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Supplemental Information

Sonic Hedgehog Effectively Improves Oct4-Mediated Reprogramming of Astrocytes into Neural Stem Cells

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Supplemental Information

1. Supplemental data

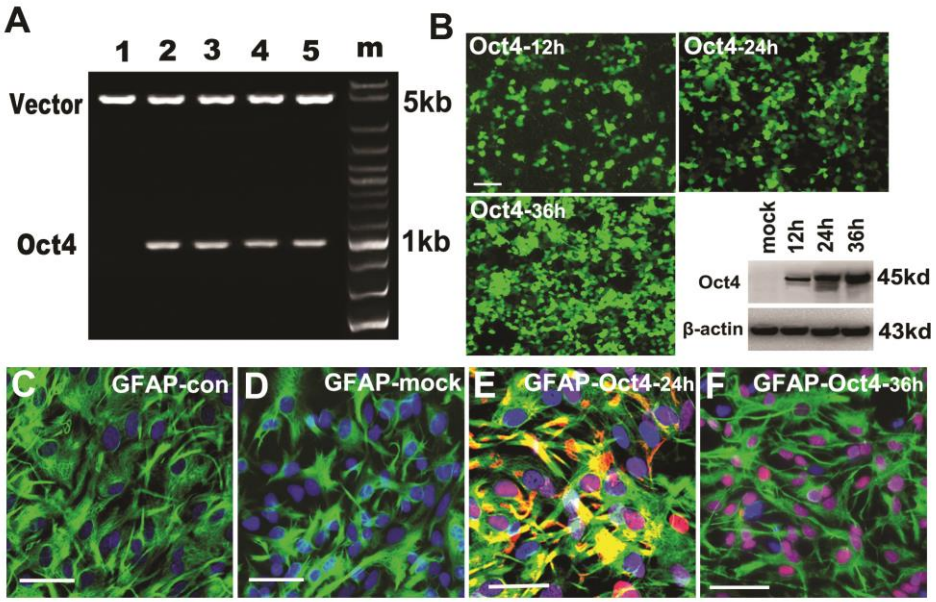

Figure S1 Identification of the Oct4 lentiviral vector and transduction of astrocytes. (A) The recombinant Oct4-pGMLV lentiviral vector from four clones was identified by restriction endonuclease digestion. (B) Identification of Oct4 expression in 293T cells transfected with Oct4 lentiviral vector for 12, 24, and 36 h, no immunostaining photomicrographs are shown for the mock-transfected cells, since Oct4 expression was undetectable in these cells. (C) Untransduced astrocytes. (D) Astrocytes infected with mock lentiviral vector. (E, F) Astrocytes infected with lentivirus encoding Oct4 for 24 and 36 h. Scale bars=100 μm.

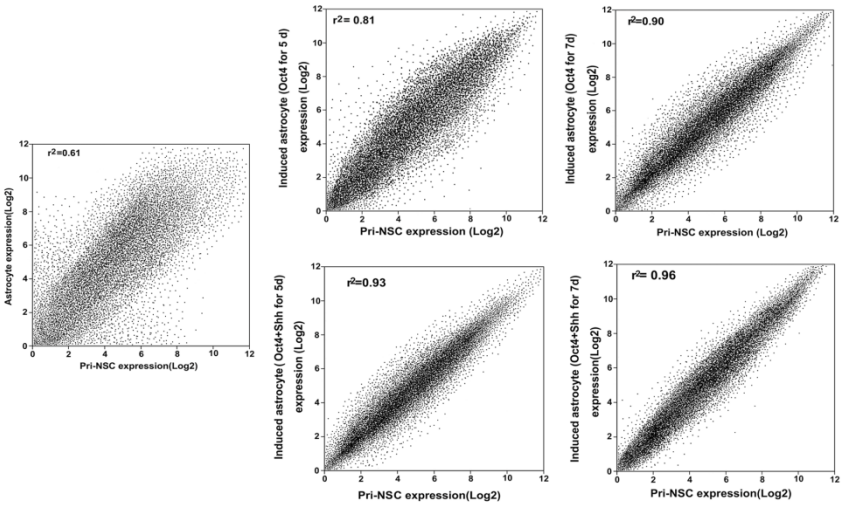

Figure S2 Correlation of Scatterplot analysis of global gene expression profiles of primary NSCs, induced astrocytes with Oct4/Shh or Oct4 alone, and astrocytes. The gene expression levels are indicated on a Log2 scale. Pearson's correlation coefficients (r) are indicated.

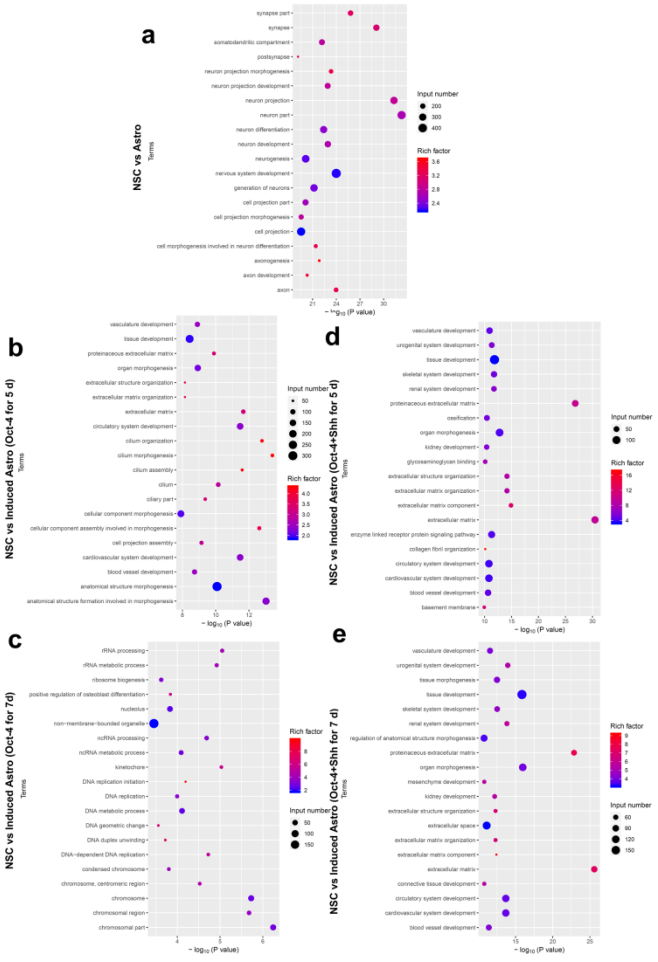

Figure S3 Gene ontology (GO) enrichment analysis of the upregulated genes between

primary NSC and induced astrocytes with Oct4/Shh or Oct4 alone or astrocytes. The upregulated genes were analyzed for enrichment by gene-set enrichment analysis. (a) Enrichment analysis of the upregulated genes between astrocyte and primary NSCs. (b-c) Enrichment analysis of the upregulated genes between induced astrocytes (Oct4 for 5d and 7d, respectively) and primary NSCs. Green represents GFAP. (d-e) Enrichment analysis of the upregulated genes between induced astrocytes (Oct4+Shh for 5d and 7d, respectively) and primary NSCs. Green represents GFAP, red represents Oct4.

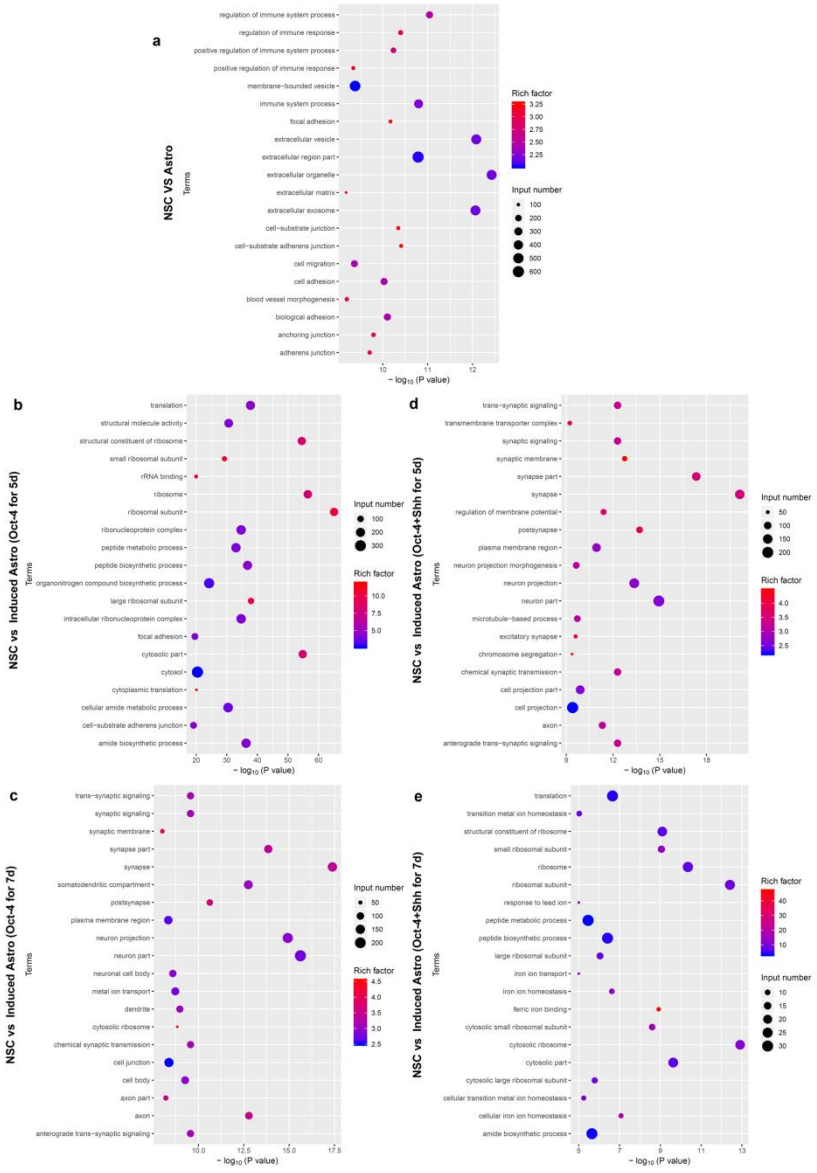

Figure S4 Gene ontology (GO) enrichment analysis of the downregulated genes between primary NSC and induced astrocytes with Oct4/Shh or Oct4 alone or

astrocytes. (a) Enrichment analysis of the downregulated genes between astrocyte and primary NSCs. (b-c) Enrichment analysis of the downregulated genes between induced astrocytes (Oct4 for 5d and 7d, respectively) and primary NSCs. (b-c) Enrichment analysis of the downregulated genes between induced astrocytes (Oct4+Shh for 5d and 7d, respectively) and primary NSCs.

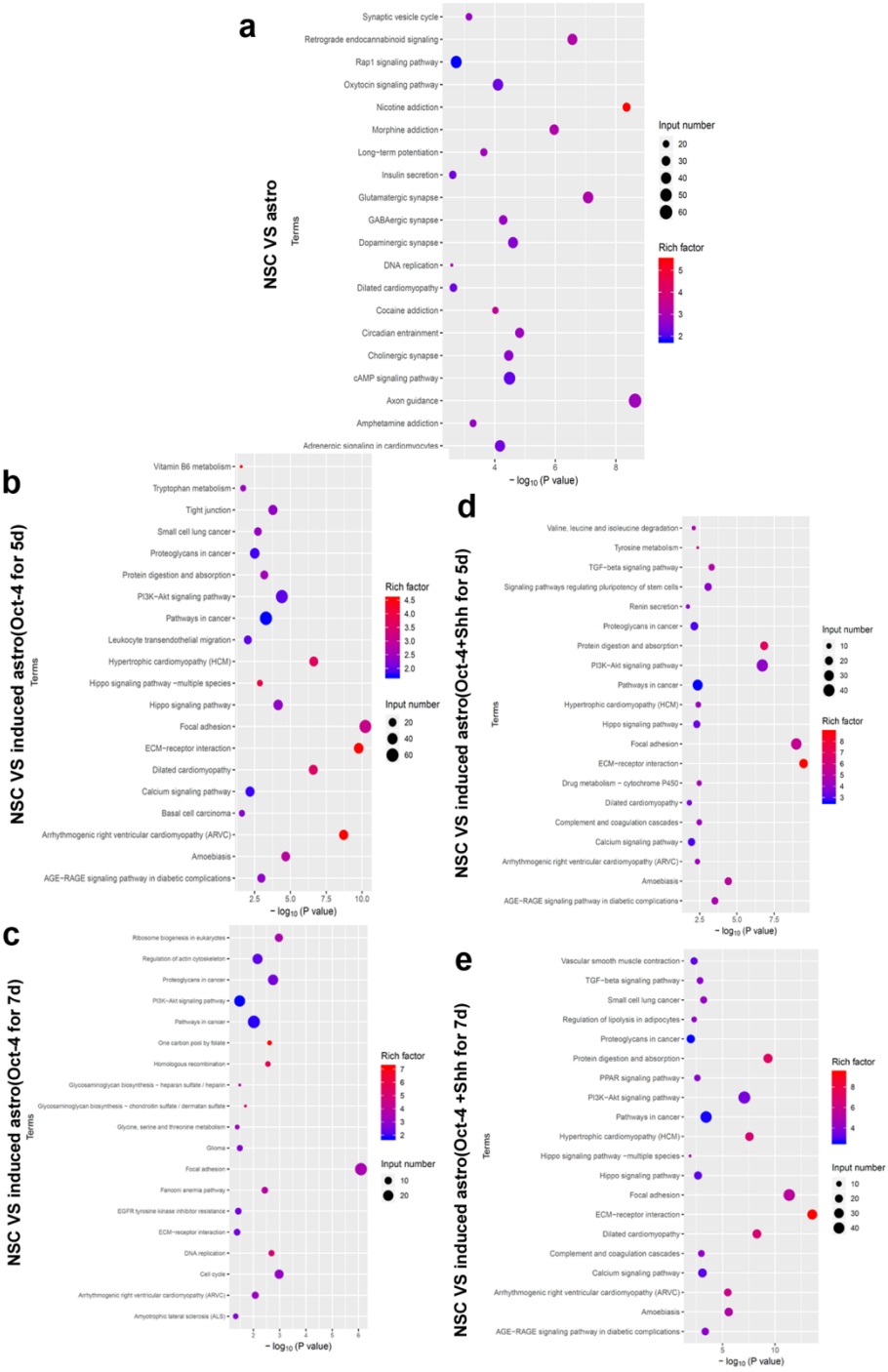

Figure S5 Gene ontology (GO) enrichment analysis of the upregulated genes for

KEGG pathway between primary NSCs and induced astrocytes with Oct4/Shh or Oct4 alone or astrocytes. (a) The upregulated genes for KEGG pathway between astrocyte and primary NSCs. (b-c) The upregulated genes for KEGG pathway between induced astrocytes (Oct4 for 5d and 7d, respectively) and primary NSCs. (d-e) The upregulated genes for KEGG pathway between induced astrocytes (Oct4+Shh for 5d and 7d, respectively) and primary NSCs.

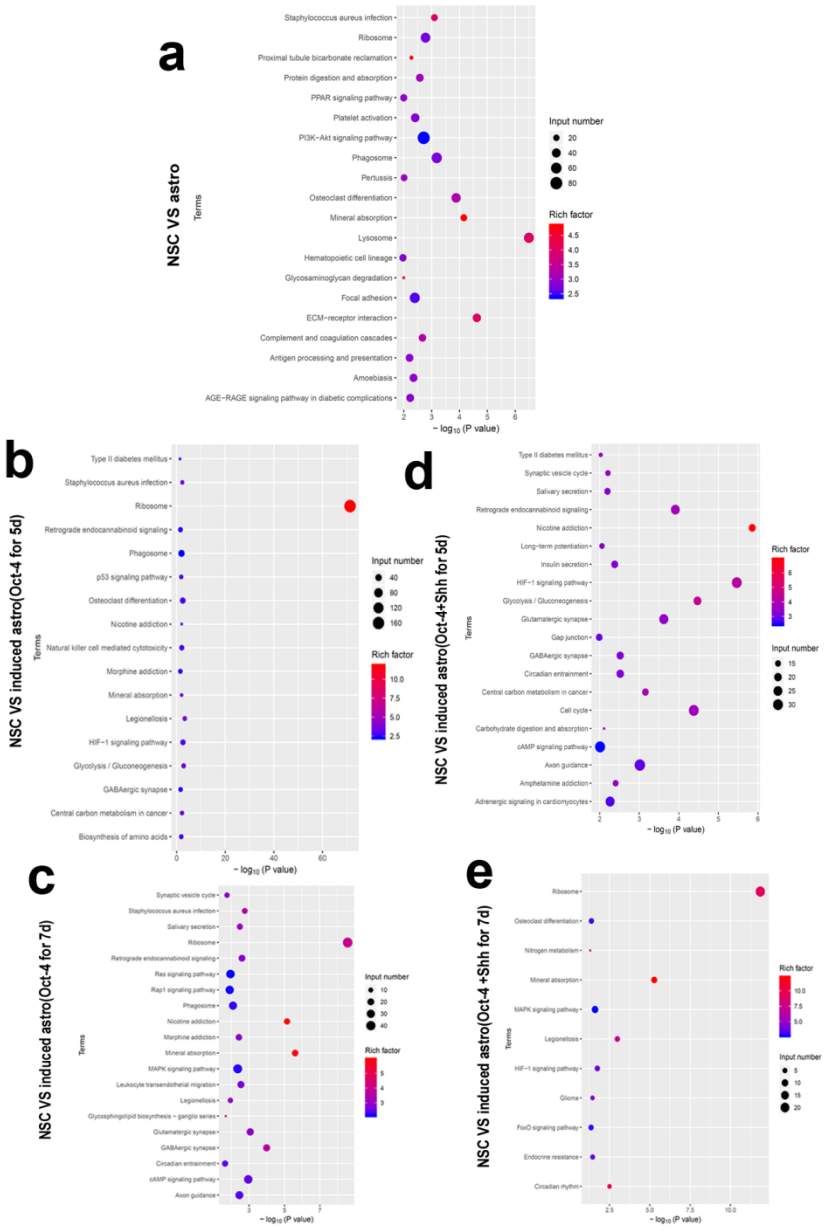

Figure S6 Gene ontology (GO) enrichment analysis of the downregulated genes for KEGG pathway between primary NSCs and induced astrocytes with Oct4/Shh or Oct4 alone or astrocytes. (a) The downregulated genes for KEGG pathway between

astrocyte and primary NSCs. (b-c) The downregulated genes for KEGG pathway between induced astrocytes (Oct4 for 5d and 7d, respectively) and primary NSCs. (d-e) The downregulated genes for KEGG pathway between induced astrocytes (Oct4+Shh for 5d and 7d, respectively) and primary NSCs.

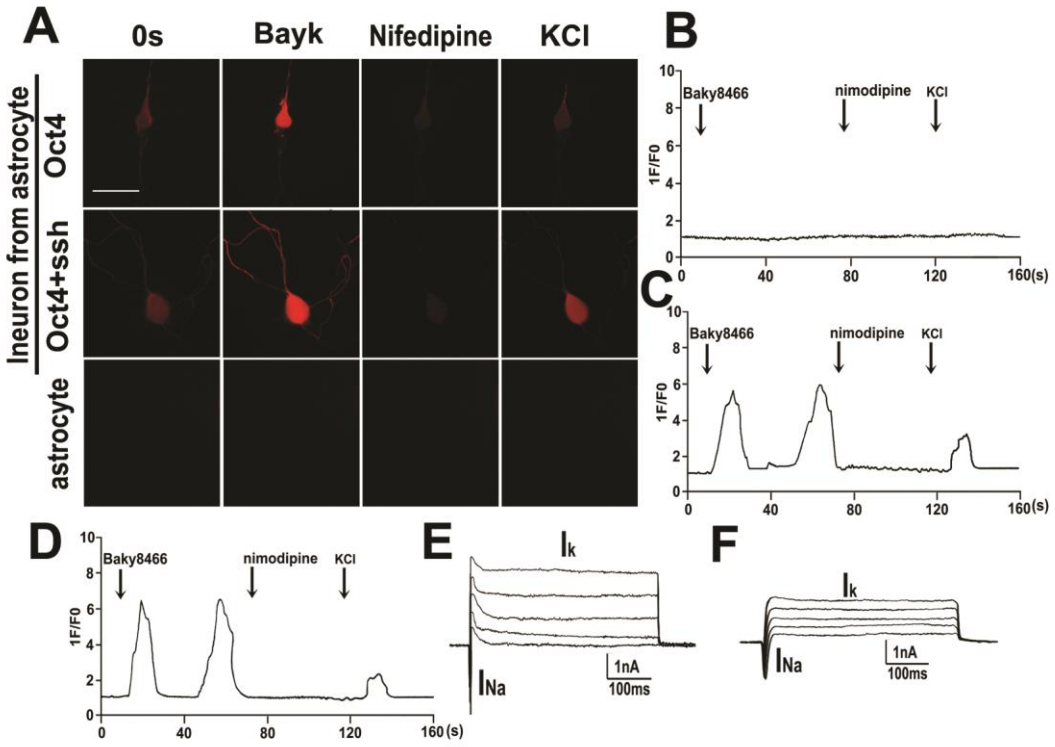

Figure S7 Functional characteristics of the neurons derived from induced astrocytes with Oct4 and Shh. (A) Changes in the calcium response in astrocytes or differentiated neurons derived from iNSCs (induced with Oct4 alone, or with Oct4/Shh) with the indicated treatments. Scale bars=25μl (B-D) the ratio of the cell fluorescence intensity at the indicated time to the fluorescence intensity of the above-mentioned astrocytes, differentiated neurons from iNSCs induced with Oct4 alone, and with Oct4 and Shh, respectively. BayK (10 mM), nifedipine (5 mM), KCl (100 mM) administered at the indicated time points. (E-F) Representative traces showing Na^+ and K^+ current recorded from differentiated neurons derived from iNSCs induced with Oct4/Shh with Oct4 alone. The total number of cells tested in each experiment was approximately 10; representative results are presented.

Table S1 The sequences of primer sets used for RT-PCR and QPCR analysis.

Primer sets used for RT-PCR and qPCR

Gene	Forward primer	Reverse primer
Nestin	5′-TGCAGCCACTGAGGTATCTG-3′	5′-CAGTTCCCACTCCTGTGGTT-3′
Olig2	5′-GGCGGTGGCTTCAAGTCATC-3′	5′-TAGTTTCGCGCCAGCAGCAG-3′
CD133	5′-CTTGGCATCGCGTTTGG-3′	5′-GAGCCCGCAAGTCTCTGTAATT-3′
Pax6	5′-GGCAACCTACGCAAGATGGC-3′	5′-TGAGGGCTGTGTCTGTTCGG-3′
A2B5	5′-ACATCGAGATCGCCACCTAC-3′	5′ -ACATCACATCCTTGTGCTCC-3′
GFAP	5′-GCGCTCAATGCTGGCTTCAA-3′	5′-ACGCAGCCAGGTTGTTCTCT-3′
Oct4	5′-CGTTCTCTTTGGAAAGGTGTTC-3′	5′-ACACTCGGACCACGTCTTTC-3′
Sox2	5′-GCAGTACAACTCCATGAC-3′	5′-CGAGTAGGACATGCTGTA-3′
Ptc	5-AACAAAAATTCAACCAAACCTC-3′	5′-TGTCTTCATTCCAGTTGATGTG-3
GLI2	5′-CGCCTGGAGAACTTGAAGAC-3′	5′-TTCTCATTGGAGTGAGTGCG-3′
CyclinD1	5′-CTCCCCACGATTTCATCGAA-3′	5′-GTGCATGTTTGCGGATGATC-3′
BLBP	5′-AGGGCAAGGATGGTAGATGC-3′	5′-ACCGTTGTTTTGGTCACATT-3′
Sox10	5′-GCTGAACGAGAGTGACAAGC-3′	5′-ATGGGCTGATCTCCCCGATGT-3′
Nanog	5′-TCTCCTCCGCCTTCCTCT-3′	5′-TTGCCTCTGAAACCTATCCTTG-3′
Klf4	5′-AGGAGCCCCAAGCCAAAGAG-3′	5′-ACAAGTGTGGGTGGCTGTTCT-3′
Zbtb46	5′-CAGAGAAGAACTGCTGTGTACGG-3′	5′-CAGACAGAAGTTGGCATGGTAGC-3′
Bmi	5′-CATTGGGCCATAGTTTGTTAATCTCAA-3′	5′-CCAATATGGCATTGTACAACAAGC-3′
Hes5	5′-TCACCAGGGCCGCCAGAGGCCG-3′	5′-ATGGCCCCAAGTACCGTGGCGG-3′
STMN	5′-CCAGAATTCCCCCTTTCCCC-3′	5′-CCAGCTCTTCAAGACCTCA-3′
TGFβ	5′-CAGAGAAGAACTGCTGTGTACGG -3′	5′-CAGACAGAAGTTGGCATGGTAGC-3′
GAPDH	5′-GTATGTCGTGGAGTCTACAG-3′	5′-GAGTTGTCATATTTCTCGTGGT-3′

Supplemental data 1 Differential gene expression between primary NSCs and astrocytes.

Supplemental data 2 Differential gene expression between primary NSCs and induced astrocytes (Oct4 for 5 d)

Supplemental data 3 Differential gene expression between primary NSCs and induced astrocytes (Oct4 for 7 d)

Supplemental data 4 Differential gene expression between primary NSCs and induced astrocytes (Oct4/Shh for 5 d)

Supplemental data 5 Differential gene expression between primary NSCs and induced astrocytes (Oct4/Shh for 7 d)

2. Supplemental Experimental materials and Procedures

Main reagents

Dulbecco's modified Eagle's medium (DMEM) with high glucose (6 g/L), DMEM/F12, normal donkey serum, fetal calf serum (FCS), G5 supplement, TRIzol,

and trypsin were purchased from Invitrogen (USA); poly-L-lysine (PLL), 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), HEPES, ethylenediamine tetraacetic acid (EDTA), bovine serum albumin (BSA), retinoic acid (RA), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), bromodeoxyuridine (BrdU), penicillin, streptomycin, RA, and D-Hanks were purchased from Sigma-Aldrich (USA); a Shh peptide was purchased from R&D (USA); NU9516 was purchased from MCE (USA); GDC-0941 was purchased from Selleck(China); USF2 was purchased from Origene (USA); anti-nestin antibody, anti-glial fibrillary acidic protein (GFAP) antibody, anti-Pax6 antibody, anti-CD133 antibody, anti-SYN antibody, anti-GABA antibody, anti-PSD-95 antibody, anti-vGluT1 antibody, anti-Oct4 antibody, anti-GLT1 antibody and horseradish-conjugated secondary antibody were obtained from Abcam (USA); anti-NeuN antibody was purchased from(Merck); anti-Tuj-1 antibody, anti-CNP antibody, anti-actin antibody, and rabbit anti-BrdU antibody were purchased from Millipore (USA); anti-Nolz1 polyclonal antibody was purchased from Biocompare (USA); anti-Ptch antibody, anti-Gli2 antibody anti-cyclinD1 antibody, anti-vGluT1 antibody, anti-MAP2 antibody, anti-Smurf2 antibody, anti-CDk2 antibody, anti-Nozl antibody, anti-PI3K antibody, anti-Sox2 antibody, anti-Homer1 antibody, and anti-pPI3K antibody were purchased from CST (USA) and a 4',6-diamidino-2-phenylindole (DAPI) staining kit, Alexa Fluor 488-conjugated donkey anti-mouse IgG, CMFDA, and Alexa Fluor 594-conjugated goat anti-rabbit antibody were purchased from Molecular Probes (USA). An RNA isolation kit was obtained from Promega (USA). A bicinchoninic acid (BCA) kit and RNeasy Mini Kit were purchased from Qiagen (Germany). An RT-PCR kit was purchased from Takara (Japan).

Production of Oct4 recombinant Lentivirus

To produce lentiviral particles, rat Oct4 cDNA was first amplified using a high-fidelity DNA polymerase and subsequently cloned into a pGMLV-based lentiviral vector as previously described by Xu et al [58]. The Oct4 primer sequences for RT-PCR are shown in Table S1. After verifying correctness of constructed vectors,

pGMLV lentiviral vectors containing Oct4 cDNA were transfected into 293T cells using Lipofectamine 2000 transfection reagent to package the Oct4 pseudotyped virus. The virus-containing supernatant was collected after 36 to 48 h of transfection, filtered through a 0.45 μm syringe filter, and further concentrated by ultracentrifugation to establish virus stocks. Subsequently, the virus stocking solution was aliquoted 50 μl per tube and stored at −80 °C for the subsequent experiments.