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Supplemental Information

Synergic Functions of miRNAs Determine Neuronal Fate of Adult Neural Stem Cells

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SUPPLEMENTAL INFORMATION

Supplemental Figures and Figure Legends

Figure S1. Related to Figure 1 and Figure 2.

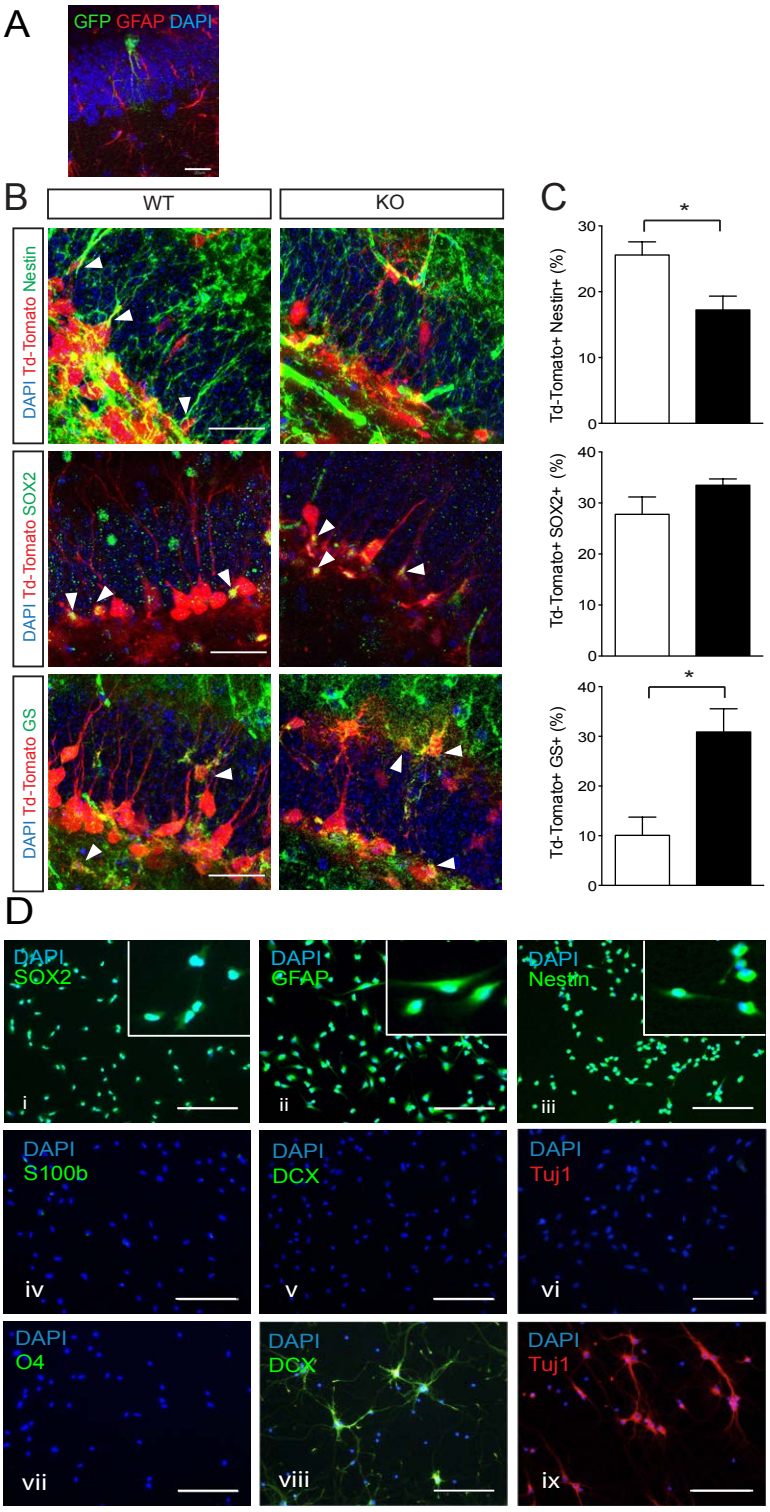

Figure S1. Characterization of adult neural stem cells subject of the study *in vivo* and *in vitro*. A: Radial glial cell (type 1 aNSC) labelled with Split-Cre virus 28 days post injection in CAG CAT GFP reporter mice. Scale bar = 20µm. B-C: Representative micrographs (B) and quantification (C) of

recombined Td-Tomato+ cells from *Dicer*$^{wt/wt}$ *Td-Tomato*$^{flox/wt}$ (WT) and *Dicer*$^{flox/flox}$ *Td-Tomato*$^{flox/wt}$ (KO) mice co-expressing Nestin (upper panel), SOX2 (middle panel) or GS (bottom panel) 2 months after Split-Cre virus injections. Data expressed as mean +/-SEM. N= 4-6 mice per group. Scale bar = 20µm. Unpaired-*t-test* *$p < 0.05$. D: aNSCs cultured in proliferative conditions express stem cell markers such as SOX2 (i), GFAP (ii) and Nestin (iii). aNSCs do not express markers of glial differentiation such as S100b (iv) and O4 (vii); or markers of neuronal differentiation such as DCX (v) and Tuj1 (vi). Mouse Adult Neurons show the staining for DCX (viii) and Tuj1 (ix) as positive controls. Scale bar = 50µm.

Figure S2. Related to Figure 2 and Figure 3.

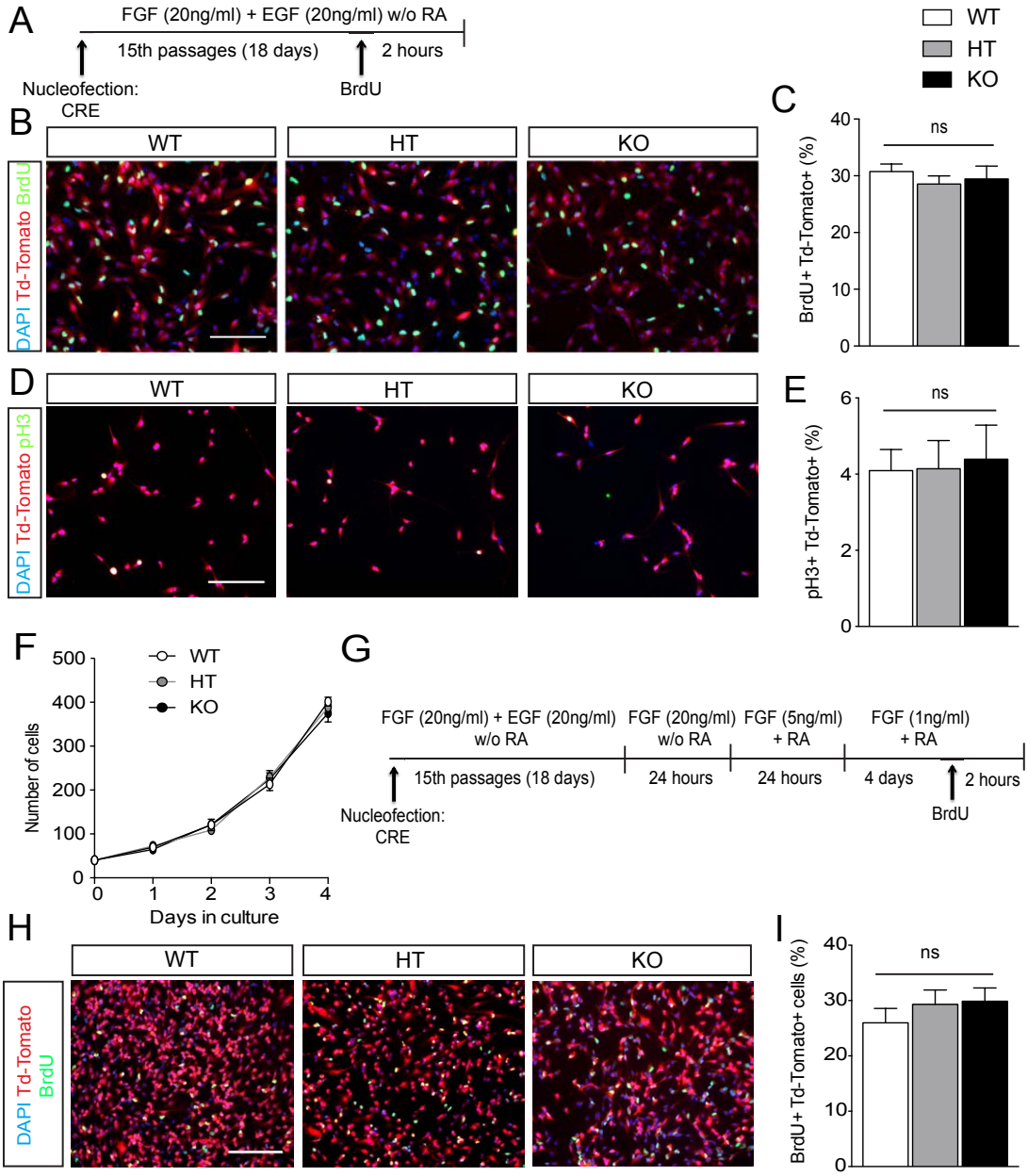

Figure S2. Dicer/miRNA depletion does not affect aNSCs proliferation *in vitro*. A: Schematic representation of the procedure used to assess proliferation after Dicer ablation. B and D: Representative micrographs showing Td-Tomato+ aNSCs from *Dicer*$^{wt/wt}$ *Td-Tomato*$^{flox/wt}$ (WT), *Dicer*$^{flox/wt}$ *Td-Tomato*$^{flox/wt}$ (HT) and *Dicer*$^{flox/flox}$ *Td-Tomato*$^{flox/wt}$ (KO) mice expressing BrdU (B and H) or pH3 (D). C and E: Percentage of Td-Tomato+ cells expressing BrdU after a 2 hours pulse (C) or pH3 (E). F: Growth curve representing the number of cells per field along days of aNSCs under proliferating conditions. G: Schematic representation of the procedure used to assess proliferation under differentiating conditions. I: Percentage of Td-Tomato+ cells expressing BrdU. Scale bar = 50µm. Data expressed as mean +/-SEM. N= 3 independent experiments containing 3 replicates. One-way ANOVA Bonferroni as *post-hoc*.

Figure S3. Related to Figure 2.

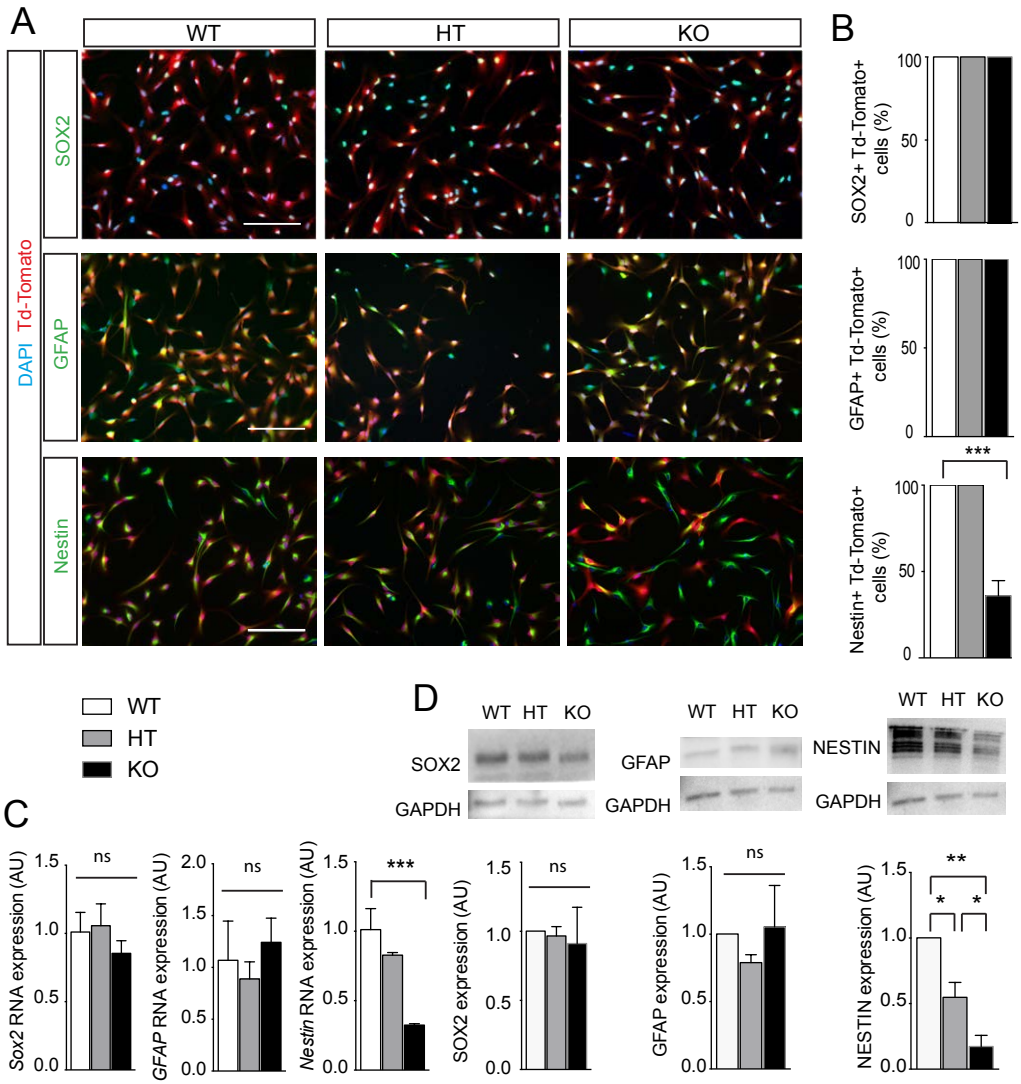

Figure S3. Dicer/miRNA depletion does not affect the expression of aNSCs markers Sox2 and GFAP but impairs Nestin expression *in vitro*. A: Representative micrographs showing recombined aNSCs (Td-Tomato+) from *Dicer*$^{wt/wt}$ *Td-Tomato*$^{flox/wt}$ (WT), *Dicer*$^{flox/wt}$ *Td-Tomato*$^{flox/wt}$ (HT) and *Dicer*$^{flox/flox}$ *Td-Tomato*$^{flox/wt}$ (KO) mice expressing SOX2 (upper panels), GFAP (middle panels) and Nestin (bottom panels). B: Percentage of Td-Tomato+ cells expressing aNSCs markers (SOX2, GFAP and Nestin). C: Relative *Sox2*, *GFAP* and *Nestin* mRNA quantification with qPCR from recombined aNSCs. D: SOX2, GFAP and NESTIN protein quantification from recombined aNSCs cultures. Scale bar = 50µm. Data expressed as mean +/-SEM. N= 3 independent experiments containing 3 replicates. One-way ANOVA Bonferroni as *post-hoc* * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Figure S4. Related to Figure 3.

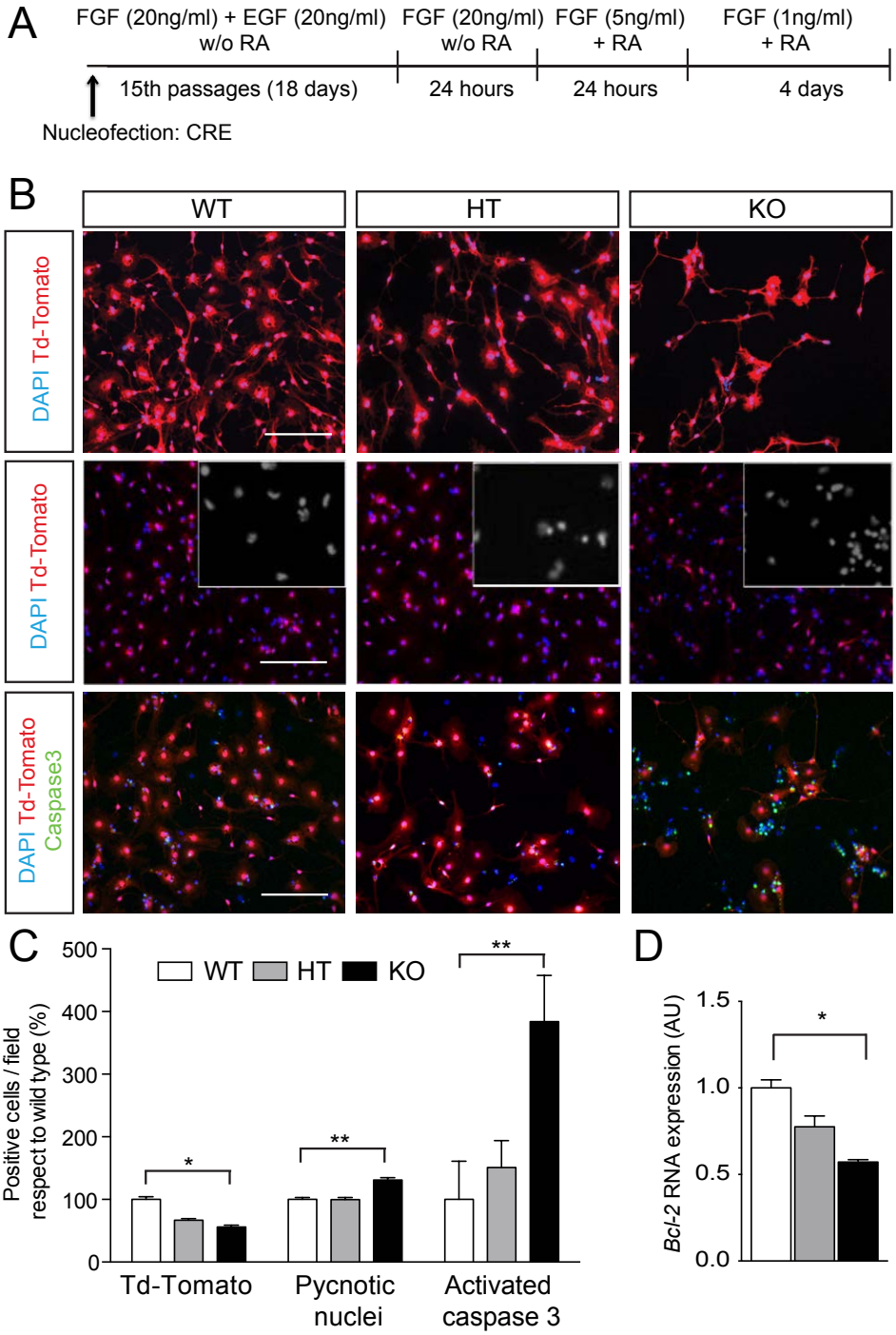

Figure S4. *Dicer*/miRNA depletion increases apoptosis of hippocampal aNSCs upon differentiation *in vitro*. A: Schematic representation of the procedure used to assess survival after Dicer ablation. B: Representative micrographs showing Td-Tomato+ aNSCs after 6 DIV with growth factors titration from *Dicer*$^{wt/wt}$ *Td-Tomato*$^{flox/wt}$ (WT), *Dicer*$^{flox/wt}$ *Td-Tomato*$^{flox/wt}$ (HT) and *Dicer*$^{flox/flox}$ *Td-Tomato*$^{flox/wt}$ (KO) mice that are surviving (upper pannels), dying with pycnotic nuclei (middle pannels) and expressing activated caspase 3 (bottom panels). C: Percentage of Td-Tomato+ cells, pycnotic nuclei and Td-Tomato+ cells expressing activated caspase 3 per field normalized per WT aNSCs after 6DIV. D: Relative *Bcl-2* mRNA quantification with qPCR from recombined aNSCs after 6DIV. Scale bar = 50µm. Data expressed as mean +/-SEM. N= 3 independent experiments containing 3 replicates. One-way ANOVA Bonferroni as *post-hoc* *p < 0.05; **p < 0.01; ***p < 0.001.

Figure S5. Related to Figure 5.

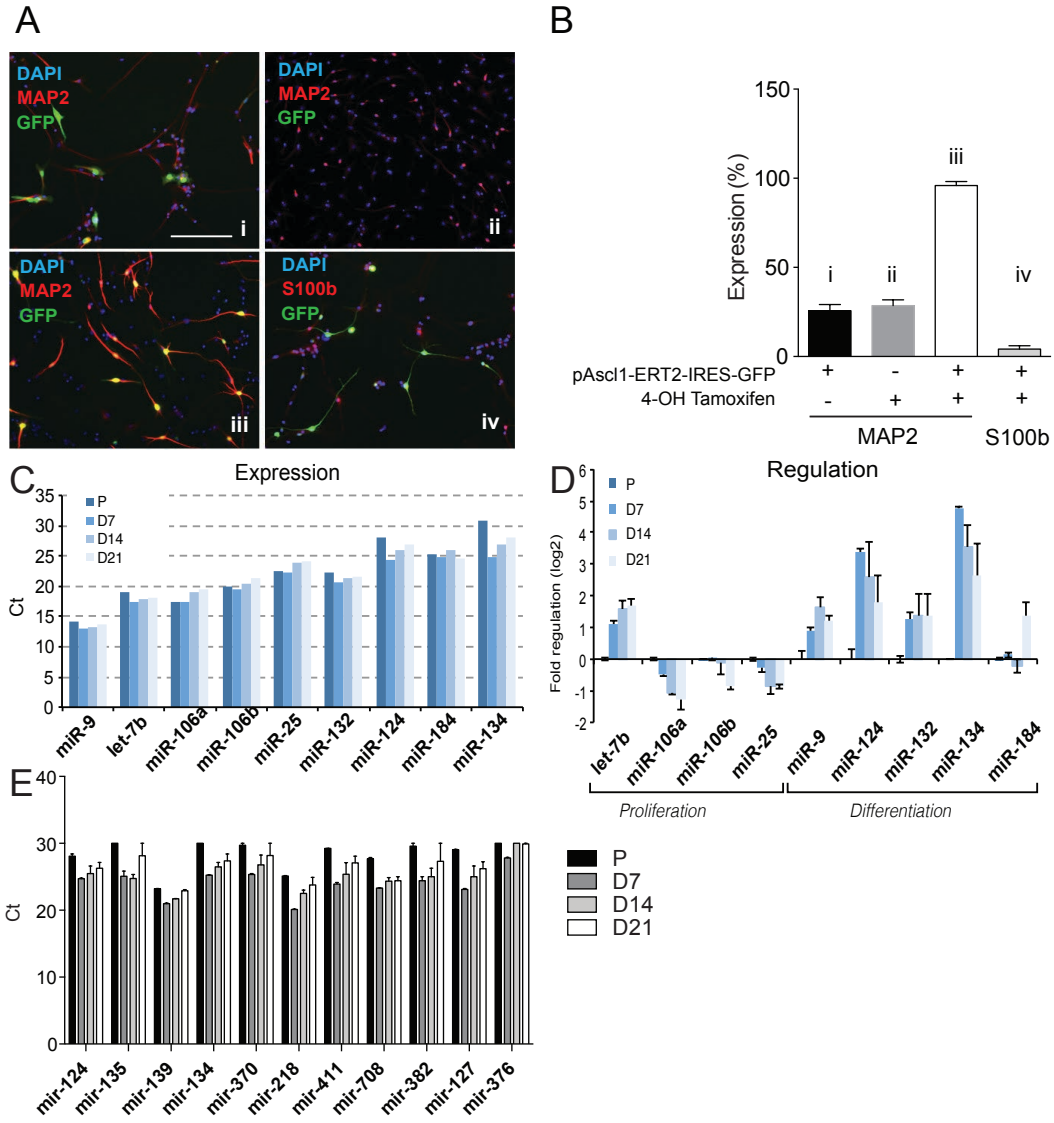

Figure S5. Expression of neurogenesis-induced miRNAs in differentiated hippocampal aNSCs using an inducible retrovirus expressing Ascl1 (*Ascl1-ERT2-IRES-GFP*). After 6 DIV, upon 4-OH tamoxifen administration, 95% of infected cells (GFP+) differentiate to neurons. A: Representative micrographs showing the efficiency of neuronal differentiation upon Ascl1 expression in hippocampal aNSCs at 6 DIV. B: Percentage of MAP2 or S100b expressing cells respect to GFP+ infected cells (i, iii and iv) or DAPI (ii) under different conditions at 6 DIV. Scale bar = 50µm. C: Expression levels of miRNAs known to be involved on neurogenesis as reported by Schouten et al., 2012. D: Fold regulation of known miRNAs during neuronal differentiation. E: Expression levels of selected miRNAs in this study during proliferation (P) or differentiation after 7 (D7), 14 (D14) and 21 (D21) DIVs. Data expressed as mean +/-SEM. N= 3 independent experiments containing 3 replicates.

Figure S6. Related to Figure 6.

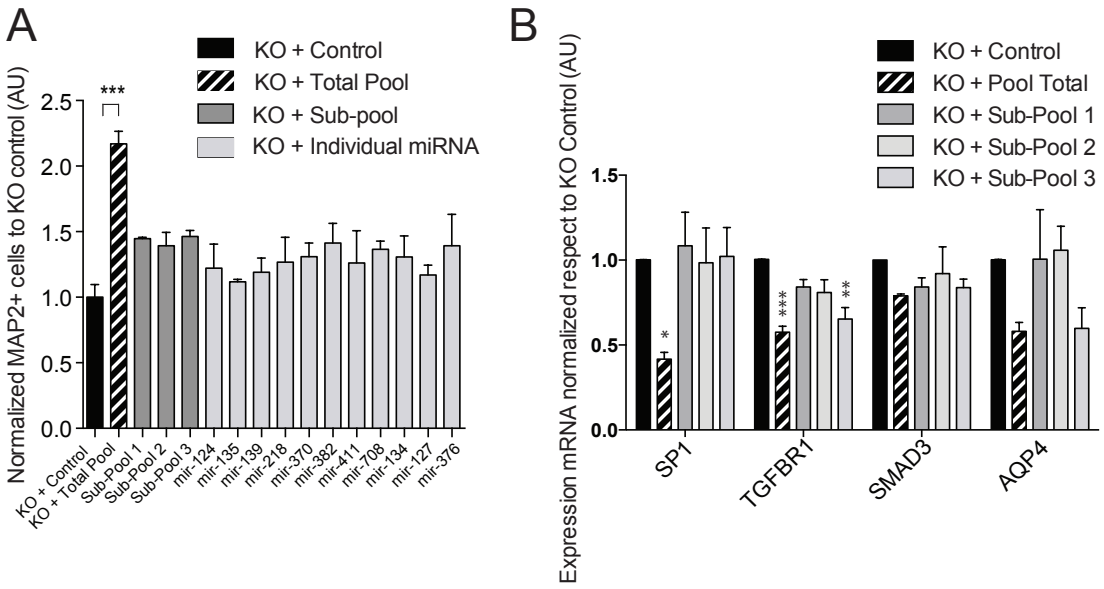

Figure S6. A pool of eleven miRNAs synergistically rescues Dicer-cKO impairment of adult neurogenesis. A: Proportion of KO aNSCs expressing MAP2 upon transfection of 250nM of Sub-Pool 1 (220nM scrambled RNA + 25nM mir-124-3p + 25nM mir-135a-5p), 250nM of Sub-Pool 2 (75nM scrambled RNA + 25nM mir-139-5p + 25nM mir-218-5p + 25nM mir-411-5p + 25nM mir-134-5p + 25nM mir-370-3p + 25nM mir-382-5p + 25nM mir-708-5p), 250nM of Sub-Pool 3 (220nM scrambled RNA + 25nM mir-127-3p + 25nM miR-376b-3p), or each miRNA alone (225nM scrambled RNA + 25nM specific miRNA) respect to KO control. B: Expression of mRNA with qPCR of negative regulators of neuronal differentiation or astrocyte inducers from recombined KO aNSCs transfected with 250nM of scrambled RNA, 250nM of Total Pool or Sub-pools after 6 DIV. Data expressed as mean +/-SEM. N= 3 independent experiments containing 3 replicates. One-way ANOVA Bonferroni as *post-hoc* *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Figure S7. Related to Figure 6.

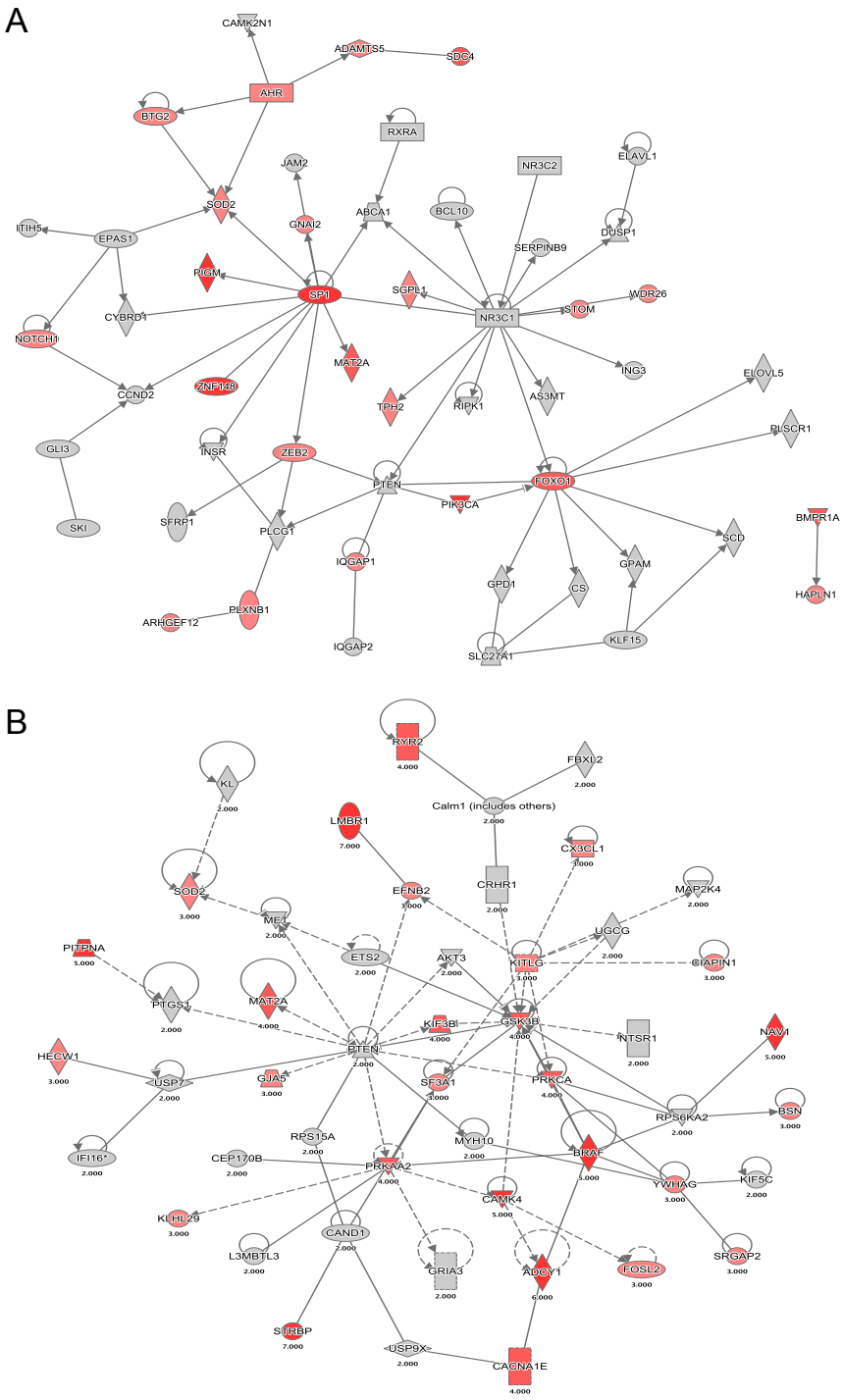

Figure S7. Gene networks of astrocytes and neurons. Representation of connected predicted targets of at least 2 of the eleven miRNAs (previously selected with Mirwalk 2.0) that are a hallmark of astrocytes (A) and neurons (B) as described by Cahoy et al., 2008. Genes targeted by 3 or more miRNAs are indicated in red (more intensity = more miRNAs are targeting the selected gene).

Table S1. Related to Figure 5. Predicted targets of at least two of the eleven miRNAs selected with Mirwalk 2.0.

Table S2. Related to Figure 5. Predicted targets of at least five of the eleven miRNAs selected with Mirwalk 2.0. Predicted targets known to have a role on neuronal and astrocyte differentiation are indicated.

Table S3. Related to Figure 7. Proteomics expression raw data.

Supplemental experimental procedures

Animals

Genotyping of mice carrying the *Dicer*^{flox} allele were performed by PCR following published protocols (Andl et al., 2006); and Td-Tomato according to Jackson laboratories instructions. Animals were 6-8 weeks-old at the time of the Split-Cre viral injections, or 6-8 weeks old for aNSCs preparation.

Virus injection

8 weeks-old mice were anesthetized with isoflurane and 1 µl of virus mix (Split-Cre N-Cre:C-Cre) per DG was stereotaxically injected at the following coordinates: −2.0 anterior/posterior, ±1.6 medial/lateral, and −1.9 to −2.1 dorsal/ventral relative to bregma (in millimeters) as previously described (Beckervordersandforth et al., 2014). One month later, mice received one BrdU intraperitoneal injection per day (50mg/kg) during 5 days. Animals were sacrificed 10 days or 1 month after BrdU injections.

Mice were anesthetized with intraperitoneal administration of ketamine (90mg/kg) and xylazine (5-7mg/kg), and subsequently perfused with PBS followed by 4% paraformaldehyde (PFA). Brains were harversted, postfixed overnight in 4% PFA, and then equilibrated in 30% sucrose. 40 µm brain sections were generated using a sliding microtome and were stored in a −20°C freezer as floating sections in 48 well plates filled with cryoprotectant solution (glycerol, ethylene glycol, and 0.2 M phosphate buffer, pH 7.4, 1:1:2 by volume).

aNSC preparation and culture

DG was isolated from 8-10 mice at the age of 6-8 weeks per genotype for each aNSCs culture preparation. After dissection in Hanks Balanced Salt Solution (HBSS, Gibco) medium, the tissue was enzymatically dissociated with papain (2,5U/ml), dispase (1U/ml) and DNaseI (250U/ml) for 20 min at 37°C. During incubation, the tissue was repeatedly triturated with a fire polished Pasteur pipette. The cell suspension was centrifuged at 130 g for 5 min and the pellet was re-suspended in buffer solution (1x HBSS, 30mM Glucose, 2mM HEPES pH 7,4, 26mM NaHCO3) followed by a centrifugation at 130 g for 5 min. aNSCs were isolated using 22% Percoll gradient solution. After further centrifugation for 5 min at 130 g the cell pellet was re-suspended in 2 ml of culture medium containing Neurobasal (Invitrogen), Glutamax (Invitrogen), 1% penicillin and streptomycin (Invitrogen), B27 without retinoic acid (Invitrogen), FGF (20ng/ml; PeproTech) and EGF (20ng/ml; PeproTech). The dissociated DG tissue was plated into PDL/Laminin (Sigma/Roche) coated wells and incubated at 37°C with 5% CO2. To further remove excess debris, the growth medium was exchanged 24 hours later. Every 2 days half of the growth medium was exchanged with fresh medium to replenish the growth factors. aNSCs were passaged once they reached 80% confluence.

Proliferation medium: Neurobasal, Glutamax, 1% penicillin and streptomycin, B27 without retinoic acid, supplemented with FGF (20ng/ml) and EGF (20ng/ml). 10 µM BrdU (Sigma-Aldrich) was added to aNSCs in proliferation medium 2 hours before fixation.

Neuronal differentiation: aNSCs were plated at 1,2*10E4 cells/cm2 in culture medium supplemented with FGF (20ng/ml) 24 hours. Then medium was exchanged with medium containing B27 with retinoic acid and FGF (5ng/ml) for 24 hours and FGF (1ng/ml) during the next four days. Cells were differentiated in culture for 6 Days in vitro (DIV).

Astrocyte differentiation medium: aNSCs were plated at a density of 1,2*10E4 cells/cm2 in growth medium with 10% FBS without growth factors for 6 DIV.

Retrovirus-mediated inducible neuronal differentiation: viral construct expressing Ascl1-ERT2 and infections conditions were previously described (Braun et al., 2013). Neuronal differentiation was induced by growth factor withdrawal in the presence of 0.5 mM OH-TAM (Sigma) for 2 days. The medium was changed every 2–3 days. Cells were fixed at 7, 14 or 21 days after the exposition to OH-TAM.

MiRNA administration: aNSCs from Dicerflox/flox Td-Tomatoflox/wt (KO) mice were transfected under proliferating conditions using Amaxa (Lonza) with 250nM of scrambled RNA, Total Pool (25nM of each miRNA), 250nM of Sub-Pool 1 (220nM scrambled RNA + 25nM mir-124-3p + 25nM mir-135a-5p), 250nM of Sub-Pool 2 (75nM scrambled RNA + 25nM mir-139-5p + 25nM mir-218-5p + 25nM mir-411-5p + 25nM mir-134-5p + 25nM mir-370-3p + 25nM mir-382-5p + 25nM mir-708-5p), 250nM of Sub-Pool 3 (220nM scrambled RNA + 25nM mir-127-3p + 25nM miR-376b-3p), each miRNA alone (225nM scrambled RNA + 25nM specific miRNA) or a pool of 10 miRNA by the withdrawal of individual miRNAs (25nM scrambled RNA + 225nM of 10 miRNA).

MiRNA mimics used for Nucleofection

miRIDIAN microRNA	Reference Dharmacon
mmu-miR-135a-5p	C-310411-05-0002
mmu-miR-376b-3p	C-310616-07-0002
mmu-miR-139-5p	C-310568-07-0002
mmu-miR-218-5p	C-310576-05-0002
mmu-miR-411-5p	C-310822-01-0002
mmu-miR-127-3p	C-310397-07-0002
mmu-miR-134-5p	C-310409-05-0002
mmu-miR-370-3p	C-310619-07-0002
mmu-miR-382-5p	C-310607-05-0002
mmu-miR-708-5p	C-310987-01-0002
mmu-miR-124-3p	C-310391-05-0002

MiRNA antagomirs used for Nucleofection

miRIDIAN microRNA Harpin Inhibitor	Reference Dharmacon
mmu-miR-135a-5p	IH-310411-07-0002
mmu-miR-376b-3p	IH-310616-08-0002
mmu-miR-139-5p	IH-310568-08-0002
mmu-miR-218-5p	IH-310575-07-0002
mmu-miR-411-5p	IH-310822-02-0002
mmu-miR-127-3p	IH-310397-08-0002
mmu-miR-134-5p	IH-310409-07-0002
mmu-miR-370-3p	IH-310619-08-0002
mmu-miR-382-5p	IH-310607-07-0002
mmu-miR-708-5p	IH-310987-02-0002
mmu-miR-124-3p	IH-310390-07-0002

Immunofluorescence

The immunofluorescence staining on brain slices was performed on sections covering the entire dorsal hippocampus (Bregma, -1.06 to -2.18 mm, Paxinos and Franklin, 2001). Sections were washed with 0.1M PBS during 40 min and pretreated with 2N HCL at 30,2°C for 30 min. After extensive washings with 0.1M PBS, sections were permeabilized with 0.3% PBS-T (PBS-Triton X-100) for 10 min followed with 20 min with 0.1% PBS-T. Sections were blocked during 2 h with 0.1% PBS-T and 5% FBS at room temperature (RT) followed by incubation with primary antibodies in a blocking solution overnight at 4°C. The next day, after washing extensively with 0.1% PBS-T sections were subsequently incubated for 1 h with the corresponding secondary fluorescent antibodies (1/1000; Goat or donkey Alexa 488, 568, and 647nm, Invitrogen). Sections were counterstained with Hoechst (1:300), mounted and cover slipped with mowiol reagent.

The immunofluorescence staining on cell cultures was performed after fixing aNSCs for 30 min with 4% paraformaldehyde (PFA) followed by extensive washings with PBS during 30 min. Cells were washed three times with PBS 0,1% Triton X-100 (PBS-T) and blocked during 2 hours with PBS-T containing 5% normal goat serum (Vector laboratories), followed by overnight incubation with primary antibodies. To detect BrdU incorporation, cells were pretreated with 2M HCl for 30 min at 37°C followed by washing with borate buffer, pH 8.5, for 30 min, before being subjected to immunofluorescence. The next day, after washing extensively with PBS-T, cells were incubated with secondary antibodies. Cells were mounted in mounting medium and counterstained with fluorescent nuclear dye DAPI (Invitrogen).

Primary Antibodies used for immunofluorescence

Antibody	Host	Company	Catalog	Dilution
BrdU	rat	Abcam	ab-6326	1:200
DCX	rabbit	Abcam	ab18723	1:1000
DCX	goat	Santacruz	SC8066	1:200
GFAP	rabbit	Dako	Z-0334	1:1000
S100b	mouse	Sigma	S2532	1:250-1:500
NeuN	mouse	Millipore	MAB377	1:250
GS	mouse	Millipore	MAB302	1:400
SOX2	rabbit	Millipore	AB5603	1:200-1:500
Nestin	mouse	Millipore	MAB353	1:250
Nestin	rat	BD-Pharmigen	556309	1:200
pH3	rat	Abcam	AB10543	1:500

Primers used for gene expression analysis by quantitative PCR

Primer Name	Sequence
Actin Fw	GGCTGTATTCCCCTCCATCG
Actin Rv	CCAGTTGGTAACAATGCCATGT
Aqp4 Fw	GAGTCACCACGGTTCATGGA
Aqp4 Rv	CGTTTGGAATCACAGCTGGC
Bcl-2 Fw	GGTATGCACCCAGAGTGATGC
Bcl-2 Rv	ATGCCTTTGTGGAACTATATGGC
Dicer Fw	CCTGACAGTGACGGTCCAAAG
Dicer Fw 2	GGAACGCTAACACATCTACCT
Dicer Rv	CATGACTCTTCAACTCAAACT
Dicer Rv 2	AGAGTCCATCCTGTCCTTGA
GAPDH Fw	TGCACCACCAACTGCTTAGC
GAPDH Rv	GGCATGGACTGTGGTCATGAG
GFAP Fw	GGGGCAAAAGCACCAAAGAAG
GFAP Rv	GGGACAACTTGTATTGTGAGCC
Nestin Fw	CCCTGAAGTCGAGGAGCTG
Nestin Rv	CTGCTGCACCTCTAAGCGA
S100b Fw	CTGGAGAAGGCCATGGTTGC
S100b Rv	CTCCAGGAAGTGAGAGAGCT
Smad3 Fw	CTGGGCCTACTGTCCAATGT
Smad3 Rv	CATCTGGGTGAGGAC CTTGT
Sp1 Fw	GGCAATAATGGGGGTAGCGG
Sp1 Rv	CAAGCTGGCAGAACTGATGGC
Tgfbr1 Fw	CATTCACCACCGTGTGCCAAATGA
Tgfbr1 Rv	ACCTGATCCAGACCCTGATGTTGT

Quantification of miRNAs

MiRNA profiling was performed on 200 ng of total RNA for each sample and Megaplex reactions were run with 12 cycles of pre-amplification. PreAmp products were diluted 1:4 in TE and 9 µl of the diluted reactions were loaded on microRNA cards. Arrays were run on a ViiA 7 Real-Time PCR system (Thermo Fisher). Raw data were exported to excel to be further analyzed. First, miRNA expression was normalized by using scaling factors calculated by median-centering the Cq values of the house-keeping genes included in each array (namely: U6, sno135, sno202, U87 and Y1). Undetermined values or Cq values >30.01 were arbitrarily set to 30.01, without any further normalization. Next, the scaled Cq values were centered on the median expression over all samples (Proliferating aNSCs, differentiating at DIV7, DIV14, DIV21). These values were used for hierarchical clustering analysis in Figure 6.

MiRNA quantification from FACS sorted Td-Tomato+ cells two months after the Split-Cre injection in WT and Dicer cKO mice was performed using the miScript Primer Assay (Qiagen) following the manufacturer's instructions.

***In silico* analysis**

Predicted target analysis was performed with the miRWalk 2.0 platform with highly restrictive parameters: seed sequence length of 7 bp, p value <0.01 and predicted targets for at least 8 databases including *miRWalk, MicroT4, miRanda, miRBridge, miRDB, miRMap, miRNAMap, PICTAR2, PITA, RNA22, RNAhybrid* and *Targetscan*).

Gene Ontology (GO) analysis was performed with DAVID Bioinformatics Resources v6.8 to classify the significantly dysregulated proteins into biological processes (BP).

Expression proteomics experiments

Sample preparation

Transfected WT aNSCs with a pool of miRNA inhibitors (250nM) or control RNA were collected after 6 DIV in differentiation conditions (3 independent experiments). At the end of the experiment, cells where lysed with RIPA buffer and 60ug of proteins were collected from all the samples to isobarically label them using TMT sixplex kits (Thermofisher Scientific) following the protocol suggested by the vendor. Tags 126 to 128 *m/z* were used for the control samples (3 replicates); tags 129 to 131 *m/z* were used for the miRNA inhibitor treated samples (3 replicates). Briefly, samples were reduced with dithiothreitol (DTT), alkylated with iodoacetamide (IAA) and then labeled using the TMT tags. After pooling the six conditions, half of the total protein content (180ug) was loaded on a monodimensional

gel-electrophoresis (1DGE). The whole lane was then cut into 12 slices and in-gel protein digestion was performed, according to (Shevchenko et al., 2006). The recovered samples were dried under nitrogen steam then re-dissolved with 50ul of 3% acetonitrile (ACN) with 0.1% formic acid for LC-MS/MS analysis.

LC-MS/MS analysis

The tryptic peptide mixture was analyzed using a Synapt G2 QToF instrument equipped with nanoACQUITY liquid chromatographer and a nanoSpray ion source. Peptides were separated on a BEH nanobore column (75um IDX25cm length) using a linear gradient of ACN in water from 3 to 55% in three hours, followed by a washout step at 90%ACN (10 min) and a reconditioning step to 3% for 20 min. Flow rate was set to 300 nL/min. Spray voltage was set to 1.6kV, cone voltage was set to 28V, spray gas was set to 0.3 L/min. Survey spectra were acquired over the 50-1600 *m/z* scan range. Multiply charged ions (2+,3+ and 4+) between 300 and 1400 *m/z* were selected as precursors for Data Dependent Acquisition (DDA) tandem mass analysis and fragmented in the Trap region of the instrument. Collision energy (CE) values were automatically selected by the software using dedicated charge-state dependent CE/*m/z* profiles. Every 60 seconds a single LeuEnk (2ng/ml) MS scan was acquired by the LockMass ion source for spectra recalibration.

Data analysis

Acquired raw datafiles were processed using PLGS software (Waters Inc.) to recalibrate the mass spectra and to generate the precursor-fragment peaklist. Protein identification and quantification were performed by interrogating the SwissProt database using MASCOT Server software (Matrixscience) (Shevchenko et al., 2006). The search parameters were set as follows, quantification: TMT6plex; fixed modifications: carbamidomethyl (C), TMT6plex (K); variable modifications: acetyl (K), acetyl (N-term), TMT6plex (N-terminus); deamidated (NQ), methyl (DE), oxidation (M), phospho (ST), phospho (Y); peptide tolerance: 50ppm; fragment tolerance: 0.5Da; maximum allowed missed cleavages: 2. At least 2 peptides were required for a positive protein identification and quantification. Protein expression ratio was normalized by the average ratio off all the peptides assigned to proteins.

Supplemental references

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Table S2. Predicted targets of at least five of the eleven miRNAs selected with Mirwalk 2.0. Related to Figure 5.

Predicted targets of > 5 miRNAs												
Gene name	**mmu-miR-124-3p**	**mmu-miR-127-3p**	**mmu-miR-135a-5p**	**mmu-miR-134-5p**	**mmu-miR-139-5p**	**mmu-miR-218-5p**	**mmu-miR-370-3p**	**mmu-miR-382-5p**	**mmu-miR-411-5p**	**mmu-miR-708-5p**	**mmu-miR-376b-3p**	**Number of miRNAs**
Adcy1	Adcy1		Adcy1		Adcy1	Adcy1	Adcy1	Adcy1				6
Afap1	Afap1		Afap1				Afap1		Afap1	Afap1		5
Amotl1	Amotl1			Amotl1		Amotl1	Amotl1			Amotl1		5
Aqp4			Aqp4			Aqp4	Aqp4	Aqp4		Aqp4	Aqp4	6
Arhgef6	Arhgef6		Arhgef6				Arhgef6	Arhgef6			Arhgef6	5
Arih1	Arih1		Arih1		Arih1			Arih1		Arih1		5
Bcl2l13	Bcl2l13				Bcl2l13			Bcl2l13	Bcl2l13	Bcl2l13		5
Braf	Braf			Braf		Braf		Braf		Braf		5
Camk4				Camk4	Camk4	Camk4	Camk4				Camk4	5
Cask	Cask					Cask	Cask	Cask		Cask		5
Cntn2	Cntn2		Cntn2			Cntn2	Cntn2			Cntn2	Cntn2	6
Coa5			Coa5	Coa5	Coa5	Coa5				Coa5		5
Csnk1g1			Csnk1g1		Csnk1g1		Csnk1g1		Csnk1g1	Csnk1g1		5
D430041D05Rik			D430041D05Rik		D430041D05Rik		D430041D05Rik	D430041D05Rik		D430041D05Rik		5
Dcbld2				Dcbld2	Dcbld2	Dcbld2			Dcbld2	Dcbld2		5
Ddx3x	Ddx3x		Ddx3x		Ddx3x		Ddx3x	Ddx3x				5
Dhfr	Dhfr			Dhfr		Dhfr	Dhfr				Dhfr	5
Dio2	Dio2		Dio2	Dio2				Dio2	Dio2	Dio2		6
Dock5	Dock5		Dock5	Dock5		Dock5			Dock5	Dock5		6
Eif5b			Eif5b				Eif5b	Eif5b		Eif5b	Eif5b	5
Eri1			Eri1		Eri1		Eri1		Eri1	Eri1		5
Faf2	Faf2				Faf2	Faf2	Faf2			Faf2		5
Fut9			Fut9		Fut9	Fut9		Fut9	Fut9		Fut9	6
Gm608			Gm608	Gm608	Gm608		Gm608			Gm608		5
Grk1			Grk1	Grk1	Grk1		Grk1			Grk1		5
Homer2	Homer2				Homer2		Homer2	Homer2			Homer2	5
Hook3	Hook3		Hook3	Hook3		Hook3	Hook3	Hook3		Hook3		7
Hs3st3b1				Hs3st3b1	Hs3st3b1	Hs3st3b1	Hs3st3b1	Hs3st3b1				5
Hus1				Hus1		Hus1	Hus1	Hus1		Hus1		5
Iws1	Iws1			Iws1			Iws1		Iws1	Iws1	Iws1	6
Kcna1	Kcna1			Kcna1	Kcna1	Kcna1	Kcna1				Kcna1	6
Kcnc1	Kcnc1			Kcnc1	Kcnc1	Kcnc1	Kcnc1			Kcnc1	Kcnc1	7
Kmt2a	Kmt2a		Kmt2a			Kmt2a	Kmt2a		Kmt2a			5
Limd2	Limd2				Limd2	Limd2	Limd2	Limd2				5
Lmbr1				Lmbr1		Lmbr1	Lmbr1	Lmbr1	Lmbr1	Lmbr1	Lmbr1	7
Lpar2			Lpar2	Lpar2	Lpar2	Lpar2	Lpar2					5
Lpp	Lpp		Lpp		Lpp		Lpp			Lpp	Lpp	6
Lrrc15		Lrrc15	Lrrc15	Lrrc15	Lrrc15		Lrrc15					5
Luzp1	Luzp1			Luzp1	Luzp1		Luzp1			Luzp1		5
Man2a1	Man2a1		Man2a1			Man2a1				Man2a1	Man2a1	5
Map3k1	Map3k1				Map3k1		Map3k1		Map3k1	Map3k1		5
Mat2a	Mat2a		Mat2a	Mat2a	Mat2a					Mat2a		5
Mtmr1			Mtmr1			Mtmr1		Mtmr1		Mtmr1	Mtmr1	5
Nfib	Nfib			Nfib	Nfib			Nfib			Nfib	5
Pcnx	Pcnx				Pcnx		Pcnx	Pcnx		Pcnx		5
Pdpk1	Pdpk1				Pdpk1		Pdpk1	Pdpk1		Pdpk1		5
Pdzd2				Pdzd2		Pdzd2	Pdzd2			Pdzd2	Pdzd2	5
Pigm			Pigm		Pigm	Pigm		Pigm		Pigm		5
Pik3c2a	Pik3c2a		Pik3c2a	Pik3c2a		Pik3c2a			Pik3c2a			5
Pitpna	Pitpna			Pitpna	Pitpna		Pitpna			Pitpna		5
Ppm1h	Ppm1h		Ppm1h	Ppm1h			Ppm1h	Ppm1h				5

Ppp1r9a			Ppp1r9a		Ppp1r9a	Ppp1r9a	Ppp1r9a	Ppp1r9a		Ppp1r9a		6
Prdm16		Prdm16	Prdm16		Prdm16	Prdm16	Prdm16		Prdm16			6
Prkca	Prkca			Prkca	Prkca		Prkca	Prkca				5
Ptpn14	Ptpn14		Ptpn14	Ptpn14	Ptpn14		Ptpn14	Ptpn14	Ptpn14	Ptpn14		8
Rab6b			Rab6b		Rab6b		Rab6b	Rab6b		Rab6b		5
Sema3d			Sema3d	Sema3d	Sema3d	Sema3d		Sema3d				5
Sema5a	Sema5a		Sema5a		Sema5a	Sema5a			Sema5a	Sema5a		6
Sertad2	Sertad2		Sertad2	Sertad2		Sertad2	Sertad2					5
Sertad4	Sertad4		Sertad4			Sertad4	Sertad4			Sertad4		5
Setd7	Setd7		Setd7		Setd7		Setd7			Setd7	Setd7	6
Slc44a5	Slc44a5		Slc44a5	Slc44a5	Slc44a5					Slc44a5		5
Slc4a8	Slc4a8		Slc4a8	Slc4a8	Slc4a8					Slc4a8	Slc4a8	6
Slc5a3	Slc5a3				Slc5a3	Slc5a3	Slc5a3	Slc5a3		Slc5a3		6
Slc7a14	Slc7a14		Slc7a14	Slc7a14		Slc7a14				Slc7a14		5
Smad3		Smad3	Smad3		Smad3		Smad3			Smad3		5
Smcr8	Smcr8			Smcr8		Smcr8	Smcr8			Smcr8		5
Sp1	Sp1		Sp1	Sp1		Sp1				Sp1		5
Srp54b	Srp54b		Srp54b			Srp54b	Srp54b	Srp54b				5
Sspn	Sspn		Sspn		Sspn	Sspn		Sspn				5
Ssr1			Ssr1	Ssr1		Ssr1	Ssr1		Ssr1			5
St8sia1	St8sia1			St8sia1			St8sia1		St8sia1	St8sia1	St8sia1	6
Stk38l			Stk38l			Stk38l	Stk38l	Stk38l		Stk38l	Stk38l	6
Tbl1xr1			Tbl1xr1	Tbl1xr1		Tbl1xr1		Tbl1xr1			Tbl1xr1	5
Tgfbr1	Tgfbr1		Tgfbr1					Tgfbr1	Tgfbr1	Tgfbr1		5
Tnrc6b	Tnrc6b			Tnrc6b	Tnrc6b	Tnrc6b	Tnrc6b					5
Tspan11	Tspan11			Tspan11	Tspan11	Tspan11	Tspan11			Tspan11		6
Twsg1	Twsg1		Twsg1		Twsg1	Twsg1	Twsg1	Twsg1				6
Ubn2	Ubn2				Ubn2	Ubn2	Ubn2			Ubn2		5
Xpo7			Xpo7	Xpo7	Xpo7	Xpo7				Xpo7		5
Zdhhc21			Zdhhc21	Zdhhc21	Zdhhc21				Zdhhc21	Zdhhc21		5
Zfand3	Zfand3	Zfand3			Zfand3		Zfand3	Zfand3				5
Zfp148					Zfp148		Zfp148		Zfp148	Zfp148	Zfp148	5
Zfp275	Zfp275		Zfp275	Zfp275			Zfp275				Zfp275	5
Zfp704	Zfp704		Zfp704		Zfp704		Zfp704	Zfp704				5
Zfyve27	Zfyve27		Zfyve27	Zfyve27	Zfyve27					Zfyve27		5
Zkscan8	Zkscan8				Zkscan8	Zkscan8	Zkscan8			Zkscan8		5
Zmat3	Zmat3		Zmat3	Zmat3	Zmat3				Zmat3			5

Genes involved in neurogenesis or gliogenesis predicted targets of > 5 miRNAs												
Gene name	**mmu-miR-124-3p**	**mmu-miR-135a-5p**	**mmu-miR-382-5p**	**mmu-miR-134-5p**	**mmu-miR-411-5p**	**mmu-miR-218-5p**	**mmu-miR-370-3p**	**mmu-miR-708-5p**	**mmu-miR-139-5p**	**mmu-miR-376b-3p**	**mmu-miR-127-3p**	**References**
Aqp4		Aqp4	Aqp4			Aqp4	Aqp4	Aqp4		Aqp4		Zeng et al., 2007
Braf	Braf		Braf	Braf		Braf		Braf				Kaul et al., 2013
Dio2	Dio2	Dio2	Dio2	Dio2	Dio2			Dio2				Guadaño-Ferraz et al., 1997
Kcna1	Kcna1			Kcna1		Kcna1	Kcna1		Kcna1	Kcna1		Olsen and Sontheimer, 2008
Kmt2a	Kmt2a	Kmt2a			Kmt2a	Kmt2a	Kmt2a					Huang et al., 2015
Nfib	Nfib		Nfib	Nfib					Nfib	Nfib		Deneen et a., 2006
Ppm1h	Ppm1h	Ppm1h	Ppm1h	Ppm1h			Ppm1h					Shen et al., 2014
Prdm16		Prdm16			Prdm16	Prdm16	Prdm16		Prdm16		Prdm16	Chuikov et al., 2010
Sema5a	Sema5a	Sema5a			Sema5a	Sema5a		Sema5a	Sema5a			Li et al., 2012
Smad3		Smad3					Smad3	Smad3	Smad3		Smad3	Zhang et al., 2010
Sp1	Sp1	Sp1		Sp1		Sp1		Sp1				Michinaga et al., 2013
St8sia1	St8sia1			St8sia1	St8sia1		St8sia1	St8sia1		St8sia1		Sim et al., 2011
Tgfbr1	Tgfbr1	Tgfbr1	Tgfbr1		Tgfbr1			Tgfbr1				Stipursky et al., 2007
Twsg1	Twsg1	Twsg1	Twsg1			Twsg1	Twsg1		Twsg1			Sun et al., 2010
Number predicted targets	11	10	7	7	6	8	8	8	6	4	2	