Supporting Information

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SI Methods

Constructs. The pGL3Basic vector harboring the promoter region of hsa-miR-34a was kindly provided by M. Oren (The Weizmann Institute, Israel). The predicted p53 binding site was deleted by PCR with two overlapping primers:

34a prom del Fwd 5′-CGGCGGCGGGCTCTGCCTGGGGTTCCGAGCCGGGCTGCTCT-3′; and
34a prom del Rev: 5′-GAAGCAGCCCGGCTCGGAACCCCAGGCAGAGCCCGCCGCCG-3′.

The pcDNA3 vector harboring the primary (pri)-hsa-miR-34a was also kindly provided by M. Oren (The Weizmann Institute, Israel). To express human miR-34b and 34c in cell cultures by transfection, their genomic sequence was amplified from genomic DNA by PCR using the following primers:

hsa-miR-34b,c -HindIIIR 5′-GGCCAAGCTTCAGCCATGGTAGGGCGTCCC-3′; and
has-miR-34b,c -XhoIR 5′-GGCCCTCGAGGCACATTGATGATGCACAGG-3′.

The resulting 720-bp fragments were cloned in pcDNA3 (Invitrogen).

Human synaptotagmin I and syntaxin 1A 3′ UTRs were amplified by PCR from human genomic DNA using the following primers:

hSyt-I 3′UTR Fwd 5′-GGCCTCTAGAGCCAAAACAGACAGCTAGTG-3′ and hSyt-I 3′UTR Rev 5′-GGCCAGATCTACTGTCTTGTGGCCTGGAAA-3′; and
hStx-1A 3′UTR Fwd 5′-GGCCTCTAGAAGCCACCCAAACTGCCACTC-3′ and hStx-1A 3′UTR Rev 5′-GGCCTCTAGATGTTGGTCGACGGGTAAAAC-3′.

The fragments (600 bp and 1,160 bp, respectively) were cloned in pGL3Control vector (Promega).

The miR-34a–predicted target sites on human synaptotagmin I 3′ UTR were deleted by excluding the mir34a binding sites from the 3′ UTR cloning. The first part of the 3′ UTR was cloned using the following primers:

hSYT 34a delA F 5′-GGCCGCTAGCGATGCTTGATTTAGGAGGAGT-3′; and
hSYT 34a delA F 5′-GGCCTCTAGATGAGTGCTATCCACTGAGGA -3′.

The resulting 170-bp fragment was cloned in pGl3 control vector and a second fragment of the human synaptotagmin I 3′ UTR (of about 250 bp) was cloned downstream of the first fragment, giving rise to a 3′ UTR with a 60-bp deletion encompassing the two mir34a predicted binding sites. The following primers were used for this second cloning:

hSYT 34a delB F 5′-GGCCTCTAGAGGTGTGTGTAGCACAAACAG-3′; and
hSYT 34a delB F 5′-GGCCGCTAGCACTGTCTTGTGGCCTGGAAA-3′.

The miR-34a-predicted target site on human syntaxin 1A 3′ UTR was deleted by excluding the mir34a binding site from the 3′ UTR cloning. The first part of the 3′ UTR was cloned using the following primers:

hSTX 34a delA F 5′-GGCCGCTAGCCTCTGGCTCAGAGCACCCTCCCT-3′; and
hSTX 34a delA F 5′-GGCCTCTAGAGACTGAGACTCAGAGCTGGG -3′.

The resulting 380-bp fragment was cloned in pGl3 control vector and a second fragment of the human syntaxin 1A 3′ UTR (of about 640 bp) was cloned downstream of the first fragment, giving rise to a 3′ UTR that starts 80 bp downstream of the first mir34a binding site and with a 10-bp deletion encompassing the second mir34a predicted binding sites. The following primers were used:

hSTX 34a delB F 5′-GGCCTCTAGAAGGCTCAGGCTGCCATG-3′; and
hSTX 34a delB F 5′-GGCCGCTAGCCAAAATGGGCAGCTGGTTGT-3′.

Cell Culture and Transfection. SAOS-2 cells and HEK 293E cells were grown in DMEM High glucose, 10% FBS, penicillin (100 U), and streptomycin (100 μg) (Invitrogen). Cells were transfected by Lipofectamine 2000 or Lipofectamine LTX and PLUS reagent according to the manufacturer's protocols (Invitrogen). Primary cortical neurons cultures were prepared from embryonic day (E) 17.5 embryos mouse. Briefly, cortices were harvested and cut into smaller pieces. Tissue was incubated with 1× Trypsin (Invitrogen) for 20 min at 37 °C and then DNaseI (40 U/mL, Sigma) was added. After washing with Neurobasal medium (Invitrogen) containing 10% FCS, penicillin (100 U), streptomycin (100 μg) (Invitrogen), and 1× Glutamax (Invitrogen), tissue was resuspended by gentle pipetting. The cell suspension was passed through a 100-μm cell strainer and cells were counted with a hemocytometer. Cells were diluted to the appropriate concentration and then plated in aforementioned medium on poly-D-lysine–coated plates. After 1 h, medium was replaced with Neurobasal medium (Invitrogen) containing B27 supplement (Invitrogen), penicillin (100 U), streptomycin (100 μg) (Invitrogen), and 1× Glutamax (Invitrogen). Cortical neurons were transfected with PremiR-34a (30 nM) or anti–miR-34a (100 nM), or FAM-labeled Negative Control (Ambion) or miRCURY LNA Knockdown probe (Exiqon) using the SiPORT neoFX transfection agent (Ambion) according to the manufacturer's instructions. In some experiments we used the following plasmid: to overexpress mir-34a and Syt-1 we used OmicsLink miRNA Expression and OmicsLink Expression Clone (GeneCopoeia), respectively. After the times indicated, cells were harvested for protein and RNA extraction.

Chromatin Immunoprecipitation. Cells were plated in 150-mm dishes (4.5×10^6 cells per dish), and after different treatments were cross-linked with 1% formaledehyde for 10 min at room temperature, crosslinking was stopped by incubating cells with glycine (0.125 M) for 5 min at room temperature. After washing with ice-cold PBS, cells were harvested in lysis buffer (50 mM Hepes-KOH pH 8.0, 1 mM EDTA, 140 mM NaCl, 25% (vol/vol) glycerol, 0.5% Nonidet P-40, 0.25% Triton X-100) plus protease inhibitors. The nuclei were collected by centrifugation and resuspended in wash buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 200 mM NaCl) plus protease inhibitors. After centrifugation, the nuclei were resuspended in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl pH 8.1). Cell lysates were sonicated to obtain chromatin fragments of 700 bp. After centrifugation for 10 min to remove any cell debris, 200 μg of total proteins was precleared with protein G-agarose/salmon sperm DNA (Upstate) for 2 h at 4 °C. Then, the precleared extracts were incubated with monoclonal

p73 (Imgenex), or anti-p73 ab4 mixture (Labvision), or anti-IgG (2 μg) (BD Pharmingen) overnight at 4 °C, followed by incubation with protein G-agarose/salmon sperm DNA for 2 h at 4 °C. The immune complexes were washed three times with low-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, 0.15 M NaCl), three times with high-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, 0.5 M NaCl), twice with LiCl salt wash buffer (1 mM EDTA, 10 mM Tris-HCl, 0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate), and twice with TE buffer. The precipitates were extracted twice using immunoprecipitation elution buffer (1% SDS, 0.1 M NaHCO3) and NaCl (5 M) were added to the total eluates and incubated at 65 °C overnight to reverse the formaldehyde cross-linking. DNA fragments were purified by phenol-chloroform extraction and ethanol precipitation, and dissolved in sterile water. DNA samples were then analyzed with 30 cycles of PCR to amplify miR-34a promoter sequences (95 °C for 30 s, 63 °C for 30 s, 72 °C for 30 s), 36 cycles to amplify the miR-34b and miR-34c promoters and 30 cycles to amplify p21 promoter fragments.

RNA Extraction and Real-Time PCR. Total RNA from cells or tissue was isolated using TRIzol (Invitrogen) according to the manufacturer's instructions. RNA samples were treated with RNase-free DNase I (Invitrogen). Total RNA was reverse transcribed using SuperScript III reverse transcriptase and oligo(dT) primer (Invitrogen). Quantitative RT-PCR (qRT-PCR) was performed in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems) with SYBR green ready mix (Applied Biosystems) and specific primers. For miRNA detection, RNA was reverse-transcribed using a TaqMan MicroRNA Reverse Transcription kit and qRT-PCR was performed with TaqMan universal master mix (Applied Biosystems) and specific primers for miR-34a. Sno202 was used as internal control (Applied Biosystems). The expression of each gene and miRNA was defined from the threshold cycle (Ct), and relative expression levels were calculated by using the 2^{-Ct} method after normalization with reference to expression of housekeeping gene (*GAPDH* or *Sno202*).

Western Blot. Proteins were extracted with RIPA buffer containing mixture inhibitors (Roche) and separated on SDS-polyacrylamide gel. Membranes were blocked with TBS-0.1% Tween 5% milk and incubated overnight with primary antibody, washed, and then incubated with the appropriate horseradish peroxidase-conjugated secondary antiboby. Proteins were visualized with the Super Signal chemiluminescence kit (Thermoscientific). The following antibodies were used: anti-synaptotagmin I (1:1,000; Abcam), anti-synapsin II (1:5,000; Abcam), anti-GAP-43, clone GAP-7B10 (1:4,000; Sigma), anti-PSD95 (1:1,000; Cell Signaling), anti-Syntaxin 1A (1 g/mL; Abcam), anti-Actin (1:2,000; Santa Cruz), p73 (2 g/mL; Imgenex), p53 (C-19) (1:1,000; Santa Cruz), Bcl-2 (1:500; BD Biosciences), cleaved caspase-3 (1:1,000; Cell Signaling), p21 (H-164) (1:500; Santa Cruz), and anti-GAPDH (1:5,000; Santa Cruz).

Bioinformatics. Bioinformatics analysis on synaptotagmin I and syntaxin 1A 3′ UTR was performed using TargetScan 5.0 and PicTar software, available at http://www.targetscan.org/ and http://pictar.mdc-berlin.de, respectively.

Luc Assay. Saos-2 cells or HEK 293E cells were plated in 12-well plates (1×10^5 and 1.5×10^5 per well, respectively). After 24 h, pGL3control vectors (200 ng) were cotransfected with precursor (pre)-miR-34a (30 nM) or Scrambled and Renilla luciferase pRL-CMV vector (10 ng), using Lipofectamine 2000. Luciferase activities were measured 24 h after transfection using a Dual Luciferase Reporter Assay System (Promega); light emission was measured over 10 s using an OPTOCOMP I luminometer. Efficiency of transfection was normalized using Renilla luciferase activity.

Laser -Microdissection. Coronal or sagittal brain sections (10 μm) were prepared using a cryostat at −20 °C and mounted on PEN-membrane slides (PALM). After sectioning, the slides were fixed in 100% ethanol and then hydrated through a series of 95%, 70%, and 50% ethanol solutions prepared with DEPC–H_2O. Slides were stained with Cresyl violet acetate for 40 s. Then, slides were dehydrated in 70%, 95%, and 100% ethanol. Finally, sections were immersed in xylene for 5 min and air-dried and subjected to laser-capture microdissection. Different regions of the cortex, cerebellum, and hippocampus were laser microdissected on a PALM MicroBeam C (Zeiss).

Flow Cytometry. Flow cytometry was performed as described in Copani et al (1) and Kruman et al. (2). Briefly, cortical neurons were harvested after treatment with 0.025% trypsin for 3 min at 37 °C and then, after addition of 10% FCS in PBS, the cells were centrifuged, washed with PBS, and fixed in cold 70% (vol/vol) ethanol. The harvesting of cortical cells using these conditions led to high yields of undamaged cells. After fixation, cells were washed with PBS, treated for 15 min at 37 °C with RNase (100 μg/mL) and then stained with propidium iodide staining (10 μg/mL in the dark for 30 min). The samples were then analyzed using a FACScan flow cytometer (Becton Dickinson).

Alzheimer's Disease Samples. RNA was extracted from snap-frozen brain tissue from the London Neurodegenerative Disease brain bank. Controls were from the hippocampus of patients classified as normal brain with no clinical dementia (age: 89.25 ± 10.7). Alzheimer samples were from the hippocampus of patients with definite Alzheimer's disease (Braak stage 5–6; age 83.00 ± 8.7).

Immunofluorescence. Postnatal day (P) 2 brains were fixed in 4% paraformaldehyde and embedded in paraffin. The sections were dewaxed, rehydrated in graded alcohols, and rinsed in distilled water. The sections were blocked in 10% goat serum in 0.1% PBS-Tween (vol/vol), incubated for 10 min with DAPI and mounted for confocal microscopy. Two different images of each section or cortical cells were taken and the number of cells labeled was counted ($n = 3$ $p73^{-/-}$ compared with $n = 3$ control littermates).

Primers for ChIP.

miR-34a Fwd 5′-AGGTGGAGGAGATGCCGCTGTC-3′;
miR-34a Rev 5′-GGAGCTTGGCTGCAGGACTCC-3′;
miR-34bc Fwd 5′-CCCTGAAAGGTGCCTTCCTTG-3′;
miR-34bc Rev 5′-GCTTGTTCTTAGGGTTGCTGTTG-3′;
p21 Fwd 5′-GGGTCTGCTACTGTGTCCTC-3′;
p21 Rev 5′-GCAGAGGATGGATTGTTCATCT-3′;

Primers for Real-Time PCR.

TAp73 Fwd 5′-GCACCTACTTTGACCTCCCC-3′;
TAp73 Rev 5′-GCACTGCTGAGCAAATTGAAC-3′;
ΔNp73 Fwd 5′-ATGCTTTACGTCGGTGACCC-3′;
ΔNp73 Rev 5′-GCACTGCTGAGCAAATTGAAC-3′;
p53 Fwd 5′-CTCTCCCCCGCAAAAGAAAAA-3′;
p53 Rev 5′-CGGAACATCTCGAAGCGTTTA-3′;
TAp63 Fwd 5′-CACCCAGACAAGCGAGTTC-3′;
TAp63 Rev 5′-TTCCAGAAAATCCCAGATATGC-3′;
ΔNp63 Fwd 5′-CTGGCAAAACCCTGGAAG-3′;
ΔNp63 Rev 5′-CAACATGTTAGCAGTGAGACTGG-3′;
Synaptotagmin I Fwd 5′-AAGGAGATTCCAAAAGGAAC-AA-3′;
Synaptotagmin I Rev 5′-TTTTGGTTCAAGCGGAATG-3′;
Synaptotagmin IV Fwd 5′-ATGGCTCCTATCACCACCAG-3′;
Synaptotagmin IV Rev 5′-AAGACGAGGCCAAAAGCAC-3′;
GAP-43 Fwd 5′-AGGAGAAGAAGGGTGAAGGG-3′;
GAP-43 Rev 5′-ATCAGTGACAGCAGCAGGCA-3′;

Synapsin I Fwd 5′-CCACAGCCAGTCTTCATCC-3′;
Synapsin I Rev 5′-GGTCGTTGTTGAGCAGGAG-3′;
Synapsin II Fwd 5′-CCTTGGAGATTATGACATCAAGGT-3′;
Synapsin II Rev 5′-GCCACCAGGTTAAGCTCTGA-3′;
PSD95 Fwd 5′-AGTCTGTGCGAGAGGTAG-3′;
PSD95 Rev 5′-GGATGAAGATGGCGATAGG-3′;
Sintaxin 1A Fwd 5′-GAACCCTGCCATCTTTGC-3′;
Sintaxin 1A Rev 5′-ATCTCACTGAGGGCCTGCT-3′;
GAPDH Fwd 5′-CAATGAATACGGCTACAGCAAC-3′;
GAPDH Rev 5′-AGGGAGATGCTCAGTGTTGG-3′;
human Actin Fwd 5′-TCACCCACACTGTGCCCATCTAC-GA-3′;
human Actin Rev 5′-CAGCGGAACCGCTCATTGCCAAT-GG-3′;
human pri-miR-34a Fwd 5′-CAACCAGCTAAGACACTGC-CAA-3′;
human pri-miR-34a Rev 5′-CCTCCTTGCATCCTTTCTTTC-CT-3′;
human pre-miR-34a Fwd 5′-TGGCAGTGTCTTAGCTGGT-TG-3′;
human pre-miR-34a Rev 5′-GGCAGTATACTTGCTGATT-GCTT-3′;
human U6 snRNA Fwd 5′-ctcgcttcggcagcaca-3′;
human U6 snRNA Rev 5′-AACGCTTCACGAATTTGCG-T-3′;
human Syt-1 Fwd 5′-tgaagtacctttgaacaaatcca-3′;
human Syt-1 Rev 5′-GCATCGTTCTTGCCAATCTT-3′;
human STX-1A Fwd 5′-GAAGCACAGTGCCATCCTG-3′;
human STX-1A Rev 5′-TGTTTGCTGTCTTCTTTATGTC-G-3′;
mouse c-fos Fwd 5′-CATCATCTAGGCCCAGTGGC-3′;
mouse c-fos Rev 5′-AGGAACCAGACAGGTCCACATCT-3′;
mouse pre-miR-134 Fwd 5′-GGGTGTGTGACTGGTTGAC-CA-3′;
mouse pre-miR-134 Rev 5′-GGGTTGGTGACTAGGTGG-CC-3′.

1. Copani A, et al. (1999) Mitotic signaling by beta-amyloid causes neuronal death. *FASEB J* 13:2225–2234.
2. Kruman II, et al. (2004) Cell cycle activation linked to neuronal cell death initiated by DNA damage. *Neuron* 41:549–561.

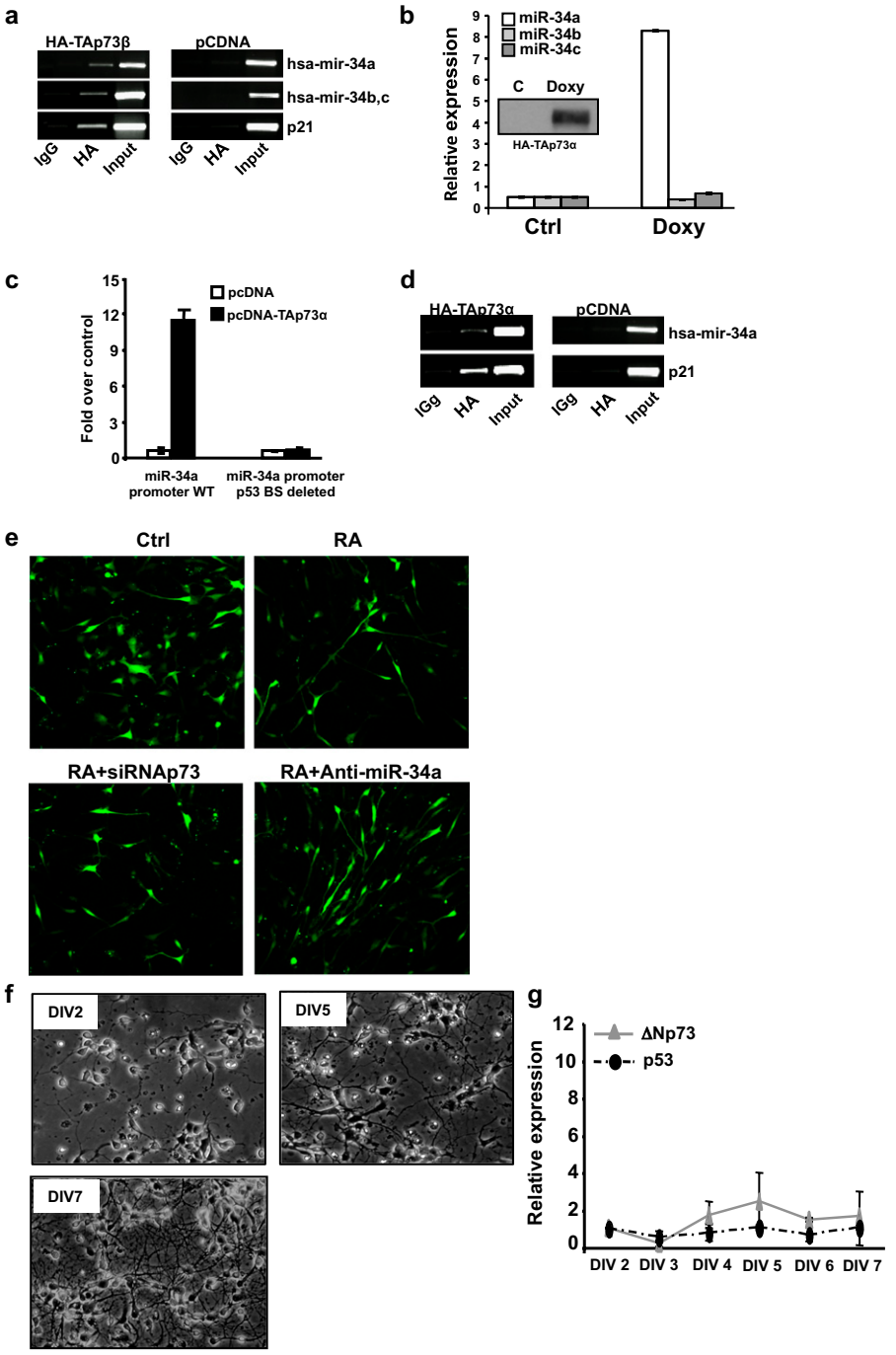

Fig. S1. TAp73α and TAp73β both increase miR-34a expression. (*A*) ChIP assay on Saos-2 cells transfected with empty vector or pcDNA-HA-TAp73β. Protein-chromatin complexes were immunoprecipitated with anti-HA or IgG as control antibody. RT-PCR was performed with primers designed against has-miR-34a, or p21 promoter regions encompassing a predicted or validated p53 binding site. Input refers to PCR carried out on samples before immunoprecipitation. (*B*) SAOS-2-TAp73α inducible cell lines were treated with Doxycyclin for 24 h to overexpress the human TAp73α protein. Endogenous levels of miRs-34a, -34b, and -34c were assayed by real-time PCR. (*C*) A p53-binding site on the miR-34a promoter between −1,472 and +551 bp was analyzed for TAp73 responsiveness. The miR-34a promoter region was cloned upstream of a luciferase reporter gene and increased luciferase activity results when TAp73α is expressed in SAOS-2 cells. Deletion of the p53-binding site abolished TAp73 mediated up-regulation. (*D*) ChIP assay on Saos-2 cells transfected with empty vector or pcDNA-HA-TAp73α. Protein-chromatin complexes were immunoprecipitated with anti-HA or IgG as control antibody. RT-PCR was performed as in *A*. (*E*) Representative images of neurite outgrowth in the SHSY-5Y cell line. Cells were untreated or treated with retinoic acid (RA) in the absence or presence of siRNAp73 or anti–miR-34a. After 48 h, cells were fixed and analyzed for neurite extension as described in *SI Methods*. (*F*) WT primary mouse cortical neurons were isolated from E16 and allowed to differentiate in vitro. Phase-contrast microscopy of cortical neurons during in vitro differentiation at days in vitro (DIV) -2, -5, and -7 is shown. (*G*) ΔNp73 and p53 expression does not change during in vitro terminal differentiation of cortical neurons. E17 cortical neurons were cultured in vitro and RNA was extracted at the indicated time points. Expression of ΔNp73 and p53 was quantified by real-time PCR. Data represent mean ± SD of three independent experiments. Magnification: *E*, 10×; *F*, 10×.

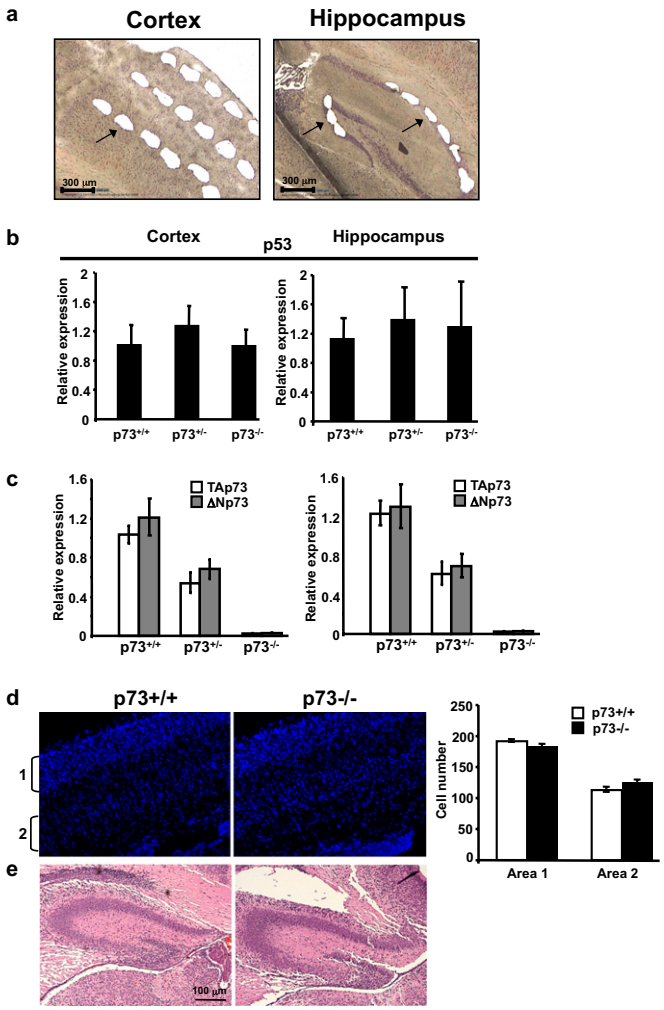

Fig. S2. p53, TAp73, and ΔNp73 expression and cell number in the brains of $p73^{+/+}$ and $p73^{-/-}$ mice. (*A*) Laser-capture microdissection from Cresyl violet-stained sections from cortex and hippocampus. Arrows indicate areas captured. (*B* and *C*) Expression of p53, TAp73, and ΔNp73 in the cortex and hippocampus isolated from $p73^{+/+}$, $p73^{+/-}$, and $p73^{-/-}$ mice, respectively (n = 5). Data represent mean ± SD of three different experiments. Data are normalized to the housekeeping gene GAPDH and are expressed relative to $p73^{+/+}$. (*D*) Cortical sections from $p73^{+/+}$ and $p73^{-/-}$ mice (age, P2), stained with DAPI. Cell number was determined by counting the DAPI-stained cells in two different layers of the cortex (indicated as 1 and 2). Magnification: ×10. Data represent mean ± SD of three independent experiments. (*E*) $p73^{-/-}$ mice show normal hippocampal structure at P2. Representative H&E sections are shown.

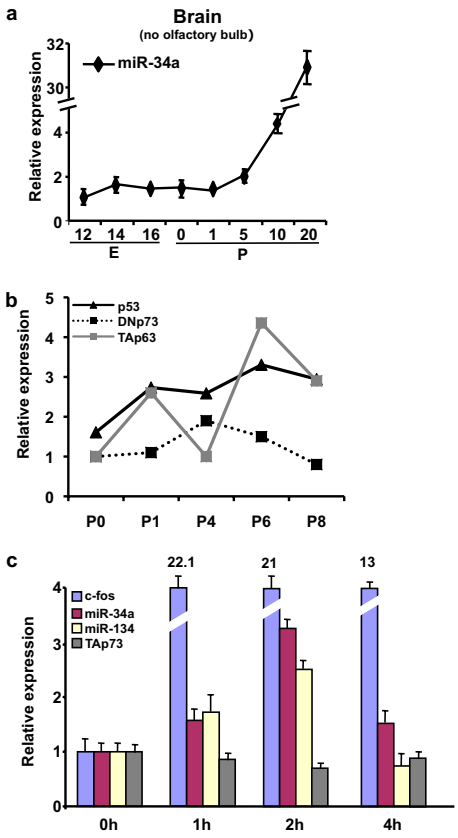

Fig. S3. Expression of miR-34a during brain development. (*A*) Whole brain (minus the olfactory bulb) was isolated from WT mice (n = 4 per point) and expression of miR-34a was evaluated during brain development by real-time PCR. E, embryonic; P, postnatal. (*B*) The cerebellum was isolated from WT mice (n = 4 per point) and expression of p53, TAp63, and ΔNp73 was evaluated during cerebellar development by real-time PCR at birth (P0) and subsequent postnatal days. (*C*) Cortical neurons were treated with KCl (55 mM) and the indicated transcripts were assayed by real-time PCR. C-fos (out of scale) and miR-134 have been evaluated as a marker of KCl activation. Results suggest a TAp73-independent expression of miR-34a following induction of neuronal activity. Data represent mean ± SD of three independent experiments.

a

		Binding side number
SYN2	synapsin II	1
SYT4	synaptotagmin IV	1
SYNJ1	synaptojanin 1	1
SYT1	synaptotagmin I	3
RIMS3	regulating synaptic membrane exocytosis 3	1
VAMP2	vesicle-associated membrane protein 2 (synaptobrevin 2)	3
Stx1A	syntaxin 1A	1
GRM7	glutamate receptor, metabotropic 7	1
GRID1	glutamate receptor, ionotropic, delta 1	1

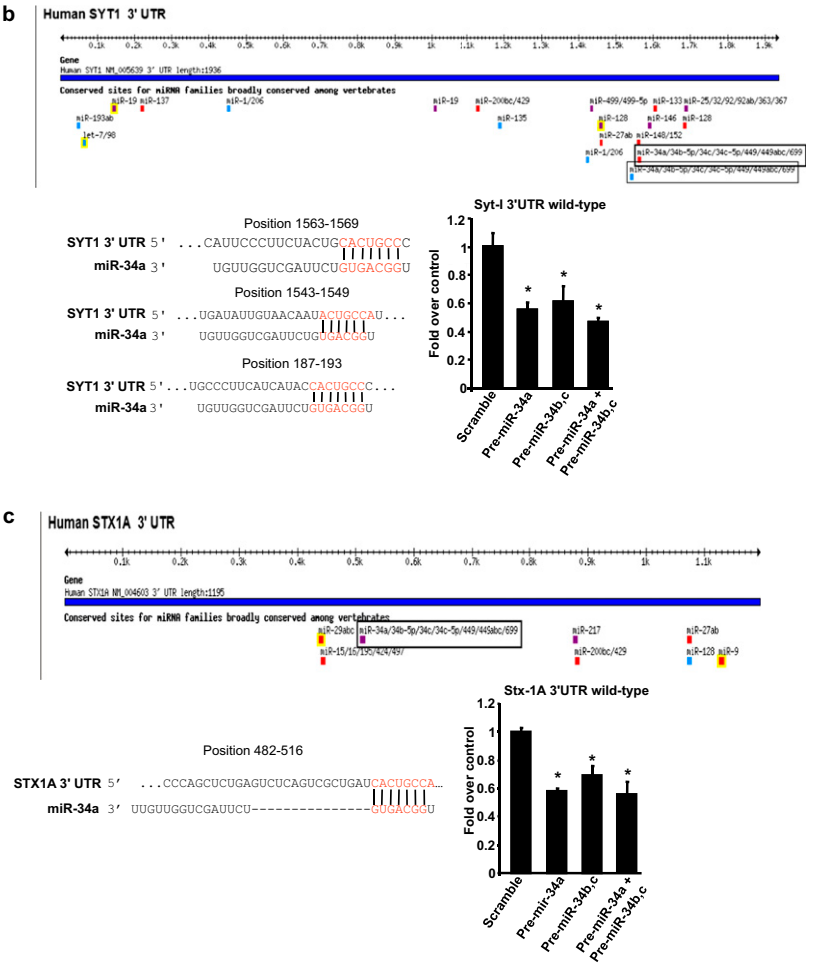

Fig. S4. Bioinformatics analysis of predicted miR-34a targets. (*A*) Several synaptic proteins were identified as potential targets of miR-34a using TargetScan 5.0 and PicTar software. (*B* and *C*) Computational analysis of synaptotagmin 1 and syntaxin 1A 3′ UTRs was performed with TargetScan 5.0 software. Insertion of the Syt-I 3′ UTR (*B*) or Stx1A 3′ UTR (*C*) upstream of a luciferase reporter gene leads to diminished luciferase activity in the presence of precursor pri-miR-34a, -b, and -c. The assay was performed in HEK 293E cells. Data represent mean ± SD of three different experiments. **P* = 0.03.

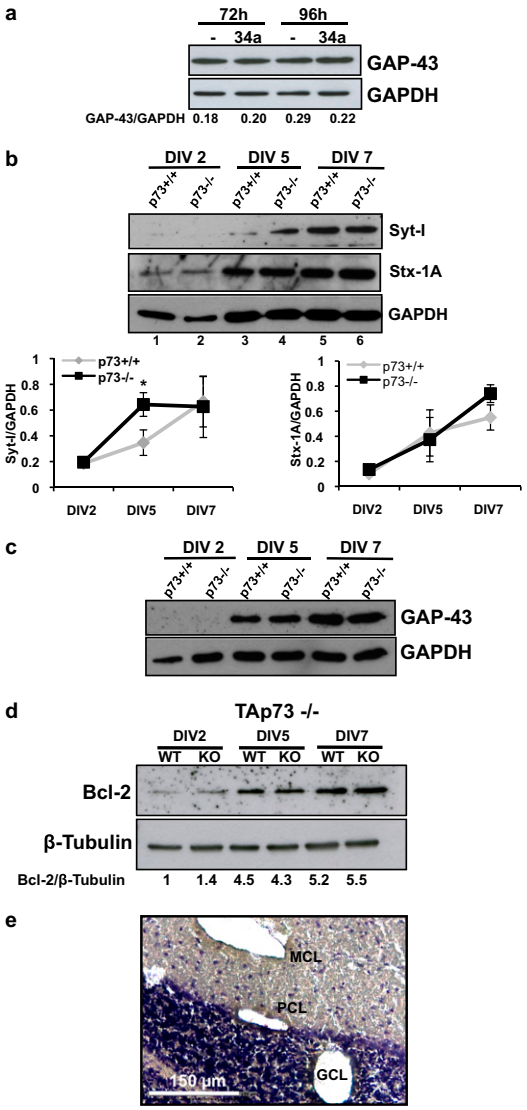

Fig. S5. Expression of GAP-43 does not change during ectopic expression of miR-34a. (*A*) Cortical neurons (DIV5) were transfected with precursor miR-34a (34a) or scrambled negative control (–) for 72 or 96 h and the cell lysates were analyzed for GAP-43 expression. Numbers below the Western blot show densitometry analysis of GAP-43 relative to GAPDH. Data are representative of three different experiments. (*B*) Expression of Syt-1 and Stx-1A in cortical neurons from $p73^{+/+}$ and $p73^{-/-}$ mice during terminal differentiation. GAPDH was used as loading control. Graphs below the Western blots show the relative levels of synaptic protein normalized to GAPDH. Data represent mean ± SD of three different experiments. *$P = 0.05$ (two-tailed Student's *t* test). (*C*) GAP-43 expression in $p73^{+/+}$ and $p73^{-/-}$ mice during terminal differentiation of cortical neurons. (*D*) Bcl-2 expression in WT and $TAp73^{-/-}$ mice during in vitro terminal differentiation of cortical neurons. In this experimental model, Bcl2 is independent of miR-34a. (*E*) Laser-capture microdissection from Cresyl violet stained sections from cerebellum. GCL, granular cell layer; MCL, molecular cell layer; PCL, Purkinje cell layer. (Scale bar, 150 μm.)

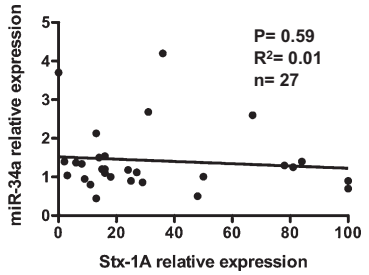

Fig. S6. No statistically significant correlation was observed for Stx-1A. Alzheimer hippocampal samples and relative controls are the same as described in Fig. 4.

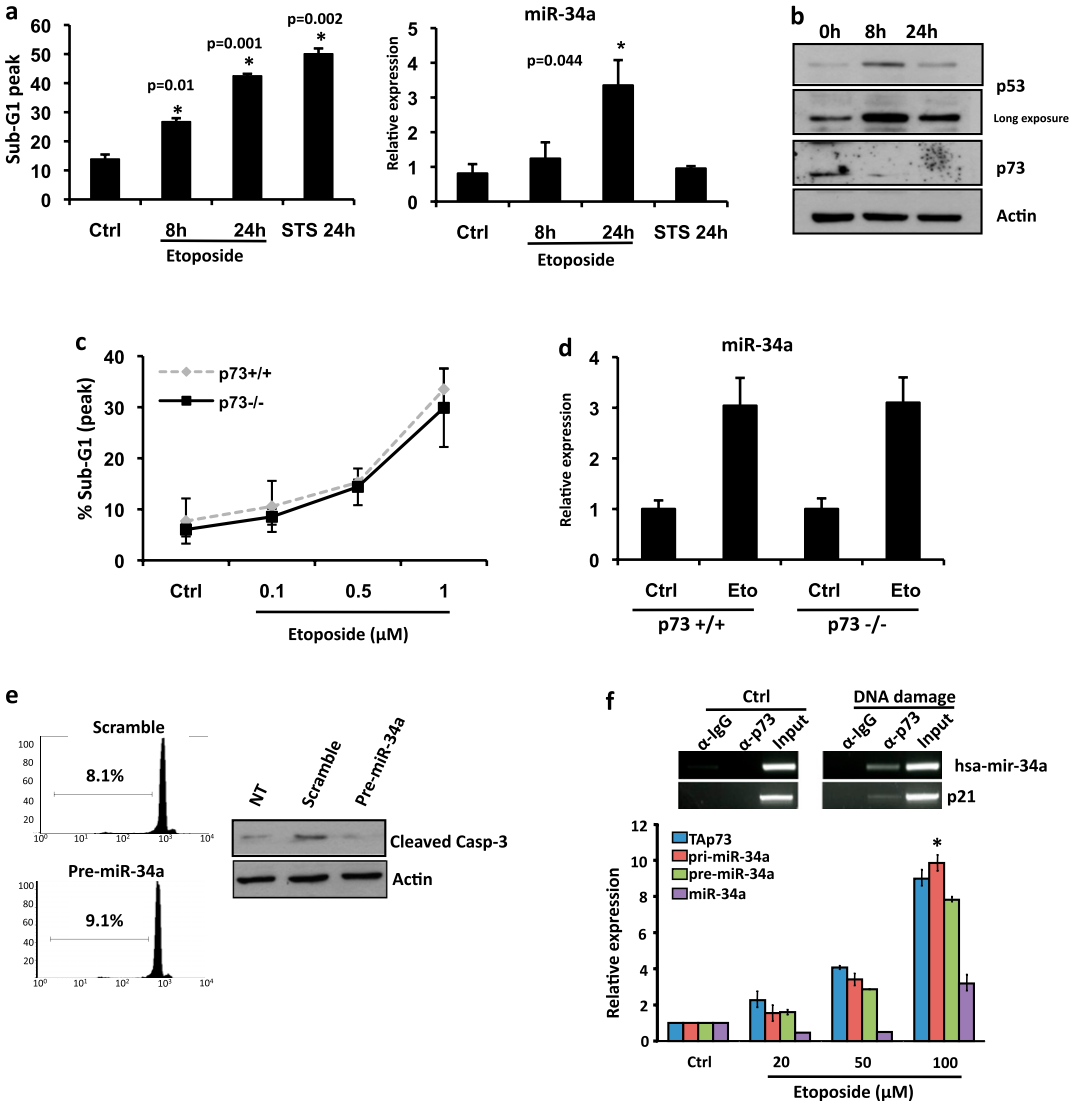

Fig. S7. miR-34a and cortical neurons apoptosis. To evaluate whether miR-34a could also play a role in the apoptotic processes in the developing nervous system, cortical neurons were treated with two different stimuli: etoposide to induce DNA damage, and staurosporine, a protein kinase inhibitor. DIV5 WT cortical neurons were treated with Etoposide or Staurosporine (STS). (*A*, *Left*) Apoptosis and miR-34a (*Right*) expression was evaluated by propidium iodide staining and real-time PCR, respectively. Data represent mean ± SD of three different experiments and *P* values were obtained using two-tailed Student's *t* test. (*B*) Western blot analysis of p53 and p73 in WT cortical neurons treated as in *A*. Actin was used as loading control. (*C* and *D*) p73 is not required for apoptosis and expression of miR-34a after DNA damage. DIV5 cortical neurons from $p73^{+/+}$ and $p73^{-/-}$ mice were treated with the indicated concentrations of etoposide, and apoptosis (*C*) and miR-34a expression (*D*) were evaluated as in *A*. (*E*) Cortical neurons were transfected at DIV5 with scrambled control or pre-miR-34a. Cells were harvested after 48 h and analyzed for apoptosis by propidium iodide staining and FACS analysis and by Western blot for cleaved caspase-3. Actin was used as loading control. Data are from a representative of three different experiments. (*F*) miR-34a and its precursors are induced after DNA damage in the absence of p53. The $p53^{-/-}$ H1299 cell line was treated with different concentrations of etoposide and total RNA was extracted after 24 h. Endogenous levels of TAp73, pri-miR-34a, pre-miR-34a and miR-34 were evaluated by real-time PCR (*Lower*, histogram). Data represent mean ± SD of three different experiments. **P* = 0.05 (two-tailed Student's *t* test). ChIP assay on H1299 cells after DNA damage (*Upper*). Protein-chromatin complexes were immunoprecipitated with anti-p73 or IgG as control antibody. RT-PCR was performed with primers designed against has-miR-34a or p21 promoter regions encompassing a predicted and validated p53 binding site. Input refers to PCR carried out on samples before immunoprecipitation.