Supporting Information

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SI Text

Materials. Mouse anti-PSA-NCAM antibody was a generous gift from Tatsunori Seki (Juntendo University, Tokyo) (1). *SIRT1-EGFP*, *SIRT1H355Y-EGFP*, and *SIRT1mtNLS-EGFP* were described previously (2). *SIRT1* and *SIRT1H355Y* in *pShuttle-IRES-hrGFP* (Clontech) were constructed by standard techniques. The *pact-N-CoR-FLAG* and *pCMX-N-CoR* were generous gifts from Shunsuke Ishii (RIKEN Tsukuba Institute, Ibaraki, Japan) and Ronald M. Evans (The Salk Institute, La Jolla, CA), respectively (3, 4). Lentiviral vector plasmids were kindly provided by Jeffery Milbrandt (Washington University School of Medicine, St. Louis) (5). The *SIRT1-siRNA* used in the *in utero* electroporation experiment was prepared in the *mU6pro* vector (6). This expressed a short hairpin RNA 5′-GACCAAGCAACAAACAACAA*ucaagag*UUGUUGUUUGUUGCUUGGUC-3′ that targeted nucleotides 1645–1664 of the coding region of mouse *SIRT1* cDNA (GenBank accession number AY377984). The underlined sequence in lowercase corresponds to the loop region. *Notch1-ICD* cDNA corresponding to the amino acid sequence (1753–2531) of *Notch1* was obtained by PCR from mouse brain cDNA. The primer sequences used for the PCR were as follows: forward 5′-ATGCAGCATGGCCAGCTCTGGTTC-3′; reverse 5′-GTCGACATTTAAATGCCTCTGGAATGTGGG-3′. The amplified cDNA was sequenced and subcloned into the *pIRES-hrGFP* vector. The deduced amino acid sequence of the cloned *Notch1-ICD* was identical to that of reported Notch1 (AK090118), except that N2078 was changed to I. The mouse *Hes1* promoter (−4 − +50) (7), which contains an RBP-J-binding site (−5 − −79), was obtained by PCR using primers 5′-CTCGAGAAAGTTACTGTGGGAAAGAAAGTT-3′ and 5′-AAGCTTAGGTAGACAGGGGATTCCGCTGTT-3′ from a mouse genome library (BD Biosciences). The amplified PCR fragment was sequenced and subcloned into the *pGL3* vector. The *12XCSL* luciferase reporter was a generous gift from Urban Lendahl (Karolinska Institute, Stockholm) (8). The primer sequences used for ChIP assays were as follows: *Hes1* promoter forward 5′-TGATTGACGTTGTAGCCTCC-3′, reverse 5′-AGATCCTGTGTGATCCGCAG-3′; *Hes5* promoter forward 5′-AAAGGCAGCATATTGAGGCG-3′, reverse 5′-CAGCACGCCAGCCCTATATA-3′: *β-actin* promoter forward 5′-AAAATGCTGCACTGTGCGGC-3′, reverse 5′-AGGAGCTGCAAAGAAGCTGT-3′. For cultured NPCs, 10 μM BrdU (Wako), 60 μM splitomicin (Calbiochem), 5 mM nicotinamide (Wako), 50 μM sirtinol (Biomol), 80 μg/ml LIF (Millipore), or 80 μg/ml BMP-4 (R & D Systems) was used.

Fluorescence Microscopy Analysis of Living Cells. Two days after nucleofection, cells were transferred into a poly-L-ornithine-coated dish under the differentiation conditions and incubated in the incubator on inverted microscope (TE2000-E; Nikon). The fluorescent images were collected every 5 min by using a CCD camera (ORCA-AG; Hamamatsu Photonics) and were analyzed with AQUACOSMOS software (Hamamatsu Photonics).

RT-PCR. Quantitative RT-PCR was carried out by using SuperScript III and One-Step RT-PCR System with Platinum TaqDNA polymerase (Invitrogen). Normalization was performed by PCR of *β-actin*. The primer sequences used for PCR were as follows: *SIRT1* forward 5′-GCATGCATGGAACCTTTGCCTCATCTACA-3′, reverse 5′-GATTACCCTCAAGCCGCTTA-3′; *nestin* forward 5′-AGAAAGTGGTCTGGACACAG-3′, reverse 5′-TGGGTATTGGCTCTCCTCTT-3′; *N-CoR* forward 5′-TTGGATCTGCCACAGCTTCA-3′, reverse CACTCCCCTGTTTGGACTCTT-3′; *Hes1* forward 5′-CAGCCAGTGTCAACACGACAC-3′, reverse 5′-TCGTTCATGCACTCGCTGAG-3′; *GAPDH* forward 5′-CTTCACCACCATGGAGAAGGC-3′, reverse 5′-GGCATGGACTGTGGTCATGAG-3′; *β-actin* forward 5′-GACCTGACAGACTACCTCAT-3′, reverse 5′-AGACAGCACTGTGTTGGCAT-3′.

Western Blotting. Dissociated neurospheres were resuspended in CelLytic-M Extraction Reagent (Sigma) with protease inhibitor mixture (Nakalai Tesque) and then sonicated. For the separation of nuclear and cytoplasmic protein fractions, the Nuclear/Cytosol Fractionation kit (BioVision Research Products) was used as directed by the manufacturer. Mouse anti-GAPDH (Chemicon) and rabbit anti-lamin A/C (Cell Signaling Technology) antibodies were used as cytoplasmic and nuclear markers, respectively. COS cells transfected with *N-CoR* and/or *SIRT1* were homogeneized in CelLytic-M with Protease Inhibitor Mixture, and insoluble matter was removed by centrifugation in a microfuge. Brains isolated from E16.5 embryonic ddY mice were minced, and brain cells were triturated with a Pasteur pipette and collected by centrifugation. Cells were cultured in the differentiation conditions for 3 h, collected by centrifugation, and then homogenized in CelLytic-M solution with Protease Inhibitor Mixture. Cell homogenates were centrifuged at 100,000 × *g* for 30 min at 4°C to remove insoluble matter. GST fusion protein of full-length SIRT1 was expressed in *Escherichia coli* and purified on glutathione-Sepharose. Glutathione-Sepharose bound GST-SIRT1 or control Glutathione-Sepharose was incubated with cell homogenates overnight at 4°C, washed four times with 50 mM Tris·HCl (pH7.5), 150 mM NaCl and 0.1% Triton X-100 (wash buffer), and then analyzed by Western blotting. For immunoprecipitation, cell homogenates were added with anti-SIRT1 antibody or control IgG and incubated overnight at 4°C. Immunoprecipitants were bound to Protein A/G PLUS-Agarose (Santa Cruz Biotechnology), washed four times with wash buffer, and then analyzed by anti-N-CoR antibodies (Santa Cruz Biotechnology or Upstate Biotechnology). To strip bound antibodies, membrane filters were treated with Re-Blot plus (Chemicon).

Preparation of Lentivirus. The protocol for preparing the lentivirus was described previously (5). Eighty percent confluent HEK293 cells in six-well dishes were transfected with packaging, envelope glycoprotein, and *FSP-si-SIRT1* plasmids by using Lipofectamine 2000 (Invitrogen). The culture medium of the transfected cells (12 ml) was collected and subjected to ultracentrifugation, and the pellet was resuspended in 100 μl of Hanks' Balanced Salt Solution (Invitrogen). We defined this resulting virus preparation as 1 unit of lentivirus.

Immunocytochemistry and Immunohistochemistry. E14.5 and 8-week-old mouse brains were used. BrdU was administered to adult mice for 2 weeks via drinking water to label dividing NPCs. The primary antibodies were as follows: rabbit or guinea pig anti-SIRT1, mouse anti-NeuN (Chemicon), rabbit polyclonal anti-GFAP (DAKO), mouse monoclonal anti-GFAP (Chemicon), mouse anti-PSA-NCAM, rabbit anti-NG2 (Chemicon), rabbit anti-S100B (DAKO), rabbit anti-πGST (MBL), mouse

anti-nestin (Chemicon), rabbit anti-Hes1 (Santa Cruz Biotechnology), goat anti-Hes5 (Santa Cruz Biotechnology), mouse anti-BrdU (Sigma), mouse anti-Tuj1 (Sigma), and mouse anti-O4 (Chemicon). The secondary antibodies were Alexa Fluor 488 anti-mouse and anti-guinea pig IgG (Molecular Probes), Alexa Fluor 594 anti-rabbit and anti-guinea pig IgG, and AMCA anti-rabbit IgG (The Jackson Laboratory). For nuclear staining, samples were incubated with 1 μM Hoechst 33342 dye (Wako) for 10 min before mounting. These samples were examined by confocal microscopy (LSM-510; Zeiss or R2100AG2, Bio-Rad). The differentiation of neurospheres was evaluated by the number of neurospheres containing Tuj1$^+$ (N) and/or GFAP$^+$ cells (A). In some experiments, the number of spheres containing O4$^+$ cells (O) was also counted. More than 100 spheres were examined in each experiment. Data are indicated as the mean $\pm$ SEM. Differences between each condition were compared by using one-way ANOVA, followed by Tukey–Kramer honestly significant difference (HSD) tests. P values <0.01 were considered to be significant.

Luciferase Assay. Plasmid DNA (0.8 μg per well) was transfected with *PRL-TK* (Promega) (20 ng per well) to HEK293 cells on a 24-well dish by using Lipofectamine 2000 (Invitrogen). The luciferase activity was assessed by using the Dual-Luciferase Reporter Assay System kit (Promega). Values were means $\pm$ SEM. Similar results were obtained from three independent experiments.

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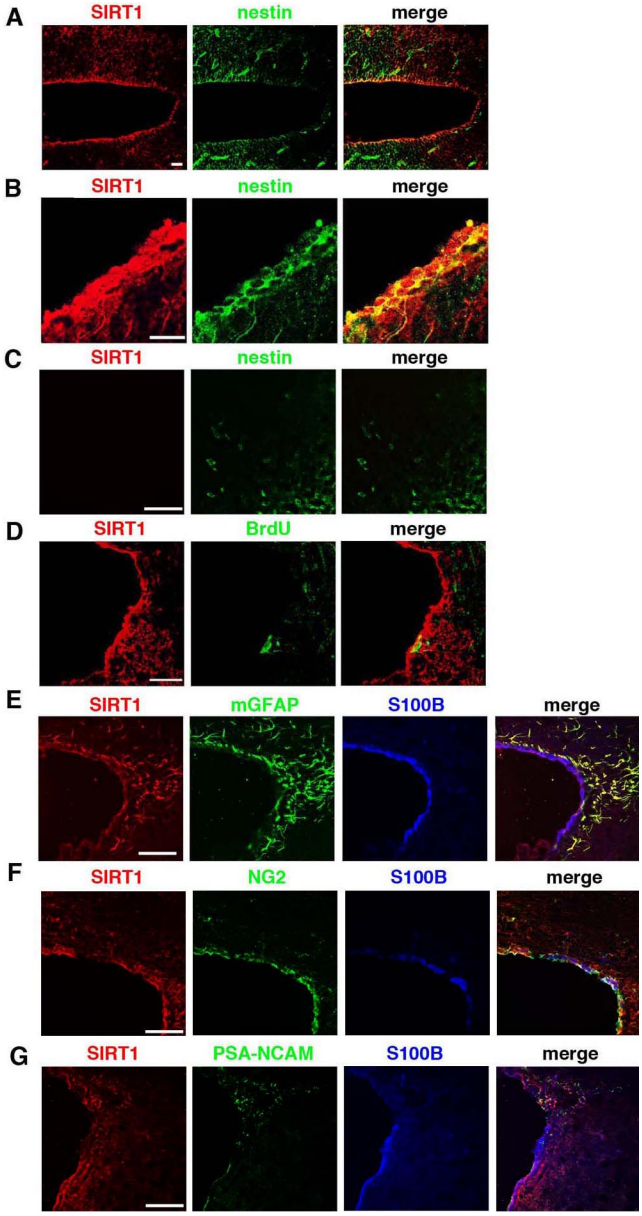


Fig. S1. Expression of SIRT1 in the adult brain. (*A* and *B*) Double immunostaining of SIRT1 (red) and nestin (green) in SVZ region. (*C*) Double immunostaining of SIRT1 (red) and nestin (green) in the hippocampus. (*D*) Double immunostaining of SIRT1 (red) and BrdU (green). (*E–G*) Triple immunostaining of SIRT1 (red) and S100B (blue) with monoclonal GFAP (green) (*E*), NG2 (green) (*F*), or PSA-NCAM (green) (*G*) antibodies. (Scale bars: 50 μm.)

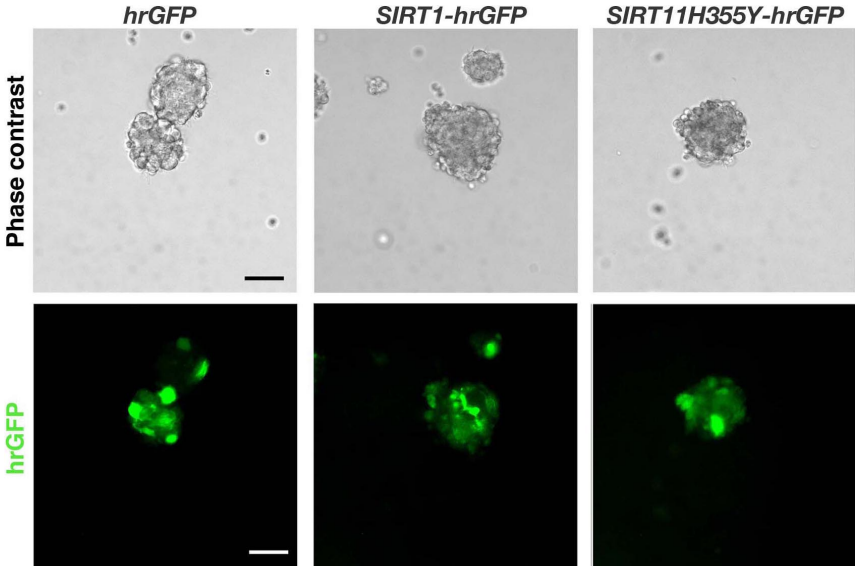


Fig. S2. Expression of hrGFP in NPCs. Expression of hrGFP in neurospheres 2 d after electroporation with *pIRES-hrGFP* (control vector), *SIRT1*, or dominant-negative *SIRT1H355Y* in the *pShuttle-IRES-hrGFP* vector. hrGFP$^+$ spheres were used for the differentiation assay. (Scale bars: 50 μm.)

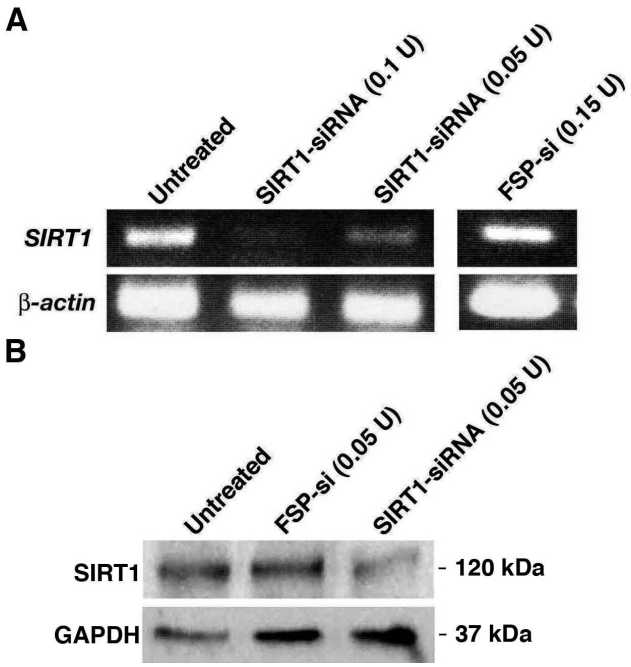


Fig. S3. Suppression of SIRT1 by *SIRT1-siRNA* lentivirus. (*A*) RT-PCR. mRNAs were isolated from cells 2 d after the infection. 0.1 U indicates a 10-μl aliquot of lentivirus suspension (see *Methods*). (*B*) Western blot analysis. Neurospheres were infected with 0.05 units of virus and analyzed 2 d after the infection. *SIRT1-siRNA* lentivirus decreased the expression of SIRT1 protein by ≈55%.

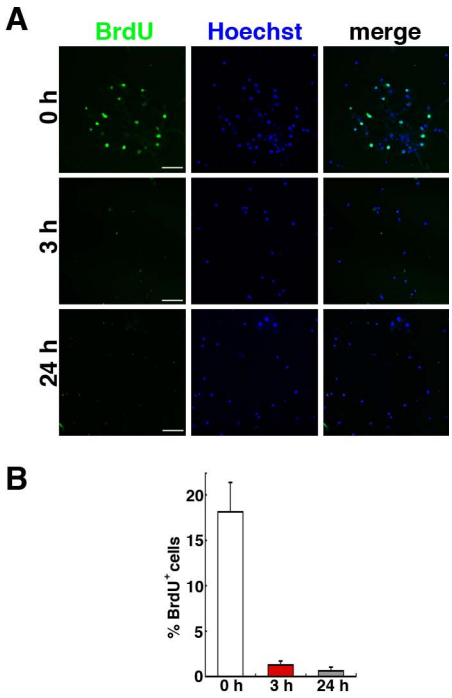


Fig. S4. Change of the number of BrdU+ cells. (*A*) Dissociated cells from P1 neurospheres were transferred onto coverslips and 10 μM BrdU was administered. Cells were cultured in the differentiation conditions for 0, 3, and 24 h and then cultured in MHM medium containing EGF and bFGF. Total incubation period of each condition was 24 h. Cells were immunostained with an anti-BrdU antibody. Nuclei were stained by Hoechst 33342. (Scale bars: 50 μm.) (*B*) The number of $BrdU^+$ cells in each visual field. Values are means ± SEM. from 10 independent fields.

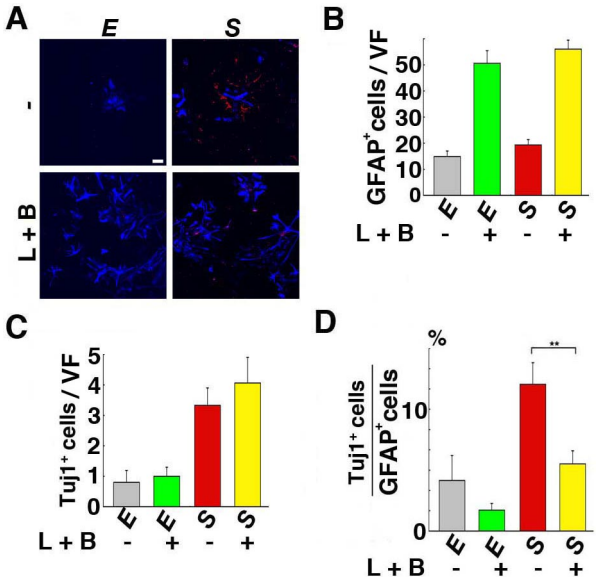


Fig. S5. Effect of overexpression of SIRT1-EGFP on astrocyte differentiation promoted by LIF and BMP-4. (*A*) Dissociated spheres were transfected with *EGFP* (*E*) or *SIRT1-EGFP* (*S*) and then differentiated in differentiation conditions in the presence or absence of LIF (L) and BMP-4 (B). After differentiation, cells were immunostained with anti-Tuj1 (red) and anti-GFAP (blue) antibodies. (Scale bar: 50 μm.) (*B*) Average number of GFAP$^+$ cells in each visual field (VF). (*C*) Average number of Tuj1$^+$ cells in each visual field. (*D*) The ratio of the number of Tuj1$^+$ cells to that of GFAP$^+$ cells in each visual field. Values are means $\pm$ SEM from 15 independent fields. **, $P < 0.01$.

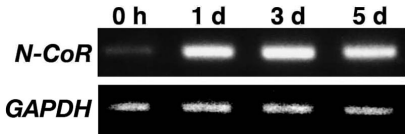


Fig. S6. Expression of *N-CoR* mRNA in neurospheres. *N-CoR* mRNA expression was examined by RT-PCR.

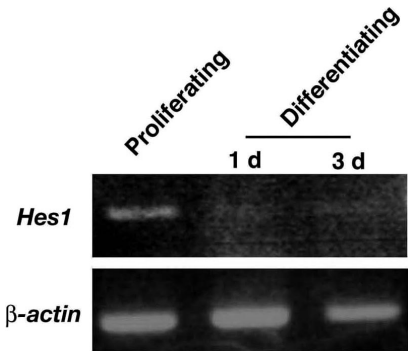


Fig. S7. Decrease of *Hes1* mRNA in neurospheres after differentiation. *β-Actin* was the internal control.

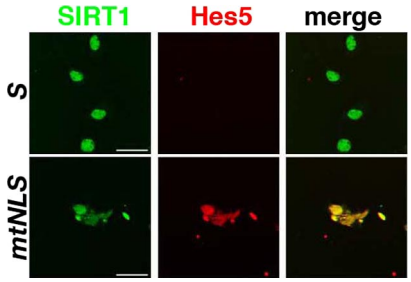
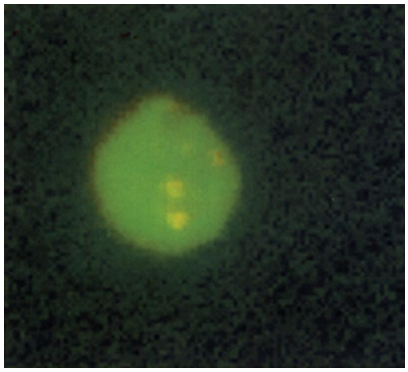
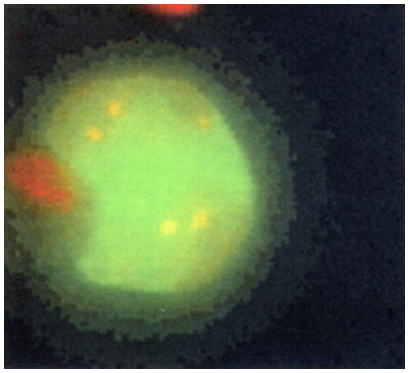


Fig. S8. SIRT1 suppresses Hes5 expression. NPCs electroporated with *SIRT1-EGFP* (*S*) or *SIRT1mtNLS-EGFP* (*mtNLS*) were cultured for 24 h in differentiation conditions and then immunostained with an anti-Hes5 antibody. (Scale bars, 20 μm.)



Movie S1. Time-lapse image of a neurosphere cell expressing SIRT1-EGFP. The video image was captured after the cell was transferred into differentiation conditions. The video corresponds to the image sequence in Fig. 2*D*. Total elapsed time is 6 h. Bar, 10 μm.

Movie S1 (MOV)



Movie S2. Time-lapse Image of neurosphere cells expressing SIRT1mtNLS-EGFP. Two cells in a cluster of three cells express SIRT1mtNLS-EGFP. The video image was captured as described in Movie S1. The video corresponds to the image sequence in Fig. 2E. Total elapsed time is 4.5 h. Bar, 10 μm.

Movie S2 (MOV)