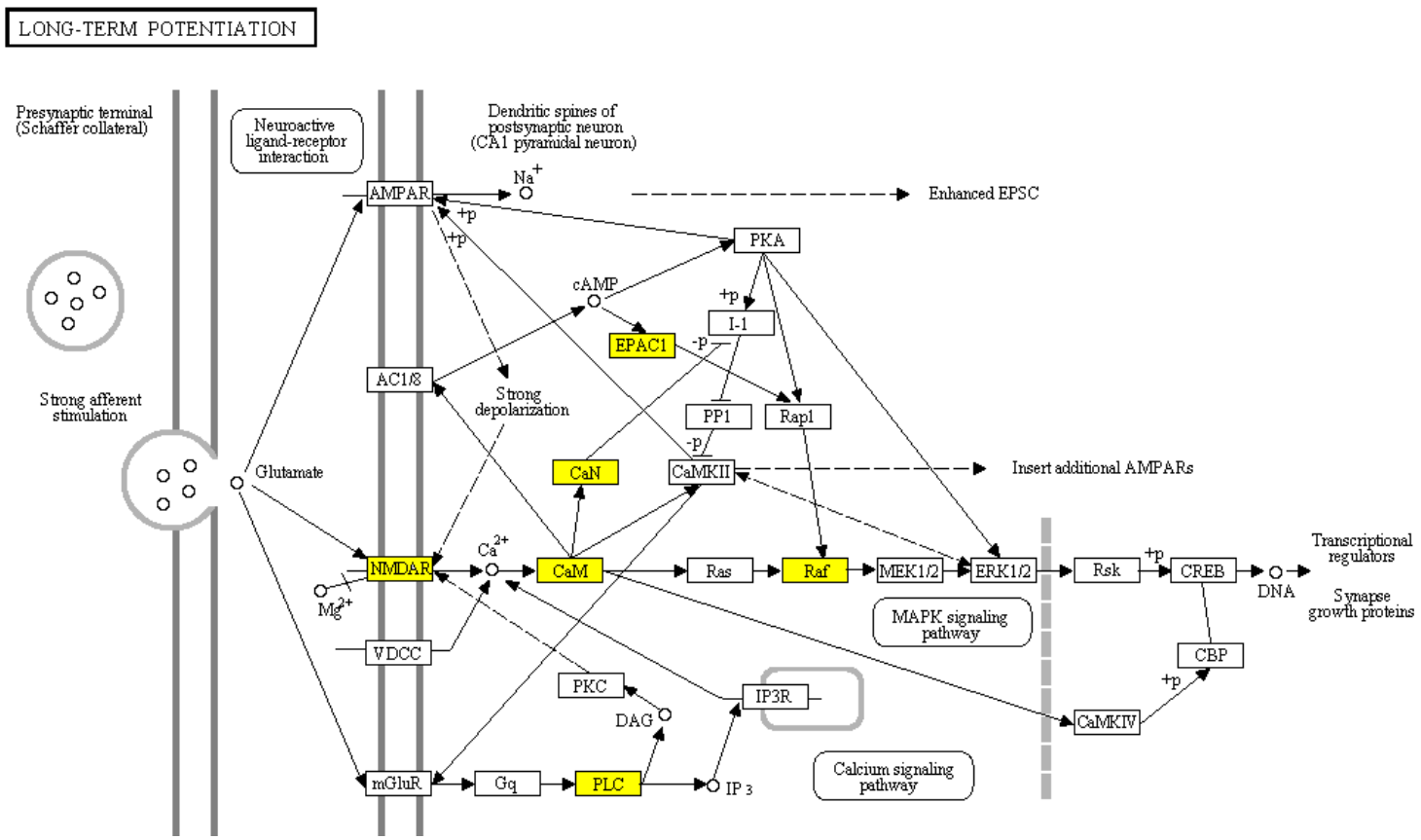
| | | miR-17 | miR-328 | miR--134 | miR-107 | miR-652 | miR-382 | DGCR8 | DROSHA | DICER |
|---|---|---|---|---|---|---|---|---|---|---|
| **miR-17** | Correlation | 1 | .470** | .405** | .434** | .141 | .089 | -.046 | -.094 | .359** |
| | p value | | .000 | .000 | .000 | .240 | .466 | .700 | .442 | .002 |
| **miR-328** | Correlation | .470** | 1 | .588** | .855** | .089 | .315** | .099 | -.023 | .226 |
| | p value | .000 | | .000 | .000 | .463 | .008 | .407 | .852 | .058 |
| **miR-134** | Correlation | .405** | .588** | 1 | .676** | .321** | .267* | .066 | .185 | .117 |
| | p value | .000 | .000 | | .000 | .006 | .025 | .584 | .127 | .331 |
| **miR-107** | Correlation | .434** | .855** | .676** | 1 | .112 | .300* | .114 | .036 | .206 |
| | p value | .000 | .000 | .000 | | .354 | .012 | .342 | .768 | .085 |
| **miR-652** | Correlation | .141 | .089 | .321** | .112 | 1 | .550** | .049 | .091 | .139 |
| | p value | .240 | .463 | .006 | .354 | | .000 | .685 | .467 | .255 |
| **miR-382** | Correlation | .089 | .315** | .267* | .300* | .550** | 1 | .311** | .035 | .297* |
| | p value | .466 | .008 | .025 | .012 | .000 | | .009 | .779 | .014 |
| **DGCR8** | Correlation | -.046 | .099 | .066 | .114 | .049 | .311** | 1 | .083 | .084 |
| | p value | .700 | .407 | .584 | .342 | .685 | .009 | | .504 | .492 |
| **DROSHA** | Correlation | -.094 | -.023 | .185 | .036 | .091 | .035 | .083 | 1 | .251* |
| | p value | .442 | .852 | .127 | .768 | .467 | .779 | .504 | | .040 |
| **DICER** | Correlation | .359** | .226 | .117 | .206 | .139 | .297* | .084 | .251* | 1 |
| | p value | .002 | .058 | .331 | .085 | .255 | .014 | .492 | .040 | |

* Two-tailed Pearson Correlation is significant at the 0.05 level;** 0.01 level

**Supplement 3.** Schizophrenia-relevant KEGG pathways predicted to be regulated by up-regulated microRNA in BA46. **(A)** Axon guidance, **(B)** Long-term potentiation. Highlighted boxes indicate genes predicted to be targeted by differentially expressed microRNA.



B


LONG-TERM POTENTIATION
Presynaptic terminal (Schaffer collateral)
Neuroactive ligand-receptor interaction
Dendritic spines of postsynaptic neuron (CA1 pyramidal neuron)
Strong afferent stimulation
Glutamate
AMPAR
Na+
Enhanced EPSC
+p
PKA
cAMP
I-1
EPAC1
-p
AC1/8
Strong depolarization
PP1
Rap1
CaN
CaMKII
Insert additional AMPARs
NMDAR
Ca2+
CaM
Ras
Raf
MEK1/2
ERK1/2
Rsk
CREB
DNA
Transcriptional regulators
Synapse growth proteins
Mg2+
MAPK signaling pathway
VDCC
CBP
PKC
IP3R
DAG
CaMKIV
mGluR
Gq
PLC
IP3
Calcium signaling pathway

**Supplement 4.** Over-represented KEGG Pathways predicted to be regulated by up-regulated microRNA in BA46: Multi-hit analysis.

| KEGG ID | Term | Genes | N | Fold Enrichment | EASE | FDR |
|---|---|---|---|---|---|---|
| hsa04810 | Regulation of actin cytoskeleton | FGFR2, FGF5, ENAH, PDGFB, DIAPH2, MRAS, WASF1, DIAPH3, SSH2, WASF2, FGF11, PIP5K1B, ABI2, ARPC4, ITGB1, VCL, PTK2, PFN2, TIAM2, ITGB8, ARPC2, TIAM1, RAC1, PPP1R12A, PDGFD, FGF1, PIK3R1, FN1, ROCK1, ROCK2, ARHGEF7, MYLK2, ACTN2, PPP1CA, ITGA5, CFL2, PDGFRA, CRK, MYLK, MYH10 | 40 | 1.995879 | 2.71E-05 | 0.033143 |
| hsa05200 | Pathways in cancer | FGF5, PDGFB, PPARG, MITF, FGF11, PTEN, CCNE1, MAX, PAX8, FAS, FGF1, CHUK, AKT3, CSF2RA, PLD1, CTBP2, RUNX1T1, CDK6, CTNNA2, RAD51, CCDC6, HIF1A, VEGFA, PDGFRA, MAPK9, LAMC1, FGFR2, EGLN3, PML, BCL2L1, ITGB1, TPM3, IGF1R, PTK2, BCL2, RAC1, NKX3-1, RUNX1, PIK3R1, FN1, COL4A1, EPAS1, VHL, CREBBP, TGFBR2, COL4A6, STAT3, DVL1, RASSF5, CDKN1A, LAMA3, ETS1, RASSF1, CRK | 54 | 1.76617 | 3.05E-05 | 0.037299 |
| hsa04360 | Axon guidance | ABLIM1, ABLIM3, ITGB1, PTK2, ROBO1, RAC1, NFAT5, PPP3CB, UNC5D, ROBO2, PPP3CA, NFATC1, ROCK1, ROCK2, DPYSL5, EPHA5, EPHA4, EPHA7, SEMA6C, SEMA4G, SEMA6D, RGS3, CFL2, SEMA4C, SEMA4B, SRGAP2 | 26 | 2.162202 | 2.71E-04 | 0.330008 |
| hsa04350 | TGF-beta signaling pathway | LTBP1, ROCK1, E2F5, ROCK2, SMAD6, SMAD5, CREBBP, TGFBR2, BMPR2, RPS6KB2, RPS6KB1, SMAD1, ACVR2A, ACVR2B, ZFYVE16, SMURF2, CHRD, CUL1, TFDP1, BMPR1A | 20 | 2.466172 | 3.05E-04 | 0.371764 |
| hsa04510 | Focal adhesion | PDGFB, COL2A1, ITGB1, PTEN, VCL, IGF1R, PTK2, PDPK1, ARHGAP5, ITGB8, BCL2, RAC1, PPP1R12A, SHC1, PDGFD, COL11A1, AKT3, PIK3R1, SHC4, FN1, COL4A1, ROCK1, ROCK2, MYLK2, ACTN2, COL4A6, PPP1CA, LAMA3, ITGA5, VEGFA, PDGFRA, MAPK9, LAMC1, CRK, MYLK | 35 | 1.868033 | 3.63E-04 | 0.442317 |
| hsa04720 | Long-term potentiation | CREBBP, GRM1, ITPR1, PPP1CA, RPS6KA3, PLCB4, GRIA2, CAMK4, GRIN2C, CAMK2D, PPP1R12A, PPP3CB, PRKACB, PPP3CA, CACNA1C, PLCB1, CALM1 | 17 | 2.681962 | 3.73E-04 | 0.454211 |
| hsa04722 | Neurotrophin signaling pathway | YWHAZ, MAPKAPK2, RPS6KA3, BDNF, YWHAH, CAMK4, MAP3K3, MAPK14, BCL2, GAB1, RAC1, NTRK2, CAMK2D, SH2B3, MAPK9, SORT1, SHC1, SH2B1, CRK, FRS2, PIK3R1, AKT3, CALM1, SHC4 | 24 | 2.076358 | 8.98E-04 | 1.091615 |