Summary of supplementary information

The Supplementary Information contains some experimental methods (Supplementary methods), some supplementary results of the experimental (Supplementary figure1-9), antibody information (Supplementary table1) used in western and Immunofluorescence staining, primer information (Supplementary table2, 3) and the contribution of each author.

Supplementary methods

Stereotaxic injections

The mice were anesthetized with 20ng/ml AVER(0.02ml/g) (Aladdin) delivered through intraperitoneal injection and placed in a stereotaxic frame. The area around the incision was trimmed, and then, treated the meninges with 3% hydrogen peroxide (Aladdin) and find the bregma to locate the origin. Virus were injected bilaterally into the DG of the dorsal hippocampi using the following coordinates: (from bregma) anterior = -1.75mm, lateral = $\pm 1.75mm$, (from skull surface) height = -2.06mm. A 2 µl volume was injected stereotaxically over 10 min (injection speed: 0.20μ l/min) using a 5 µl 33s gauge Hamilton syringe. To limit reflux along the injection track, the needle was maintained in situ for 10 minutes, slowly pulled out. The skin was closed using silk suture. Mice were single-housed and monitored during recovery.

Novel Object Recognition

Mice were habituated in the room for behavior detection for 1d. For novel-object recognition training, the mice were handed into a black Plexiglas rectangular chamber $(31 \times 24 \text{ cm})$, height 27 cm), and two identical objects were presented to mice to explore for 20 min, after which mice were returned to the home cage. Twenty-four hours later, one object was replaced by one novel object and the mouse was again placed in the chamber 6min. The novel object had the same height and volume as the object it replaced, but different shape and appearance. To control for odor cues, the open field arena and the objects were thoroughly cleaned with ethanol, dried, and ventilated between mice. Exploration of the objects was defined as sniffing of the objects (with nose contact or head directed toward the object) within a 2-cm radius of the objects. Sitting or standing on the objects was not scored as exploration. Behavior was recorded from cameras positioned above the training chamber.). The discrimination index (DI) was computed as DI = (novel-object exploration time – familiar-object exploration time/total exploration time) × 100. The discrimination ratio (DR) of novel-object was computed as DR = (novel-object exploration time /total exploration time) × 100.

Water maze

Mice were habituated in the room for behavior detection for 1d. The water maze consisted of a circular pool (120 cm diameter) filled with water $(22 \pm 2 \, ^{\circ}C)$ surrounded by Curtains. The platform (10 cm wide) was located approximately 1 cm below the water level. On the first 4 d (d, days), the mice were trained to locate a visible platform. Each mouse was given four trials (at different quadrants) per day. Each trial began by placing the mouse in the water, near and facing the wall of the pool. The starting points for each subject were chosen randomly from any of the three quadrants other than the one with the platform. Each subject was allowed 60 s to find the platform and, if it failed to reach the platform within 60 s, it was guided by the experimenter to the platform and allowed to stay on the platform for 20 s. The mice that did not reach the platform during a trial were assigned a latency of 60 s. Upon removal from the maze, the mice were dried with fresh towel, placed on a warm platform and returned to their home cages. After the visible platform test was completed, the mice were then tested with no platform, each subject was allowed 60 s to find the platform and record the data with the

camera. The entire travel of the mouse was tracked and analyzed off-line with EthoVision XT software. The number of mice through the platform area during the probe trial or the time of mice arrival in platform during learning was used as a measure of spatial memory retention. Mice with more than 20 s of floating during learning acquisition were excluded from analysis.

Frozen section

Three weeks after stereotaxic injection, mice transcranial perfusion with 4% paraformaldehyde (PFA), brains were removed and postfixed for a further 16 h in 4% PFA and then transferred to 30% sucrose in PBS until saturated. The brain was embedded in Tissue-Tek O.C.T. compound. Coronal frozen slices 30 µm thick were prepared on a freezing microtome (Leica) and stored the brain slices in the brain freezing medium which contains 30% sucrose and 30% ethanediol, 11.36% Na2HPO4 and 2.4% NaH2PO4.







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Figure S7







Supplementary figure legends

Figure S1. Related to Figure 2. a-e Flow cytometry detection of percentage of cy5-positive cells at 48h post transfection with mock(a), mc-cy5(b), m153-cy5(c), ic-cy5(d), i153-cy5(e). **f** Quantitative RT-PCR detection of miR-153 in L-NSC+mc and L-NSC+m153. U6 was used as an internal control for the normalization in quantitative RT-PCR. **g** Quantitative RT-PCR detection of wpre in E-NSCs-ctrl and E-NSC-Lenti-sp-153-wpre (infection with Lenti-sp-153-wpre virus). **h** Quantitative RT-PCR detection of neuron-related genes and glial cell-related genes in the neural differentiation of E-NSCs-ctrl or E-NSC-Lenti-sp-153-wpre. **i** Immunofluorescence analysis of neural differentiation with E-NSCs-ctrl or E-NSC+Lenti-sp-153-wpre as evaluated by MAP2 (red), GFAP (green) and Ho.33342 (blue). Scale bar: 50 μm. **j** Percentage of MAP2-positive or GFAP-positive cells in neural differentiation. Data information: The data shown are the mean±SEM, n=3; ANOVA; ***p<0.001, **p<0.01. Abbreviations: L-NSCs, late NSCs; E-NSCs, early NSCs; mc, control mimics; m153, miR-153 mimics; ic, control inhibitor; i153, miR-153 inhibitor; Lenti-sp-153-wpre, Lenti-sponge-153-wpre.

Figure S2. Related to Figure 3. Quantitative RT-PCR detection of miR-153 in L-NSC+mc, L-NSC+m153, L-NSC+Dll4 and L-NSC+m153+Dll4 groups; Data shown are the mean±SEM, n=3; U6 was used as an internal control for the normalization in quantitative RT-PCR. Abbreviations: L-NSCs, late NSCs; E-NSCs, early NSCs; mc, control mimics; m153, miR-153 mimics.

Figure S3. Related to Figure 4. a The sequence of miR-153-3p and the binding sites of miR-153-3p in 3'UTR of Hey2 and Jag1 in different species. **b** Western blot assay for measuring the HEY2 protein levels normalized to β-TUBULIN in ctrl, shHey2-1 and shHey2-2 groups of NSCs. **c** Quantitative RT-PCR detection of neuron-related genes (*Tubb3, Map2, NeuN*) and glial cell-related genes (*Gfap, S100β*) in neural differentiation of ctrl, shHey2-1 and shHey2-2 groups. **d** Western blot assay for measurements of JAG1, HEY1 and HES1 proteins in ctrl, shJag1-1 and shJag1-2 groups of NSCs, normalized to β-TUBULIN. **e** Quantitative RT-PCR detection of neuron-related genes and glial cell-related genes in neural differentiation of ctrl, shJag1-1 and sh Jag1-2 groups. **f** Quantitative RT-PCR detection of miR-153 in L-NSCs+mc, L-NSCs+m153, L-NSCs+m153+H, L-NSCs+m153+J and L-NSCs+m153+H+J groups. Data information: The data shown are the mean±SEM, n=3; ANOVA; ***p<0.001, **p<0.01, *<0,05. Abbreviations: L-NSCs, late NSCs; E-NSCs, early NSCs; mc, control mimics; m153, miR-153 mimics; H, Hey2; J, Jag1.

Fig. S4 Related to Figure 5. a Exploration time for different objects in young and aged mice; young, n = 9; aged, n = 9. **b**, **c** Movement distance and speed of young and aged mice during the water maze test; young, n = 10; aged, n = 10. **d**, **e** Percentage of BrdU+ cells expressing either DCX, NEUN, GFAP, or S100 β ; n = 3 brains; ANOVA; ****p<0.0001, ***p<0.001, *p<0.05. Data information: The data shown are the mean±SEM. Abbreviations: young mice, 8-10 weeks old; aged mice, 16-18 months old; NOR, novel object recognition.

Fig S5. Related to Figure 6. a Percentage of GFP positive cells in hippocampus three weeks post injection AAV-ctrl or AAV-sp-153; n = 3 brains. **b** Exploration time for different objects for the AAV-ctrl or AAV-sp-153 mice; AAV-ctrl, n=9; AAV-sp-153, n=9. **c** Movement distance and speed of the AAV-ctrl or AAV-sp-153 mice during the water maze test; AAV-ctrl, n = 11; AAV-sp-153, n = 10. **d**, **e** Percentage of GFP⁺/BrdU⁺ cells expressing either DCX, NEUN, GFAP, or S100 β ; n = 3 brains; ANOVA; **p<0.01, *p<0.05. Data information: The data shown are the mean±SEM. Abbreviations: AAV-sp-153, AAV-sponge-153; young mice, 8-10 weeks old; NOR, novel object recognition.

Fig S6. Inhibition of miR-153 with Retro-sp-153 decreases hippocampal neurogenesis and impairs the cognitive function of young mice. a Schematic of virus injection into the hippocampi of young mice and the process diagram of detection. **b** Percentage of GFP positive cells in hippocampus three weeks post injection Retro-ctrl or Retro-sp-153; n = 3 brains. **c** Immunofluorescence analysis of neurons in the hippocampi of Retro-ctrl or Retro-sp-153 young mice, as evaluated by GFP (green), DCX (red), NEUN (red), and Ho.33342 (blue). Scale bar: 50 µm. **d** Immunofluorescence analysis of astrocytes in the hippocampi of Retro-ctrl or Retro-sp-153 mice, as evaluated by GFP (green), GFAP (red), S100β (red), and Ho.33342 (blue). Scale bar: 50 µm. **e**, **f** Percentage of GFP⁺ cells expressing either DCX, NEUN, GFAP, or S100β; n = 3 brains; ANOVA; ***p<0.001, *p<0.05. **g** Exploration time for different objects and discrimination index and discrimination ratio of novel objects; Retro-ctrl, n=9; Retro-sp-153, n=12; Student's t test; ***p<0.001. **h** Platform crossings and the amount of time for the mice to first reach the platform area during the water maze test; Retro-ctrl, n=8; Retro-sp-153, n=11; Student's t test; *p<0.05. **i** Movement distance and speed of the Retro-ctrl or Retro-sp-153, n=11; Student's t test; Retro-sp-153, n=11. Data information: The data shown are the mean±SEM Abbreviations: Retro-sp-153, Retro-sp-153. Abbreviations: young mice, 8-10 weeks old; NOR, novel object recognition; MWM, Morris water maze.

Fig S7. Related to Figure 7. a Percentage of GFP positive cells in hippocampus three weeks post injection AAV-ctrl or AAV-sp-153; n = 3 brains. **b** Exploration time for different objects for the AAV-ctrl or AAV-153 mice during testing; AAV-ctrl, n=7; AAV-153, n=11. **c**, **d** Movement distance and speed of AAV-ctrl and AAV-153during the water maze test; Data shown are the mean \pm SEM; AAV-ctrl, n = 7; AAV-153, n = 8. **e**, **f** Percentage of GFP+/BrdU+ cells expressing either DCX, NEUN, GFAP, or S100 β ; n = 3 brains; ANOVA; ***p<0.001, **p<0.01. **g** Immunofluorescence analysis of NSCs in the hippocampi of AAV-ctrl or AAV-153 mice, as evaluated by GFP (green), Ki67 (red), SOX2 (white), and Ho.33342 (blue). Scale bar: 50 µm. **h** Percentage of GFP⁺ cells expressing either SOX2/Ki67; Data shown are the mean percentage \pm SEM; n = 3 brains. **i** Quantitative RT-PCR detection of the NSCs markers *Sox2*; AAV-ctrl, n=14; AAV-153, n=14; and *Nestin*; AAV-ctrl, n=11; AAV-153, n=10; in the hippocampi of young or aged mice. Data

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information: The data shown are the mean±SEM. Abbreviations: aged mice, 16-18 months old; NOR, novel object recognition.

Fig S8. Retro-153 increases adult neurogenesis in the hippocampus and enhances the cognitive ability of aged mice. a Schematic of retro virus injection into the hippocampi of aged mice and the process diagram of detection. **b** Percentage of GFP positive cells in hippocampus three weeks post injection Retro-ctrl or Retro-153; n = 3 brains. **c** Quantitative RT-PCR detection of miR-153 in the hippocampi of Retro-ctrl or Retro-153 mice; Retro-ctrl, n=9; Retro-153, n=9; Student's t test; *******p<0.001. **d** Immunofluorescence analysis of neurons in the hippocampi of Retro-ctrl or Retro-153 young mice, as evaluated by GFP (green), DCX (red), NEUN (red), and Ho.33342 (blue). Scale bar: 50 μm. **e** Immunofluorescence analysis of astrocytes in the hippocampi of Retro-ctrl or Retro-153 mice, as evaluated by GFP (green), GFAP (red), S100β (red), and Ho.33342 (blue). Scale bar: 50 μm. **f**, **g** Percentage of GFP⁺ cells expressing either DCX, NEUN, GFAP, or S100β; n = 3 brains; ANOVA; ******<0.01, ***p**<0.05. **h** Exploration time for different objects and discrimination index and discrimination ratio of novel objects; Retro-ctrl, n=8; Retro-153, n=8; Student's t test; ***p**<0.05. **i** Platform crossings and the amount of time for the mice to first reach the platform area during the water maze test; Retro-ctrl, n=8; Retro-153, n=8; Student's t test; ***p**<0.05. **j** Movement distance and speed of the Retro-ctrl or Retro-153 mice during the water maze test; Retro-ctrl, n=8; Retro-153, n=8. Data information: The data shown are the mean±SEM. Abbreviations: aged mice, 16-18 months old; NOR, novel object recognition; MWM, Morris water maze.

Fig S9. MiR-153 could not convert astrocytes into neurons. a Schematic of transdifferentiation of astrocytes with miR-153 overexpression. **b** Quantitative RT-PCR detection of miR-153 in astrocytes. **c** Immunofluorescence analysis of transdifferentiation of astrocytes after 4 days transfection with mc or miR-153, as evaluated by GFAP (green), MAP2 (red), and Ho.33342 (blue). Scale bar: 50 µm. **d** Schematic of neural differentiation of NSCs co-culture with astrocytes. **e** Quantitative RT-PCR detection of miR-153 in astrocytes. **f** Immunofluorescence analysis of neural differentiation of NSCs co-culture with astrocytes transfection with mc or m153, as evaluated by MAP2 (red), GFAP (green) and Ho.33342 (blue). Scale bar: 50 µm. **j** Percentage of MAP2-positive or GFAP-positive cells in neural differentiation. **h** Schematic of transdifferentiation of astrocytes co-culture with NSCs. **i** Quantitative RT-PCR detection of miR-153 in NSCs. **g** Immunofluorescence analysis of neural transdifferentiation of astrocytes co-culture with NSCs transfection with mc or m153, as evaluated by MAP2 (red), GFAP (green) and Ho.33342 (blue). Scale bar: 50 µm. **j** Percentage of MAP2-positive or GFAP-positive cells in neural differentiation. **h** Schematic of transdifferentiation of astrocytes co-culture with NSCs. **i** Quantitative RT-PCR detection of miR-153 in NSCs. **g** Immunofluorescence analysis of neural transdifferentiation of astrocytes co-culture with NSCs transfection with mc or m153, as evaluated by MAP2 (red), GFAP (green) and Ho.33342 (blue). Scale bar: 50 µm. Data information: The data shown are the mean±SEM; n=3.

Supplementary table 1. Antibodies list

Antibody	Company	Cat	Concentration
β-tubulin	Bioworld	ap0064	1:2 000
Hey2	Proteintec	10597-1-AP	1:1 000
Hey1	Abcam	ab154077	1:1 000
Hes1	Abcam	ab71559	1:1 000
NICD	Cell Signaling technology	41478	1:1 000
Jag1	Cell Signaling technology	26208	1:1 000
Map2	Abcam	ab32454	1:3 000
Gfap	Millipro	MAB360	1:1 000
NeuN	Millipro	ABN78	1:1 000
Dcx	Abcam	ab18723	1:1 000
S100β	Abcam	ab11178	1:1 000
BrdU	Abcam	ab6326	1:1 000
Sox2	R&D systems	AF2018	1:1 000
Ki67	Abcam	ab15580	1:1 000

Supplementary table 2. Primers list1

Application	Gene	Forward primer	Reverse primer	The efficacy	The value	The value
				of primer	of Ct in cell	of Ct in tissues
QPCR	Gapdh	AGGTCGGTGTGA	TGTAGACCATGT	108.0%	17-19	14-16
		ACGGATTTG	AGTTGAGGTCA			
QPCR	Tuj1	TAGACCCCAGCG	GTTCCAGGTTCC	98.9%	24-27	/
		GCAACTAT	AAGTCCACC			
QPCR	Map2	GGTCACAGGGCA	TGTTCACCTTTC	102.4%	21-24	/
		CCTATTCA	AGGACTGC			
QPCR	NeuN	GGGTATGGGTAG	GTGGAAGGTTTC	97.0%	28-31	21-23
		GATTGGGG	ACTACAACAGA			
QPCR	Dcx	GGCCAAGAAGGT	AGCAACGCATCA	100.8%	/	23-25
		ACGTTTCTAC	AAACTACGAA			
QPCR	Gfap	ACCAGCTTACGG	CCAGCGATTCAA	97.8%	25-28	18-20
		CCAACAG	CCTTTCTCT			
QPCR	S100β	TGGTTGCCCTCAT	CCCATCCCCATC	111.5%	21-24	22-23.5
		TGATGTCT	TTCGTCC			
QPCR	Sox2	GCGGAGTGGAAA	CGGGAAGCGTGT	104.3%	/	21-22.5
		CTTTTGTCC	ACTTATCCTT			
QPCR	Nestin	CTGCAGGCCACT	GACCCTGCTTCT	106.7%	/	26-28
		GAAAAGTT	CCTGCTC			
QPCR	Wpre	CCTTTTACGCTAT	CGTCAGCAAACA	109.1%	19-23	/
		GTGGATACGCTG	CAGTGCACACC			
QPCR	Hey2	AGTCAACCCCAT	TGGCATCCGAAG	110.6%	28-29	/
		GTCGCCTAT	AGCAGAATC			
QPCR	Jag1	CCTCGGGTCAGTT	CCTTGAGGCACA	104.5%	25-26	/
		TGAGCTG	CTTTGAAGTA			
QPCR	miR-	Obtained from	Obtained from	99.2%	20-27	14-27
	153	RiboBio company	RiboBio company			
QPCR	U6	Obtained from	Obtained from	103.5%	14-16	11-13
		RiboBio company	RiboBio company			

Supplementary table 3. Primers list2

Application	Gene	Forward primer	Reverse primer
PCR	Pre-miR-153	TTTGAGCTGCTCCTTGTAATGTGTC	AAAAATTAGACTCCAAGCTGGGCGT
Sponge	Sponge-	AATTCGATCACTTTTACTCTATGCAA	GATCCTTGCATAGAGTAAAAGTGAT
	miR-153	CCGGATCACTTTTACTCTATGCAAG	CCGCTTGCATAGAGTAAAAGTGATC
		CGGATCACTTTTACTCTATGCAAG	CGGTTGCATAGAGTAAAAGTGATCG
ShRNA	ShHey2-1	CCGGCCTCTCAGATTATGGCAAGAA	AATTCAAAAACCTCTCAGATTATGG
		CTCGAGTTCTTGCCATAATCTGAGA	CAAGAACTCGAGTTCTTGCCATAAT
		GGTTTTTG	CTGAGAGG
ShRNA	ShHey2-2	CCGGCTCTCAGATTATGGCAAGAAA	AATTCAAAAACTCTCAGATTATGGC
		CTCGAGTTTCTTGCCATAATCTGAG	AAGAAACTCGAGTTTCTTGCCATAA
		AGTTTTTG	TCTGAGAG
ShRNA	ShJag1-1	CCGGACATCTGCCAGCGGTCCTAAT	AATTCAAAAAACATCTGCCAGCGGT
		CTCGAGATTAGGACCGCTGGCAGAT	CCTAATCTCGAGATTAGGACCGCTG
		GTTTTTTG	GCAGATGT
ShRNA	ShJag1-2	CCGGGCTCTGTCTTAACAGTGGCTT	AATTCAAAAAGCTCTGTCTTAACAG
		CTCGAGAAGCCACTGTTAAGACAGA	TGGCTTCTCGAGAAGCCACTGTTAA
		GCTTTTTG	GACAGAGC

DECLARATION OF CONTRIBUTIONS TO ARTICLE



Manuscript Number:	Journal Name:	
	Cell Death & Differentiation	(the 'Journal')
Proposed Title of the Contribution:		
		(the 'Contribution)
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Authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Any person who cannot be shown to have made a substantial contribution to the article cannot be listed as an author in the final version. The name of any person who is deemed to have made a minor contribution can, however, appear in the Acknowledgments section of the article.

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