Supplementary Data

Materials and Methods

Cell culture SK-N-AS, G401, U251, WiDr, Hela, were obtained from the American Type Culture Collection (Manassas). We monitored the morphology of these cells by microscopy and confirmed their morphology was the same as original. No further authentication was performed during experiments.

Cell growth and cell cycle assay Stable SH-SY5Y cells established by expressing control shRNA or shRNA-2 for NeuroD1 were seeded to 96-well plate. A cell growth assay was performed by using cell counting kit-8 (Dojindo Laboratories, Kumamoto, Japan). For cell cycle assay, $2x10^6$ stable knockdown SH-SY5Y cells were collected, fixed in 80% ethanol over 24 h at -20℃. The cells were then treated by RNaseA (Sigma; working concentration: 16 μg/ml) at 37℃ for 30min, followed by propidium iodide staining (Sigma; working concentration: 50 μg/ml) at RT 5 min. After filtering by a 40 μm filter, sample was ready for cell cycle analysis. Cell-cycle distribution was calculated by ModFit LT software.

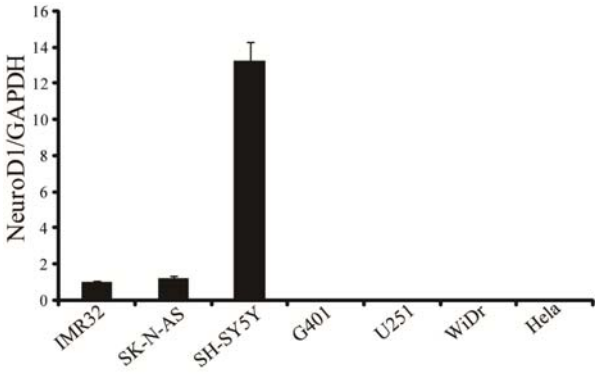
Supplementary Table I. Primer sequences for PCR experiments

Primer name	Forward primer sequence(5'-3')	Reverse primer sequence(5'-3')	Tm(□)	Product size
mouse NeuroD1	GCAGCTCTGGAGCCCTTCTT	GCGGCACCGGAAGAGAAGAT	56	218bp
human NeuroD1	GGAAACGAACCCACTGTGCT	GCCACACCAAATTCGTGGTG	55	248bp
human Slit2	GGGGACAGCTGTGATCGAGA	CACCTCGTACAGCCGCACTT	56	251bp
mouse GAPDH	GGTGGTGAAGCAGGCATCTG	GGAGGCCATGTAGGCCATGA	56	220bp
human GAPDH	ATCATCCCTGCCTCTACTGG	CCCTCCGACGCCTGCTTCAC	56	188bp
mouse TH(tyrosine hydroxylase)	GCCCAGTTCTCGCAGGACAT	CACGAAGTAGACGGGCTGGT	56	267bp
human MYCN	CGACCACAAGGCCCTCAGTA	CAGCCTTGGTGTTGGAGGAG	56	140bp
ChIP-1 for E3,4,5	CGTGAGTGAGCAGAGTCCAG	GTGGGCTCCGGAAGCTAGAA	56	141bp
ChIP-2 for E6	CCCTCGGAGCAGCAAGCTAA	GATCGCCAGCACTAACCCCA	56	120bp
ChIP-3 for E1,2	GCTGCGGCTTATCTGGGAGA	GGGTGGGATCCACTACTCCA	56	99bp

Supplementary Table II. shRNA sequences for RNAi experiments

shRNA clone	**shRNA sequence(5'-3')**
human NeuroD1 shRNA-1	CCGGGCACAATTTGAGCAATTCATTCTCGAGAATGAATTGCTCAAATTGTGCTTTTT
human NeuroD1 shRNA-2	CCGGGCCTTGCTATTCTAAGACGCACTCGAGTGCGTCTTAGAATAGCAAGGCTTTTT
mouse NeuroD1 shRNA-1	CCGGGCGTTGCCTTAGCACTTCTTTCTCGAGAAAGAAGTGCTAAGGCAACGCTTTTTG
mouse NeuroD1 shRNA-2	CCGGGCTCAGCATCAATGGCAACTTCTCGAGAAGTTGCCATTGATGCTGAGCTTTTTG
human Slit2 shRNA-1	CCGGCCTCACCTTAATTCTTAGTTACTCGAGTAACTAAGAATTAAGGTGAGGTTTTTG
human Slit2 shRNA-2	CCGGCCTGGAGCTTTCTCACCATATCTCGAGATATGGTGAGAAAGCTCCAGGTTTTTG

Supplementary Figure 1

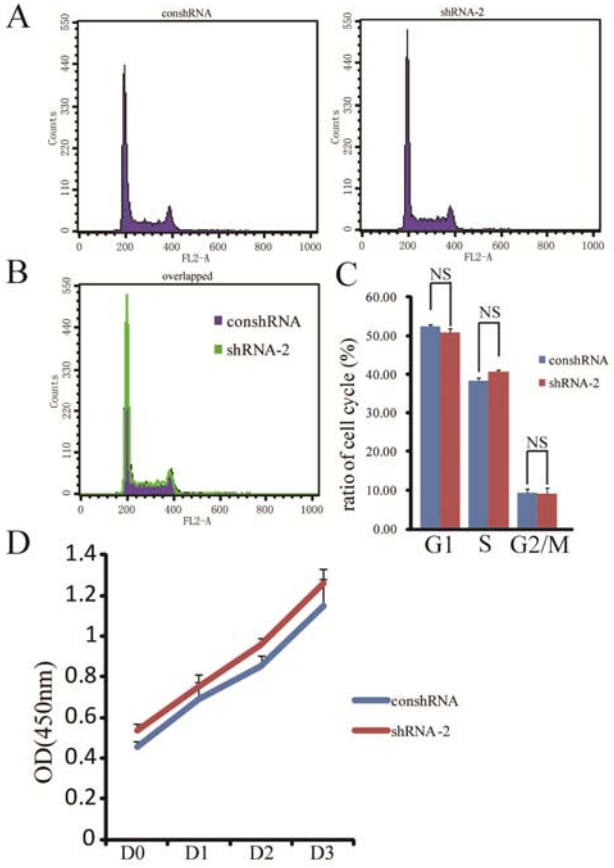


Supplementary Fig. 1

Expression of NeuroD1 in NB cell lines and other tumor cell lines. Real-time PCR results are shown. IMR32, human NB cells with *MYCN* amplification; SK-N-AS and SH-SY5Y, human NB cells without *MYCN* amplification; G401, human Wilms' tumor cells; U251, human glioblastoma cells; WiDr, human colorectal adenocarcinoma cells; Hela, human cervical cancer cells.

.

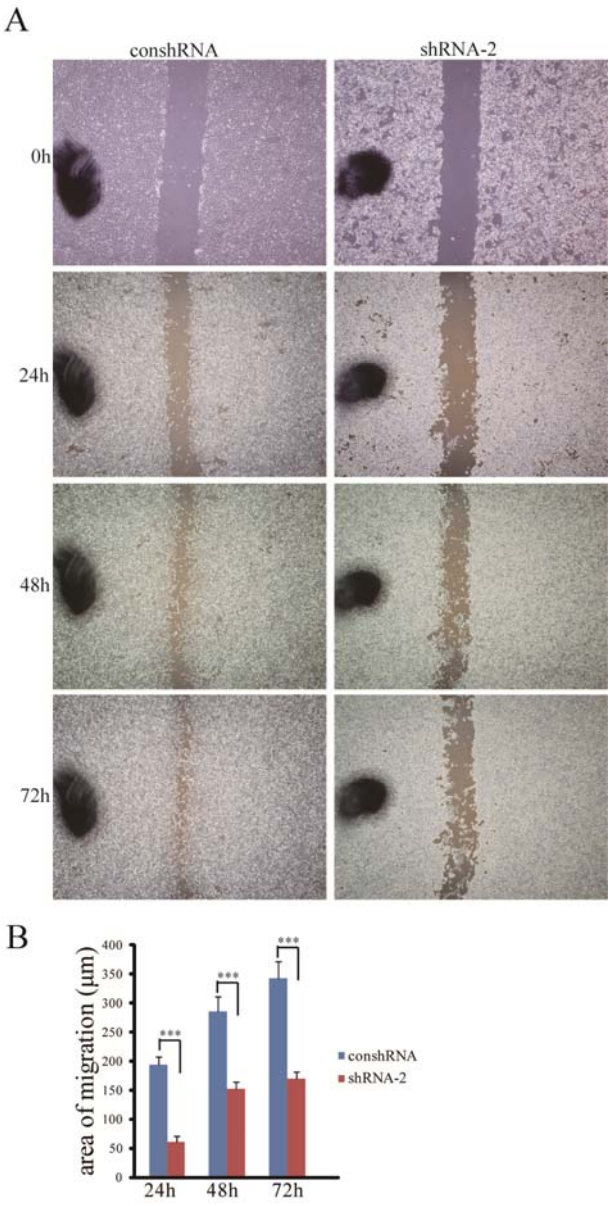
Supplementary Figure 2



Supplementary Fig. 2

Cell-cycle analysis and growth assay of the human NB line SH-SY5Y, which stablely expressed conshRNA or NeuroD1 shRNA. **A-C,** DNA content and estimation of cell-cycle distribution was determined by FACS analysis. **D,** Cell growth was evaluated by growth assay kit up to 3 days after seeding.

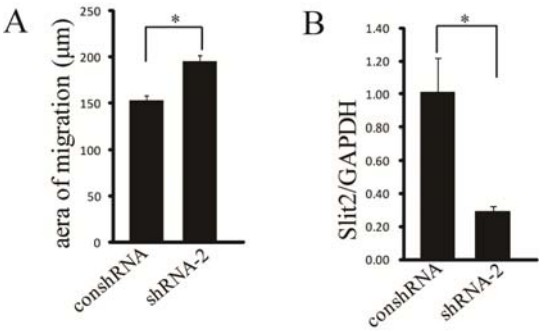
Supplementary Figure 3



Supplementary Fig. 3

Stable knockdown of NeuroD1 could lead to motility inhibition of human NB cell line SH-SY5Y. **A,** Wound-healing assay of SH-SY5Y cells stably expressing conshRNA or NeuroD1 shRNA-2 was performed, and data were collected at 24 h, 48 h, 72 h after a scratch was made. **B,** Area of migration was calculated. Significance was obtained at each time point. ***p<0.001 (Student's *t*-test)

Supplementary Figure 4



Supplementary Fig. 4

Directional cell migration was stimulated in a monolayer using an *in vitro* wound-healing assay. Slit2 knockdown alone can also enhance the motility of SH-SY5Y. *p<0.05 (Student's *t*-test).