

SUPPLEMENTARY DATA

TNF α -induced Up-regulation of *Ascl2* Affects the Differentiation and Proliferation of Neural Stem Cells

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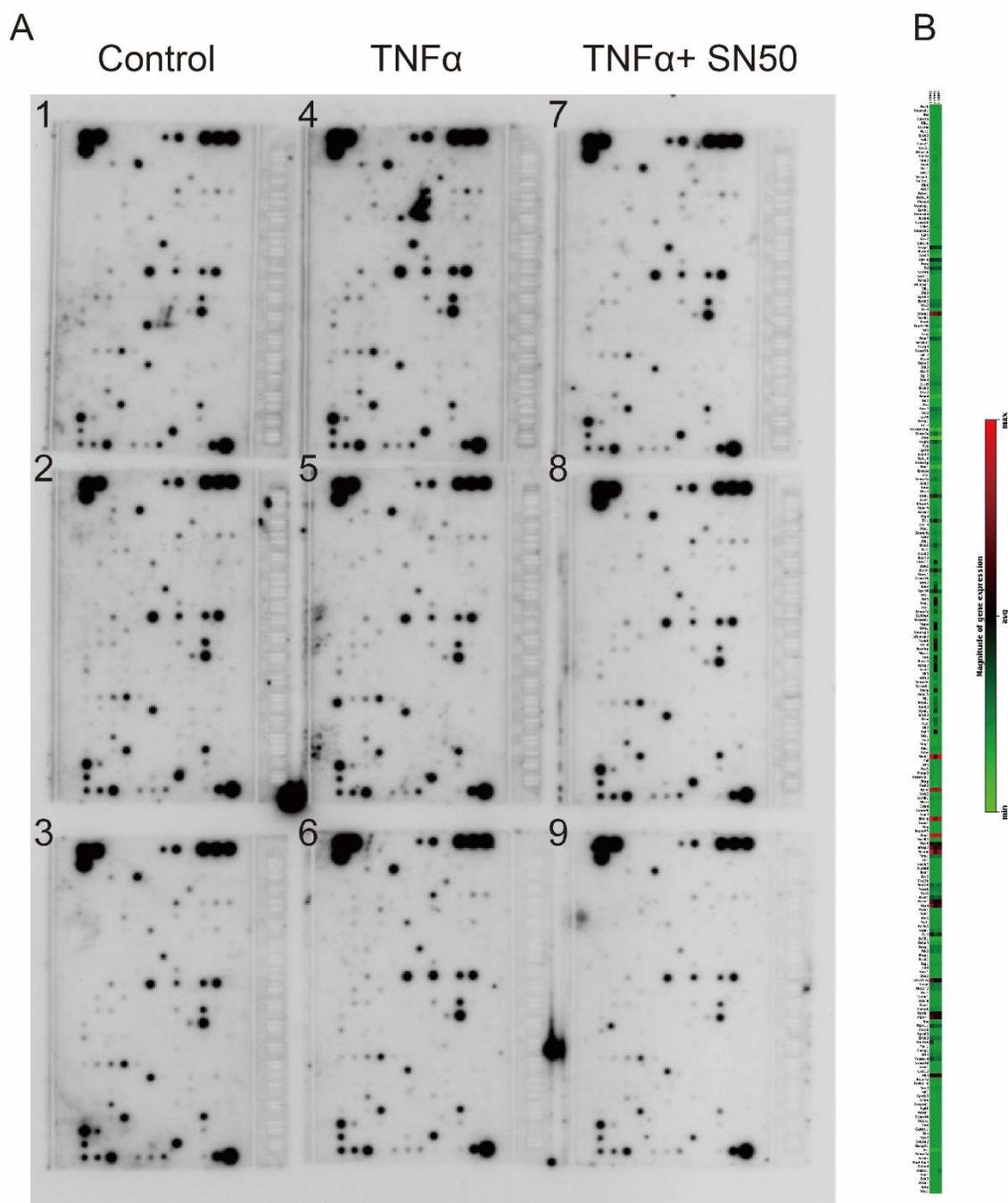
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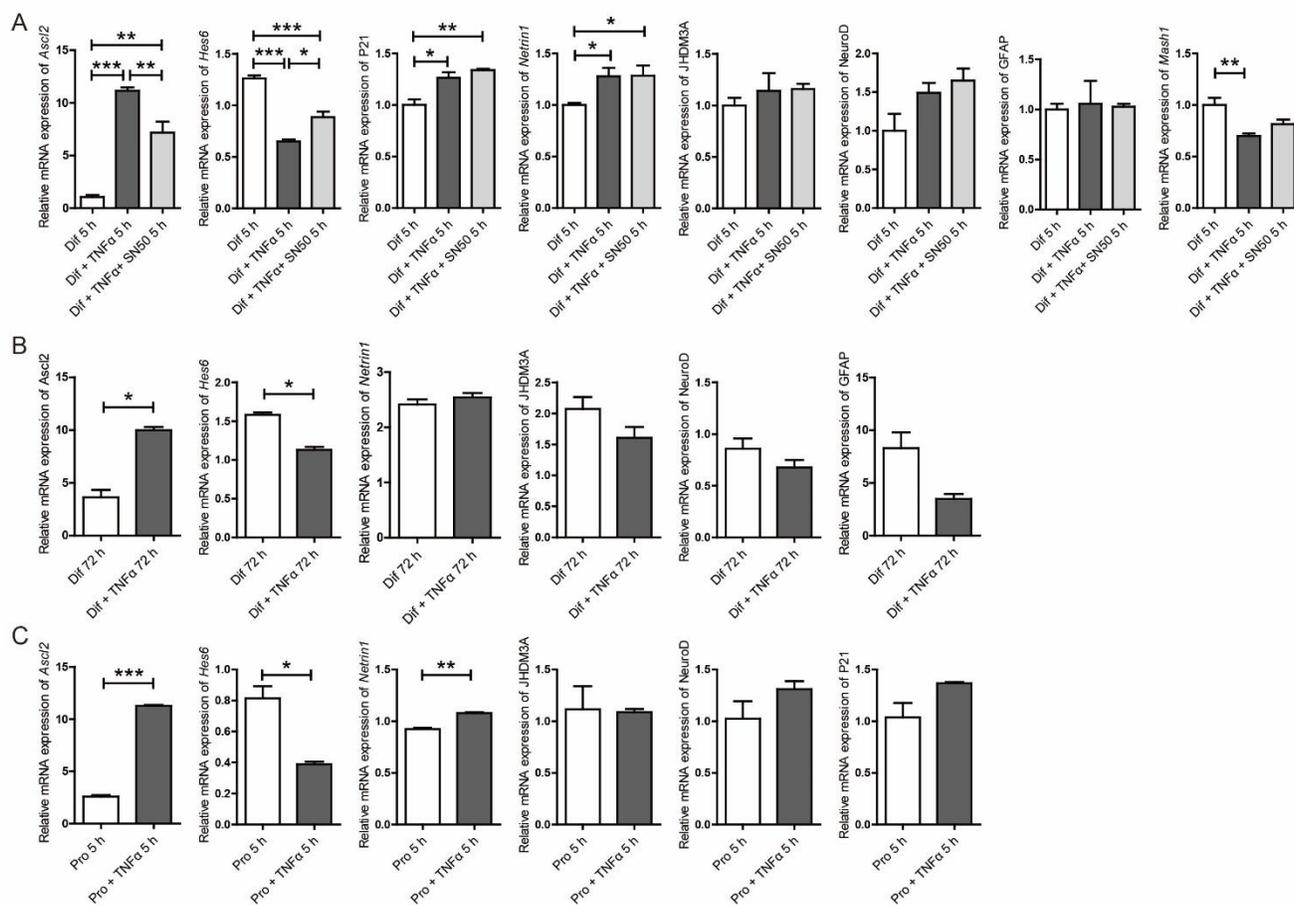
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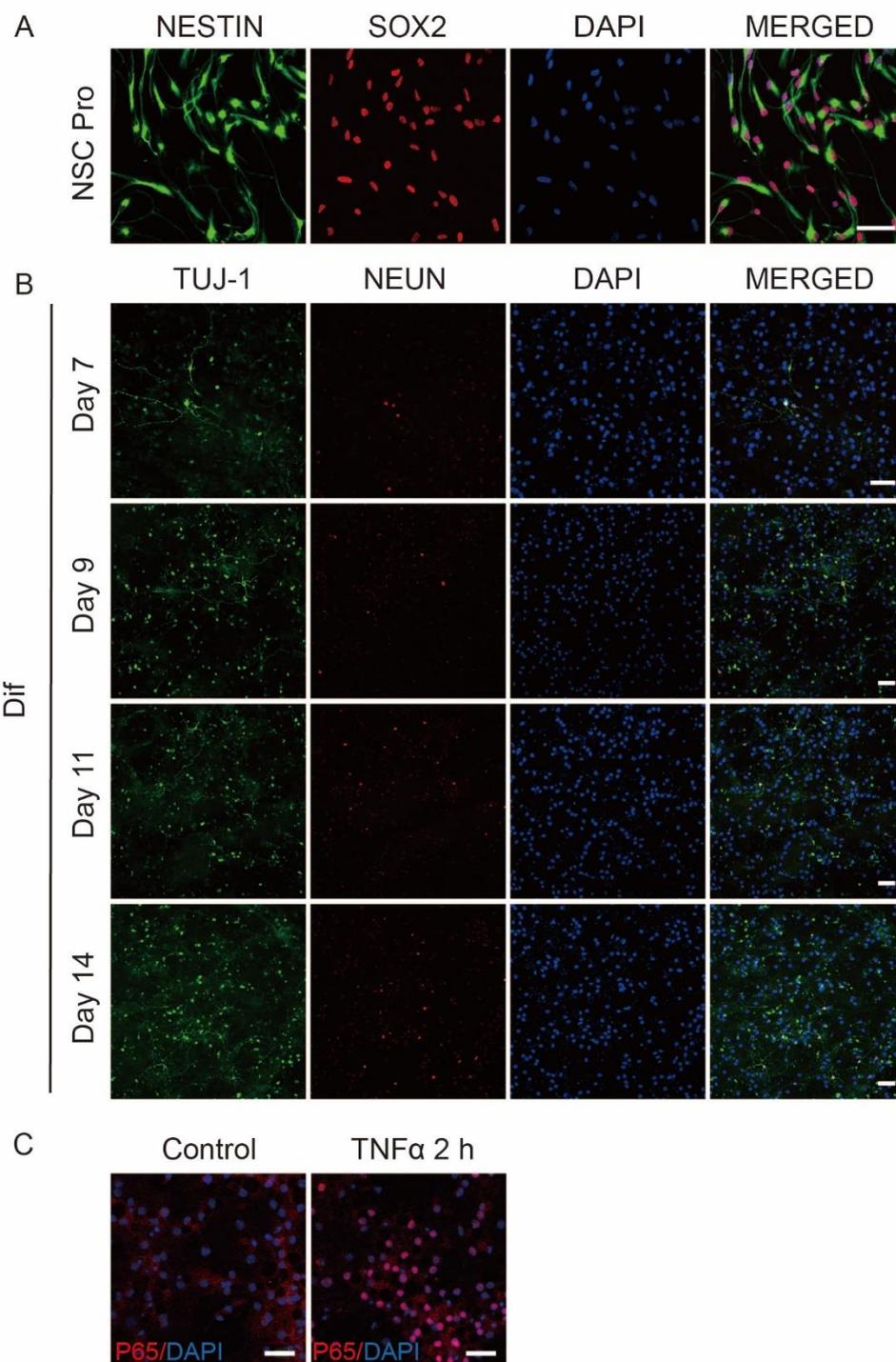
Supplementary Figure 1. Transcriptional changes in stem cell-related genes downstream of TNF α treatment in murine NSCs cultured in a differentiation condition. Related to Figure 1. (A) NSCs of passage number 7 were cultured as monolayer in a differentiation medium. 1, 2, and 3 refer to control group without TNF α treatment; 4, 5, and 6 refer to NSCs treated with 20 ng/ml TNF α for 5 h; 7, 8, and 9 refer to NSCs treated with 20 ng/ml TNF α and 50ug/ml SN50 for 5 h. (n=3). **(B)** Heatmap shows expression of 281 genes related to neural stem cells plus internal control *Gapdh* in the three groups.

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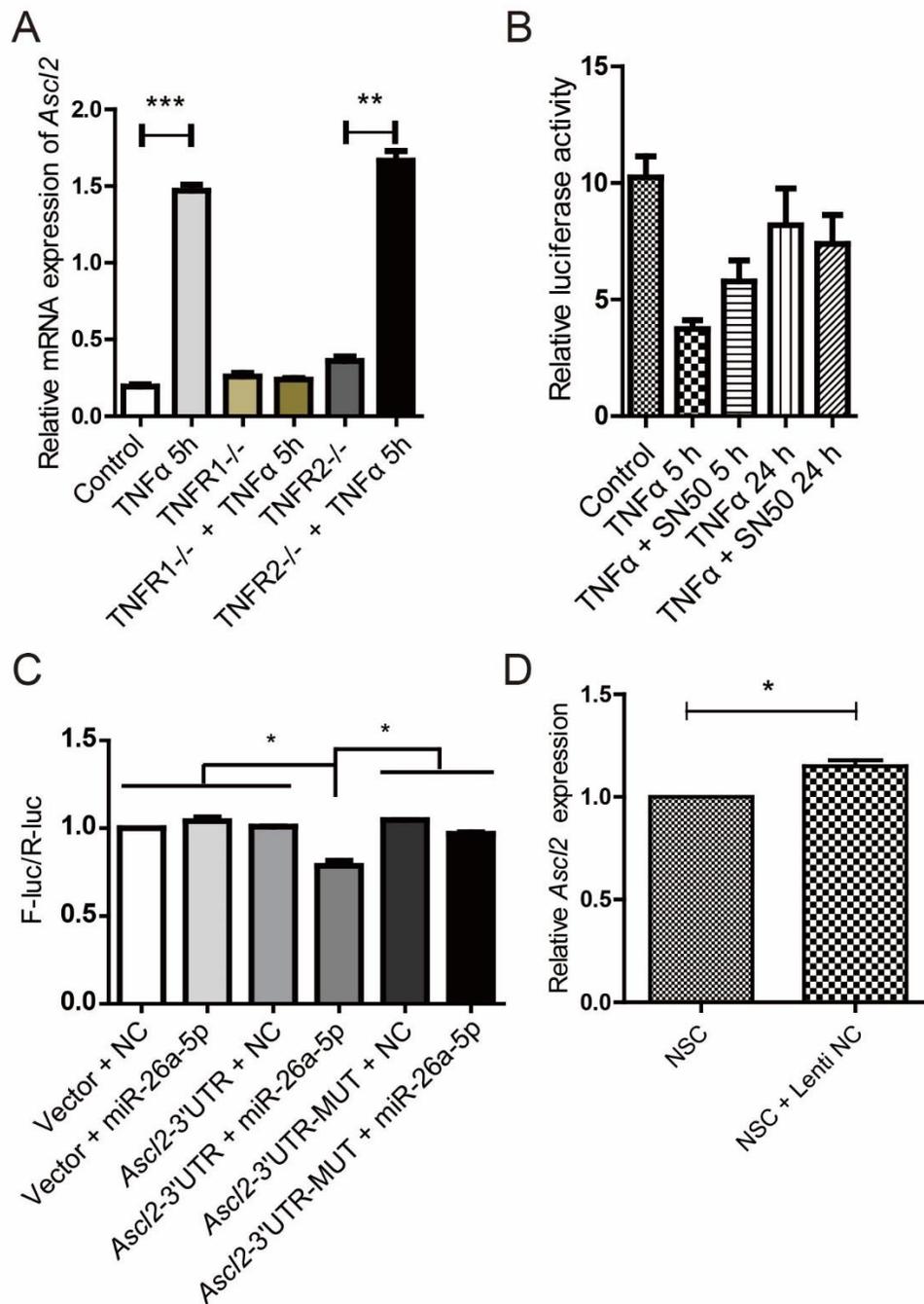
Supplementary Figure 2. qPCR quantification of the mRNA expression levels for *Ascl2*, *Hes6*, *P21*, *Netrin1*, *JHDM3A*, *NeuroD*, *GFAP*, and *Mash1* under different conditions. (A) Differentiation condition for 5 h with or without TNF α and SN50. (n=3). (B) Differentiation condition for 72 h with or without TNF α . (n=3). (C) Proliferation condition for 5 h with or without TNF α . (n=3).

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Supplementary Figure 3. Characterization of neural stem cells and activation of NF- κ B pathway after TNF α treatment. (A) Monolayer murine NSCs were cultured in proliferation medium, and the cells were positive for NSC markers NESTIN and SOX2. Pro, proliferation. Scale bars, 50 μ m. **(B)** Monolayer murine NSCs were subjected to a differentiation condition for 7, 9, 11 and 14 days. Early neuronal marker TUJ-1 and mature neuron marker NEUN were stained. Dif, differentiation. Scale bars, 50 μ m. **(C)** Monolayer murine NSCs were treated with TNF α (20 ng/ml) for 2 h. After TNF α treatment, NF- κ B translocated from cytoplasm to nucleus, as evidenced by P65 staining.

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Supplementary Figure 4. Expression levels of *Ascl2* after TNF α , miRNA, and virus treatment. (A) NSCs isolated from TNFR1^{-/-} and TNFR2^{-/-} mice were used to determine whether TNFR1 or TNFR2 was involved in regulation of *Ascl2* by TNF α . *Ascl2* expression level analyzed by realtime PCR. (n=3). (B) NSCs cells were transfected with pGL3-*Ascl2* Promoter (-968 to +444bp) together with Renilla vector. The next day treat cells with TNF α and/or SN50 for 5 h or 24 h. 24 h after treatment, the cells are lysed for Dual luciferase assay. Firefly luciferase activities were examined and normalized to Renilla luciferase activities. (n=3). (C) 293T cells were transfected with empty control vectors, *Ascl2* 3'UTR luciferase construct or *Ascl2* 3'UTR mutation luciferase construct together with miR-26a-5p mimics. Forty-eight hours following transfection, cells were collected and lysed. Firefly luciferase activities were examined and normalized to Renilla luciferase activities. (n=3). (D) *Ascl2* expression increased in NSCs infected with lentivirus. (n=3).

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Ascl2 promotor sequence cloned into pGL3 vector

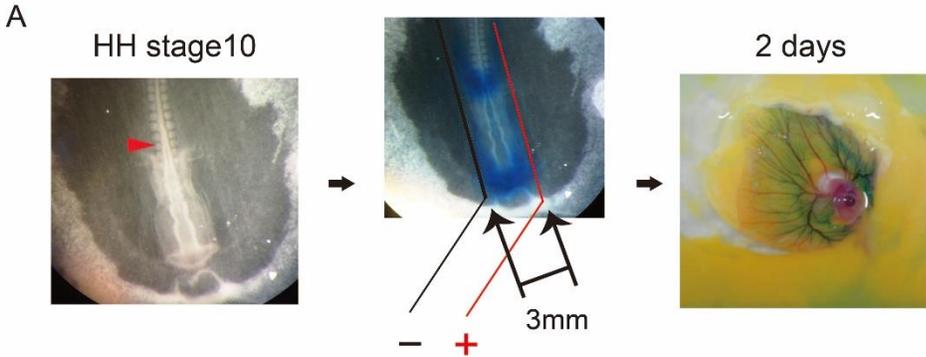
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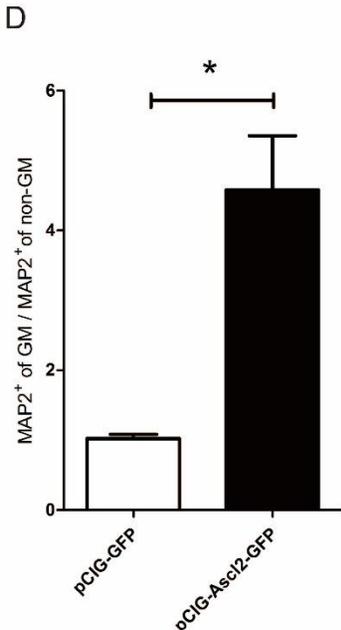
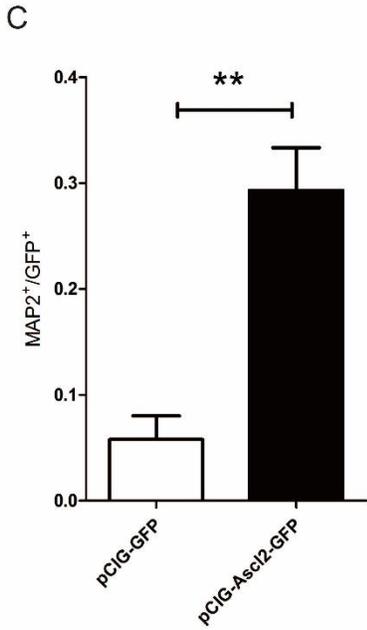
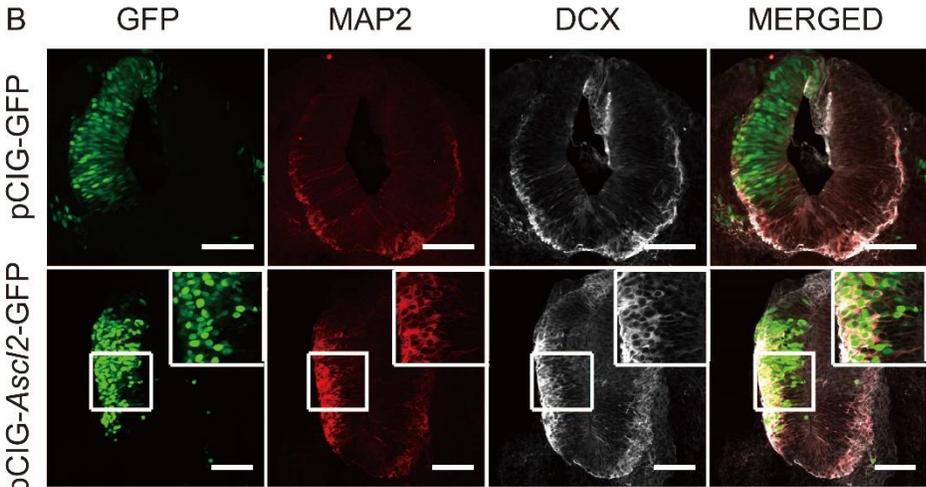
Predicted NFκB Binding site: **CATGAAATTTCCCAT**; Transcript start site: **G**

Supplementary Figure 5. Luciferase Reporter Assay. Software LASAGNA-Search 2.0 predicts a NF-κB binding site in *Ascl2* promotor.

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Supplementary Figure 6. The impact of *Ascl2* overexpression on NSC differentiation *in ovo*. Related to Figure 7. (A) Schematic representation of *in ovo* electroporation of *Ascl2*. (B) Expression of neuronal marker MAP2 at 24 h post-electroporation. (C) Proportion of MAP-positive cells among GFP-positive cells. (n=6). (D) Rate of MAP-positive cells of genetically modified (GM) side over non-genetically modified (non-GM) side. (n=6)



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Supplementary Table 1. Primer sequences used for quantitative real time PCR. Related to Figure 1 and supplementary Figure 2.

Gene ID	Primer sequence (5' to 3')	Product Size(bp)
Gapdh: NM_138654.3	TCAACAGCAACTCCCCTCTTCCA	117
	ACCACCTGTGTGCTGTACCGTATT	
Pten: NM_008960.2	AATCCCAGTCAGAGGCGCTATGT	137
	GATTGCAAGTCCGCCACTGAACA	
Ascl2: NM_008554	ACCTGCTTCTAGCCCAAGAAACCT	139
	TGCAAGGTCCGGAAGATGGAAGAT	
NeuroD: NM_010894.2	ACCTTGCTACTCCAAGACCCAGAA	139
	TTTGCAGAGCGTCTGTACGAAGGA	
GFAP: NM_001131020	GGAAATTGCTGGAGGGCGAAGAAA	163
	TGGTGAGCCTGTATTGGGACAACCT	
P21: NM_007669	TTGTACAAGGAGCCAGGCCAAGAT	119
	ACCCACTAAGTGCTTTGACACCCA	
Mash-1: NM_008553	ACGACTTGAACCTCTATGGCGGGTT	100
	AAGTCCAGCAGCTCTTGTTCCCTCT	
JHDM3A: NM_172382	GGCTTCAACCATGGCTTCAACTGT	194
	TCAATAACCGTGCTGTCCTTCCCA	
Hes6: NM_019479	TCACTGAAGCTGCTCCTCGTTTGT	80
	ATTCGGTTGGAGCATCGATGGGAT	
Ascl2 promotor: NC_000073.6	CGCGGTACCTTGAGAGCACACTTCTG	1407
	CGCGGGATCCTACGAGTTCTGGTGC	

Note: the red part indicates kpnI and BamHI site respectively.

Supplementary Table 2. Primers and probes for miR-26a-5p Taqman quantitative real-time PCR. Related to Figure 2.

U6	FO	ATTGGAACGATACAGAGAAGATT
	RE	GGAACGCTTCACGAATTTG
	PR	TGCGCAAGGATGACACGCA
mmu-miR-26a-5p	FO	TCGCCGTTCAAGTAATCCAG
	RE	CAGAGCAGGGTCCGAGGTA
	PR	ACCCGAGAGCCTATCCTGGA
mmu-miR-26b-5p	FO	CGCCGTTCAAGTAATTCAGG
	RE	CAGAGCAGGGTCCGAGGTA
	PR	CGTTCGCTCTGGACCCGAGAC
mmu-miR-26b-3p	FO	CAGTGCTGCCTGTTCTCCATTAC
	RE	TATGGTTGTTACGACTCCTTCAC
	PR	CCCTATCCAACCATACAGACGAGCC
mmu-miR-26a-1-3p	FO	CAGTGCTGCCTATTCTTGTTAC
	RE	TATGGTTGTTACGACTCCTTCAC
	PR	CCCTATCCAACCATACAGACCGTGC
mmu-miR-26a-2-3p	FO	CAGTGCTGCCTGTTCTTGATTAC
	RE	TATGGTTGTTACGACTCCTTCAC
	PR	CCCTATCCAACCATACAGACGAAACA

Note: FO, forward primer; RE, reverse primer; PR, probe.