Supporting Information

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SI Materials and Methods

Human Tissue Samples. Micro RNA (miRNA) microarray analysis was performed on RNA samples obtained from the Stanley Medical Research Institute (SMRI) (1). The SMRI Array Collection consists of age-matched samples from the dorsolateral prefrontal cortex (DL-PFC) (Brodmann's Area 46) from 34 control (CON), 35 schizophrenic (SCZ), and 31 bipolar (BPD) subjects. Samples were collected by designated medical examiners in a standardized manner, and all samples were collected between the years 1995 and 2002. Samples were excluded if there was significant structural brain pathology upon examination or if the individuals were over age 65, had a documented IQ < 70, or exhibited focal neurological symptoms or CNS disease before death. Control samples from individuals under age 30 or who had a history of drug abuse in the previous year were also excluded. Subsequent experiments were performed using DL-PFC (Brodmann's Area 46) from 15 control and 16 age- and sex-matched schizophrenic subjects obtained from the Harvard Brain Tissue Resource Center (HBB) (http://www.brainbank.mclean.org) (2). HBB tissue was provided as fresh-frozen blocks. All RNA was extracted in TRIzol (Invitrogen) using a standard phenol:chloroform process. The integrity and quality of all RNA samples used for the array and quantitative real-time PCR (qPCR) experiments was verified by either gel (intact 18S and 26S bands) or Agilent Bioanalyzer analysis to measure RNA integrity.

Primate Tissue Samples. Male African green monkeys (*Chlorocebus sabaeus*) were treated with a subchronic dose of phencyclidine (PCP) (0.3 mg/kg) or saline intramuscularly daily for 14 d. Seven days after the final drug treatment, brain tissue was perfused with saline and 2-mm tissue punches from the DL-PFC were removed and snap-frozen. The samples used for both microarray and qPCR analysis consisted of six control and five PCP-treated individuals. All primate procedures were approved by the Yale University Institutional Animal Care and Use Committee.

NR1 Hypomorphic Mouse Tissue Samples. Mouse experiments were approved by the Pfizer Institutional Animal Care and Use Committee. Littermate mice from a mixed (C57BL/6 × 129/Ola × DBA/2) background carrying wild-type or hypomorphic ($^{-/-}$) NR1 alleles were obtained from Taconic at 6–8 wk of age. At 4–5 mo of age, mice were killed and the prefrontal cortex was dissected out and snap-frozen.

MicroRNA Microarrays. Human samples from the SMRI collection were analyzed using custom microarrays (Ocean Ridge Biosciences) containing 854 probes against human mature miRNA sequences from the Sanger 12.0 mirBASE. Primate and mouse samples were analyzed using a universal mammalian array (Ocean Ridge Biosciences) containing probes against 892 human, 697 mouse, and 388 rat mature miRNA sequences from the Sanger 13.0 database.

All arrays contained 30 negative control probes, 12 mismatch controls, and 11 spiking controls. The microarrays were produced for Ocean Ridge Biosciences by Microarrays Inc., and consisted of epoxide glass substrates spotted in triplicate with each probe. RNA samples were 3′ end-labeled with Alexa-647 fluorescent dye using the Rapid Labeling Kit (Invitrogen). Labeled low molecular weight RNA samples were hybridized to the miRNA microarrays according to conditions recommended in the Rapid Labeling Kit manual. The microarrays were scanned on an Axon Genepix 4000B scanner, and data were extracted using GenePix V4.1 software. Spot intensities were obtained for all features on each microarray by subtracting the median local background from the median local foreground for each spot. Detection thresholds for each array were determined by calculating the mean intensity of the negative control spots and adding five-times the SD of the background (nonspot area). The spot intensities and the threshold (T) were transformed by taking the log (base 2) of each value. Normalization was performed using the invariant set probe method (3): a set of 96 miRNAs with a relatively high expression value and low SD across all 100 samples was identified and used to normalize miRNA intensities across all human chips. A similar process was used to normalize the primate and mouse arrays. Analysis was performed using R software (v2.9.1).

Microarray Data Analysis. To identify miRNAs with altered expression in schizophrenic and bipolar disorder, an ANCOVA model was fit using diagnosis (CON, SCZ, BPD) and sex as main effects, and brain pH and age as covariates. The comparison of each disease group to control was conducted under this model to adjust for the effects of age, sex, and brain pH. False-discovery rate (FDR) values were computed using the resulting *P* values. The FDR values presented represent *P* values corrected for a 5% FDR. A linear regression model was used to identify association between miRNA expression levels and the demographic details (drug use, antipsychotic treatment) provided by SMRI.

Results from animal models were analyzed using a Student *t* test to compare drug treatment (primate) or genotype (NR1 mice) for individual miRNA probes, with the same threshold and FDR analyses as was used for human miRNA analysis.

Reverse Transcription and qPCR. Reverse transcription before qPCR was performed using either the SuperScript III First-Strand Synthesis system (Invitrogen) with random hexamer priming to produce cDNA from a starting quantity of 3 μg of total RNA per sample, or the Multiscribe miRNA Reverse Transcription kit (Applied Biosystems) using miRNA-specific primers from a starting quantity of 400 ng total RNA per sample. All reverse transcription was performed using standard protocols from the respective manufacturers. qPCR was performed using inventoried Taqman miRNA and mRNA assays (Applied Biosystems) with standard ABI protocols and reagents. For human samples, miRNA qPCR reactions were performed using both the SMRI and HBB samples, but mRNA qPCR reactions were only performed on HBB samples because of the limited amount of SMRI RNA available. NR1 hypomorph qPCRs were performed using both the six samples per genotype that had been used in the microarray and an additional four samples per genotype that had not been arrayed. Primate qPCRs were performed on the same samples used for the microarrays because of limited sample quantities. All samples were run in quadruplicate using RNU6B as an endogenous miRNA control and 18S (human) or GAPDH (rodent) as an endogenous mRNA control, after determining that these endogenous controls did not differ in abundance between the groups under comparison. Relative abundance was determined using the comparative ΔCt method. Statistical analyses were performed with a one- or two-way ANOVA and Student *t* test using JMP statistical software (SAS).

Network Pathway Analysis. We used the Ingenuity Pathway Analysis (IPA; www.ingenuity.com) core analysis tool to analyze the biological significance of the miR-132 target genes as predicted by the target prediction database TargetScan (www.targetscan.org). Mir-132 target genes were populated into the IPA Knowledge

Base, which consists of molecular interactions, regulatory events, gene-to-phenotype associations, and chemical knowledge that provide the building blocks for pathway mapping and network construction. The IPA Functional Analysis Tool was used to calculate a P value using a right-tailed Fisher's exact test to determine statistically significant overrepresentation of genes in known canonical pathways. A P value of < 0.05 was set as the significance cutoff for enriched pathways identified, and networks were constructed by IPA with miR-132 target genes. IPA identified three significant pathways from the list of 754 genes: axonal guidance ($P = 0.00025$), glioma signaling ($P = 0.007$), and hepatocyte growth factor signaling ($P = 0.017$). There were 19 genes common to all three pathways (Database S2*B*) representing potential "driver" genes central to multiple signaling networks altered in both disorders.

For comparison, the enriched pathways and significant networks were overlaid with publicly available gene-expression profiles of DL-PFC tissue from two schizophrenia cohorts from the SMRI (arrays A and C) (4).

Expression of miR-132 Targets in CON, BPD, and SCZ mRNA Microarray Datasets. Gene-expression microarray analysis of tissues from control, bipolar, and schizophrenic subjects from two SMRI cohorts was evaluated for overlap with miR-132 target genes. Raw signal intensities for each probeset from the SMRI array A (BA46 from 34 CON, 32 SCZ) and array C (BA46 from 29 CON, 21 SCZ), as contained in the cel files available at http://www.stanleygenomics.org (4), were combined and renormalized using the robust multi-array analysis method. The normalized log base 2 signal intensities for all probesets on the Human Hu133 plus 2.0 arrays for the subjects profiled were used in the analysis. Statistical analysis was performed using Partek Genomics Suite (Partek). Principal component analysis of mRNA data on control subjects from SMRI arrays A and C across all genes on the Human Hu133 array indicated an identical gene-expression pattern in the control subjects from SMRI arrays A and C, compared with brain regions BA9 and BA10 (5). Therefore, a meta-analysis was used to combine subjects from SMRI arrays A and C for further statistical analysis. An ANCOVA model was fit for each probeset with age and brain pH as covariates, and sex and diagnosis as main effects, for identification of genes differentially expressed between control and schizophrenia subjects. The comparison of control to schizophrenia was performed under the ANCOVA model to adjust for the effect of age, sex, and brain pH level. FDR values were computed for each probe set on the resulting P values. The FDRs were calculated based on J. Storey's algorithm, which estimates the q-values for a given set of P values (6). The q-value of a test measures the proportion of false positives incurred (FDR) when that particular test is called significant. These datasets were then evaluated for overlap with putative miR-132 targets to identify miR-132 targets relevant to schizophrenia.

Functional Target Analysis. To identify cortical gene-expression profiles that were enriched for the gene targets of miR-132, a gene set enrichment analysis was run in Nextbio (www.nextbio.com). TargetScan-predicted gene targets of miR-132 were run through a bioset correlation analysis against all 4,404 studies in Nextbio, and results were filtered by keyword "cortex." The top-scoring result was a gene-expression study of developing mouse PFC. To obtain a gene-level analysis, probesets were mapped to genes using IPA (v8.5), using averaged fold-change to resolve duplicate probes. The resulting gene set was reloaded into Nextbio for integration into the bioset correlation of miR-132 target genes.

3′ UTR Luciferase Reporter Analysis of miR-132 Targets. Genes that were both predicted miR-132 targets and up-regulated in DL-PFC from schizophrenic subjects were analyzed by qPCR, as described above, and by functional reporter assays. In the reporter assays, 3′ UTR fragments (1–1.5 kb) from genes of interest were cloned into the psiCHECK2 vector (Promega), and luciferase assays were performed as previously described (7). Briefly, HEK293 cells were cotransfected with plasmids carrying the precursor genomic sequences for either miR-132 or one of two control miRNAs (let-7 or miR-125b), along with the 3′ UTR construct, containing firefly luciferase, and Renilla luciferase. The Dual-Glo Luciferase assay (Promega) was used to measure 3′ UTR expression 2 d later. The firefly luciferase and Renilla luciferase were determined, and the relative expression in the miR-132-treated cells was compared with that of psiCHECK2 empty vector control as well as let-7c and miR-125b-transfected cells.

The 3′ UTR reporters were PCR-amplified from human and rat cDNA with the following primers and cloned into psiCHECK2 following XhoI/NotI digestion:

human DNMT3a 5′-ATGCTCGAGGGGGGCAAACTGAGGTAGCGACA-3′, 5′-ATAGCGGCCGCCACGGGCCCGACCACCTCAT-3′;
human DPYSL3 5′-ATGCTCGAGCTCAGCCCCTGAGTCTGACTCA-3′, 5′-ATAGCGGCCGCAATTTCATAGTTATTTTAATAACC-3′;
human GATA2 5′-ATGCTCGAGATGGACGTCGAGGACCGGGCA-3′, 5′-ATAGCGGCCGCCACAAAAAGTATTTTATTATTC-3′.

Western Blot Analysis of miR-132 Targets. HEK293T cells were infected with recombinant lenti-vectors generated as previously described (7). Stable transgene expression was assessed by UV-microscopic detection of a mCherry reporter gene. Expression levels of miR132 were assessed by reverse transcription and qPCR using a miR132 Taqman assay. Nuclear protein extracts ($n = 7$) from $\sim 2 \times 10^6$ cells were obtained using a NE-PER kit (Pierce; 78833) and quantified by the BCA method (Pierce; 23227). Twenty micrograms of total protein was resolved on a polyacrylamide gel (BioRad; 567–1125) and transferred to nitrocellulose paper. Rabbit polyclonal antibodies for the detection of DNMT3a (1:750, #3598), GATA2 (1:500, #4595), β-actin (1:5,000, #4970), and Histone H3 (1:3,000, #4499) were obtained from Cell Signaling Technology (expression of DPYSL3 could not be accurately determined because of lack of a reliable antibody). All primary antibody hybridizations were performed at 4 °C overnight after blocking with Tris-buffered saline containing 5% milk (0.05 g/mL) and 0.5% Tween-20. An HRP-conjugated anti-Rabbit (1:3,000; Millipore AP307P) secondary antibody and enhanced chemiluminescence (GE Healthcare; RPN2106V2) were used for detection. Relative optical density was assessed using ImageJ64 (National Institutes of Health).

Developmental and NMDA Regulation of miR-132 Expression. The developmental time course of the expression of miR-132 and its mRNA targets were determined using male and female offspring of C57BL/6J mice (Jackson Laboratory). Five to six samples per time point were collected at E15 and E18 and P1, P7, P14, P21, P28, and P60. The telencephalon was collected at E15, the frontal cortex at E18 and P1, and the PFC at all later stages. RNA was extracted and assayed for the expression of miR-132, *Dnmt3a*, *Dpysl3*, and *Gmfb*, as described above. Before performing the target assays, the endogenous controls RNU6B and GAPDH were run using known amounts of cDNA from each developmental time point to ensure that endogenous control levels did not vary by stage of development.

The effect of MK-801 on miR-132 was determined by two methods. In the first experiment, adult male C57BL/6J mice (2–3 mo of age, six to seven mice per dose) received daily intraperitoneal injections of either saline or one of three doses of MK-801

(0.1, 0.3, or 0.6 mg/kg, dissolved in saline) for 5 d. Forty minutes after the final injection on day 5, the mice were killed and the PFC was collected and snap-frozen on dry ice. In the second experiment, C57BL/6J pups were injected intraperitoneally with either saline or 0.1 mg/kg MK-801 dissolved in saline once daily from P4–P17 (day of birth: P0) (8, 9). Mice were allowed to mature to P14 (no injection of saline or MK-801 on the day of tissue collection) or P60, at which point the PFC was collected and RNA was extracted for miR-132 analysis.

Expression of four miR-132 target genes in P14 and P60 developmentally treated mice was performed using either Taqman assays, as described above (*p250GAP*, *Gata2*, *Dpysl3*, rodent GAPDH), or SYBR Green (BioRad) using standardized *Gapdh* qPCR primers (Qiagen) and a custom set of qPCR primers designed to specifically target the isoform of *Dnmt3a* associated with the long 3′ UTR containing the putative miR-132 binding sites (F: 5′-TAC ATC AGC AAA CGG AAA CG-3′; R: 5′-AGA CTC TCC AGA GGC CTG GT-3′). Because of age-dependent differences in expression (as determined in untreated mice, above), qPCR data were analyzed two separate ways. First, to determine effects of age (P14 vs. P60) on gene expression, ΔΔCt was determined using the average ΔCt of all samples from both age groups (Fig. S2*C*). A second analysis to determine the effect of treatment (saline vs. MK-801) was performed by calculating separate ΔΔCts for each age group (Fig. S2*D*). All experiments were approved by the Scripps Florida Institutional Animal Care and Use Committee.

In Vivo Antipsychotic Treatment in Rats. This experiment was approved by the Pfizer Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (53–57 d old) were implanted with subcutaneous Alzet osmotic minipumps (2ML4) delivering vehicle (1% acetic acid), 0.25 mg/kg haloperidol, or 5 mg/kg risperidone. After 21 d, rats were killed by CO_2 exposure. Blood was collected via cardiac puncture for measurement of drug levels. The frontal cortex was collected, frozen rapidly on dry ice, and stored at −80 °C until analysis. All animal experiments were performed in Association for Assessment and Accreditation of Laboratory Animal Care facilities and approved by the representative Institutional Animal Care and Use Committee.

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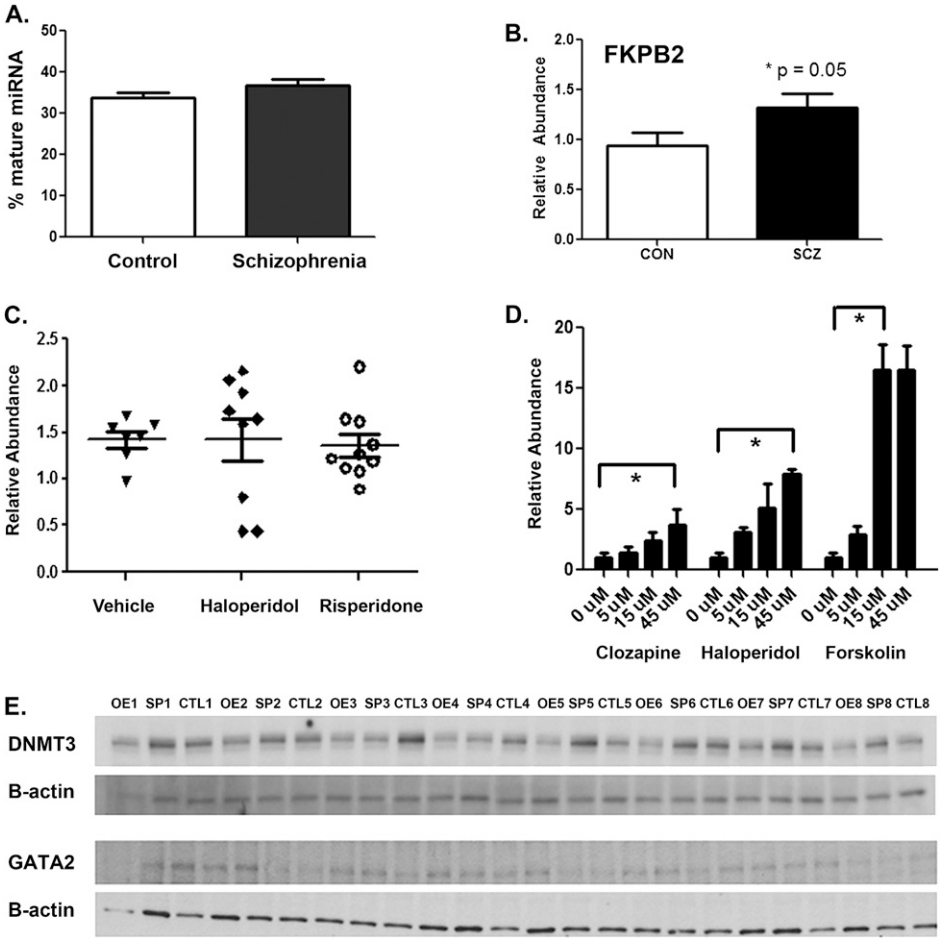
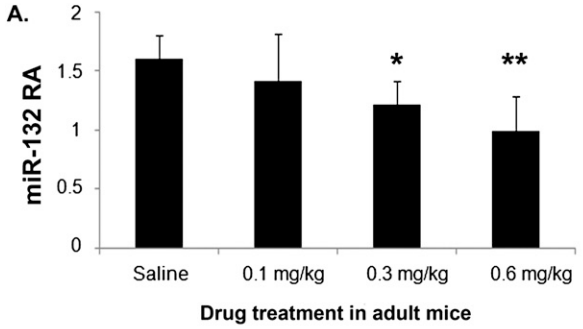
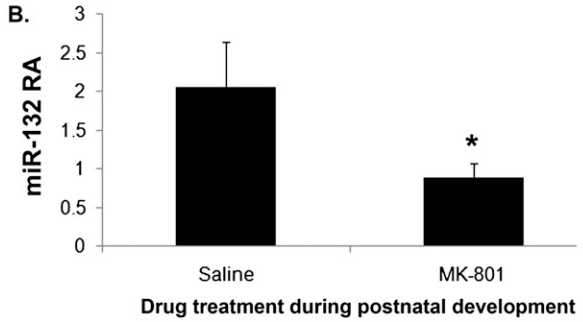


Fig. S1. (*A*) Analysis of small RNA in HBB CON and SCZ samples. To identify any alterations in miRNA processing, the total small RNA (< 200 nucleotides) fraction and proportion of mature miRNAs (~22 nt) in the HBB control and SCZ samples were measured using Agilent's Small RNA II kit. RNA was diluted to 100 ng/μL, and the gel-dye mix, ladder, and RNA samples were prepared and loaded onto Small RNA chips per Agilent instructions. The chips were run on an Agilent 2100 Bioanalyzer using the Small RNA II protocol. The graph shows the percent of total small RNA (< 200 nucleotides) compared of the mature miRNA fraction (~22 nt). Error bars = SEM. (*B*) FKBP2 expression is significantly up-regulated in the PFC of schizophrenic subjects from the HBB samples. Error bars = SEM. Effect of long-term treatment with haloperidol (mean serum level = 1.65 ng/mL) or risperidone (mean serum level = 57.1 ng/mL) on miR-132 expression in the PFC of male rats. (*C*) Effect of clozapine, haloperidol, or forskolin on miR-132 expression in SH-SY5Y cells. To determine the potential effect of antipsychotic treatment on SCZ-associated miRNAs, human neuronal SH-SY5Y cells (ATCC) were treated with the antipsychotics clozapine or haloperidol. Cells were plated in six-well plates at a density of 4×10^5 cells per well. Twenty-four hours after plating, cells were treated with 5, 15, or 45 μM of clozapine, haloperidol (Sigma Aldrich), or forskolin (LC Laboratories) in 1% DMSO, or 1% DMSO (control). RNA was collected 1 h (forskolin) or 24 h (clozapine, haloperidol) later and assayed for miR-132 abundance. All experiments were performed with three biological replicates. Error bars = SEM, $^*P < 0.05$. (*D*) Effect of miR-132 dysregulation on target protein levels in vitro. HEK-293 cells were transduced with a lentiviral vector carrying a control (scrambled) sequence, a miR-132 precursor hairpin expression cassette, or a sponge directed against miR-132. Protein levels of DNMT3A, GATA2, and β-actin were determined by Western blot, and were normalized to β-actin.





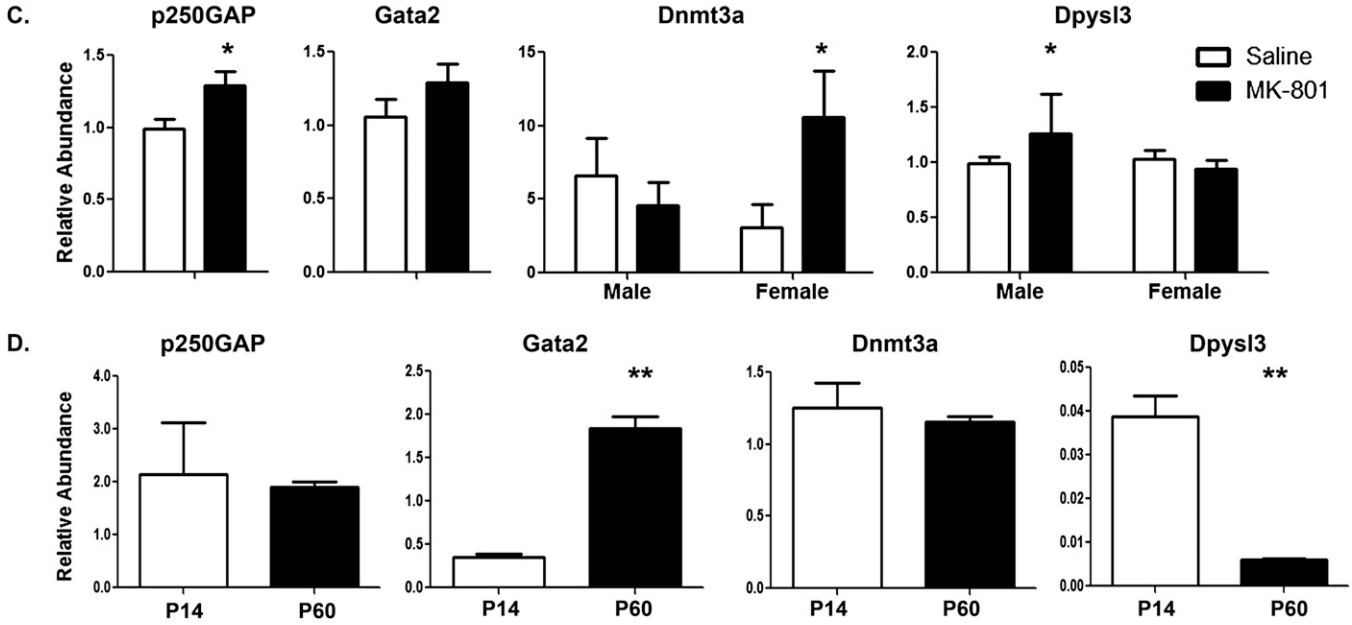


Fig. S2. Effect of MK-801 on miR-132 and miR-132 target expression. (*A*) Daily (5-d) treatment of adult mice with MK-801 resulted in a dose-dependent reduction in prefrontal cortex miR-132 expression. $*P < 0.05$, $**P < 0.01$. (*B*) Chronic daily low-dose treatment of postnatal mice with MK-801 from P4–P18 resulted in a significant reduction of prefrontal miR-132 expression in adulthood (P60) ($P = 0.03$). Error bars = SEM. (*C*) Effect of developmental MK-801 inhibition on adult expression of miR-132 target genes. Expression levels of four miR-132 targets were measured in prefrontal cortex from 2-mo-old mice who had received either saline ($n = 11$ male, 8 female) or MK-801 (0.1 mg.kg, $n = 15$ male, 10 female) from P4–P17. Developmental treatment with MK-801 resulted in significant adult up-regulation of *p250GAP* in both sexes ($P = 0.04$), up-regulation of *Dnmt3a* in females ($P = 0.04$), and up-regulation of *Dpysl3* in males ($P = 0.02$). Error bars = SEM, $*P < 0.05$. (*D*) Effect of age on expression of miR-132 targets in developmentally treated mice. Expression of *Gata2* and *Dpysl3* changed significantly from P14 ($n = 8$ saline, 10 MK-801) to P60. In contrast to the data presented in Fig. 3, *Dnmt3a* did not show a significant change, likely because of the strong sex treatment effects observed in P60 mice. Error bars = SEM, $**P < 0.01$; $*P < 0.05$.

Table S1. Demographic data for human samples from the SMRI dataset

Group or *P* value	*n*	Age (y)	Sex (M/F)	Suicide status (Y/N)	Alcohol use (Y/N)	Drug use (Y/N)	Smoke (Y/N)	PMI (h)	RI	Brain pH
Groups										
Schizophrenia	35	42.6 (8.5)	26/9	7/28	25/10	19/14	23/4	31.4 (15.5)	5.9 (4.2)	6.5 (0.2)
Bipolar	31	44.9 (11.0)	15/16	14/17	26/4	22/9	14/6	36.6 (18.1)	8.7 (7.2)	6.5 (0.3)
Control	34	43.8 (7.4)	25/9	0/34	16/18	5/29	9/9	6.61 (0.8)	3.7 (2.6)	6.6 (0.3)
P value										
Overall	100	0.57	**0.045**	**<0.0001**	**0.003**	**<0.0001**	**0.04**	0.17	**0.0008**	**0.035**
Schizophrenia control		0.56	0.94	**0.0059**	**0.039**	**0.0003**	**0.011**	0.61	0.074	**0.031**
Bipolar control		0.62	**0.037**	**<0.0001**	**0.0009**	**<0.0001**	0.21	0.069	**0.0002**	**0.021**

Boldface indicates demographic variables that are significantly different from Control subjects. PMI, post-mortem interval (hrs); RI, RNA Integrity number.

Table S2. Demographic data for human samples from the HBB dataset

Group or *P* value	*n*	Age (y)	Sex (M/F)	PMI (h)
Group				
Schizophrenia	16	56.8 (16.9)	8/8	17 (5.3)
Control	15	59.9 (13)	7/8	20 (3.0)
P value				
Overall	31	0.58	0.97	0.08

Dataset S1. Complete ANCOVA results from the SMRI miRNA microarray

Dataset S1 (XLS)

Dataset S2. Overlap of SCZ- and BPD-associated miRNAs

Dataset S2 (XLS)

(*A*) In the SMRI array, miRNAs with an uncorrected *P* value < 0.05 showed significantly greater overlap between SCZ and BPD than would be expected by chance. However, only those miRNAs with an FDR-corrected *P* value < 0.05 were subsequently validated by qPCR analysis. FC, fold-change. (*B*) Top-ranked, shared miRNAs had 754 common putative protein-coding targets, which were analyzed with IPA, resulting in three significantly overrepresented signaling pathways that had 19 genes in common. The miRNAs used for analysis were miR-132, miR-320, miR-135, miR-105, miR-449, and miR_17-5p.

Dataset S3. Linear regression of SMRI miRNA microarray data to SMRI demographic data

Dataset S3 (XLS)

Dataset S4. IPA identified 26 biological pathways significantly overrepresented by miR-132 targets

Dataset S4 (XLS)

We used the IPA core analysis tool to analyze the biological significance of the miR-132 target genes as predicted by the target prediction database TargetScan (www.targetscan.org). For comparison, the enriched pathways and significant networks were overlaid with publicly available gene expression profiles of DL-PFC tissue (BA46) from two schizophrenia cohorts from the SMRI (arrays A and C) (4).

Dataset S5. Predicted miR-132 targets significantly up-regulated in the SMRI A + C mRNA microarray datasets

Dataset S5 (XLS)

Raw signal intensities for each probeset from the SMRI array A (BA46 from 34 CON, 32 SCZ) and array C (BA46 from 29 CON, 21 SCZ), as contained in the cel files available from http://www.stanleygenomics.org, were combined and conormalized using the robust multiarray analysis method and compared predicted miR-132 targets. There were significant male/female differences, with many more genes meeting the FDR significance cutoff in females. It is unclear whether these results may underlie some of the sex differences that have been reported in psychiatric disorders, or if these differences represent demographic disparities between the sexes (1).

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Dataset S6. MiR-132 targets changed in mouse prefrontal cortex between postnatal weeks 2 and 4

Dataset S6 (XLS)

Dataset S7. Results of mouse and primate prefrontal cortex miRNA microarrays

Dataset S7 (XLS)

Multiple NMDA hypofunction models have been used to study endophenotypes similar to those observed in schizophrenia. To evaluate the molecular underpinnings of these models, which have primarily been characterized at the behavioral level, we used miRNA microarrays to quantify miRNA expression patterns in the PFC of a murine and a primate animal model.

(*A*) In the first experiment, we measured miRNA expression differences between wild-type and NR1 hypomorphic mice, which express only 5–15% of normal NMDAR1 subunit levels (1). We identified three miRNAs—miR-675*, miR-465b-5p, and miR-671–3p—that had an FDR-corrected P value < 0.05. No significant overlap between the miRNAs altered in the NR1 hypomorph and either SCZ or BPD were observed. qPCR analysis of additional NR1 hypomorphic mouse PFC samples confirmed that SCZ-associated miRNAs, including miR-132, were not affected by NR1 genotype. Moreover, the previously reported reduction of miR-219 in NR1 hypomorphs was not observed in the present experiment (2). However, the sample size was small ($n = 6$ per genotype). Moreover, we found that miRNA expression was highly variable within genotypes, and multidimensional scaling of all probe intensities (stress = 0.055) showed little separation by genotype. Strain background may play a more significant role than NR1 genotype in regulating miRNA expression, as mice used in the experiment were on a mixed B6 × 129 × DBA background, with each strain represented by different alleles in individual mice. Previous studies have shown that ∼15% of brain-expressed miRNAs differ among inbred mouse strains (3). Finally, the NR1 hypomorph model is severely deficient in NMDA signaling from before birth, whereas the developmental MK-801 model described above experienced relatively mild NMDA inhibition for a limited period of postnatal development, perhaps explaining the miRNA expression differences between the two models. Our miRNA expression data suggest that moderate postnatal inhibition of NMDA signaling may be more consistent with molecular abnormalities in schizophrenic patients than almost complete NMDA inhibition.

(*B*) We extended our comparative analysis to a primate model of NMDA hypofunction. Primates that are withdrawn from chronic treatment with the NMDA antagonist PCP exhibit significant deficits in prefrontal-mediated behavior and dopamine utilization that persist even after PCP treatment has ceased (4). In the present study, male African green monkeys (*C. sabaeus*) were treated with a subchronic dose of PCP (0.3 mg/kg) or saline intramuscularly daily for 14 d. Seven days after the final drug treatment, brain tissue was perfused with saline and 2-mm tissue punches from the DL-PFC were removed and snap-frozen. The samples used for both microarray and qPCR analysis consisted of six control and five PCP-treated individuals. All primate procedures were approved by the Yale University Institutional Animal Care and Use Committee.

There was no significant (FDR-corrected) effect of drug treatment on the expression of any miRNAs. In addition, qPCR analysis of several schizophrenia-associated miRNAs, including miR-383, miR-132, and miR-219–5p, did not show differential miRNA expression between the control and PCP-treated primates. The lack of significant miRNA differences between treatment groups may be associated with the length of the PCP withdrawal period (1 wk), suggesting that chronic PCP treatment, although producing enduring cognitive deficits, may not induce sustained differences in miRNA expression following cessation of treatment. Additionally, the sequence-specific applicability of the mammalian miRNA arrays to green monkey RNA is unknown, as green monkey miRNAs have not been sequenced. However, given the high level of miRNA sequence conservation among mammals, many are likely to be identical to known mouse and human sequences (5, 6). The microarrays used for the primate samples contained exact-match probes for 334 known *Macaca mulatta* miRNAs, including 38 of the miRNAs drawn from the list of most significant SCZ- or BPD-associated miRNAs. To correct for the effect of potential sequence disparities on the array results, statistical analysis was performed by two methods, once using only the 233 of the 334 *M. mulatta* probes expressed above threshold, and then using all 590 mammalian probes that were expressed above threshold.

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