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**Supplemental Information**

**MiR-135a-5p Is Critical for Exercise-Induced Adult Neurogenesis**

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Figure S1 related to Figure 1

A

Target name	EXP1 Ct values		EXP2 Ct values		EXP3 Ct values	
	STD	RUN	STD	RUN	STD	RUN
miR-135a	24,142	32,448	20,975	21,225	19,784	19,696
miR-375	30,814	35,344	27,745	28,186	26,388	25,953
miR-203	32,996	34,865	28,829	29,491	27,756	27,136
miR-190	23,909	24,855	21,233	21,528	17,267	21,292
miR-381	29,31	29,699	25,307	25,398	25,04	24,774
miR-350	28,429	28,709	24,308	24,452	24,542	23,719
miR-340-3p	26,755	26,95	21,624	21,862	21,343	20,338
miR-31	29,469	29,351	25,332	26,311	23,661	23,305
<b>Normalizers</b>						
U87-001712	22,620	22,193	20,092	19,034	20,203	18,246
Y1-001727	22,126	21,635	19,849	20,545	18,988	17,653
snoRNA135-001230	22,995	22,45	13,905	14,005	14,403	12,984
snoRNA202-001232	16,457	16,336	20,2	19,495	19,845	18,458

B

Target name	STD1	STD2	STD3	RUN1	RUN2	RUN3	t-test (paired)
miR-135a	0,348	0,542	1,337	0,001	0,219	0,366	0,123
miR-190	0,003	0,005	0,014	0,000	0,002	0,005	0,113
miR-203	0,057	0,346	0,454	0,037	0,141	0,235	0,147
miR-375	0,409	0,453	7,653	0,158	0,178	0,121	0,383
miR-381	0,001	0,002	0,005	0,000	0,001	0,002	0,140
miR-350	0,010	0,027	0,035	0,006	0,012	0,011	0,130
miR-340-3p	0,018	0,054	0,049	0,011	0,023	0,023	0,100
miR-31	0,009	0,026	0,091	0,007	0,006	0,030	0,257

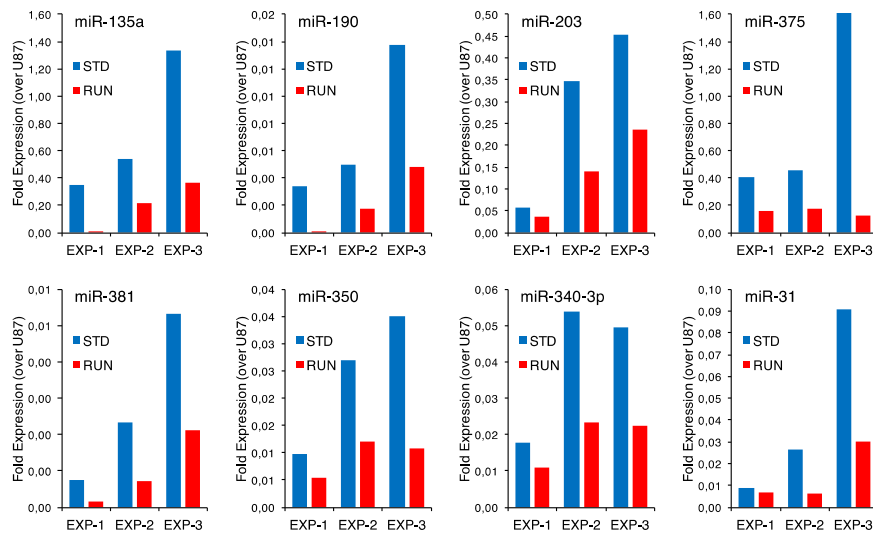
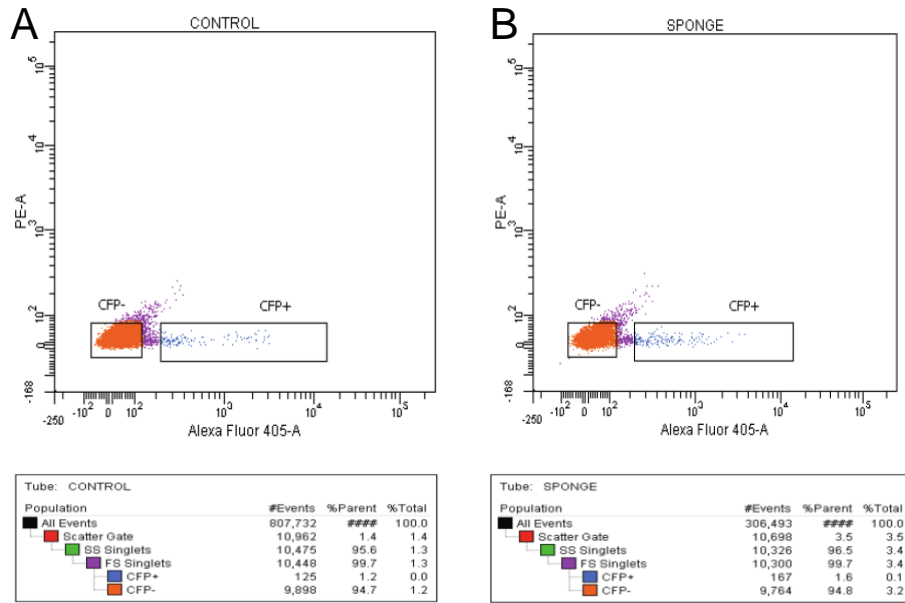


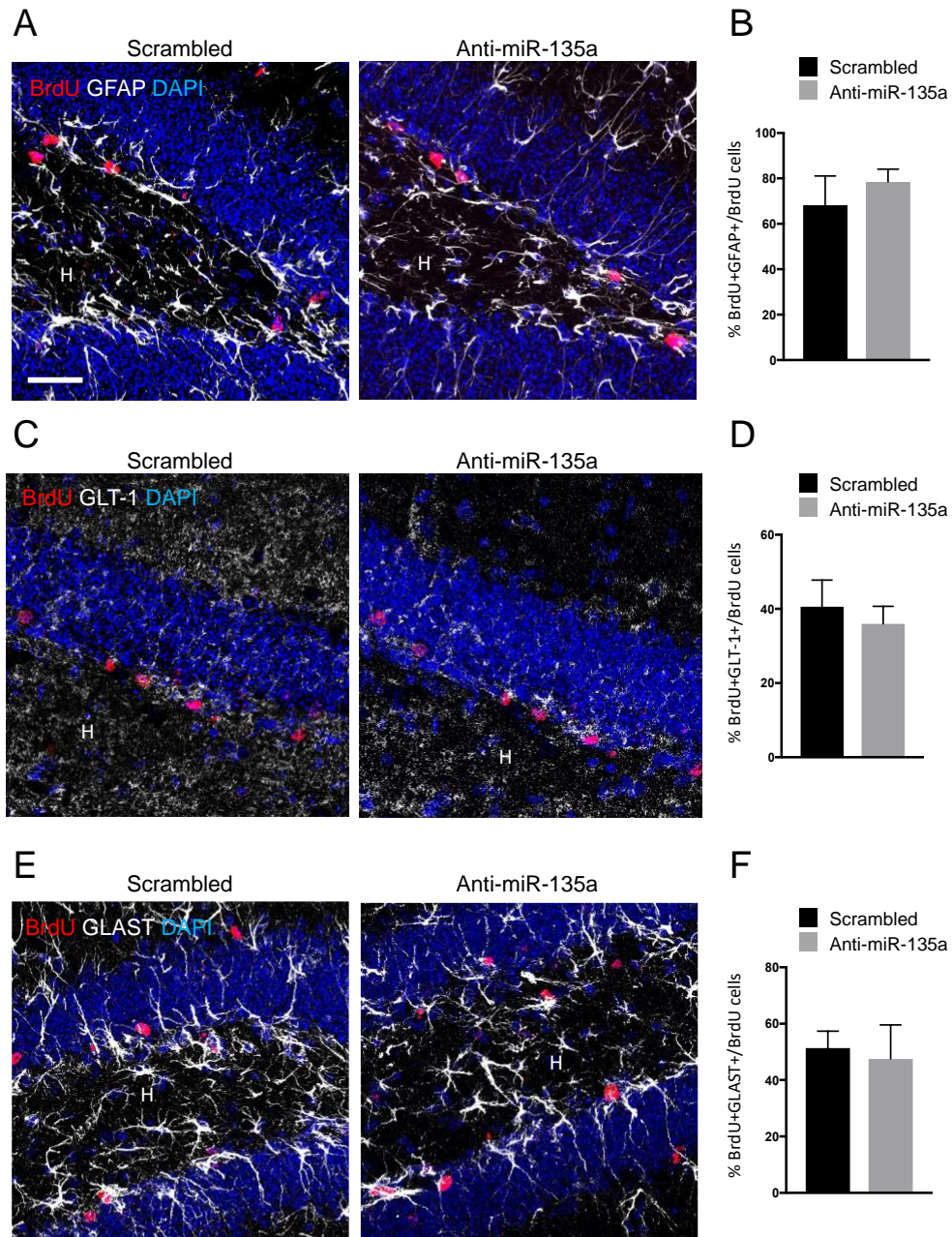
Figure S1. Fold change expression of miRNAs in *Nestin*-CFPnuc positive NPCs sorted from adult hippocampus of mice in standard (STD) or running (RUN) conditions for 10 days. (A) Expression levels of miRNAs and normalizers (Ct values) by TaqMan Low Density Array (TLDA) from each independent experiment (EXP; n=3 independent biological replicas each of them containing a pool of *Nestin*-CFPnuc positive cells isolated from 8 mice, per condition). (B) Normalized values from each independent experiment and plots.

**Figure S2 related to Figure 3**



**Figure S2. Inhibition of miR-135a increases the number of *Nestin*-CFPnuc positive NPCs *in vivo*.** (A-B) Fluorescent sorting of CFPnuc positive cells from 6-week-old *Nestin*-CFPnuc mice after 10 days upon stereotaxic injections of lentiviruses encoding control (A), or miR-135a (B) sponge in the hippocampal DG of adult mice. n= 10 mice per group, pooled before sorting.

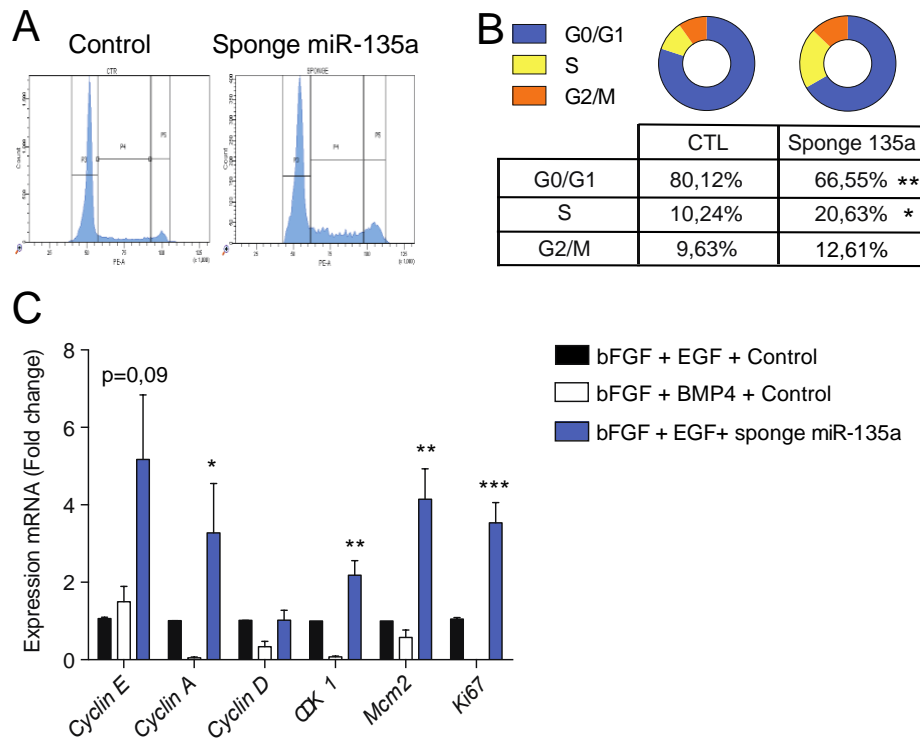
Figure S3 related to Figure 5



**Figure S3. Transient inhibition of miR-135a does not alter the astroglial cell markers in hippocampal SGZ.**

(A-F) Representative micrographs showing BrdU (red), GFAP (A), GLT-1 (C) or GLAST (E) (white) cells in hippocampal SGZ of 6-week-old C57BL/6 mice, injected with control scrambled or anti-miR-135a, subject to BrdU administration (2 injections every day per 5 days) and sacrificed 2 weeks after the last BrdU injection. (B) Quantification of the proportion BrdU+GFAP+ (B), BrdU+GLT-1+ (D) or BrdU+GLAST+ over total BrdU+ cells. H, hilus. Data are expressed as mean ± SEM, n = 7 mice per group. Scale bars, 25 μm.

**Figure S4 related to Figure 6**



**Figure S4. Downregulation of miR-135a regulates proliferation and cell cycle -dependent genes in NPCs *in vitro*.**

(A) Representative cell cycle analysis of propidium iodide staining by flow cytometry. (B) Percentage of NPCs in G0/G1, S and G2/M phases upon infection with viral-encoded control or sponge for miR-135a *in vitro*. (C) Quantification of relative mRNA expression of cell cycle-dependent genes normalized to *Actin* in NPCs cultured in proliferative (bFGF+EGF), or quiescence (bFGF+BMP4) media upon transduction with viral-encoded control, and in proliferative media upon transduction with viral-encoded miR-135a sponge. Data are expressed as mean  $\pm$  SEM, n = 3 independent experiments containing three replicates. One-way ANOVA Bonferroni as post hoc. \*p< 0.05, \*\*p< 0.01, \*\*\*p< 0.001.

**Table S1 related to Figure 1**

**miRNome expression analysis in *Nestin*-CFPnuc positive NPCs sorted from adult hippocampus of mice in standard (STD) or running (RUN) conditions for 10 days.**

**Table S2 related to Figure 6**

**Proteomics data upon miR-135a overexpression in NPCs *in vitro*.**

Fold change of significantly differentially expressed proteins (cutoff > 1.5-fold or < 0.5-fold) and Gene ontology analysis, upon miR-135a overexpression (sh-miR-135a), compared to scrambled control. Blue and Red proteins indicate common protein found in both Table S2 and S3

**Table S3 related to Figure 6**

**Proteomics data upon miR-135a inhibition in NPCs *in vitro*.**

Fold change of significantly differentially expressed proteins (cutoff > 1.5-fold or < 0.5-fold) and Gene ontology analysis, upon miR-135a inhibition (sponge miR-135a) compared to scrambled control. Blue and Red proteins indicate common protein found in both Table S2 and S3

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Contact for reagent and resource sharing

“Further information and requests for resources and reagent should be directed to and will be fulfilled by the Lead Contact, Davide De Pietri Tonelli ([davide.depietri@iit.it](mailto:davide.depietri@iit.it)).

### Primary NPC culture

Hippocampal NPCs were prepared and expanded as described previously from 8-10 6-week-old C57BL/6J mice (Babu et al., 2011) and kept in culture up to 10 passages (Pons-Espinal et al., 2017). Briefly, NPCs were plated into PDL/Laminin (Sigma/Roche) coated wells in culture medium containing Neurobasal (Invitrogen), Glutamax (Invitrogen), 1% penicillin and streptomycin (Invitrogen), B27 without retinoic acid (Invitrogen), bFGF (20ng/ml; PeproTech) and EGF (20ng/ml; PeproTech) and incubated at 37°C with 5% CO<sub>2</sub>. Every 2 days half of the growth medium was exchanged with fresh medium to replenish the growth factors. NPCs were passaged once they reached 80% confluence.

To induce quiescence,  $1.2 \times 10^4$  cells/cm<sup>2</sup> were plated into normal proliferation medium (EGF and bFGF, both at 20 ng/mL; PeproTech), and, after 16 h, fresh NPC medium was added without EGF and with 50 ng/mL BMP4 (R&D Systems) and 20 ng/mL bFGF (Martynoga et al., 2013).

### Virus injection

8-week-old mice were anesthetized with isoflurane and 1.5 µl of virus 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 (Pons-Espinal et al., 2017). Virus used: *mmu-miR-135a-5p sponge*: MISSION® Lenti microRNA Inhibitor Mouse (MLTUD0048, Sigma); *mmu-miR-135a-5p overexpression*: MISSION® Lenti microRNA Human (HLMIR0200, Sigma) or *control lentivirus*: MISSION® TRC2 pLKO.5-puro Non-Mammalian shRNA Control (SHC202, Sigma). Mice were single-housed under standard conditions or in cages equipped with running wheels for 10 days. Mice received 3 BrdU intraperitoneal injections per day (100 mg/kg) (one every 2 hours) and sacrificed 24 hours later.

Histology: 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). 40 µm brain sections were generated using a microtome and were stored with cryoprotectant solution.

### Synthetic oligos injection

6-week-old mice were anesthetized with isoflurane and 1.5 µl of synthetic oligos 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 (Pons-Espinal et al., 2017). Oligos used: miRCURY LNA miRNA Custom Power Inhibitor I-MMU-MIR-135A-5P and NEGATIVE CONTROL (339146, Qiagen).

Mice were single-housed under standard conditions. To assess the effect of miR135 inhibition, a first group of mice (n=4) were sacrificed 48 hours after the injection and the DG dissected for RNA extraction and miR135 quantification. 24 hours after the oligos injection another set of animals received 2 BrdU intraperitoneal injections per day for 5 days (50 mg/kg) (one every 12 hours). 5 mice were then sacrificed 2 hours after the last BrdU injection and the remaining 7 were sacrificed 2 weeks after the last BrdU injection (in total, 21 days after oligos injection). Brains were collected as previously described and used for RNA extraction or immunofluorescence analysis.

### **MiRNA administration**

MiRNA mimics (Qiagen): Syn-mmu-mir-135a-5p, MSY0000147; Syn-mmu-miR-203-3p, MSY0000236; or Syn-mmu-miR-190a-5p, MSY0000220.

MiRNA antagonists (Qiagen): Anti-mmu-miR-135a-5p, MIN0000147; Anti-mmu-miR-203-3p, MIN0000236; or Anti-mmu-miR-190a-5p, MIN0000220.

To constitutively overexpress or inhibit miR-135a-5p, NPCs were infected at MOI=5 with lentiviruses and selected with 1 µg/ml puromycin (Sigma). Cell cycle distribution was monitored by propidium iodide staining of cells and fluorescence-activated cell sorting (FACS).

### **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.

To detect Ki67 immunostaining, citrate buffer 10 mM pH = 6 treatment during 10 min at 95 °C was used. Sections were blocked during 2 h with 0.1% PBS-T and 5% NGS 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 NPCs 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).

### **List of antibodies used:**

<b>Antibody</b>	<b>Host</b>	<b>Company</b>	<b>Catalog</b>	<b>Dilution</b>
BrdU	rat	Abcam	ab6326	1:200
KI67	rabbit	Abcam	ab15580	1:250
GFAP	rabbit	Dako	Z-0334	1:1000
GFP	chicken	Abcam	ab13970	1:500
GLAST	rabbit	Abcam	ab416	1:200
GLT-1	rabbit	Abcam	ab41621	1:200
DCX	rabbit	Abcam	ab18723	1:1000
NEUN	mouse	Millipore	MAB377	1:250

Confocal stack images of brain slices (40µm) were obtained with the Confocal A1 Nikon Inverted SFC with 40x objective and the Zeiss Spinning Disc with a 20x objective. Cell quantification and analysis was performed using NIS-Elements



software (Nikon) and ZenBlue (Zeiss). Given that the density of the positive cells was relatively low, for each stacked-confocal image we drew and measure the area of the DG following the position of the nuclei stained with DAPI and in that particular area we counted the positive cells. To obtain the number of cells in the volume, the density of positive cells was multiplied by the thickness of the slice (40  $\mu$ m). Final cell number was corrected after checking along z-stack that no overlapping cells were counted twice.

Immunofluorescence on cell cultures was performed as previously described (Pons-Espinal et al., 2017). Images were obtained using the microscope Nikon Eclipse at 20x or 40x magnification and quantification was performed using a Cell-counter plugin in FIJI (Fiji is Just ImageJ).

### Fluorescence-Activated Cell Sorting (FACS), RNA Extraction and Analysis

For RNA extraction and cDNA preparation, six to ten *Nestin*-CFPnuc mice per condition were euthanized at the indicated time points (animal facility MTZ Dresden). DG cells were dissociated with the Neural Tissue Dissociation Kit P (Miltenyi Biotec) and FACS-sorted cells were immediately processed for RNA extraction (Walker et al., 2016). Total RNA was extracted with QIAzol protocol (Qiagen) and RNA purified with RNeasy Mini Kit, or miRNeasy Mini Kit (Qiagen) following the manufacturer's instructions. cDNA (for mRNAs) synthesis was obtained with ImProm-II reverse transcriptase (Promega); cDNA (from miRNA) was prepared with an miScript II RT kit using the HiSpec Buffer (Qiagen) according to the manufacturer's instructions. mRNA was quantified with a QuantiFast SYBR Green PCR Kit (Qiagen) on a ABI-7500 Real-Time PCR System (Applied Biosystems). Each sample was normalized to Actin levels. MiRNAs were quantified with the miScript SYBR Green PCR kit (Qiagen) following the manufacturer's recommendations on an ABI-7500 Real-Time PCR System (Applied Biosystems) or with TaqMan Array Rodent MicroRNA A Cards Set v3.0 (Thermo Fisher) following the manufacturer's recommendations with a ViiA 7 Real-Time PCR system (Thermo Fisher), for which original Ct values are available on request.

#### List of specific primers used:

Primer name	Sequence (5'-3')
Actin Fw	GGCTGTATTCCCCTCCATCG
Actin Rv	CCAGTTGGTAACAATGCCATGT
Ki67 Fw	ATTCAGTTCGCCAATCC
Ki67 Rv	GGCTCCGTCTTCATACCTAAA
Cyclin E Fw	GATCCAGAAAAAGGAAGGCAAA
Cyclin E Rv	TGAAGAAATTGCCAAGATTGACA
Cyclin A Fw	GCCTTCACCATTCATGTGGAT
Cyclin A Rv	TTGCTGCGGGTAAAGAGACAG
Cyclin D Fw	GCGTACCCTGACACCAATCTC
Cyclin D Rv	CTCCTCTTCGCACTTCTGCTC
CDK1 Fw	CAGAGCTGGCGACCAAGAA
CDK1 Rv	GATTGACCAGCTCTTCAGGATCTT
Mcm2 Fw	ATCCACCACCGCTTCAAGAAC
Mcm2 Rv	TACCACCAAACCTCTCACGGTT

#### *In situ* hybridization

Mice were anesthetized and perfused transcardially with cold 4% paraformaldehyde in 1X PBS. Brains were removed and post-fixed in the same fixative for 5 hours at 4°C. Tissues were washed several times in 1X PBS prior to de-hydration with 30% sucrose in 1X PBS, overnight (or until they sink) at 4°C and carefully dried before proceeding with the flash freezing protocol: tissues were placed in a metal beaker filled with isopentane (Sigma), located in a foam cooler or laboratory ice bucket and surrounded with crushed dry ice. 18-20 µm brain slices were collected using a cryostat and attached on glass slides. Slices were permeabilized by treating twice for 10 min with RIPA buffer (NaCl 150 mM, NP-40 1%, Na deoxycholate 0.5%, SDS 0.1%, EDTA 1 mM, Tris pH 8.0 50 mM) and post-fixed for 10 min in 4% PFA in 1X PBS, followed by washes in 1X PBS to remove the excess PFA. The positive charges in the tissue were blocked by treating slides for 15 min with acetic anhydride (Sigma) (0.25% final concentration) in triethanolamine buffer (triethanolamine 100 mM, acetic acid pH 8.0), followed by washes with 1X PBS. In order to block aspecific sites, a pre-hybridization step with 200-250 µl hybridization solution (Formamide 50%, SSC 5X, Denhardts 5X, Salmon sperm DNA (500 µg/ml, yeast RNA 250 µg/ml) was carried out. After 1 hour, a hybridization solution containing 160 nM (miR135, miR190, miR203) or 100 nM (miR9) of the DIG-labeled LNA probe (Exiqon) was added and incubated ON. The next day, slides were washed for 1 hour with a post-hybridization solution (Formamide 50%, SSC 2X, Tween-20 0.1%). For the immunological detection, slides were incubated twice for 5 min and once for 20 min at RT in buffer B1 (Maleic acid 100 mM pH 7.5, NaCl 100mM, Tween-20 0.1%) and then blocked in buffer B2 (10% Normal Goat Serum in B1) for 1 hour. The anti-DIG antibody (Roche) was diluted 1:2000 in B2 buffer and incubated ON at 4°C. For development of the color reactions, two different alkaline phosphatase substrates were used: NBT/BCIP (Roche) or Fast Red TR/Naphthol AS-MX solution (Sigma), according to manufacturer instructions. The reaction was stopped by several washes with 0.1 % Tween 20 in 1X PBS. Sections were mounted using mounting resins (Thermo Fisher) or in VECTA-Shield mounting medium (VECTOR Labs) including DAPI, and imaged using conventional bright-field microscopy or a confocal microscope with the Cy-3 filter.

## **Proteomics**

Chemicals and solvents were purchased from Sigma Aldrich (Milano, Italy), unless otherwise indicated. The NanoAcquity LC system, trapping column and Oasis SPE columns were purchased from Waters (Milford, MA, USA). The 5600+ TripleTof system, ProteinPilot, MarkerView softwares and the PepCalMix reference standard were purchased from SCIEX (Ontario, Canada).

Sample preparation and ion library creation: The ion library preparation has been previously described (Braccia et al., 2018).

Protein digestion for Sequential window acquisition of all theoretical (SWATH) mass spectra: The volume corresponding to 50 µg of proteins from each sample was incubated in 100 mM dithiothreitol (DTT) in ammonium bicarbonate 50 mM, for reduction of disulfide bonds and then alkylated with iodoacetamide (IAA). Protein content was precipitated with cold acetone. The resulting protein pellets were dried under a nitrogen stream and then dissolved in ammonium bicarbonate 50 mM. An overnight digestion was performed using trypsin. The resulting peptides were desalted on Pierce C18 Spin columns (Thermo scientific, USA), according to manufacturer's instructions. The desalted peptides were dried in a vacuum centrifuge.

LC-MS/MS analysis: Adult hippocampal NPC tryptic digests were re-dissolved in 3% ACN with 0.1% FA. As internal standard for retention time calibration, 5 µl of PepCalMix (corresponding to 250 pmoles) were spiked into each fraction. 2 µg of digest from each sample was acquired in SWATH mode (10.1038/sdata.2014.31, doi: 10.1002/pmic.201400270.) using a pre-acquired, dedicated ion library, as already described by our group (Braccia et al., 2018). For SWATH

quantification, the following criteria were set: minimum peptide confidence 90%, 50 ppm maximum mass tolerance, 30 minutes maximum RT tolerance, 6 MRM transitions per peptide and modified peptides were allowed. With these settings, we were able to quantify 3352 proteins from all the samples at 1% FDR. All the RAW data files used for protein quantification are freely available through the acquired for the present work are freely available through ProteomExchange database (Vizcaino et al., 2014, Deutsch et al., 2017) with identifier PXD009845.

### **Statistical analysis**

Data are presented as mean  $\pm$  SEM and were analyzed using Prism 6 (GraphPad). Statistical details of experiments can be found in the Results and Figure legend sections. Statistical significance was assessed with a two-tailed unpaired *t test* for two experimental groups. For experiments with three or more groups, one-way ANOVA with Bonferroni's multiple comparison test as *post hoc* was used. Mean differences were considered to be statistically significant when  $p < 0.05$ .