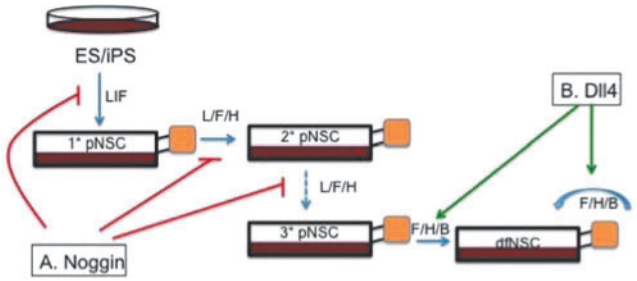
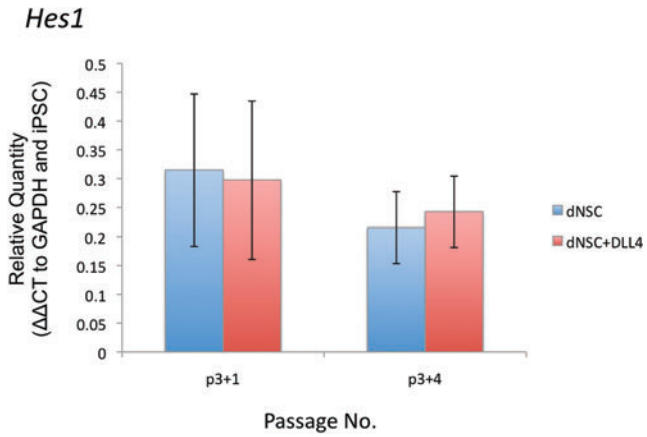
Supplementary Data

Supplementary References

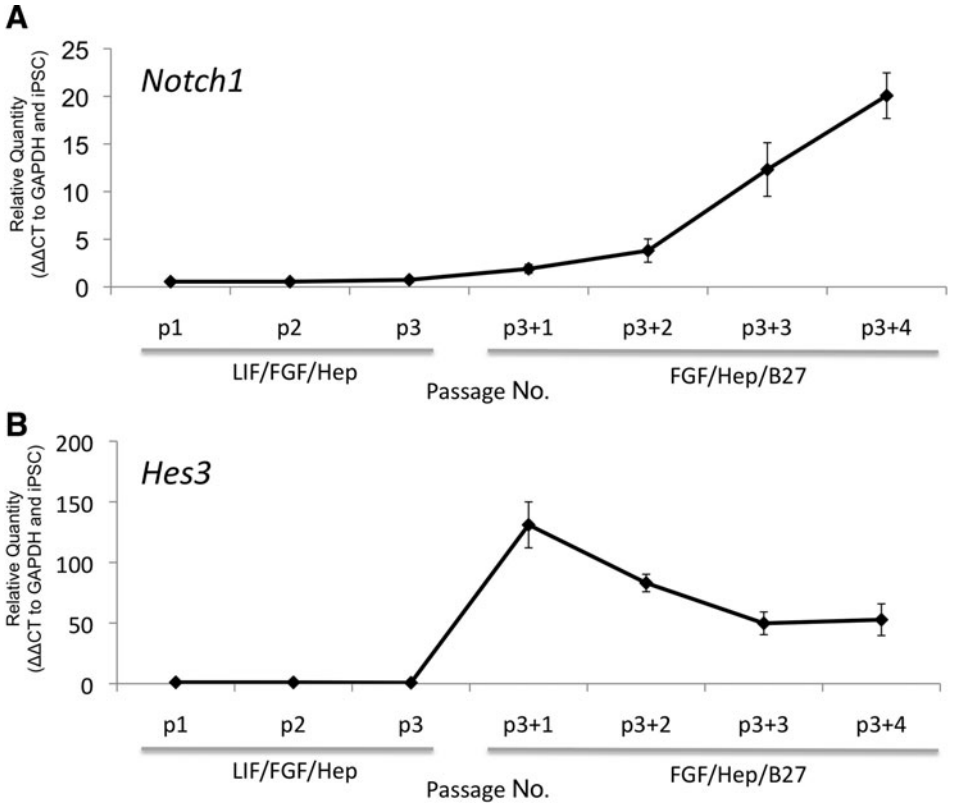
1. Nagy A, J Rossant, R Nagy, W Abramow-Newerly and JC Roder. (1993). Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. Proc Natl Acad Sci U S A 90:8424–8428.
2. Vintersten K, C Monetti, M Gertsenstein, P Zhang, L Laszlo, S Biechele, A Nagy. (2004). Mouse in red: red fluorescent protein expression in mouse ES cells, embryos, and adult animals. Genesis 40:241–246.
3. Woltjen K, IP Michael, P Mohseni, R Desai, M MIleikovsky, R Hamalainen, R Cowling, W Wang, P Liu, et al. (2009). *piggyBac* transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458:766–770.



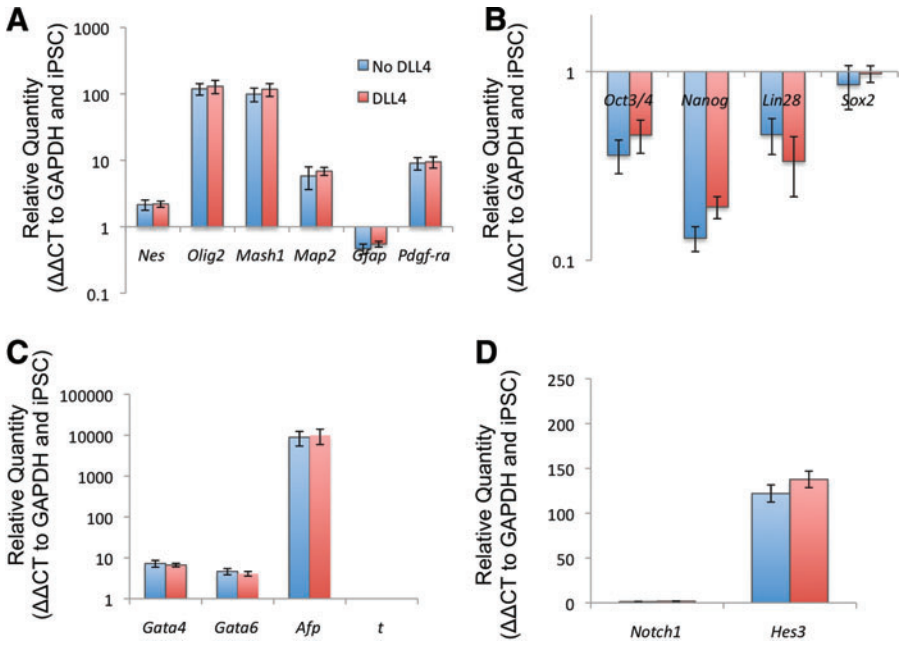
SUPPLEMENTARY FIG. S1. Schematic diagram of cell culture conditions. The default pathway of neuralization relies on the absence of cues that direct pluripotent cells to other lineages resulting in the acquisition of a neural identity. In brief, following ES/iPS cell expansion conditions, the cells were cultured in serum- free media (SFM) with LIF at low cellular density (10 cell/μL) to yield primary primitive neural stem cells (pNSCs). These pNSCs were further passaged in SFM with LIF(L), FGF2(F), and heparin(H) to generate sphere colonies of secondary, tertiary, etc. pNSCs. To generate definitive NSCs (dNSCs) the pNSCs were passaged in SFM lacking LIF and containing FGF2, heparin, and B27(B) supplement. (A) Noggin is a BMP antagonist that was used to reinforce the default pathway during the neuralization of the iPSCs. Noggin (200 ng/mL; R&D systems) was added to the culture condition during the induction and maintenance of the pNSCs. (B) NOTCH signaling has been shown to be essential for the maintenance of dNSCs, as well as to inhibit the further differentiation of these cells. Delta-like ligand 4 (DLL4, 500 ng/mL; R&D systems) was added to the definitive culture conditions to induce the NOTCH pathway. ES, embryonic stem; iPS, induced pluoripotent stem; LIF, leukemia inhibitory factor; FGF, fibroblast growth factor; BMP, bone morphogenetic proteins.



SUPPLEMENTARY FIG. S2. *Hes1* mRNA levels in iPS-dNSCs are not affected by addition of DLL4 to culture conditions. RT-PCR for HES1, a NOTCH pathway-related effector, showed no significant difference in *Hes1* expression during the initial passage (p3+1) or the fourth definitive passage (p3+4) of iPS-dNSCs regardless of NOTCH agonism (C5-4A iPSC line). RT-PCR, real-time-polymerase chain reaction.



SUPPLEMENTARY FIG. S3. *Notch1* and *Hes3* gene expression in ES-NSCs during both the primitive and definitive stage of neuralization following the default pathway conditions. RT-PCR for the NOTCH pathway genes *Notch1* and *Hes3* showed that these genes are maintained in the definitive state of ES-derived NSCs (R1 ES cell line).



SUPPLEMENTARY FIG. S4. The initial passage of iPS-dNSCs in definitive media resulted in an mRNA profile that was intermediate between primitive and definitive characteristics. Following a single passage in SFM+ FGF2, heparin, and B27, the resultant dNSCs showed an mRNA profile indicative of poor neuralization in terms of neural markers **(A)**, pluripotency markers **(B)**, nonectodermal markers **(C)**, and NOTCH pathway genes **(D)**. The addition of DLL4 did not affect the initial definitive passage (C5-4A iPSC line).

Supplementary Table S1. Cell Lines

Cell line	*Description*	*References*
ESCs		
R1	Derived from male blastocyst, hybrid of two 129 substrains (129×1/SvJ and 129S1/SV-+*p*+ *Tyr-c*Kitl*Sl-J*/+)	[1]
G4	Male blastocyst derived from the natural mating of 129S6/SvEvTac female with C57BL/6Ncr male	[2]
iPSCs		
C5	MEFs were isolated from 15.5d.p.c. ROSA26 knockin rtTA-IRES-GFP17 embryos. Single copy of transgene MKOS cassette.	[3]
C5-4A	MEFs were isolated from 15.5d.p.c. ROSA26 knockin rtTA-IRES-GFP17 embryos. Single copy of transgene MKOS cassette removed following reprogramming, daughter cell line to C5.	[3]
B1-1G	MEFs were isolated from 15.5d.p.c. ROSA26 knockin rtTA-IRES-GFP17 embryos. Single copy of transgene MKOS cassette removed following reprogramming.	[3]

ESCs, embryonic stem cells; MEF, mouse embryonic fibroblast; iPSCs, induced pluripotent stem cells.

Supplementary Table S2. TAQman Gene Expression Primers

Gene	*TAQ expression assay ID*
Acll (Mash1)	Mm03058063_m1
Afp	Mm00431715_m1
Gata4	Mm00484689_m1
Gata6	Mm00802636_m1
Gfap	Mm01253033_m1
Gapdh	4352932E
Hes1	Mm01342805_m1
Hes3	Mm00468603_m1
Nanog	Mm02019550_s1
Nestin	Mm00450205_m1
Notch1	Mm00435249_m1
Olig2	Mm01210556_m1
PDGFra	Mm00440701_m1
Sox2	Mm03053810_s1
Pouft1 (Oct4)	Mm03053917_g1
Lin28	Mm00524077_m1
CNPase	Mm01306640_m1
Map2	Mm00521988_m1
Tuj1 (bIII tub)	Mm00727586_s1

Supplementary Table S3. List of Primary Antibodies Used for Immunocytochemistry

Antibody	*Target*	*Concentration*	*Supplier*
Nestin	Neural stem cells (NSCs)	1:200	Chemicon
Olig2	Undifferentiated NSCs, All oligodendrocyte lineages, including oligoprecursors	1:1,000	Chemicon
GFAP	Astrocytes	1:100	Chemicon
PDGFra	Immature oligodentrocyes	1:50	Santa Cruz
CNPase	Mature oligodendrocytes	1:100	Chemicon
βIII Tubulin	Neurons	1:500	Millipore
Oct4 (Pou5f)	Pluripotent stem cells	1:500	Sigma
Afp	Pan-endodermal cell marker	1:100	NovusBio
Notch1	NOTCH pathway receptor	1:100	Abcam
GFP	Green fluorescing Protein	1:1,000	Aves Lab