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Materials and Methods

Animals

C57BL/6 and Td-Tomato$^{flox/wt}$ knock-in reporter mice (Jackson Laboratory stock number 007908) (*1*), were housed at Istituto Italiano di Tecnologia (IIT); Nestin-GFP mice (*2*) for the Kainic Acid experiments were housed at the Swammerdam Institute for Life Sciences, University of Amsterdam. All animal procedures were approved by IIT animal use committee and the Italian Ministry of health, or by the Commission for Animal Welfare at University of Amsterdam (DEC protocol 4925, AVD1110020184925), respectively and conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the European Community Council Directives. All mice were group-housed under a 12-h light-dark cycle in a temperature and humidity-controlled environment with ad libitum access to food and water.

aNPCs preparation and culture

Hippocampal NPCs were prepared and expanded as described previously (*3–5*) and induction of neuronal differentiation was done with a viral construct expressing Ascl1-ERT2 as previously described (*6*).

Protein extraction and Western blot

For total protein extraction, adult testis or hippocampus or cell pellets were homogenized in RIPA buffer and the protein concentration was determined using a Bradford Assay kit (Bio-Rad). For blot analysis, equal amounts of protein (30 µg) were run on homemade 10% polyacrylamide gels and transferred on nitrocellulose membranes (GE Healthcare). Membranes were probed with primary antibodies (listed in the table below) followed by HRP-conjugated secondary antibody anti-rabbit or mouse (Invitrogen, A16104, A16072; 1:2,000). LAS 4000 Mini Imaging System (GE Healthcare) was used to digitally acquire chemiluminescence signals, and the band intensities were quantified using Fiji (Macbiophotonics, Fiji is Just ImageJ) (*7*).

List of antibodies used for WB:

Antibody	Host	Company	Catalog	Dilution
MILI	Rabbit	Kind gift of Dr. Hannon		1:100
MIWI	Rabbit			1:200
ACTIN	Rabbit	Abcam	ab13970	1:1000
GADPH	Rabbit	Santa Cruz	sc-25778	1:1000
GFAP	Rabbit	Dako	Z-0334	1:1000

Virus and GapmeR injection

Virus or GapmeR injection was done as previously shown (*5*): 8 weeks-old mice Td-Tomato$^{flox/wt}$ or WT C57BL6/J were anesthetized with isoflurane, 1 µl of virus mix (Split-Cre N-Cre:C-Cre) or 1.5 µl of antisense LNA GapmeR for mili KD or negative control (MILI 339511, Control 339515, Qiagen), was stereotaxically injected in the dentate gyrus. To assess the efficacy of mili inhibition, a first group of mice (n=5) was sacrificed 48 hours after the injection and the DG dissected for RNA or protein extraction. 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). Animals were sacrificed 10 (n=5) or 30 days after oligos injection (n=7) for histological analysis, as previously described (*5*). 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 harvested, 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). Slices were used for immunofluorescence and immunohistochemical analysis.

Fluorescence-Activated Cell Sorting (FACS) and flow cytofluorometric analysis of cell cycle distribution

For RNA extraction and cDNA preparation, Td-Tomato$^{flox/wt}$ or Nestin-GFP mice were used. Six to ten Td-Tomato$^{flox/wt}$ mice were euthanized 10 or 30 days after the split cre viruses injection. DG cells were dissociated with the Neural Tissue Dissociation Kit P (Miltenyi Biotec) and FACS-sorted as previously published (*8*). FACS-sorted cells were immediately processed for RNA extraction. To measure the cell cycle length, we used propidium iodide (PI), which binds to DNA by intercalating between the bases, as previously described (*9*). Briefly, cells were trypsinized, resuspended in PBS and fixed with 70% of ethanol for 40 minutes on ice. Cells were then centrifuged, resuspended in PBS for 15 minutes and then incubated 1h at 37°C with 60 µg/ml of PI (Sigma). Cells were collected by centrifuge and resuspended in ice-cold PBS for FACS analysis.

Immunostaining analysis

The immunostaining on brain slices was performed on sections covering the entire dorsal hippocampus as previously described (*5*, *10*). To detect Ki67 staining, citrate buffer 10 mM pH = 6 treatment during 10 min at 95 °C was used. Primary antibodies are listed below, secondary fluorescent antibodies were diluted 1:1000 (Goat Alexa 488, 568, and 647nm, Invitrogen). Confocal stack images of brain slices (40µm) were obtained with the Confocal A1 Nikon Inverted SFC with 20x objective. Cell quantification and analysis was performed using NIS-Elements software (Nikon) and the Cell-counter plugin in Fiji. Immunofluorescence staining on cell cultures was performed as reported in (*5*). To detect BrdU incorporation, cells were pretreated with 2N HCl for 30 min at 37°C. Cells were mounted in mounting medium and counterstained with fluorescent nuclear dye DAPI (Invitrogen). Images were obtained using the microscope Nikon Eclipse at 20x or 40x magnification and quantification was performed using a Cell-counter plugin in Fiji. DAB staining was performed as previously reported (*11*). Briefly, sections were incubated with peroxidase block (Vectashield), permeabilized with 0.3% PBS-Triton X (PBS-T) and 0.1% PBS-T. Sections were blocked with 0.1% PBS-T and 5% Normal Goat Serum (NGS), incubated with primary antibodies and subsequently with the corresponding biotinylated secondary antibodies (1:1000 Goat anti-rabbit, Invitrogen). Signal amplification was performed using the ABC complex (Vectashield), according to manufacturer's instructions. Sections were

incubated with the solution for DAB reaction (Sigma) and counterstained with Hoechst (1:300), mounted and cover slipped with Vectashield reagent (VECTOR Labs).

List of antibodies used for IF/IHC:

Antibody	Host	Company	Catalog	Dilution
MILI	rabbit	Hannon Lab		1:100
BrdU	rat	Abcam	ab6326	1:200
KI67	rabbit	Abcam	ab15580	1:250
GFAP	rabbit	Dako	Z-0334	1:1000
NeuN	mouse	Millipore	MAB377	1:250
RPL26	rabbit	Abcam	ab59567	1:500
Nestin	mouse	Millipore	MAB353	1:250
Cleaved Caspase-3	rabbit	Cell Signaling Technology	9664	1:400

RNA extraction and real time qPCR

Total RNA was extracted from aNPCs (proliferating and differentiating conditions), or DG dissected from adult C57BL/6, Nestin-GFP or Td-Tomato$^{flox/wt}$ mice with QIAzol protocol (Qiagen) according to the manufacturer's instructions. 1 µg of total RNA was treated with DNase I (Sigma) and cDNA was synthesized using iScript cDNA Synthesis kit (Bio-Rad) or with ImProm-II reverse transcriptase (Promega). Real time qPCR was performed in duplex with Actin or Ubiquitin C as a reference gene, with QuantiFast SYBR Green PCR Kit (Qiagen) or Taqman Assay (Thermo Fisher) on ABI-7500 Real-Time PCR System (Applied Biosystems). Expression levels were determined relative to Ubiquitin C or Actin, using delta delta Ct method.

Primers listed below were designed using NCBI/UCSC Genome Browser and Primer3 software tools and then checked in PrimerBLAST for their specificity to amplify the desired genes.

Assay ID	Forward primer (5' to 3')	Reverse primer (5' to 3')
Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Mili	GGCCAGCATAAATCTCACAC	TAGCTGGCCATCAGACACTC
Miwi	TAATTGGCCTGGAGTCATCC	GAGGTAGTAGAGGCGGTTGG
Gfap	GGGGCAAAAGCACCAAAGAAG	GGGACAACTTGTATTGTGAGCC
Complement C3	CCAGCTCCCCATTACGTCTG	GCACTTGCCTCTTTAGGAAGTC
Serpina 3n	ATTTGTCCCAATGTCTGCGAA	TGGCTATCTTGGCTATAAAGGGG
Cxcl10	CCAAGTGCTGCCGTCATTTTC	GGCTGGCAGGGATGATTTCAA
Vac14	AAGTGGCTCTACCATCTCTACAT	ACAACCTCATCAGATTCGTCAGA
Lars2	CATAGAGAGGAATTTGCACCCTG	GCCAGTCCTGCTTCATAGAGTTT
Rpl13a	AGCCTACCAGAAAGTTTGCTTAC	GCTTCTTCTTCCGATAGTGCATC
Rpl17a	ATCAAGAGGGTCAAACCTTCGT	CCACCATTATACCGCCGGAA
Cyclin A	GCCTTCACCATTCATGTGGAT	TTGCTGCGGGTAAAGAGACAG

Cyclin D1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
Cyclin E	GATCCAGAAAAAGGAAGGCAAA	TGAAGAAATTGCCAAGATTGACA
L1 5'UTR Af	TGCCCACTGAAACTAAGGAGA	GCTTGTTCTTCAGGTGACTCTGT
L1 5'UTR Gf	CCAAACACCAGATAACTGTACACC	CGTGGGAGACAAGCTCTCTT
L1 5'UTR Tf	TGAGCACTGAAACTCAGAGGAG	GATTGTTCTTCTGGTGATTCTGTTA
L1 ORF2	CCCTCAACAGAGGAATGGAT	CCATCCATTTGGCTAGGAAT
SINE B1	TGGCGCACGCCTTTAATC	GAGACAGGGTTTCTCTGTGTAGCC
5s rRNA	ACGGCCATACCACCCTGAA	GGTCTCCCATCCAAGTACTAACCA
Ubiquitin C	ACAGACGTACCTTCCTCACC	CCCCATCACACCCAAGAACA

Small RNA library preparation

Cells were lysed in QIAzol lysis reagent and total RNA was isolated using the miRNeasy Mini kit (Qiagen), according to the manufacturer's instructions. Quantity and quality of the total RNA were measured by Nanodrop spectrophotometer and Experion RNA chips (Bio-Rad). RNA with RNA integrity number (RIN) values ≥ 9.5 were selected for the study. 1 μg of high quality RNA for each sample was used for library preparation according to the Illumina TruSeq small RNA library protocol. Briefly, 3' adapters were ligated to 3' end of small RNAs using a truncated RNA ligase enzyme followed by 5' adaptor ligation using RNA ligase enzyme. Reverse transcription followed by PCR was used to prepare cDNA using primers specific for the 3' and 5' adapters. The amplification of those fragments having adapter molecules on both ends was carried out with 13 PCR cycles. The amplified libraries were pooled together and run on a 6% polyacrylamide gel. The 145-160 bp bands (which correspond to inserts of 24-32 nt cDNAs) were extracted and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). The quality of the library was assessed by the Experion DNA 1K chips (Bio-Rad). Small RNA sequencing using HiSeq2000 (Illumina Inc., CA) was performed by the Center for Genomic Science of IIT@SEMM.

Processing of sequencing data

Illumina reads were trimmed to remove the 3′ adapter using Cutadapt, with parameters -m 25 -q 20. Since piRNA size ranges from 26 to 31 bases, all sequences with length ≤24 bases were discarded.

Reads mapped to known non-coding RNAs (RNAcentral v6.0 snoRNA, UCSC tRNA, miRBase Release 21 miRNA hairpin and mature miRNA annotation, NCBI complete ribosomal DNA unit) (*12–14*) were removed from the datasets. The comparison was performed using NCBI BLASTN v2.6.0 with parameters -max_hsps= 1, -max_target_seqs= 1, -perc_identity= 80, mismatches <= 1, qcovhsp >= 90 (*15*). Reads were aligned on the non-repeat-masked UCSC release 9 of the mouse genome (MM9) (*16*) using bowtie2 (*17*) v2.2.6 with the sensitive preset option and allowed a maximum of 100 alignments. All the reads that aligned to the genome were retained and used for subsequent analysis. piRNA clusters were identified collapsing overlapped piRNA sequences (piRBase Release 1) (*18*) into one cluster (mergeBed with preset options) (*19*). piRNA clusters and all the reads that aligned to the genome were intersected (intersectBed with option -f 1). Intersection files were then parsed using a custom perl script in order to evaluate alignment counts. Differential expression was assessed using DESeq2 (*20*). piRNA clusters were considered differentially expressed when the adjusted p-value was ≤ 0.05, and down- and up-regulation was established in the range of ≤ -1 to ≥ 1 log2 fold-change, respectively.

qPCR of piRNAs

Total RNA enriched in the fraction of small RNAs, was extracted using miRNeasy Mini kit following the manufacturer's instructions from aNPCs, DG extracted from C57BL6/J or Td-Tomato$^{flox/wt}$ mice. cDNA was obtained using the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher) according to the manufacturer's instructions and quantified using the Custom TaqMan Small RNA Assay (Thermo Fisher) on a ABI-7500 Real-Time PCR System (Applied Biosystems). Each sample was normalized to U6 snRNA level (Thermo Fisher). Cluster sequences used for probe design are listed in Table S1.

MILI Knock down *in vitro*

To constitutively knockdown mili expression *in vitro*, aNPCs were infected at MOI=5 with a lentivirus encoding for shMILI (plKO.1, Sigma), or control lentivirus (SHC202, Sigma) both decorated with an eGFP reporter. GFP-positive cells were first selected by FACS after three passages, and then plated in proliferating or differentiating media, as previously described.

***in silico* piRNA targets prediction**

For piRNA targets analysis, we divided the sequencing data in one set of 100 piRNA clusters enriched in proliferating aNPCs (DIF0) and a second set of 198 clusters specifically expressed at DIF4/7 stage.

The Differential Expression analysis for piRNAs mapping on REs showing trends for enrichment of piRNAs mapping on repeat elements (REs) in DIF4 and DIF7 compared to DIF0 was done using EdgeR software package (*21*).

Identification of piRNA targets was divided in: piRNAs mapping on REs only / piRNAs mapping on GENCODE elements / piRNAs mapping on REs within GENCODE elements / unannotated piRNAs / piRNAs clusters.

Gene Ontology analysis for piRNAs mapping on GENCODE protein-coding genes (but NOT mapping on REs) has been done with the R package GOFuncR (https://bioconductor.org/packages/release/bioc/html/GOfuncR.html)

Protein synthesis assay

To quantify the protein synthesis rate of cells, we used the Global Protein Synthesis Assay Kit (FACS/Microscopy), Red Fluorescence kit (abcam), following the manufacturer's instructions. Briefly, cells in proliferating or differentiating media (DIF7) were treated with Cycloheximide as an inhibitor of protein synthesis, for 30 min at 37°C. Media were replaced with fresh aliquots containing Protein Label (400X) diluted to 1X final concentration and the cells were incubated for additional 30 min at 37°C. Negative control cells were not incubated with the protein label. Samples were analyzed by FACS for red fluorescence generated by *de novo* synthesized protein during click reaction. Translation rate is directly proportional to emitted fluorescence. Cells emitting fluorescence lower than 10^3 were considered negative (P3), higher than 10^4 were considered positive (P2).

Cell survival assay

Control and MILI KD aNPCs were plated at the same concentration (50k cells/well), in coated multi-well of 12 wells. Cells were harvested and counted using Trypan Blue (Gibco) at different time points: proliferation (DIF0), 4 and 7 days after induction of differentiation (DIF4 and 7). The number of cells was normalized on the number of plated cells.

Kainic Acid Administration, single cell suspension and enrichment of aNPCs by FACS

Kainic Acid to elicit tonic, non-convulsive epileptic seizures, was administered as described before (*11*). Briefly, 50nL of 2.22mM Kainic Acid dissolved in PBS (pH 7.4) was injected bilaterally into the hippocampus at the following coordinates (AP -2.0, ML +/- 1.5, DV -2.0 mm) (between 9AM and 1PM). Control animals were administered saline (pH 7.4). Bilateral dentate gyri from 3 animals per condition were pooled to allow sufficient recovery of NPCs. A single cell suspension was created using a Neural Tissue Dissociation kit (Miltenyi Biotec), according to the manufacturers protocol. In order to enrich aNPCs from the DG, we used the endogenous GFP expression driven by the Nestin promotor in combination with FACS. Propium Iodide (5µg/mL) was added to the single cell suspension to assess cell viability. Cells were sorted using a FACSAriatm III system (BD) with 488nm excitation laser. Cell duplets were removed based on forward and side scatter and viable cells were selected based on PI negativity. GFP-positive (corrected for autofluorescence) cells were sorted (≅ 50000 cells/pool) and collected in PBS containing 1% FBS. Trizol LS (Thermo Scientific) was added and after resuspension samples were snap-frozen and stored at -20°C.

Immunofluorescence, STED nanoscopy and particle analysis

Confocal and Stimulated Emission Depletion (STED) nanoscopy were performed as previously reported (*22*, *23*). aNPCs were plated on glass coverslips 24 h before fixation. Cells were fixed with PFA 4%, permeabilized with PBS-T 0.1%, blocked 1 hour at room temperature with PBS-T 0.1% NGS 5% and incubated according to the dilution suggested by the manufacturer's instructions with 0.01 µg/ml rabbit polyclonal antibody against the N terminus of RPL26 (Abcam) for 1 h at room temperature. Cells were washed extensively and incubated with the secondary antibody goat anti–rabbit ATTO-647N (0.8 µg/ml; Sigma) for 45 min. Nuclei were stained while mounting the coverslip with DAPI-Prolong antifade (Invitrogen). Confocal and STED images were acquired at 23°C with a modified TCS SP5 STED-CW gated (Leica Microsystems, Mannheim, Germany) operated with Leica's microscope imaging software. The microscope has been customized with a second pulsed STED laser line at 775nm. The beam originates from a Onefive Katana HP 8 (NKT, Birkerød, Denmark) and pass through a vortex phase plate (RPCphotonics, Rochester, NY, USA) before entering the microscope through the IR port. The depletion laser pulses are electronically synchronized with the Leica's supercontinuum pulsed and visible excitation laser. The ATTO- 647N fluorescence was excited at 633 nm, and the fluorescence depletion was performed 775 nm. The maximal focal power of the STED beam was 200 mW at 80 MHz. Both beams were focused into the 1.4 NA objective lens (HCX PL APO 100× 1.40 NA Oil STED Orange; Leica). Fluorescence was collected by the same lens, filtered with a 775 nm notch filter, and imaged in the spectral range 660–710 nm by hybrid detector with a time gating of 1 ns. All of the images have 14-nm pixel size and 37-µs pixel dwell time.

We performed the analysis of polysome clusters in aNPCs on more than 20 images likewise different cells. Image analysis was performed using the Fiji software.

Statistical analysis

Data are presented as mean ± SEM and were analyzed using Prism 6 (GraphPad). 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. Results were considered significant when $p < 0.05$.

Supplementary figures

Fig. S1

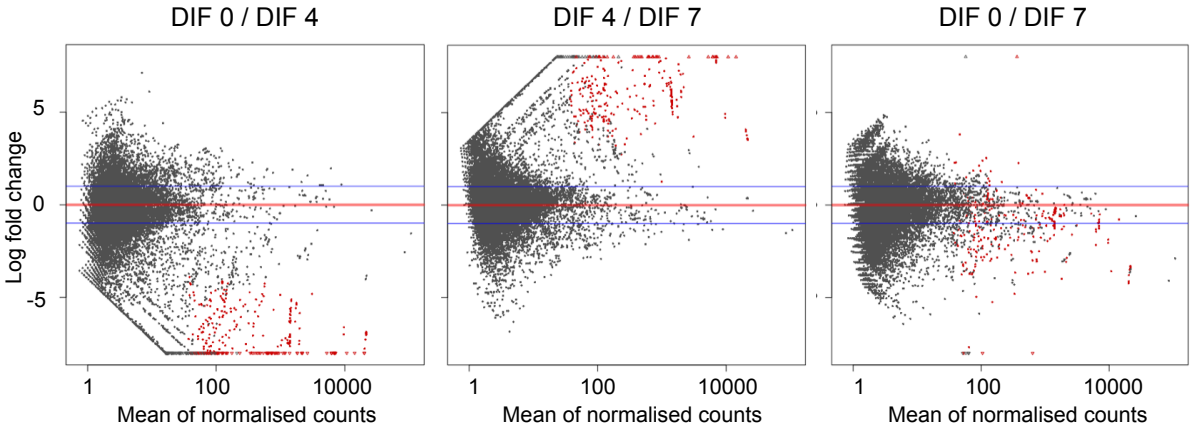


Fig. S1 Pairwise comparison of piRNA clusters in neurogenesis
Pairwise comparison of 298 piRNA clusters differentially expressed in undifferentiated aNPCs (DIF0) or upon induction of neurogenesis (DIF4-7).

Fig. S2

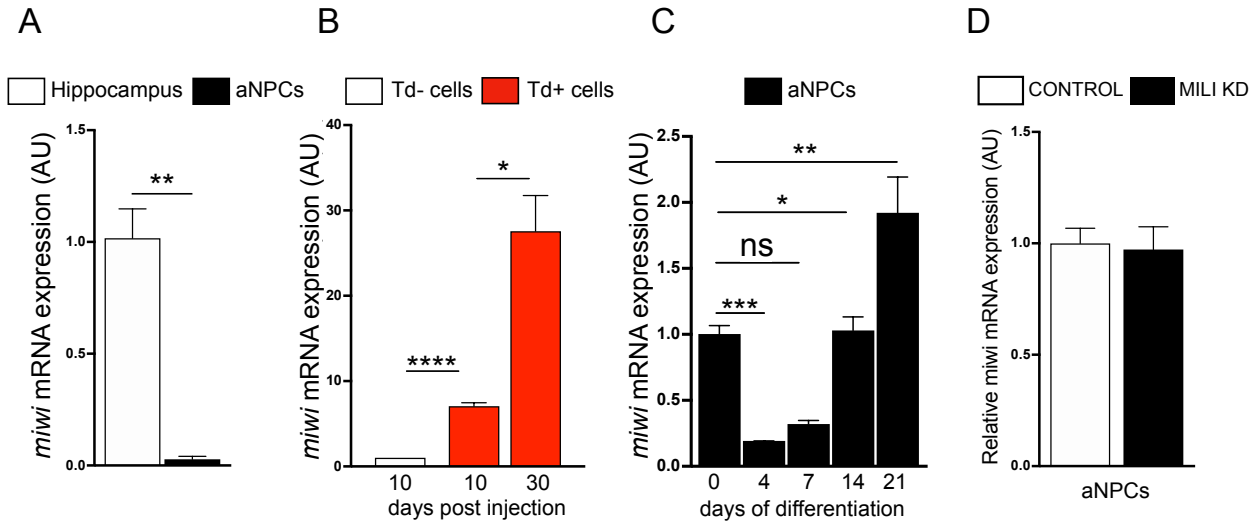


Fig. S2 Miwi expression in control aNPCs, neurons and in Mili KD aNPCs.
Relative expression of *miwi* gene in: (A) postnatal hippocampus and cultured aNPCs; (B) Td-tomato- and + cells sorted at 10 or 30 days post injection of viruses expressing Split-Cre in the postnatal mouse hippocampus; (C) aNPCs undifferentiated (DIF0) or (DIF 4-21) upon viral-mediated induction of neurogenesis; (D) undifferentiated aNPCs *in vitro* transduced with viruses transcribing a Scrambled short-hairpin (Control) and shMILI (MILI KD). Data are expressed as mean ± SEM, n = 3 independent experiments. t-Student test as post hoc: *p < 0.05, **p < 0.01, ***p< 0.001, **** p < 0.0001.

Fig. S3

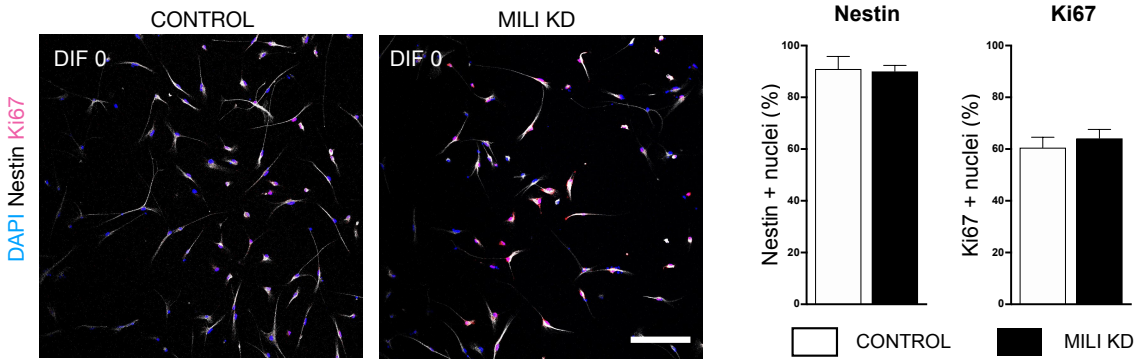


Fig S3. Mili KD does not affect aNPC stemness and proliferation
(Left) Confocal microscopy images of undifferentiated aNPCs *in vitro* transduced with viruses transcribing a Scrambled short-hairpin (Control) and shMILI (MILI KD), immuno-stained with anti-Nestin (white), or anti-Ki67 (purple) antibodies and stained for nuclear DNA with hoechst (blue). (Right) Percentage of Nestin or Ki67 positive cells over total cells. Scale bar 50 μm. Data are expressed as mean ± SEM, n = 3 independent experiments.

Fig. S4

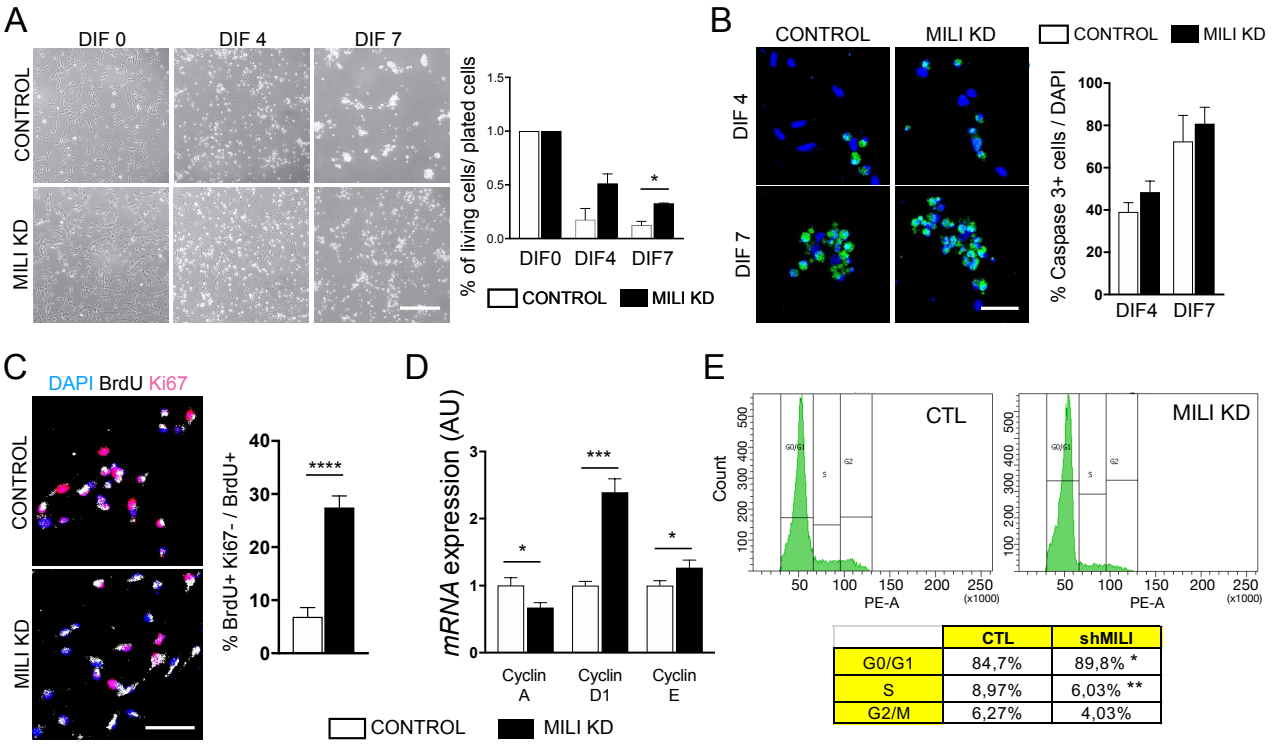
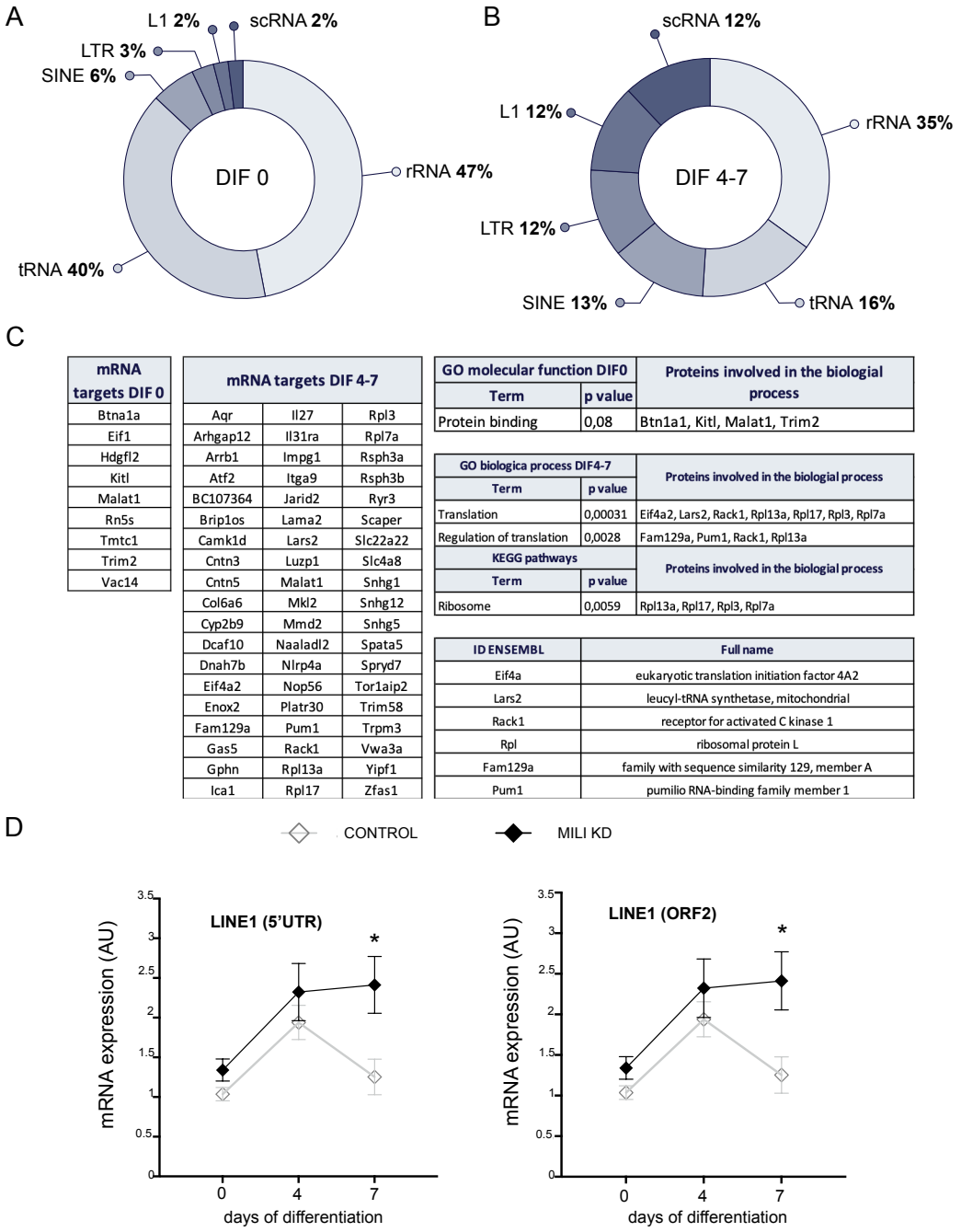


Fig S4. Mili KD increases survival and promotes cell cycle exit without affecting apoptosis in aNPCs.
(A) Light-microscopy images of aNPCs *in vitro* transduced with viruses transcribing a Scrambled short-hairpin (Control) and shMILI (MILI KD) in proliferative (DIF 0) or upon induction of differentiation (DIF 4-7). (Right) Percentage of living cells observed at each time point, normalized on the number of plated cells at DIF0. (B) Fluorescence microscopy images of differentiated (DIF 4-7) Control or MILI KD aNPCs, immunostained with anti-cleaved caspase-3 (green) and for nuclear DNA with Hoechst (blue). (Right) Percentage of cleaved caspase-3+ cells over total cells. (C) Fluorescence microscopy images of control or MILI KD aNPCs, 48h after induction of differentiation, imunostained with anti-BrdU (white) and Ki67 (purple) antibodies. (Right) Percentage of BrdU+ and Ki67- cells over BrdU+ cells. (D) Relative abundance of cell cycle-dependent genes (indicated) normalized to Actin, in Control and MILI KD aNPCs 48 hours after the induction of differentiation. (E) (Top) Representative cell cycle analysis of propidium iodide staining by flow cytometry; (Bottom) Percentage of aNPCs in G0/G1, S and G2/M phases 48h after the induction of differentiation. Scale bar, 500 µm (A) and 50 µm (B,C). Data are expressed as mean ± SEM, n = 3 independent. t-Student test as post hoc: ∗p < 0.05, ∗∗p < 0.01, ∗∗∗p< 0.001.

Fig. S5



mRNA targets DIF 0
Btna1a
Eif1
Hdgfl2
Kitl
Malat1
Rn5s
Tmtc1
Trim2
Vac14

mRNA targets DIF 4-7		
Aqr	Il27	Rpl3
Arhgap12	Il31ra	Rpl7a
Arrb1	Impg1	Rsph3a
Atf2	Itga9	Rsph3b
BC107364	Jarid2	Ryr3
Brip1os	Lama2	Scaper
Camk1d	Lars2	Slc22a22
Cntn3	Luzp1	Slc4a8
Cntn5	Malat1	Snhg1
Col6a6	Mkl2	Snhg12
Cyp2b9	Mmd2	Snhg5
Dcaf10	Naaladl2	Spata5
Dnah7b	Nlrp4a	Spryd7
Eif4a2	Nop56	Tor1aip2
Enox2	Platr30	Trim58
Fam129a	Pum1	Trpm3
Gas5	Rack1	Vwa3a
Gphn	Rpl13a	Yipf1
Ica1	Rpl17	Zfas1

GO molecular function DIF0		Proteins involved in the biologial process
Term	p value	
Protein binding	0,08	Btn1a1, Kitl, Malat1, Trim2

GO biologica process DIF4-7		Proteins involved in the biologial process
Term	p value	
Translation	0,00031	Eif4a2, Lars2, Rack1, Rpl13a, Rpl17, Rpl3, Rpl7a
Regulation of translation	0,0028	Fam129a, Pum1, Rack1, Rpl13a
KEGG pathways		Proteins involved in the biologial process
Term	p value	
Ribosome	0,0059	Rpl13a, Rpl17, Rpl3, Rpl7a

ID ENSEMBL	Full name
Eif4a	eukaryotic translation initiation factor 4A2
Lars2	leucyl-tRNA synthetase, mitochondrial
Rack1	receptor for activated C kinase 1
Rpl	ribosomal protein L
Fam129a	family with sequence similarity 129, member A
Pum1	pumilio RNA-binding family member 1

Fig S5. Noncoding RNAs and mRNAs involved in ribosome assembly and translation control are putative piRNA targets.

(A, B) Pie plots showing proportions of noncoding RNAs predicted targets of piRNAs in undifferentiated aNPCs (A, DIF0) and upon induction of neurogenesis (B, DIF4-7). (C) List of genic RNAs predicted targets of piRNAs in aNPCs (DIF0) or upon induction of neurogenesis (DIF4-7); Gene Ontology (GO) biological process and (Kyoto Encyclopedia of Genes and Genome) KEGG pathway analysis of genic piRNA targets, ID ENSEMBL and Full names of targets encoding regulators of translation are indicated. (D) Relative abundance of transcripts for LINE1 families in undifferentiated (DIF0) and differentiated (DIF4-7) aNPCs upon viral transduction of a scrambled shRNA (Control) or antisense to Mili (Mili KD). Data are expressed as mean ± SEM, n = 3 independent experiments. t-Student test as post hoc: $*p < 0.05$.

Supplementary Data Table S1.
Genomic location on mouse genome (NCBI37/mm9) and read sequence of piRNA clusters in undifferentiated aNPCs (DIF0) and upon induction of neuronal differentiation (DIF4-7).

Supplementary Data Table S2.
Genomic location on human genome (hg38) of piRNA clusters expressed in human neural stem cells (NSC) and astrocytes.

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