SUPPORTING INFORMATION

Synthetic Small Molecules That Induce Neuronal Differentiation in Neuroblastoma and Fibroblast Cells

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RT-PCR experiments

Total RNAs isolated using TRIzol (Invitrogen) from neuroblastoma and fibroblast cells treated with Nz or Nzl for different time periods were subjected to cDNA synthesis using oligo (dT) primers and a Superscript II reverse transcriptase (Invitrogen), according to the manufacturer's instructions. The resultant cDNAs were used to analyze genes of interest by RT-PCR with the Bio-Rad PCR system. The PCR primer sets targeting for the following genes were purchased from Bioneer Company (Korea).

Table S1. Oligonucleotide sequences of primers utilized for RT-PCR

Target	Forward primer	Reverse primer	PCR Cycle	References
Mouse				
NeuroD	5'- GCATGCACGGGCTGAACGC-3'	5'- GGGATGCACCGGGAAGGAAG-3'	29	Ref. 1
Mash1	5'- CAAGTTGGTCAACCTGGGTT-3'	5'- GCTCTTGTTCCTCTGGGCTA-3'	30	Ref. 2
Tuj1	5'- CTCCCTTCGATTCCCTGGTC-3'	5'- TGCTCCGAGATGCGTTTGA-3'	30	Designed by Primer-BLAST
MAP2	5'- GGATGGGCTTGTGTCTGATT-3'	5'- CTGGACCCACTCCACTCCACAAACT-3'	35	Ref. 3
NF200	5'-AGCCTGCACTACTCGCTGA-3'	5'-GGCCGTTGCTTAGGGTGTC-3'	35	Primer bank, ID-387493a1
NMDR1	5'-ATGCACCTGCTGACATTCG-3'	5'-TATTGGCCTGGTTTACTGCCT-3'	30	Primer bank, ID-26331234a1
AMPA-α1	5'-TTCTCCTGTTTTATGGGGACTGA-3'	5'-CCCTACCCGAAATGCACTGTA-3'	28	Ref. 4
GluK2	5'-ACTCTGCTACCCAATACCACG-3'	5'-AGCGGGTCTGTATGTGAGGAA-3'	30	Primer bank, ID-163659880c3
GluK4	5'-CCAAGGTCGAAGTGGACATCT-3'	5'- CTGGGGTGAAGGTTCAGGG-3'	30	Primer bank, ID-304766512c1
mGluR3	5'- CTGGAGGCCATGTTGTTTGC-3'	5'- TGTACGAACCGCCAATGACTC-3'	35	Primer bank, ID-118130617c1
mGluR5	5'-TGGCCCTCTTTATCATGGAG-3'	5'- AGGTTGACTAGGCTGCT-3'	30	Ref. 5
mGluR6	5'-GGAGGCTCATGGAGACACC-3'	5'-TTGAAGCCCTTTTGGGCAAGA-3'	30	Primer bank, ID-197333818c3
mGluR7	5'-CCAGATGTGGCAGTGTGTTC-3'	5'-CGAGTCTTGATGGCATA-3'	30	Ref. 5
GAPDH	5'- GCACAGTCAAGGCCGAGAAT-3'	5'- GCCTTCTCCATGGTGGTGAA-3'	30	Ref. 6
Human				
Tuj1	5'- CAGATGTTCGATGCCAAGAA-3'	5'- GGGATCCACTCCACGAAGTA-3'	30	Designed by Primer-BLAST
MAP2	5'- AATAGACCTAAGCCATGTGACATCC-3'	5'- AGAACCAACTTTAGCTTGGGCC-3'	32	Ref. 7
NF200	5'-TCCTACTACACCAGCCATGTC-3'	5'-TCCCCAGCACCTTCAACTTTC-3'	35	Designed by Primer-BLAST
NeuroD	5'-AGCCCCAAGGTCCTCCAA-3'	5'-CGTGCTCCTCGTCCTGAGA-3'	30	Designed by Primer-BLAST
NMDR1	5'- ACCCCAAGATCGTCAACATTG-3'	5'-GGCTAACTAGGATGGCGTAGA-3'	30	Primer bank, ID-297374810c1
GluK4	5'- GCCTCCATCGACGGATTTGA-3'	5'-GCCTCCATCGACGGATTTGA-3'	30	Designed by Primer-BLAST
mGluR6	5'- CCTGTTTGCGATACCCCAGAT-3'	5'-AGTGCCCTCACGATGTCCA-3'	30	Primer bank, ID-110611175c1
GAPDH	5'- ACAACTTTGGTATCGTGGAAGG-3'	5'- GCCATCACGCCACAGTTTC-3'	30	Primer bank, ID-378404907c2

Supplementary References

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Detailed informations for the primers taken from Primer bank are available at http://pga.mgh.harvard.edu/primerbank/index.html.

Primers designed by using software are available at http://www.ncbi.nlm.nih.gov/tools/primer-blast/.
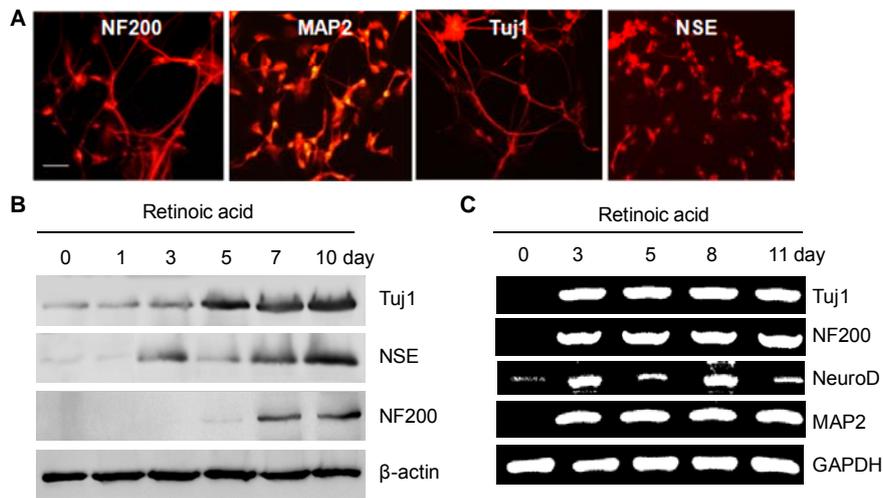
Supplementary Fig. S1 Induction of neurogenesis of SH-SY5Y cells by retinoic acid. SH-SY5Y cells were incubated with 10 µM of retinoic acid for 10 days. (A) The cells were fixed and stained with neuron-specific antibodies against Tuj1, Map2, NF200 and NSE. (B) Western blot analysis with neuron-specific markers such as Tuj1, NF200 and NSE. (C) RT-PCR analysis of neuron-specific genes such as Tuj1, NF200, NeuroD and MAP2.
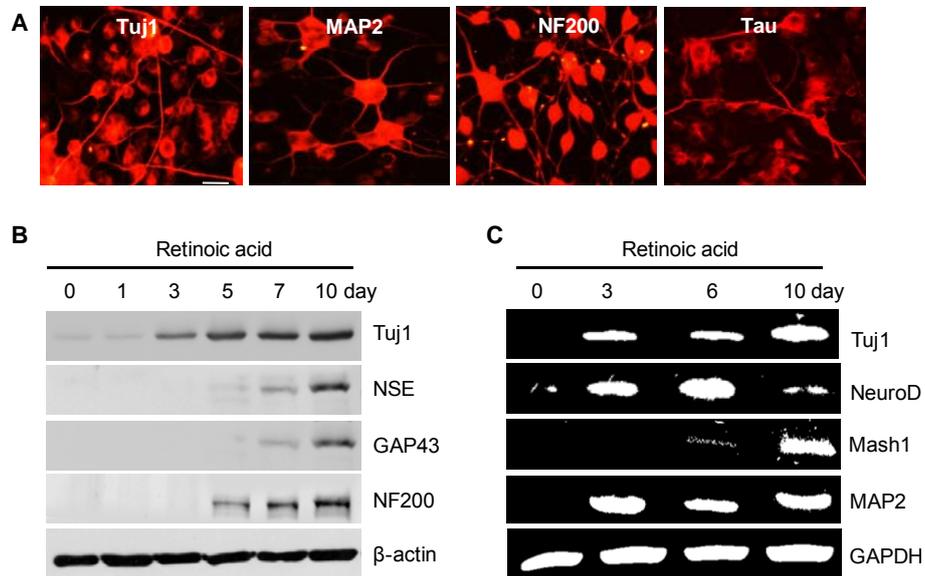
Supplementary Fig. S2 Induction of neurogenesis of Neuro-2a cells by retinoic acid. (A) Neuro-2a cells were incubated with 10 μM retinoic acid for 10 days. The cells were fixed and stained with neuron-specific antibodies against Tuj1, MAP2, NF200 and NSE. (B) Western blot analysis with neuron-specific markers such as Tuj1, GAP43, NF200 and NSE. (C) RT-PCR analysis of neuron-specific genes such as Tuj1, MAP2, NeuroD and Mash1.
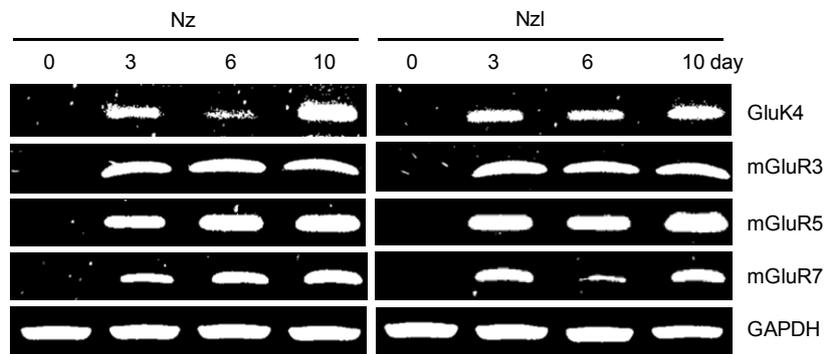
Supplementary Fig. S3 Expression of glutamate receptors in Neuor-2a cells was examined after treatment with 5 µM Nz or 5 µM Nzl by using RT-PCR.
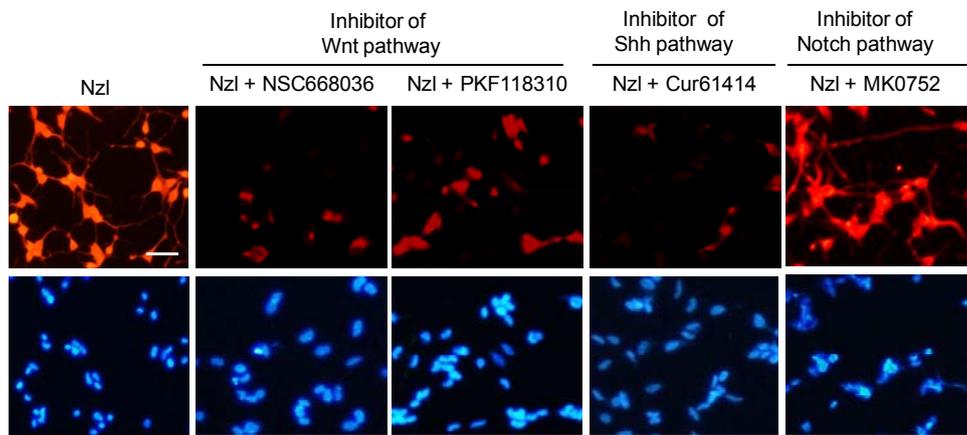
Supplementary Fig. S4 Effect of inhibitors of Wnt, Shh and Notch signaling pathways on neurogenesis of SH-SY5Y cells induced by Nzl. SH-SY5Y cells were incubated with 5 µM Nzl in the presence or absence of 20 µM NSC668036, 25 nM PKF118-310, 25 µM Cur61414, and 25 µM MK0752 for 8 days. The cells were stained with NF200 antibody. Bottom images are of DAPI stained cells (bar: 50 µm).
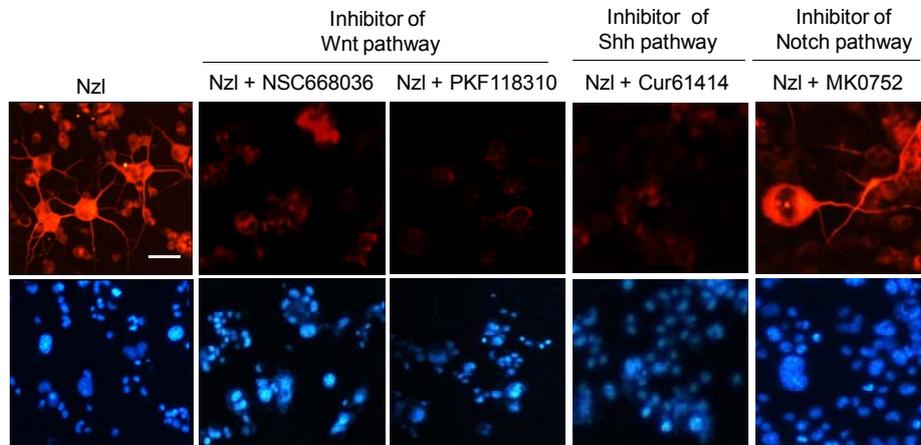
Supplementary Fig. S5 Effect of inhibitors of Wnt, Shh and Notch signaling pathways on neurogenesis of Neuro-2a cells induced by Nzl. Neuro-2a cells were incubated with 5 μM Nzl in the presence or absence of 20 μM NSC668036, 25 nM PKF118-310, 25 μM Cur61414, and 25 μM MK0752 for 8 days. The cells were stained with NF200 antibody. Bottom images are of DAPI stained cells (bar: 50 μm).
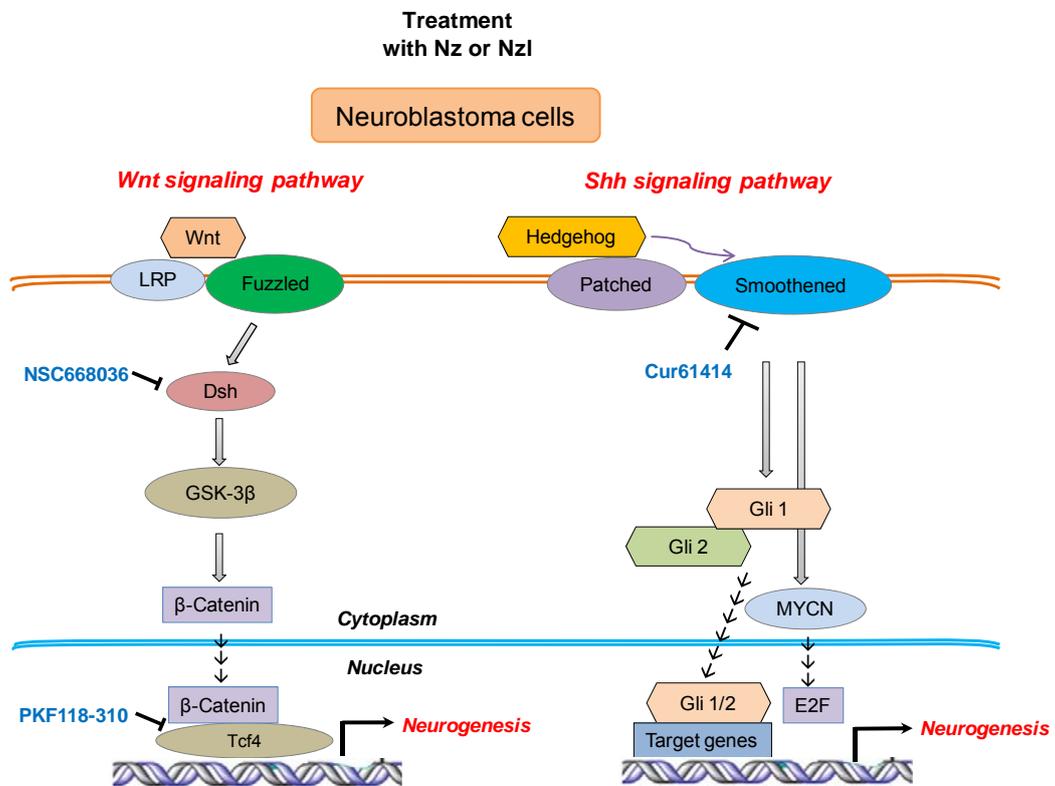
Supplementary Fig. S6 Nz and Nzl enhance neurogenesis in neuroblastoma cells by activating the Wnt and Shh signaling pathways.
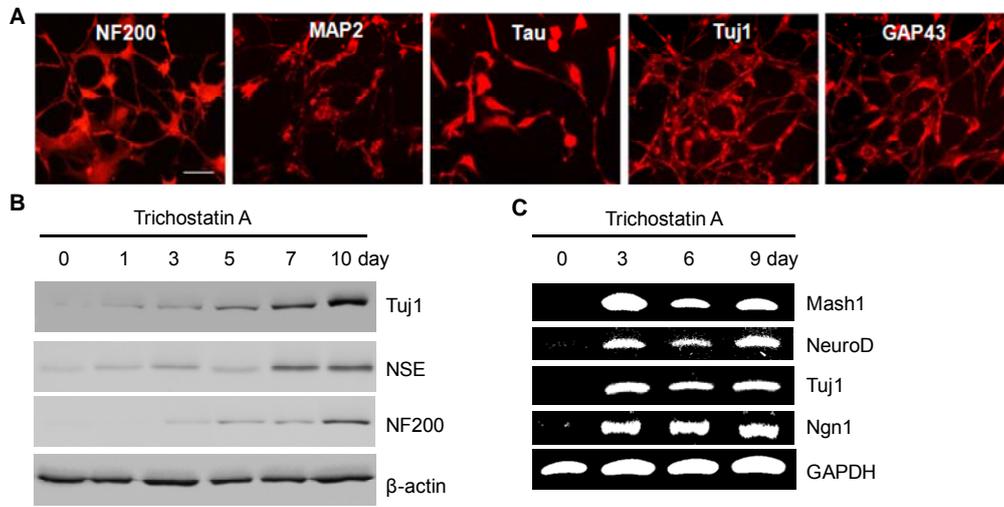
Supplementary Fig. S7 Induction of neurogenesis of NIH3T3 cells by trichostatin A. (A) NIH3T3 cells were incubated with 50 nM trichostatin A for 10 days. The cells were fixed and stained with neuron-specific antibodies against Tuj1, MAP2, NF200, Tau and GAP43. (B) Western blot analysis with neuron-specific markers such as Tuj1, NF200 and NSE. (C) RT-PCR analysis of neuron-specific genes such as Tuj1, Ngn1, Mash1 and NeuroD.

| | Inhibitor of Wnt pathway | | Inhibitor of Shh pathway | Inhibitor of Notch pathway |
| Nz | Nz + NSC668036 | Nz + PKF118310 | Nz + Cur61414 | Nz + MK0752 |
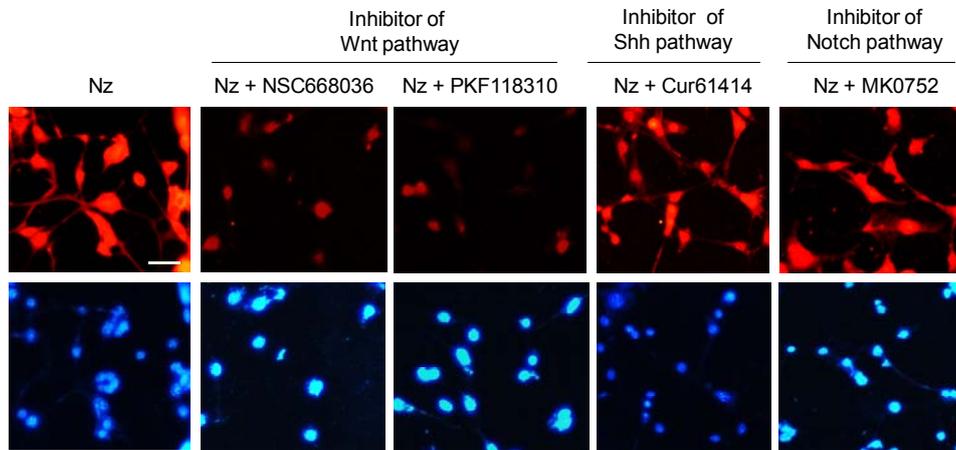
Supplementary Fig. S8 Effect of inhibitors of Wnt, Shh and Notch signaling pathways on neurogenesis of NIH3T3 cells induced by Nz. NIH3T3 cells were incubated with 2.5 μM Nz in the presence or absence of 15 μM NSC668036, 20 nM PKF118-310, 25 μM Cur61414, and 20 μM MK0752 for 8 days. The cells were stained with NF200 antibody. Bottom images are of DAPI stained cells (bar: 50 μm).
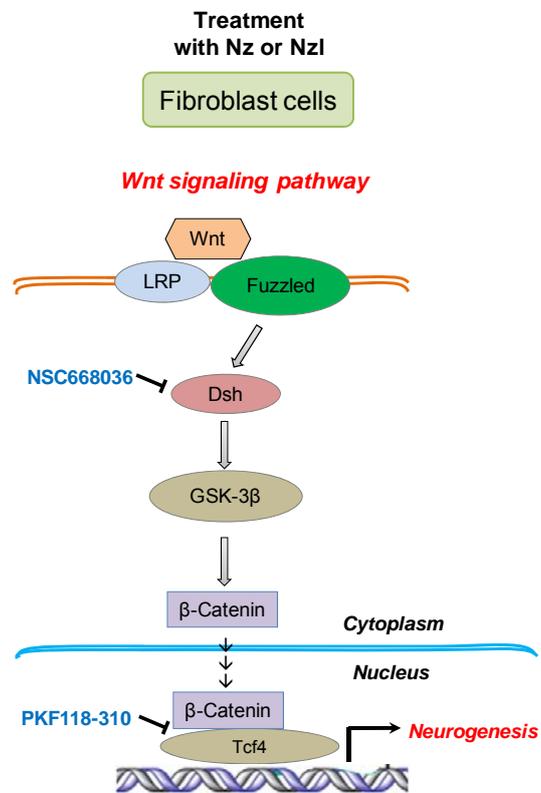
Supplementary Fig. S9 Nz and Nzl promote neurogenesis in NIH3T3 fibroblast cells mainly through activation of the Wnt pathway.