

Supplementary Table 1. Primary antibodies used in this study.

Antibodies	Working dilution	Company	Notes
Mouse monoclonal Antibody(Ab)			
Oct4	1:200	¹ Santa Cruz Biotechnology	Use only Cy3 2nd antibody
Pax6	1:100	² DSHB	
Cdh2 (N-cadherin)	1:500	Santa Cruz Biotechnology	
Neuron-specific class III beta-tubulin (TuJ1)	1:500	³ Covance	
Tyrosine Hydroxylase (TH)	1:1000	⁴ Immunostar	Incubation for 2 days at 4°C
Ki67	1:100	⁵ Novocastra	
Pax5	1:50	⁶ BD Biosciences	
human Neural Cell Adhesion Molecule (hNCAM)	1:100	Santa Cruz Biotechnology	
HuC/D	1:100	⁷ Chemicon	
Microtubule-Associated Protein 2 (MAP2)	1:200	⁸ Sigma	
Dopamine Transporter (DAT)	1:100	BD Biosciences	Blocking with 0.1% Saponin
Human Nuclei antigen (HN)	1:1000	Chemicon	
Rabbit polyclonal Ab			
Nanog	1:200	Santa Cruz Biotechnology	
Nestin #130	1:50	⁹ Dr Martha Marvin, Dr Ron McKay	
Sox2	1:100	Chemicon	
TuJ1	1:2000	Covance	
Pax2	1:100	Covance	
TH	1:500	¹⁰ Pel-freez	
GFAP	1:400	¹¹ DAKO	
Serotonin	1:4000	Sigma	
γ-aminobutyric acid (GABA)	1:700	Sigma	
GAD67	1:500	Chemicon	
Vesicular monoamine transporter 2 (VMAT2)	1:500	Pel-freez	Use only cy3 2nd antibody
Nurr1	1:500	Chemicon	0.1% SDS treatment for 5min before blocking
EN1	1:200	Chemicon	Incubation for 2 days at 4°C
Calbindin1, 28kDa (calbindin D28K)	1:250	Chemicon	
potassium inwardly-rectifying channel, subfamily J, member 6 (GIRK2)	1:200	Sigma	
Cleaved Caspase-3	1:500	¹² Cell signaling	

1. Santa Cruz Biotechnology, Santa Cruz, CA, USA
2. DSHB, U. Iowa, Iowa City, IA
3. Covance, Richmond, CA, USA
4. Immunostar, Hudson, WI, USA
5. Novocastra, Newcastle, UK
6. BD Biosciences, Franklin Lakes, NJ, USA
7. Chemicon, Temecula, CA, USA
8. Sigma, St. Louis, MO
9. Dr Martha Marvin and Dr Ron McKay, NIH, Bethesda, MD, USA
10. Pel-Freez, Rogers, AR, USA
11. DAKO, Glostrup, Denmark
12. Cell signaling Technology, Beverly, MA, USA

Supplementary Table 2. RT-PCR primer information.

Gene	Annealing Temp	Cycle	Product Size		Sequence (5'-3')
House keeping gene					
<i>G3PDH</i>	55 °C	36	474bp	Forward(F)	GCTCAGACACCATGGGGAAGGT
				Reverse(R)	GTGGTGCAGGAGGCATTGCTGA
Primers for midbrain-specific DA neurons					
<i>EN1</i>	60 °C	35	247bp	F	GCAACCCGGCTATCCTACTTATG
				R	ATGTAGCGGTTTGCCTGGAAC
<i>NURR1</i>	60 °C	35	332bp	F	TTCTCCTTTAAGCAATCGCCC
				R	AAGCCTTTGCAGCCCTCACAG
<i>LMX1A</i>	58 °C	33	150bp	F	CAGCCTCAGACTCAGGTAAAAGTG
				R	TGAATGCTCGCCTCTGTTGA
<i>LMX1B</i>	60 °C	35	253bp	F	ACGAGGAGTGTTCAGTGCGG
				R	CCCTCCTTGAGCACGAATTCG
<i>GIRK2</i>	60 °C	35	162bp	F	GCTACCGGGTCATCACAGAT
				R	ACTGCATGGGTGAAAAGAC
Primers for DA neurons phenotype					
<i>DAT</i>	55 °C	35	785bp	F	AGCAGAACGGAGTGCAGCT
				R	GTATGCTCTGATGCCGTCT
<i>VMAT2</i>	55 °C	35	301bp	F	CTTTGGAGTTGGTTTTGC
				R	GCAGTTGTGATCCATGAG
<i>AADC</i>	60 °C	35	212bp	F	CTCGGACCAAAGTGATCCAT
				R	GTCTCTCTCCAGGGCTTCCT
Primers for undifferentiated ESC/iPSCs markers					
<i>GDF3</i>	59 °C	29	631bp	F	CTTATGCTACGRAAAGGAGCTGGG
				R	GTGCCAACCCAGGTCCCGGAAGTT
<i>REX1</i>	56 °C	29	306bp	F	CAGATCCTAAACAGCTCGCAGAAT
				R	GCGTACGCAAATTAAGTCCAGA
<i>DNMT3L</i>	60 °C	29	189bp	F	CTCTCAAGCTCCGTTTCACC
				R	GTACAGGAAGAGGGCATCCA
<i>OCT3</i>	56 °C	29	168bp	F	CTTGCTGCAGAAGTGGGTGGAGGAA
				R	CTGCAGTGTGGGTTTCGGGCA
<i>NANOG</i>	56 °C	29	111bp	F	CAGAAGGCCTCAGCACCTAC
				R	ATTGTTCCAGGTCTGGTTGC
Primers for detecting mRNA for exogenous reprogramming factors					
<i>OCT3</i> (Lenti1-3)	56 °C	38	656bp	F	AGGCCTGTGACAAGCGGCCGCTC
				R	AGAGGAACTGCTTCCTTCACGACA
<i>SOX2</i> (Lenti1-3)	55 °C	38	467bp	F	TACCTCTTCCTCCCACTCCA
				R	AGAGGAACTGCTTCCTTCACGACA
<i>NANOG</i> (Lenti1-3)	55 °C	38	732bp	F	CAGAAGGCCTCAGCACCTAC
				R	AGAGGAACTGCTTCCTTCACGACA
<i>OCT3</i> (Retro1,2)	63 °C	37	138bp	F	GCACTGTACTCCTCGGTCCCTTTCCC
				R	AGGCCTGTGACAAGCGGCCGCTC
<i>SOX2</i> (Retro1,2)	65 °C	38	141bp	F	GCCCCAGCAGACTTCACATG

				R	AGGCCTGTCGACAAGCGGCCGCCTC
Primers for detecting the mRNA for endogenous reprogramming factors					
<i>OCT3</i> (Lenti1-3)	56 °C	30	113bp	F	AGTTTGTGCCAGGGTTTTTTG
				R	ACTTACCTTCCCTCCAACC
<i>NANOG</i> (Lenti1-3)	59 °C	30	194bp	F	TTTGAAGCTGCTGGGGAAG
				R	GATGGGAGGAGGGGAGAGGA
<i>OCT3</i> (Retro1,2)	56 °C	28	259bp	F	GCACTGTACTCCTCGGTCCCTTTCCC
				R	CTTCCCTCCAACCAGTTGCCCAAAC
<i>SOX2</i> (Retro1,2)	56 °C	28	300bp	F	GCCCCAGCAGACTTCACATG
				R	CGCGGTTTTTGCCTCAGTGTGGATGGG

Supplementary Figure S1. Morphological (A) and immunocytochemical (B) characteristics of the undifferentiated hiPSC/hESC colonies used in this study. The representative images were taken from the hiPSCs and hESC as indicated. The undifferentiated colonies are assembled by cells with high nucleus/cytoplasm and prominent nucleoli (inset of A), which are positive for undifferentiated ESC markers Nanog (Bi) and Oct3/4 (Bii). Inset, high-powered view of the boxed area. Ci-ii are merged with the respective DAPI-stained views. Scale bars, 30 μ m.

Supplementary Figure S2. Efficient neural induction and DA differentiation from the hiPSCs established by different reprogramming methods. A, Neural induction efficiency estimated as % colonies containing primitive neural structures from total colonies 3 weeks after co-cultures. The data represent values of 3-4 series of independent experiments. B, Expression of mRNAs specific for midbrain DA neurons (*EN1*, *NURR1*, *LMX1A*, *LMX1B*, *GIRK2*) and general DA neuron phenotypes (*DAT*, *VMAT2*, *AADC*). RT-PCR analyses were carried out in Lenti-2, Retro-2, Pro-1 hiPSCs and H9 hESCs cultures for undifferentiated and differentiated NPC cultures before (Exp) and after terminal differentiation (Diff).

Supplementary Figure S3. DA neuronal and other neural type differentiations of Lenti-1,3,4, Retro-1, Pro-2 hiPSCs and HSF6 hESCs. NPCs were derived from hiPSCs and hESCs using the protocol described in Figure 1C. Terminal differentiation of NPCs was induced. Immunocytochemical analyses for TuJ1 (neuronal), GFAP (astrocytic), TH (DA neuronal), serotonin (serotonergic neuronal), and GABA (GABAergic neuronal) were performed 15 days after terminal differentiation. Scale Bars, 30 μ m.

Supplementary Figure S4. Comparison of Oct4 expressions between undifferentiated and

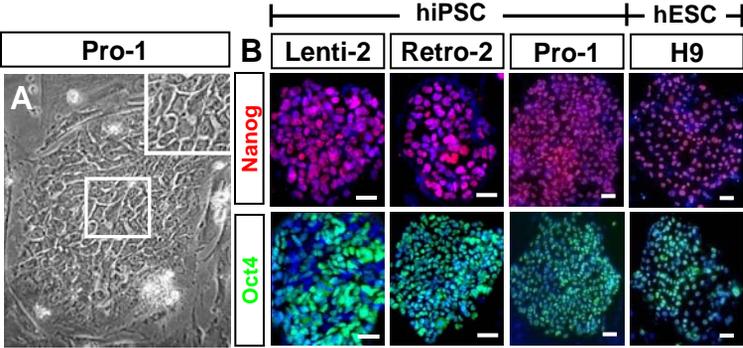
differentiated Lenti-hiPSCs. Expression levels of Oct4 in the undifferentiated Lenti-2 and their differentiated NPC progenies were directly compared by immunocytochemical (A-C) and real-time PCR (D) analyses. Shown in A and B are representative images of the Oct4 immunofluorescent staining. Graph in C represents single cell levels of Oct4 immunofluorescent intensity. Twenty Oct4-stained cells were randomly picked from each culture and their fluorescent intensities were determined by LAS image analysis (Leica). Graph D depicts relative *OCT4* mRNA levels determined by real-time PCRs (n=4). *Significantly different from undifferentiated levels at $p < 0.001$ by t-test (2 tailed).

Supplementary Figure S5. Quantitative PCR analysis to determine total, endogenous (Endo), or exogenous (Exo) reprogramming gene expression. Differentiation of Lent-2, 3, Retro-2, and Pro-2 was induced by sequential steps consisting of co-culturing with MS5 (MS5-SHH), selection and expansion of NPCs, and terminal differentiation as schematized in Figure 1C. Samples for real-time RT-PCR analyses were obtained from undifferentiated (Undiff), expanded NPC (NPC), and terminal differentiation stages (DA). The total reprogramming gene expression was determined using PCR primers recognizing common sites of *OCT4* or *NANOG* coding regions in all hiPSC lines. PCRs for detecting endogenous and exogenous reprogramming gene expression were performed using primers (shown in Suppl. Table 3) specifically designed for each cell line. Data are expressed as mean \pm SEM relative to the respective undifferentiated values in each hiPSC line, n=3-9 PCR reactions from two independent series of hiPSC differentiation.

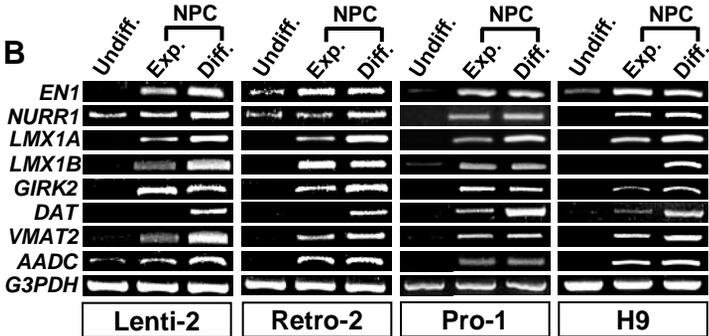
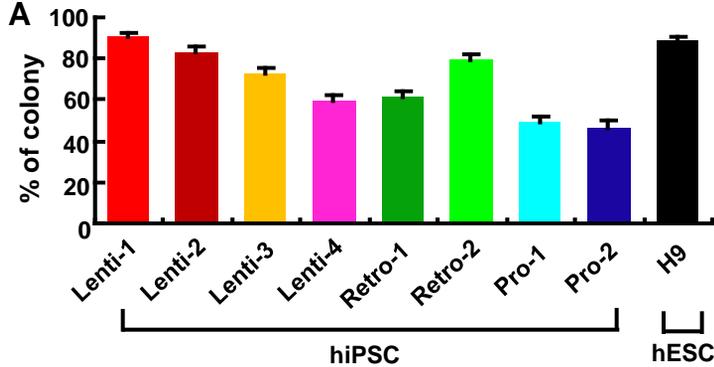
Supplementary Figure S6. A–C, Representative images for cells positive for neural stem cell-specific Nestin (A), and the proliferating cell-specific proliferating cell nuclear antigen

(PCNA; B), and M phase specific phospho-histone H3 (PHH3; C) in the rats transplanted with Pro-1-NPCs with condition 1. Dashed lines mark the borders of the graft (g) and host striatum (h). Bar = 30 μ m.

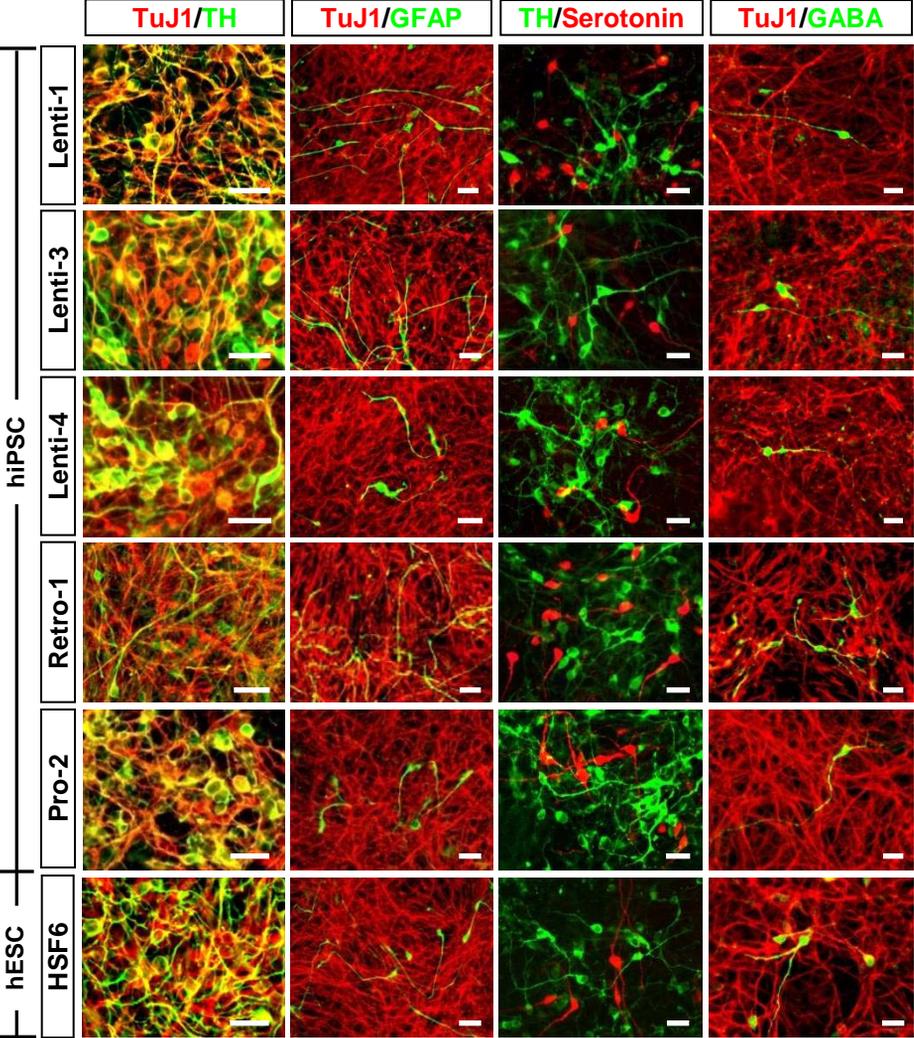
Supplementary Figure S1



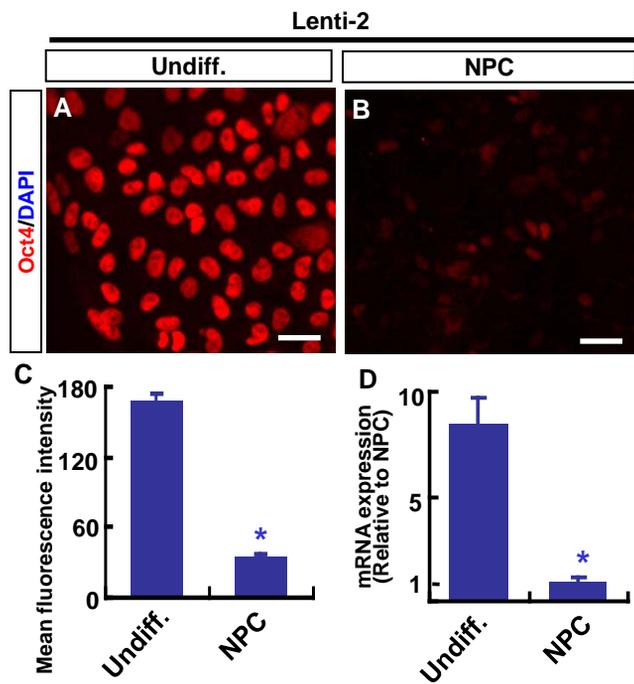
Supplementary Figure S2



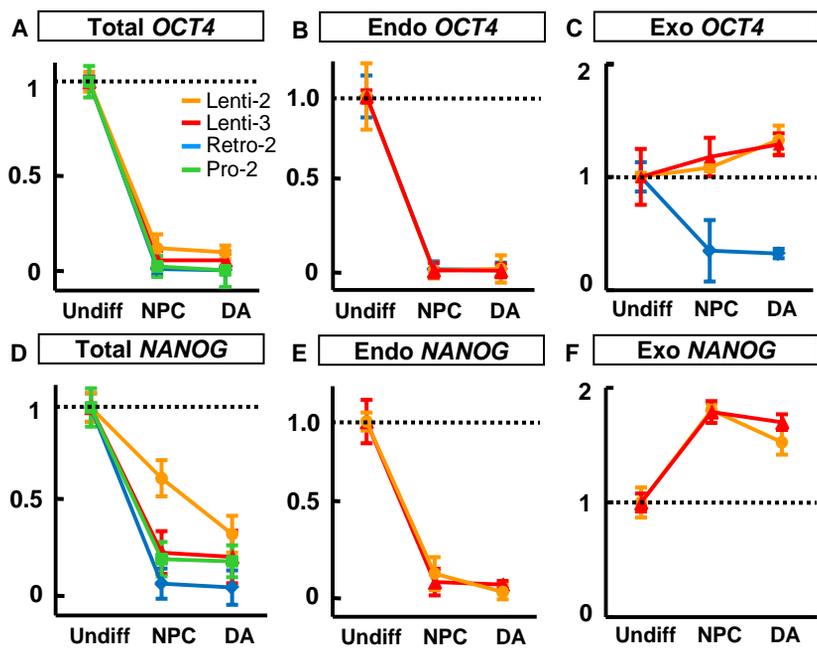
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6

