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Supplementary Information for

Polypyrimidine tract-binding protein blocks miRNA-124 biogenesis to enforce its neuronal-specific expression in the mouse

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SI Materials and Methods

Cell lines and tissue culture

The N2a neuroblastoma cell line was grown in DMEM (Fisher Scientific) supplemented with 10 % FBS (Omega Scientific). The mESC line, E14 (1), *Ptbp1*$^{loxp/loxp}$ WT and KO mESCs, were cultured on 0.1 % gelatin-coated dishes with mitotically inactivated mouse embryonic fibroblasts (MEF) (CF1, Applied StemCell, Inc.) in ESC media. ESC media consisted of DMEM (Fisher Scientific) supplemented with 15 % ESC-qualified fetal bovine serum (Thermo Fisher Scientific), 1x non-essential amino acids (Thermo Fisher Scientific), 1x GlutaMAX (Thermo Fisher Scientific), 1x ESC-qualified nucleosides (EMD Millipore), 0.1 mM β-Mercaptoethanol (Sigma-Aldrich), and 10^3 units/ml ESGRO leukemia inhibitor factor (LIF) (EMD Millipore). Mouse primary cortical cultures were prepared from gestational day 15 C57BL/6 embryos (Charles River Laboratories), as described previously (2). Briefly, cortices were dissected out into ice cold HBSS and dissociated after a 10 min digestion in Trypsin (Invitrogen). Cells were plated at 5 million cells per 78.5 cm^2 on 0.1 mg/ml poly-L-lysine coated dishes and cultured for 5 days. Neurons represented 70 – 80 % of the cells in the culture. A mouse neuronal progenitor cell line was established from cortical cells of gestational day 15 embryos generated by crossing homozygous Nestin-GFP transgenic mice to wild type C57BL/6. GFP positive cells were collected using FACS and plated on uncoated culture dishes. These NPCs were grown in DMEM/F12 supplemented with B27 (without vitamin A, Thermo Fisher Scientific), 1 mM GlutaMax and antibiotics. EGF and FGF (PeproTech) were added every day at 10 ng/ml concentration. All experiments were approved by the UCLA Institutional Animal Care and Use Committee (ARC# 1998-155-61).

PTBP protein quantification

Whole-cell lysates (WCL) were extracted in RIPA, and quantified with the bicinchoninic acid (BCA) method (3). 12 µg of WCL protein was loaded on 10 %

SDS-PAGE adjacent to a standard curve of His-PTBP recombinant protein. PTBP bands were detected with anti-PTBP1 (PTB_NT) and anti-PTBP2 (PTB_IS2) (SI Appendix, Table S4), and fluorescent (Cy3) secondary antibodies and imaged on a Typhoon Imager. Band intensities were quantified with ImageQuant software (GE Healthcare).

Subcellular fractionation, RNA sequencing and data analysis

These datasets will be described in detail elsewhere. Briefly, total Ribo-minus RNA was isolated from mESCs (line E14) that were fractionated into cytoplasmic, soluble nuclear, and chromatin pellet compartments as described previously (4-6). Paired-end libraries were constructed using the TruSeq Stranded mRNA Library Prep Kit (Illumina). The libraries were subjected to 75 nt paired-end sequencing at the UCLA Broad Stem Cell Center core facility on an Illumina HiSeq2000 to generate 200–250 million mapped reads per sample. Sequences were aligned using STAR (STAR_2.5.1b) to examine the miR-124-1 locus. Further analysis of these datasets is ongoing. The iCLIP data are described in Linares et al. (7), and are available at GEO (GSE71179).

DNA constructs

To generate chimeric pri-miRNAs, the gene splicing by overlap extension technique was used (8). Briefly, PCR products of the sequence upstream of miR-124-1 or miR-1a-2 stem loop and the sequence including the stem loop and downstream sequence of these RNAs were amplified in separate reactions. The reverse primers of the upstream products were designed to overlap the stem loop-downstream products. Fragments for miR-124-1 and miR-1a-2 were mixed and re-amplified to form chimeric pri-miRNAs. To produce pri-miRNA substrates for *in vitro* pull-down and pri-miRNA processing assays, the region including ~ 107 nt upstream of the stem loop to ~ 10 nt downstream of the stem loop for each pri-miRNA was amplified by PCR, with a forward primer containing the T7 promoter. The PCR product was then used for *in vitro* transcription. The full length human PTBP1.4 CDS was cloned into the pAAV-nEF-tRFP vector. This

construct expresses the tRFP fluorescence reporter under the nEF promoter, linked to a p2A cleavage sequence followed by a FLAG tag PTBP 1.4 (pAAV-nEF-tRFP-p2A-FLAG_PTBP1.4). The primer sequences used for cloning are shown in SI Appendix, Table S2.

RT-PCR conditions

cDNA was generated with SuperScript III RT (Thermo Fisher Scientific) and PCR was performed using Phusion DNA polymerase (Fisher Scientific). For pri-miR-124-1 (amplicon size = 315nt) and pri-miR-124-2 (269 nt), PCR reactions used 29 cycles at an annealing temperature of 65°C. For pri-miR-133a-1 (293 nt) and pri-miR-9-1 (370 nt), PCR run used 26 cycles annealing at 63°C, and 29 cycles at 66°C, respectively. For the pri-miR-294/295 cluster (393 nt), PCR runs used 27 cycles at 63°C. Primers designed to amplify pri-miR-124-3 did not produce a PCR amplicon after 34 cycles using annealing temperatures between 58°C and 68°C. The above primer pairs spanned the stem-loop region of each miRNA (spanning primer). A separate primer pair detecting a region upstream of the pre-miR-124-1 stem-loop (upstream primers (9, 10), 256nt; SI Appendix, Fig. S2A) was used in 28 cycles of PCR at an annealing temperature of 63°C. The primer sequences are shown in SI Appendix, Table S2.

Generation of WT and *Ptbp1* KO mESC

A *Ptbp1* mouse line (*Ptbp1*$^{loxp/loxp}$) carrying loxp sites flanking *Ptbp1* exon 2 was obtained from the Xiang-Dong Fu lab (UCSD, generation of this mouse will be described elsewhere) and crossed to an actin-flip recombinase mouse line to remove the neomycin selection cassette in intron 2. *Ptbp1*$^{loxp/loxp}$ mESCs were generated from this *Ptbp1*$^{loxp/loxp}$ mouse following a previously published protocol (11). Briefly, healthy late blastocysts were collected at 3.5 days post conception (dpc) and cultured overnight in KSOM medium (DMEM high glucose with 15 % KO serum, 2 mM GlutaMAX, 1 mM sodium pyruvate, 0.1 mM MEM NEAA, 0.1 mM β-Mercaptoethanol, 10^3 units/ml of LIF, 1 μM PD0325901 and 3 μM CHIR99021). Blastocysts were transferred to defined serum-free medium (1 : 1

mixture of DMEM-F12/N2 and Neurobasal/B27, 1 mM GlutaMAX, 0.5 mM sodium pyruvate, 0.1 mM MEM NEAA, 0.1 mM β-Mercaptoethanol) plus 2i/LIF (EMD Millipore) to allow attachment to the feeder layer. After outgrowth from the blastocysts, cells were disaggregated and cultured in the serum-free medium plus 2i/LIF together with MEF. Cells were monitored daily for colony formation. Cells exhibiting differentiation or atypical ES cell morphology were discarded and the healthy ES cell lines were maintained in the serum-free medium plus 2i/LIF with MEF. These *Ptbp1* $^{loxp/loxp}$ WT mESCs were used to generate *Ptbp1* KO mESCs by transduction with Cre-GFP lentivirus for 3 days. FACS selected GFP positive cells were cultured and genotyped to confirm homozygous deletion of *Ptbp1* exon 2.

Adeno-associated viruses (AAVs) and transduction of cultured cortical neurons

AAVs were packaged with serotype 9. AAV2/9 nEF-tRFP and nEF-tRFP-p2A-FLAG-PTBP1.4 were produced by co-transfection of the AAV2 genomic plasmid, pHelper and an AAV9 envelope plasmid into 293FT cells and purified on ioxodianol gradients (Optiprep, Sigma-Aldrich). Virus titer was determined by qPCR. Cultured mouse cortical neurons were transduced with AAV2/9 virus one day after plating (day *in vitro* 1), with RNA and Protein then isolated at days *in vitro* 8.

***In vitro* pri-miRNA processing assays**

Substrate RNAs for *in vitro* pull down and *in vitro* pri-miRNA processing were prepared by *in vitro* transcription as described previously (12, 13). *In vitro* processing of pri-miRNAs was carried out using either nuclear extract or DROSHA-FLAG immunoprecipitate. Nuclear extract from mESC (E14) was prepared as described previously with some modifications (14). Briefly, nuclei were purified by cell lysis in mild detergent followed by pelleting through 24 % sucrose (6). After washing, the nuclear pellet was resuspended in Buffer C (20 mM HEPES–KOH [pH 7.9], 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA [pH

8.0], 25 % glycerol) and stirred on ice for 30 min. Supernatant was taken as nuclear extract, and dialyzed against a 100-fold volume of Buffer D-200K (20 mM Tris·Cl, pH 8.0, 200 mM KCl, and 0.2 mM EDTA) for 2 hrs for each of 3 buffer changes. To prepare DROSHA-FLAG immunoprecipitates, mESC (E14) were transfected with a DROSHA-FLAG expression plasmid driven by the modified CMV promoter (DROSHA-FLAG-pCK, a gift from Dr. V. Narry Kim) using Lipofectamine 2000 (Thermo Fisher Scientific). Cells were harvested 48 hrs after transfection and sonicated in cold Buffer D-200K. Cell lysate was cleared by centrifugation at maximal speed for 15 min at 4°C. DROSHA-FLAG was immobilized on anti-Flag antibody-conjugated agarose beads (Sigma-Aldrich) and used for western blot and *in vitro* processing assays (15, 16). 30 µl reactions contained either 15 µl of nuclear extract or 15 µl of DROSHA bound beads, and 6.4 mM $MgCl_2$, 1 unit/ml of Ribonuclease Inhibitor (Thermo Fisher Scientific) and 3 ~ 5 nM of ^{32}P-UTP internally labeled pri-miRNA transcript. Reaction mixtures were incubated for 60 min at 37°C. For reconstitution experiments in Figure 4C, RNAs were first incubated with rHis-PTBP1.4 for 15 min at RT, DROSHA bound beads were then added, and incubated for 20 min at 37°C. T4 RNA ligase I (New England Biolabs) was used to label the 3'-ends of pri-miRNA substrates. Cytidine 3', 5' bis(phosphate) labeled on the 5' phosphate group with ^{32}P (pCp) was incubated with *in vitro* transcribed RNA at 16°C overnight. End labeled RNAs were gel purified after labeling, and RNA concentrations were measured by Nanodrop. The same amount of RNA was used for pri-miRNA processing assay for four different RNA substrates. After reaction, RNA was extracted with phenol, separated on 12.5 % denaturing polyacrylamide gels, adjacent to the Decade RNA marker (Thermo Fisher Scientific), and scanned by Typhoon Phospho Imager. Equal volumes of reaction products were loaded on denaturing gels for internal labeled pri-miRNA (Figure 4A), while loading volumes were adjusted in the experiment using 3'-end labeled substrate to normalize the total counts per lane (SI Appendix, Fig. S6), due to variations of 3'-end labeling efficiency. Band intensities were quantified with ImageQuant software (GE Healthcare).

Calculation pri-miRNA processing efficiency

Images were gathered on the Typhoon Imager (GE Healthcare) and band intensities quantified with ImageQuant software (GE Healthcare) and normalized by the number of U residues in the substrate that internally labeled with ^{32}P-UTP. The normalized band intensities of the processed products (pre-miRNA and 3' flanking fragment for internal labeled and 3'-end labeled substrate, respectively) were divided by intensities of the pri-miRNA substrate that had been incubated with buffer only (without nuclear extract / without DROSHA-FLAG IPs). These values were further normalized to that of WT pri-miRNA.

Recombinant His-PTBP protein

Recombinant His-PTBP protein was prepared as described previously (17, 18). Briefly, PTBP1.4 was expressed in *E.coli* Rosetta2 (DE3) with an N-terminal 6x-His Tag. Cells were harvested and resuspended in buffer containing 50 mM Tris·Cl [pH 7.5], 150 mM NaCl and 15 % glycerol. The cells were lysed by sonication and centrifuged at 18,000 rpm for 1 hr (Rotor JA20, Beckman). The supernatant of the cell lysate was supplemented with 1 M NaCl and 0.1 % polyethyleneimine (PEI) to precipitate RNA. The precipitated RNA was pelleted by centrifuging at maximal speed for 10 min at 4°C. The supernatant was brought to 2 M $(NH_4)_2SO_4$ to precipitate the proteins, and the proteins were pelleted by centrifuge at 12,000 rpm for 10 min at 4°C (Rotor JA14, Beckman). The protein pellets were resolubilized in Loading Buffer (50 mM Tris·Cl [pH 7.5], 150 mM NaCl and 15 % glycerol) and loaded on a HisTrap HP 5 ml column (GE Healthcare). The column was washed with Washing Buffer (50 mM Tris·Cl [pH 7.5], 150 mM NaCl, 15 % glycerol and 50 mM imidazole), and the target protein was eluted with Elution Buffer (50 mM Tris·Cl [pH 7.5], 150 mM NaCl, 15 % glycerol and 500 mM imidazole). The target protein was further purified on a Hiload Superdex 200 pg column (GE Healthcare) equilibrated in 40 mM HEPES-K [pH 7.9], 160 mM potassium glutamate, 0.4 mM EDTA and 2 mM DTT. Fractions containing the target protein were pooled, concentrated, and the concentrated protein added to an equal volume of glycerol for storage at -80°C.

Computational Modeling

All computational simulations were performed in COPASI (19). Ordinary differential equation (ODE) based models were constructed as depicted in Figure 6A as described previously (20). The full system of differential equations with PTBP1 meditated inhibition of miR-124 processing is as follows:

Eqn 1

$$\frac{d([\text{PTBP1}])}{d\text{t}} = -k_1 \cdot \text{PTBP1} + k_2 \cdot \text{PTBP1}_{\text{mRNA}} \cdot \left(1 - \frac{\text{PTBP2}}{k_3 \cdot \text{PTBP2}}\right) \cdot \left(1 - \frac{\text{miR124}}{k_4 \cdot \text{miR124}}\right)$$

Eqn 2

$$\frac{d([\text{PTBP2}])}{d\text{t}} = -k_5 \cdot \text{PTBP2} + k_6 \cdot \text{PTBP2}_{\text{mRNA}} \cdot \left(1 - \frac{\text{PTBP1}}{k_7 \cdot \text{PTBP1}}\right)$$

Eqn 3

$$\frac{d([\text{miR124}])}{d\text{t}} = -k_8 \cdot \text{miR124} + k_9 \cdot \text{pri\_miR124\_1} \cdot \left(1 - \frac{\text{PTBP1}}{k_{10} \cdot \text{PTBP1}}\right) + k_{11} \cdot \text{pri\_miR124\_2}$$

MiR124 denotes the mature miRNA, and pri_miR124_1 and pri_miR124_2 denote the two primary miRNAs. Simulations without PTBP1-meditated inhibition of miR-124 processing were creating by removing the PTBP1 dependent term in Eqn 3 such that pri-miR-124-1 and pri-miR-124-2 processing were modeled in the same way. miRNA and precursor concentrations were experimentally determined as shown in Table 1 and numerically defined. All other parameters were estimated using COPASI parameter estimation to minimize the distance to the remaining molecule numbers in Table 1. Each parameter was constrained between $1 \cdot 10^{-6}$ and $1 \cdot 10^{6}$ and particle swarm optimization was performed with a swarm size of 500. Fitting was terminated when the quality of fit no longer improved with repeated iterations (Figure 6B). Repeated single-cell simulations

were performed using the “parameter scan” task within COPASI. All kinetic parameters were sampled over a 4-fold range centered on the optimum parameter and a time-course was performed from ESC to the cortical neuron state (SI Appendix, Fig. S9). This process was repeated 1000 times to generate 1000 single-cell simulations with distinct parameters. The range of PTBP2 concentrations within the final population was determined, and cells where PTBP2 was present but below the PTBP1 concentration were measured as “Remaining Cells with [PTBP1]>[PTBP2]” (Figures 6E and 6F).

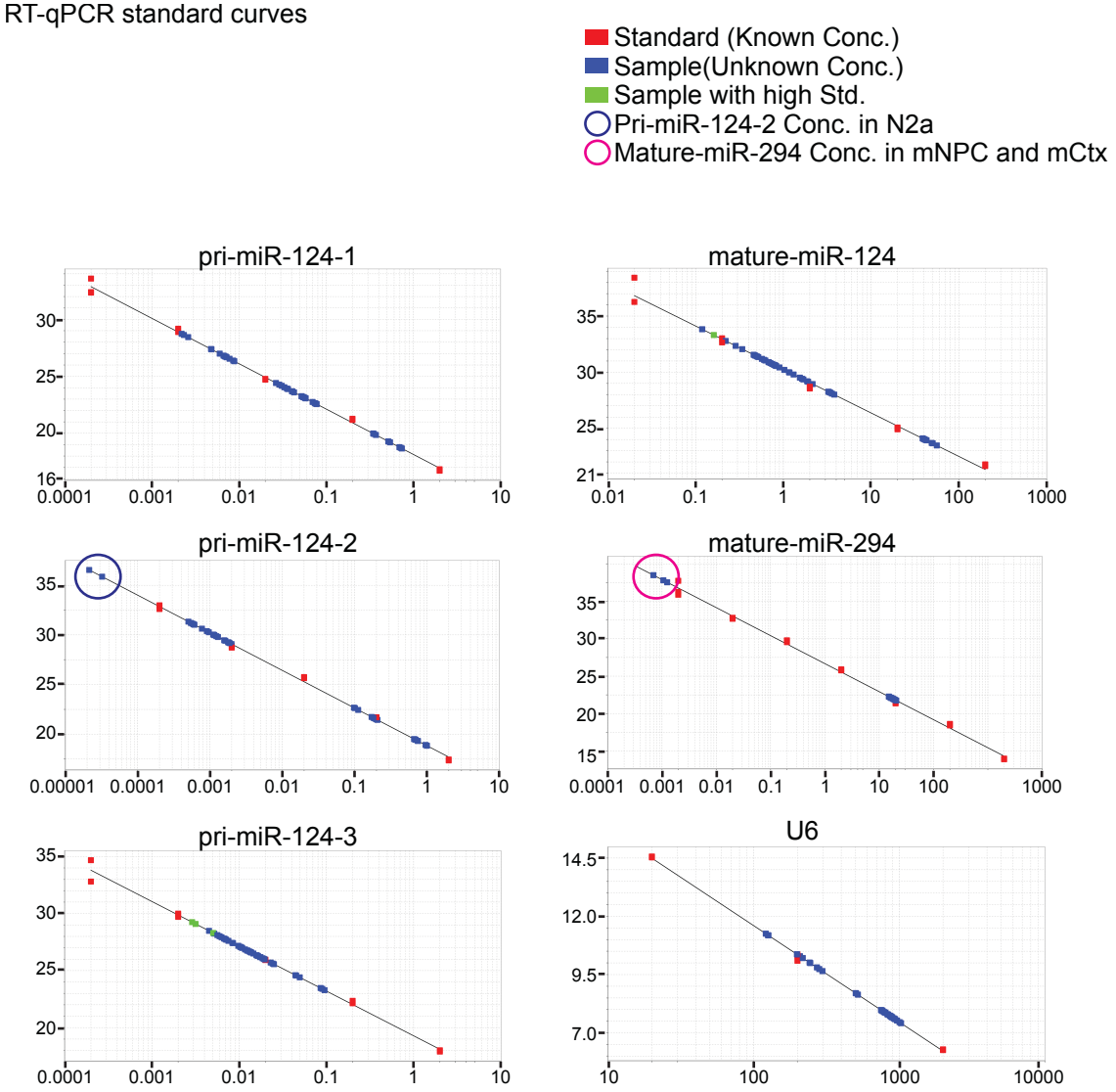


Fig. S1 (Related to Table 1). Standard curves used for RT-qPCR quantification.

Sample concentrations were determined by comparing Ct values (Y axis) to known concentration standards (X axis). Red dots denote the standard points. Blue dots denote sample points. Pri-miR-124-2 levels in N2a cells were below the lowest standard point and marked with a blue circle. Mature-miR-294 levels in mNPC and mCtx were below the lowest standard point and marked with a magenta circle.

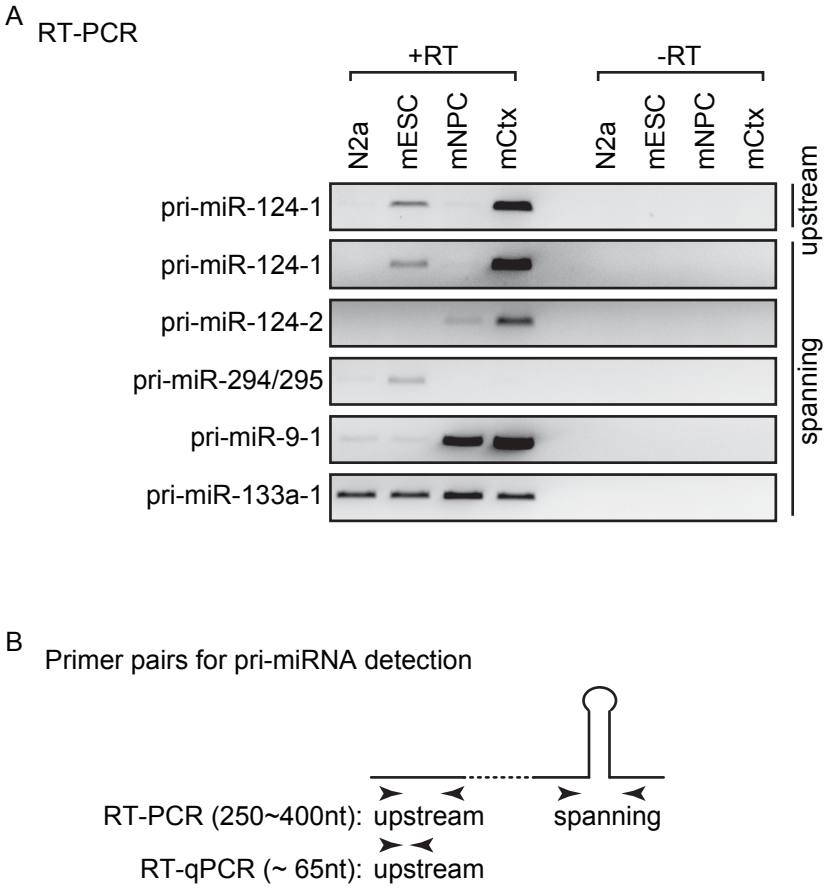


Fig. S2 (Related to Figure 1A). RT-PCR of pri-miR-124-1, pri-miR-124-2, pri-miR-294/295, pri-miR-9-1 and pri-miR-133a-1 from N1a, mESC, mNPC and mCtx.

A. Representative gel image is shown from 3 biological replicate cultures. RT-PCR products generated in the absence of reverse transcription (-RT) served as negative controls for genomic DNA contamination. Primer pairs used for each RT-PCR are indicated to the right of the gel panel. Pri-miR-124-3 was not detected in these conditions. N2a, mouse neuroblastoma cells; mESC, E14 mouse embryonic stem cells; mNPC, isolated mouse neural progenitor cells; mCtx, mouse cortical neurons, days *in vitro* 5.

B. Primer pair locations are indicated. Spanning primer pairs were used for semiquantitative RT-PCR analysis (panel A above; Figures 1A, 2C and 3A), while upstream primer pairs are used for RT-qPCR (Table 1). Pri-miR-124-1 was also analyzed with an additional primer pair for RT-PCR (upstream, top panel in A) that shares the forward primer with the upstream pair used for RT-qPCR.

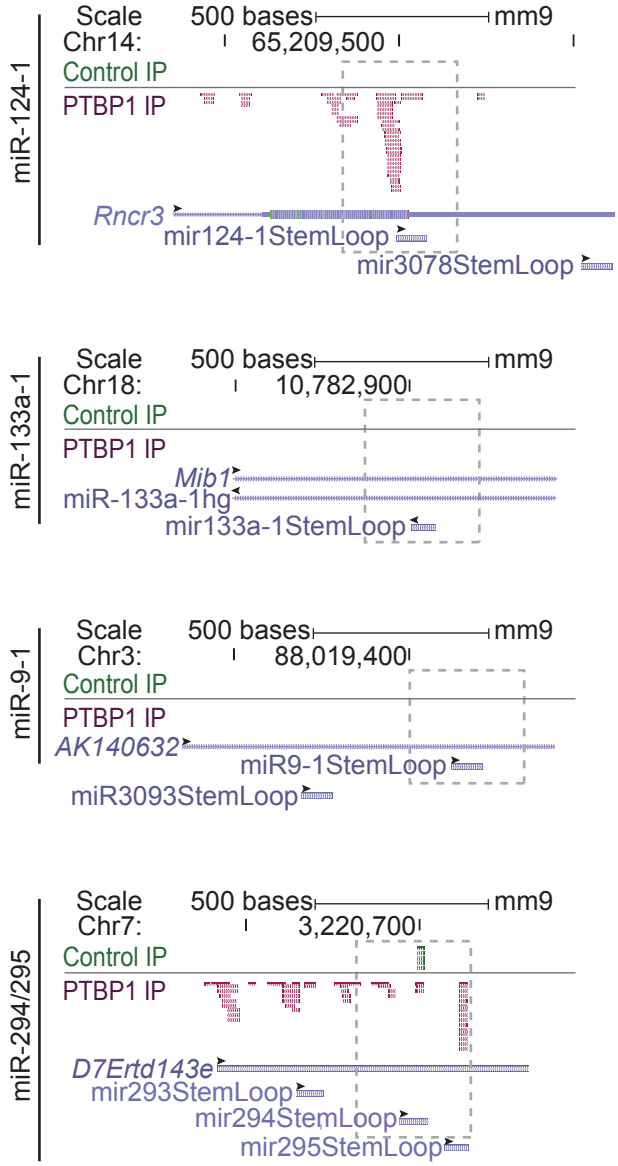
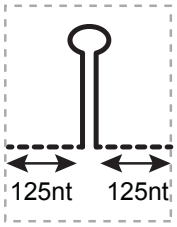


Fig. S3 (Related to Figure 2C). PTBP1 and control iCLIP tags mapping to miRNA loci in mESCs.

Genome browser tracks (~ 1 kb window) of loci used in the miRNA PTBP1-IP experiment in Figure 2C. Host transcripts for each miRNA are indicated (light purple). iCLIP tags from control IPs are marked in green and PTBP1-IP iCLIP tags are marked in magenta. Sequence intervals from - 125 nt upstream to + 125

nt downstream of each annotated miRNA stem-loop is marked with a grey dashed box. Arrowheads indicate 5’ to 3’ direction of each of RNA.

A



iCLIP tags within ±125 nt of miRNA StemLoop : 2020
PTBP1 Bound miRNA Loci : 295

pri-miR	Number of tags	% of tags	Ranking
mmu-miR-124-1	53	2.67	1
mmu-miR-5125	51	2.57	2
mmu-miR-7-1	50	2.52	3
mmu-miR-127	49	2.46	4
mmu-miR-7670	47	2.36	5
mmu-miR-433	46	2.31	6
mmu-miR-292a	41	2.06	7
mmu-miR-291a	35	1.76	8
mmu-miR-493	34	1.71	9
mmu-miR-5114	34	1.71	
mmu-miR-291b	34	1.71	
mmu-miR-294	34	1.71	
mmu-miR-431	33	1.66	10

B

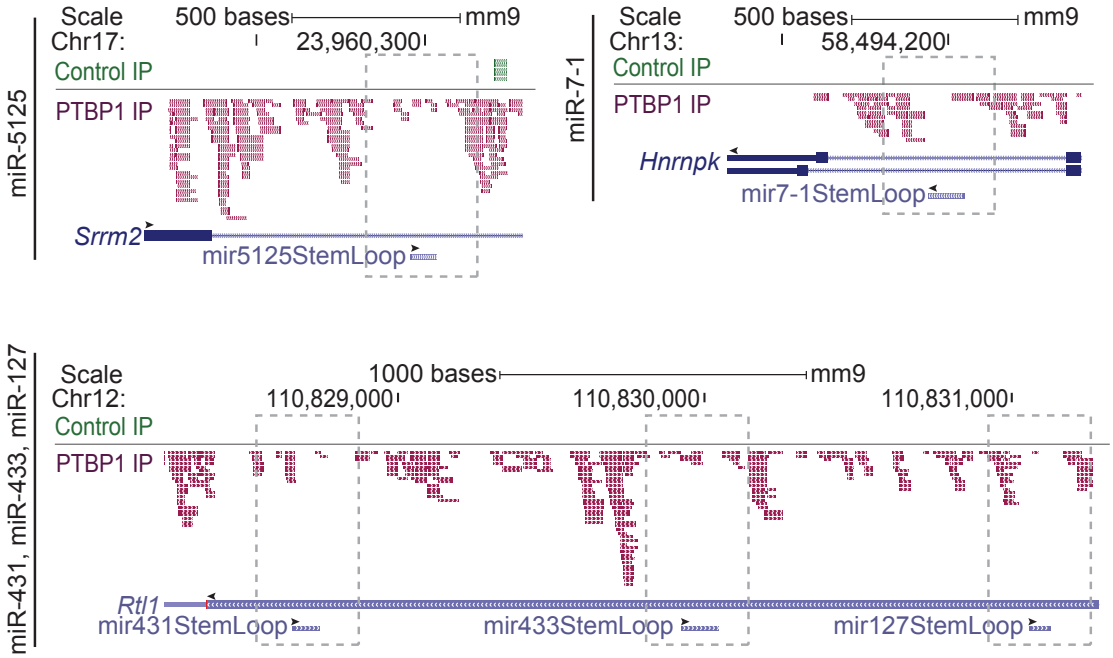


Fig. S4 (Related to Figure 2B). PTBP1 iCLIP tags mapping to miRNA loci in mESCs.

A. List of top miRNAs bound by PTBP1. The sequence intervals extending from -125 nt upstream to + 125 nt downstream of the pre-miRNA stem-loop for all annotated miRNAs (miRbase, Release 21) were assessed for PTBP1 iCLIP tags (left, miRNA stem-loop in solid line; upstream - 125 nt and downstream + 125 nt

in dashed line; assessed range for PTBP1 iCLIP tags in the grey dashed box). A total of 2020 PTBP1 iCLIP tags were found to map adjacent to 295 different miRNAs. These pri-miRNAs were ranked by the number of mapped tags and the top 10 are listed on the right panel.

B. Genome browser tracks (~ 1 kb and ~ 2 kb window) of miRNA host genes listed in A. Host transcripts for each miRNA are indicated (light purple). iCLIP tags from control IPs are marked in green and PTBP1-IP iCLIP tags are marked in magenta. The interval from - 125 nt upstream to + 125 nt downstream of each pre-miRNA stem-loop that was analyzed for miR-124-1 above is marked by a grey dashed box. Several miRNAs show extensive PTBP1 binding at more distal positions than those measured above. Arrowheads indicate the 5' to 3' orientation of each of RNA. Note that for the miR-431 locus at the bottom, miR-431, miR-433 and miR-127 are on the antisense strand, while Rtl1 is a sense transcript. All the PTBP1 iCLIP tags from this locus map in the antisense direction onto the presumptive but not annotated pri-miRNA.

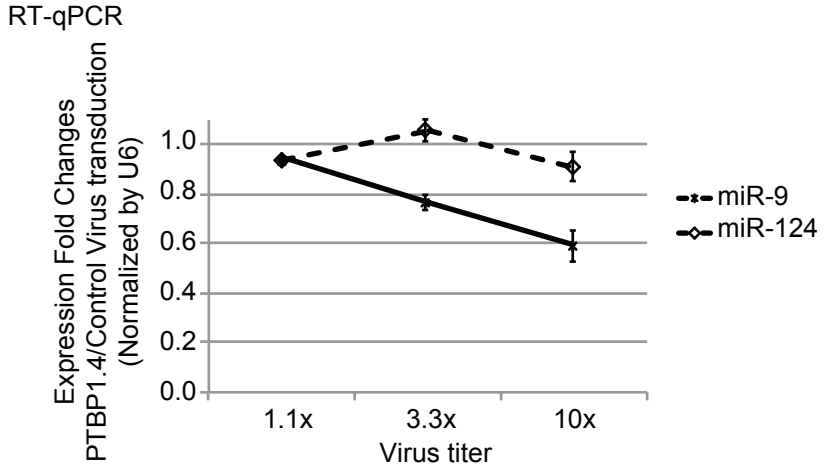
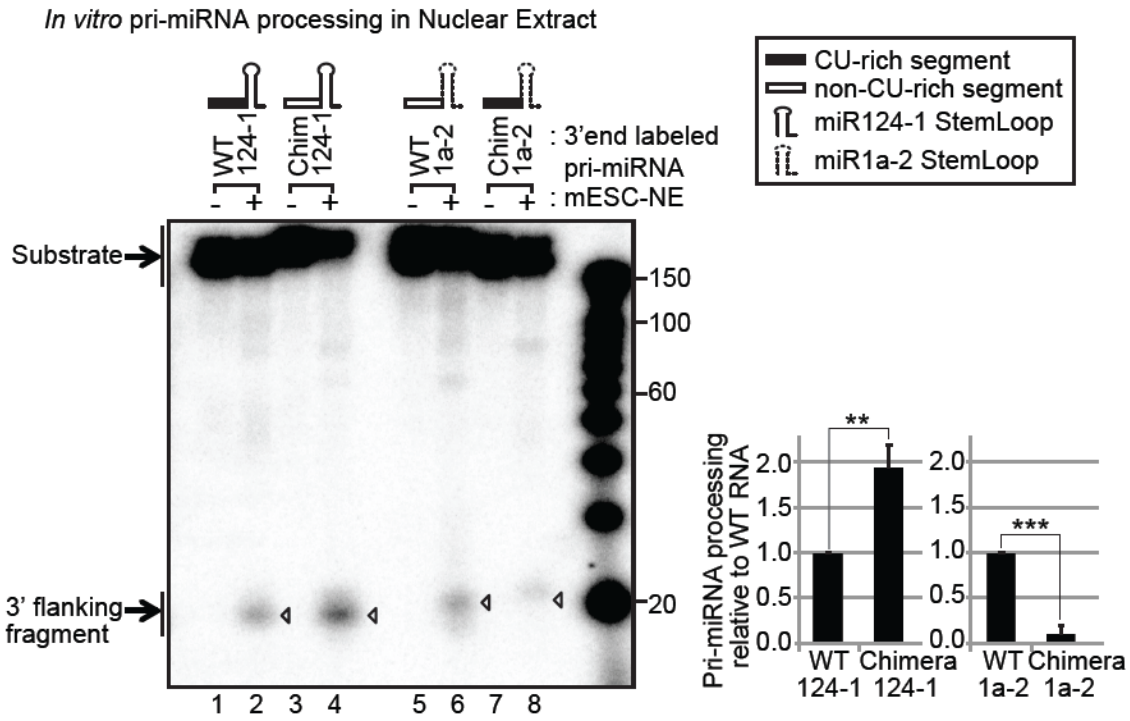


Fig. S5 (Related to Figure 3C). Dependency of miRNA levels on PTBP1-AAV titer in cortical neurons (mCtx).

Mature-miRNA levels were measured 8 days after AAV transduction (AAV2/9-nEF-tRFP-p2A-FLAG-PTBP1.4) into cultured cortical neurons. RT-qPCR values were normalized to U6 and to the control transduction (AAV2/9-nEF-tRFP). (n=2 biological replicates; error bars are s.e.m.)



Length (nt)\Pri-miR	WT124-1	Chimera124-1	WT1a-2	Chimera1a-2
Substrate	211	234	227	204
5'flanking fragment	130	153	147	124
Pre-miRNA	59	59	61	61
3'flanking fragment	22	22	19	19

Fig. S6 (Related to Figure 4A). *In vitro* processing of 3'-end labeled pri-miRNA in nuclear extract.

3'-end labeled pri-miR-124-1, pri-miR-1a-2 and chimeric substrates (~ 3 nM) were incubated in mESC nuclear extract (NE) active for DROSHA processing and cleaved products were resolved by 12.5 % Urea-PAGE. For the chimeric RNAs the upstream sequences of miR-124-1 (black box, containing the PTBP1 binding CU-rich segment) and miR-1a-2 (open box, non-CU-rich segment) were switched. The stem-loops of miR-124-1 and miR-1a-2 are indicated by solid and dashed lines, respectively. Concentrations of substrate RNAs were measured by nanodrop, and with equal concentrations used for each pri-miRNA processing reaction. Gel loading volumes were adjusted to the labeling efficiency for each

substrate. Un-processed substrates and the DROSHA processing products (3' flanking fragments) are indicated by arrows. Open arrowheads in lanes 2, 4, 6 and 8 indicate the 3' flanking fragments of pri-miR-124-1 (22nt), chimeric-miR-124-1 (22 nt), pri-miR-1a-2 (19 nt), and chimeric pri-miR-1a-2 (19 nt) released by DROSHA cleavage. Note that the 3' flanking fragments for pri-miR-1a-2 (19 nt) run more slowly on the gel than longer pri-miR-124-1 (22 nt) fragments presumably due to sequence specific effects from these short RNAs. A representative gel image is shown from 3 replicates (upper left panel). Pri-miRNA processing efficiency was calculated from the band intensity of the 3' flanking fragment divided by the intensity of the un-processed substrate (incubated with buffer only). This was compared to the WT construct in each replicate (upper right panel). (n=3 biological replicates; Student's t-test; ** $p \leq 0.01$, *** $p \leq 0.001$; error bars are s.e.m.) Substrate and expected DROSHA processing product sizes of each pri-miRNA are indicated in lower panel.

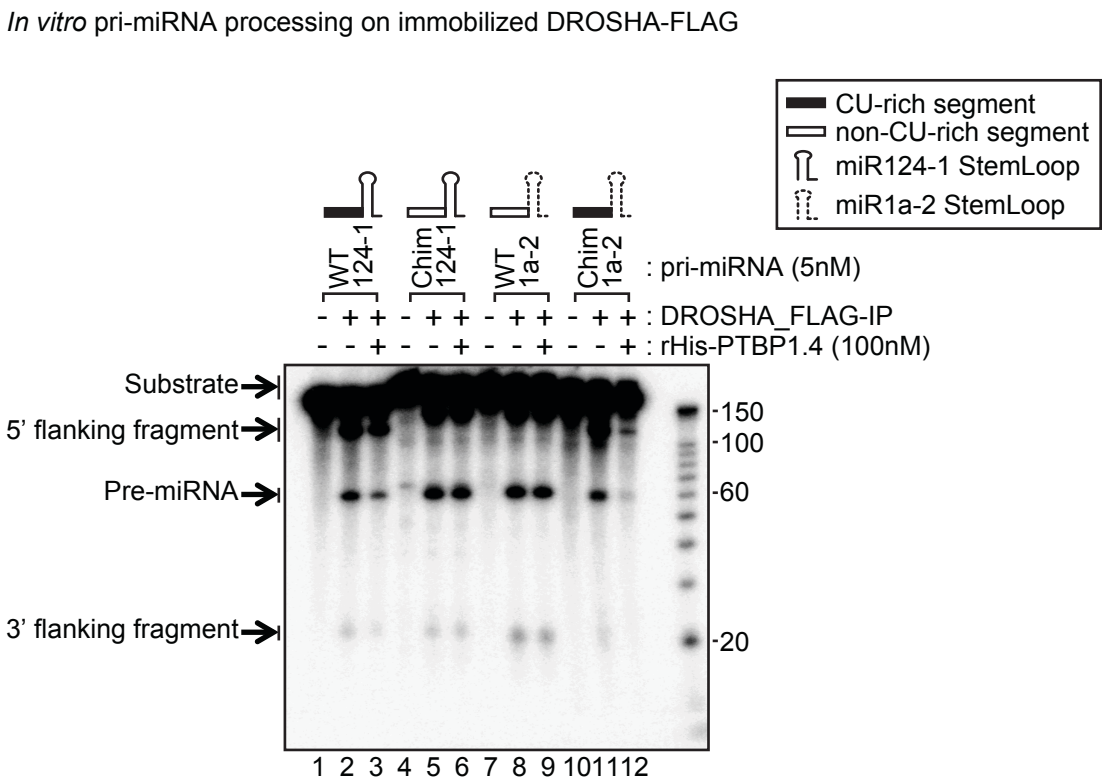


Fig. S7 (Related to Figure 4C). *In vitro* pri-miRNA processing on immobilized DROSHA-FLAG.

A longer exposure of Figure 4C allows visualization of the released 3' flanking fragments.

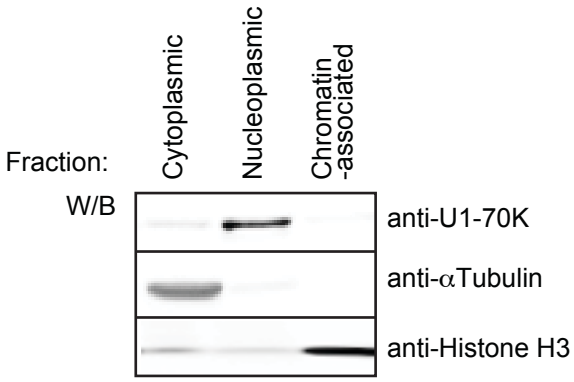


Fig. S8 (Related to Figure 5). Validation of subcellular fractionation.

Quality of fractionation was assessed by western blot for separation of diagnostic proteins; U1-70K for nucleoplasm, alpha-Tubulin for cytoplasm and Histone H3 for chromatin pellet. Gel image is one of 3 biological replicates.

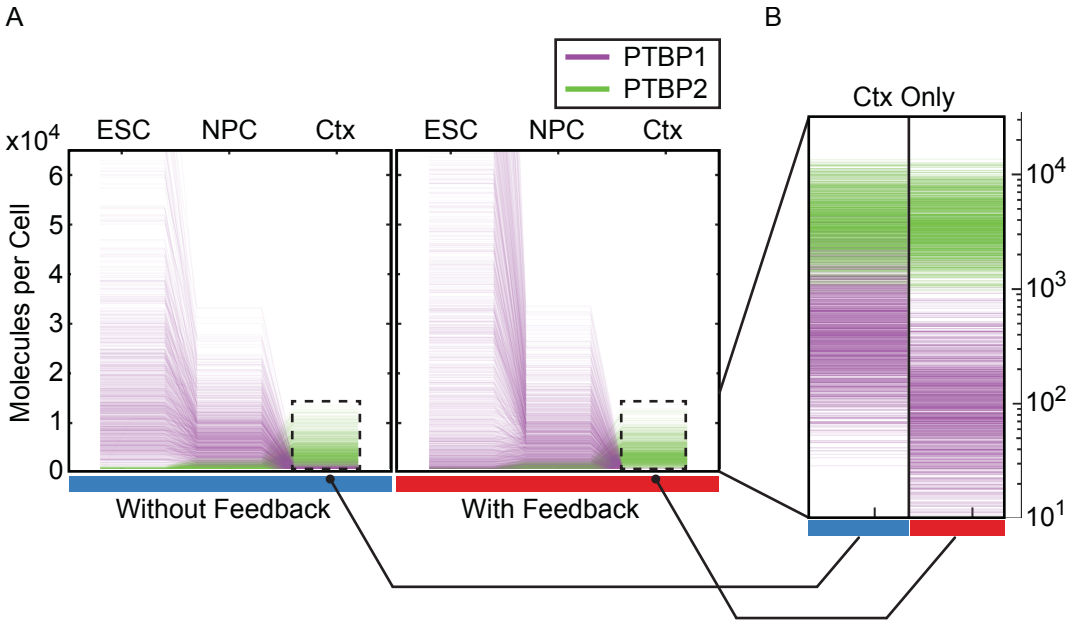


Fig. S9 (Related to Figures 6D and 6E). Single-cell model time-courses with distributed parameters.

A. PTBP1 and PTBP2 concentrations during modeled single cell differentiation from embryonic stem cell (ESC) to neuronal progenitor cell (NPC) to cortical neuron (Ctx). PTBP1 (purple) and PTBP2 (green) concentrations in 1000 single-cell simulations in the model without inhibition of miR-124-1processing (left, blue bar) and with inhibition of miR-124-1 processing (right, red bar) during a timecourse simulation of neuronal differentiation from ESC to NPC and to Ctx. Parameters were sampled from a distribution centered on the optimal parameter set identified by parameter estimation (Figures 6A and 6B) with maximum and minimum covering a 4-fold range around this parameter.

B. Expanded view of PTBP1 and PTBP2 concentrations in Ctx in the model without and with inhibition of miR-124-1 processing.

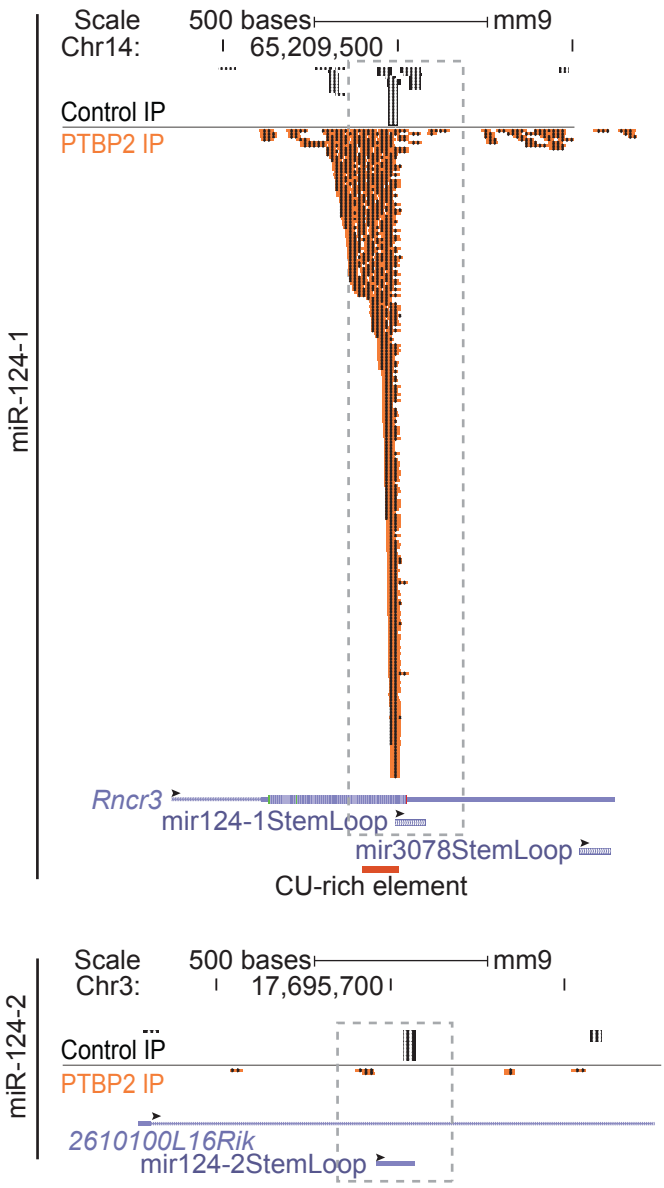


Fig. S10. PTBP2 and control iCLIP tags mapping to pri-miR-124-1 loci in mouse brain.

Genome browser tracks (~ 1 kb window) of the miR-124 loci with control (black) and PTBP2 (orange) iCLIP tags isolated from mouse brain (21). Sequence intervals from - 125 nt upstream to + 125 nt downstream of each annotated miRNA stem-loop is marked with a grey dashed box. The stem-loop of the

miRNA and the CU-rich element is indicated. Arrowheads indicate 5’ to 3’ direction of each of RNA.

Table S1. Quantification of total RNA and total protein per cell.

	N2a	mESC	mNPC	mCtx
Total RNA (pg) per cell	11.9 ± 0.5	19.1 ± 0.8	10.8 ± 0.5	10.4 ± 0.6
Total Protein (ng) per cell	0.52 ± 0.03	0.37 ± 0.02	0.44 ± 0.01	0.46 ± 0.03

Total RNA was extracted from cells, treated with DNaseI and measured by Nanodrop spectrophotometer. Total protein was extracted in RIPA buffer followed by protein BCA quantification. RNA and protein values were divided by cell number, as determined by hemacytometer counting. N2a, mouse neuroblastoma cells; mESC, E14 mouse embryonic stem cells; mNPC, mouse neuronal progenitor cells; mCtx, mouse cortical neurons. The mean ± s.d. of 3 cultures are given.

Table S2. Sequences of oligos, primers and RNAs used in this study.

Use	Target [Purpose]	Name	Sequence (5' to 3')
RT-PCR	mmu-pri-miR-124-1 (spanning)	F	CTTCCATCCTTCCTTCCTTTCC
		R	CTAAGGGCTACTTTGCGAGG
	mmu-pri-miR-124-2 (spanning)	F	CTCCAAGCCTAGAGCTCCAAG
		R	GAGGGGTCCTTGTGTCGCAC
	mmu-pri-miR-124-3 (spanning)	F	GCGAAGAAGCTGGAGCATTCGC
		R	GGATTTGTCCGCGGAGCGAG
	mmu-pri-miR-133a-1 (spanning)	F	CAACTGGTACACTAGTGTGGG
		R	CTTCCAAATAAGGTTGACAGTTGC
	mmu-pri-miR-9-1 (spanning)	F	GAGGTCTCGTCGTGATAGTGCG
		R	GGGCTGAGCAACCTTTGAAGG
	mmu-pri-miR-294/295 (spanning)	F	GGAAGTGACCCAGATTTGACAG
		R	GTTCCAGGAAACCTTCATCTGG
	mmu-pri-miR-124-1 (upstream)	F	GGCTGTTATCTCATTGTCTGTG
		R	GAAGGAAGGATGGAAGAGAGG
RT-qPCR	mmu-pri-miR-124-1 [RT]	RT	AGAAAAAGCTGGCGACGCGTG
	mmu-pri-miR-124-1 [q-PCR] (upstream)	F	GGCTGTTATCTCATTGTCTGTG
		R	GGACCACCGCATCCTTCCTAC
	mmu-pri-miR-124-1 [Standard cDNA]	Std	AACGCGATGAGCTAAGGACCACCGCATCC TTCCTACCGCAGCTCCCCCAGTCACAGAC AATGAGATAACAGCC
	mmu-pri-miR-124-2 [RT]	RT	CCCCTCTGGTACAGATCTAATTTCG
	mmu-pri-miR-124-2 [q-PCR] (upstream)	F	GCAGCAGAACACTCATGGTGTG
		R	GCCGTCCTAAGACATTTGTAAGG
	mmu-pri-miR-124-2 [Standard cDNA]	Std	CGCCCTGAATGCCGTCCTAAGACATTTGT AAGGAAAGGCAAGTCTTTAAGGACCACAC CATGAGTGTTCTGCTGC
	mmu-pri-miR-124-3 [RT]	RT	CTCCCCGAGGTAGAGAAGGGC
	mmu-pri-miR-124-3 [q-PCR] (upstream)	F	GAAGACTGCAGCAGGCCGGAG
		R	CGAGGGAGATGCAGGAGCC
	mmu-pri-miR-124-3 [Standard cDNA]	Std	GGAGCCGCGGCGAGGGAGATGCAGGAGC CGGCCGGGGTGAGCGCGCGTGCTCCGGC CTGCTGCAGTCTTC
	mmu-U6 [RT]	RT	TATGGAACGCTTCACGAATTTGCGTGT
	mmu-U6 [q-PCR]	F	GCTTCGGCAGCACATATACTAAAAT
		R	CGCTTCACGAATTTGCGTGTCAT
	mmu-U6 [Standard cDNA]	Std	TATGGAACGCTTCACGAATTTGCGTGTCAT CCTTGCGCAGGGGCCATGCTAATCTTCTC TGTATCGTTCCAATTTTAGTATATGTGCTG CCGAAGC
	mmu-miR-124-3p [Standard RNA]	Std	UAAGGCACGCGGUGAAUGCC
	mmu-miR-294-3p [Standard RNA]	Std	AAAGUGCUUCCCUUUUGUGUGU

Cloning to pAAVnEF_tRFP	p2A-FLAG_hsa-PTBP1.4	F	GCTAGCGGCTCCGGAGCCACGAACTTCTC TCTGTTAAAGCAAGCAGGAGACGTGGAAG AAAACCCCGGTCCCGACTACAAGGACGAC GACGACAAGATGGACGGCATCGTCCCAGA TATAGC
		R	GATATCCTAGATGGTGGACTTGGAGAAGG
Making Chimera pri-miRNAs	mmu-pri-Chimera-124-1	F:T71a	TAATACGACTCACTATAGGGAGACAGTCTC TGATAAGCTAATCTCCGCACTGG
		R:fs1a	CGGAGAGAGAGGCCTGTAGGCACTCCTG CGCCGGCCGATAG
		F:fs124	GGAGTGCCTACAGGCCTCTCTCTCCGTGT TCAC
		R:124	GTGCTCAGACAGCCCCATTC
	mmu-pri-Chimera-miR-1a-2	F:T712 4	TAATACGACTCACTATAGGGAGAGATATCT CCCATCCTCCCTCTCTTCC
		R:fs12 4	GTATGTGCTCTGATTCTCCTGAGGAAGGA AGAAGGAAGG
		F:fs-1a	CTCAGGAGAATCAGAGCACATACTTCTTTA TGTACC
		R:1a	CGGACATTACCTACCCAAAATACATACTTC
	Pri-miR-124-1 [Biotinylated DNA adapter]	AdtO1	5'biotin-GGGAGAATAGATAGCA
	Chimera 1+124 [Biotinylated DNA adapter]	AdtO2	5'biotin-CTACGTAGATATGAGGC
Pull down and pri-miRNA processing	T7-mmu-pri-miR-(WT)124-1 [Pull-down assay]	F	TAATACGACTCACTATAGGGAGAGATaTCT CCCATCCTCCCTCTCTTCC
		R: AdtO1	GGGAGAATAGATAGCAGTGCTCAGACAGC CCCATTC
	T7-mmu-pri-miR-Chimera124-1 [Pull-down assay]	F	TAATACGACTCACTATAGGGAGACAGTCTC TGATAAGCTAATCTCCGCACTGG
		R: AdtO2	CTACGTAGATATGAGGCGTGCTCAGACAG CCCCATTC
	T7-mmu-pri-miR-(WT)124-1	F	TAATACGACTCACTATAGGGAGAGATATCT CCCATCCTCCCTCTCTTCC
		R	GTGCTCAGACAGCCCCATTC
	T7-mmu-pri-miR-(WT)1a-2	F	TAATACGACTCACTATAGGGAGACAGTCTC TGATAAGCTAATCTCCGCACTGG
		R	CGGACATTACCTACCCAAAATACATACTTC
	T7-mmu-pri-miR-Chimera1a-2	F	TAATACGACTCACTATAGGGAGAGATaTCT CCCATCCTCCCTCTCTTCC
		R	CGGACATTACCTACCCAAAATACATACTTC
	T7-mmu-pri-miR-Chimera124-1	F	TAATACGACTCACTATAGGGAGACAGTCTC TGATAAGCTAATCTCCGCACTGG
		R	GTGCTCAGACAGCCCCATTC

Complete sequence of *in vitro* transcribed RNA	mmu-pri-miR-(WT)124-1 [Pull-down assay]		GGGAGAGATATCTCCCATCCTCCCTCTCTT CCATCCTTCCTTCCTTTCCATCCTTCCTTC TTTCTTTCCTTCCTTCCTTCTTTCTTTCCTT CCTTCCTTCTTCCTTCCTCAGGAGAAAGGC CTCTCTCTCCGTGTTCACAGCGGACCTTG ATTTAAATGTCCATACAATTAAGGCACGCG GTGAATGCCAAGAATGGGGCTGTCTGAGC ACTGCTATCTATTCTCCC
	mmu-pri-miR-Chimera124-1 [Pull-down assay]		GGGAGACAGTCTCTGATAAGCTAATCTCC GCACTGGATCCATTACTCTTCCAGAAAAGT AATCCAAATGTCCTACTAACAGCTGGGTGA GGTGGGCTGCTTCATGTTTTCACAGCTATC GGCCGGCGCAGGAGTGCCTACAGGCCTC TCTCTCCGTGTTCACAGCGGACCTTGATTT AAATGTCCATACAATTAAGGCACGCGGTG AATGCCAAGAATGGGGCTGTCTGAGCACG CCUCAUAUCUACGUAG
	mmu-pri-miR-(WT)124-1 [*In vitro* pri-miRNA processing]		GGGAGAGATaTCTCCCATCCTCCCTCTCTT CCATCCTTCCTTCCTTTCCATCCTTCCTTC TTTCTTTCCTTCCTTCCTTCTTTCTTTCCTT CCTTCCTTCTTCCTTCCTCAGGAGAAAGGC CTCTCTCTCCGTGTTCACAGCGGACCTTG ATTTAAATGTCCATACAATTAAGGCACGCG GTGAATGCCAAGAATGGGGCTGTCTGAGC AC
	mmu-pri-miR-(WT)1a-2 [*In vitro* pri-miRNA processing]		GGGAGaCAGTCTCTGATAAGCTAATCTCC GCACTGGATCCATTACTCTTCCAGAAAAGT AATCCAAATGTCCTACTAACAGCTGGGTGA GGTGGGCTGCTTCATGTTTTCACAGCTATC GGCCGGCGCAGGAGTGCCTACTCAGAGC ACATACTTCTTTATGTACCCATATGAACATT CAGTGCTATGGAATGTAAAGAAGTATGTAT TTTGGGTAGGTAATGTCCG
	mmu-pri-miR-Chimera1a-2 [*In vitro* pri-miRNA processing]		GGGAGAGATaTCTCCCATCCTCCCTCTCTT CCATCCTTCCTTCCTTTCCATCCTTCCTTC TTTCTTTCCTTCCTTCCTTCTTTCTTTCCTT CCTTCCTTCTTCCTTCCTCAGGAGAATCAG AGCACATACTTCTTTATGTACCCATATGAA CATTCAGTGCTATGGAATGTAAAGAAGTAT GTATTTTGGGTAGGTAATGTCCG
	mmu-pri-miR-Chimera124-1 [*In vitro* pri-miRNA processing]		GGGAGaCAGTCTCTGATAAGCTAATCTCC GCACTGGATCCATTACTCTTCCAGAAAAGT AATCCAAATGTCCTACTAACAGCTGGGTGA GGTGGGCTGCTTCATGTTTTCACAGCTATC GGCCGGCGCAGGAGTGCCTACAGGCCTC TCTCTCCGTGTTCACAGCGGACCTTGATTT AAATGTCCATACAATTAAGGCACGCGGTG AATGCCAAGAATGGGGCTGTCTGAGCAC

Underlines indicate sequences of T7 promoter.

Table S3. List of TaqMan miRNA assays for RT-qPCR of mature-miRNA.

Target	miRBase ID/ NCBI Accession ID	Assay ID (Thermo Fisher)
mmu-miR-124a	mmu-miR-124-3p	4427975-001182
mmu-miR-294	mmu-miR-294-3p	4427975-001056
mmu-miR-9	mmu-miR-9-5p	4427975-000583
mmu-let7-e	mmu-let-7e-5p	4427975-002406
U6 snRNA (Control)	NR_004394	4427975-001973

Table S4. List of antibodies used in this study.

Target	Antibody name (host/Source)	Used in
PTBP1	PTB_NT (Rabbit/DB lab made)	W/B, Absolute Quantification and PTB-IP
PTBP2	PTB_IS2 (Rabbit/DB lab made)	W/B and Absolute Quantification
PTBP1/2	PTB_CT (Rabbit/DB lab made)	W/B
DGCR8	NC1 (Rabbit/FG lab made)	W/B
GAPDH	GPADH 6C5 (Mouse/Thermo Fisher Scientific, AM4300)	W/B
U1-70K	SNRNP70 (Rabbit/DB lab made)	W/B
alpha-tubulin	alpha-tubulin DM1A(Mouse/Millipore, CP06)	W/B
Histone H3	Histone H3 (Rabbit/Abcam, ab1791)	W/B
-	Rabbit IgG isotype Control (Rabbit/Thermo Fisher Scientific, 02-6102)	W/B and Control-IP
rabbit IgG light chain	HRP conjugated Anti-Rabbit IgG, Light Chain Specific (Mouse/Jackson ImmunoResearch, 211-032-171)	W/B (Figure 2C)

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