SUPPLEMENTARY FIGURE /TABLE LEGENDS

Figure S1. EZ spheres remained karyotypically normal. Representative G-banding images are shown for 4.2 iPSC, 21.8 iPSC, and H9 hESC derived EZ spheres all at passage 17.

Figure S2. Rosette structures are detected following neural induction. (A) Rosettes were observed in whole, fixed 4.2 iPSC EZ spheres (passage 9) by ZO1 immunofluorescence and (B) in live, unfixed H9 hESC EZ spheres (passage 18) by light microscopy after 1 week of neural differentiation. Arrows in B point to the lumen at the apical side of the radially organized neuroephithelial cells.

Figure S3. Schematic of the differentiation protocols used. Beginning with EZ sphere formation, the multiple steps are diagramed to show the progression through several weeks of differentiation to generate motor neurons, dopamine neurons, striatal neurons, sensory neurons, and oligodendrocytes.

Figure S4. 21.8 iPSC EZ sphere-derived astrocytes show typical membrane current patterns. Potassium membrane currents and the current/voltage relationship were measured in response to 10mV steps from a resting potential of -70mV for (A) A1 type astrocytes, (B) A2 type astrocytes, and (C) complex type astrocytes. Astrocyte classifications was based on previous descriptions (Anderova et al., 2004).

Figure S5. Dual SMAD did not alter nestin expression levels in p3 H9 EZ spheres as demonstrated by (A) immunocytochemistry or (B) PCR.

Figure S6. Forebrain markers FOXG1 and OTX2 are up-regulated in 21.8 iPSC derived EZ spheres upon differentiation toward striatal neurons. Neither FOXG1 nor OTX2 were expressed in 21.8 iPSC derived EZ spheres at passage 15 prior to striatal differentiation. However, as early as 7 days into the differentiation protocol, both forebrain specific markers were up-regulated; this expression continued out to day 42 of differentiation.

Table S1. Nineteen independent stem cell lines have been used to generate EZ spheres. The reprogramming method and genes used for the generation of each line is listed. *OCT4* (O), *SOX2* (S), *Nanog* (N), *LIN28* (L), *KLF4* (K), and *c-myc* (M). Fibroblast lines are not applicable

(N/A) for the hESCs, and some iPSC lines used fibroblast cells that are not commercially available (NCA).

Table S2. Relative gene expression levels are depicted for all genes tested through passage 32. GAPDH was used as a control.

Table S3. This table lists the primer sequences and PCR conditions used for all genes tested.

Table S4. The table contains a list of the species and company for all antibodies used.

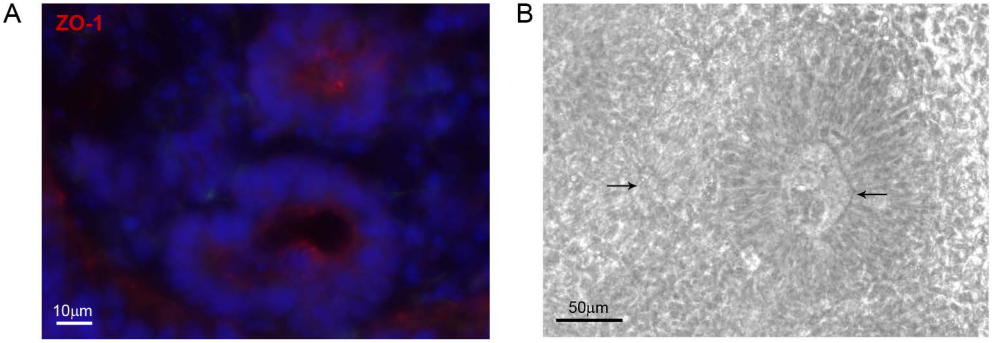
Supplementary Figure 1

iPSC 4.2 EZ p17

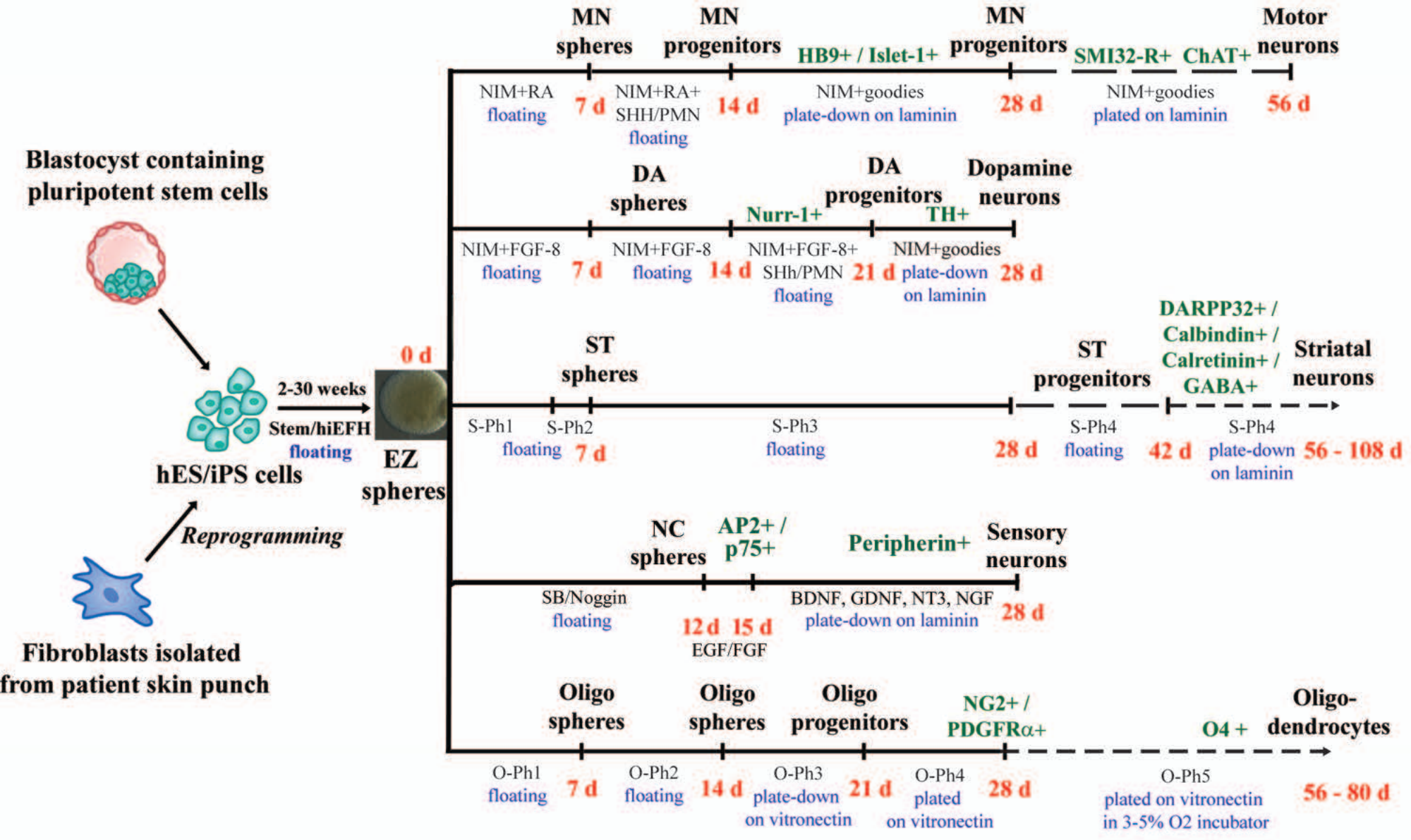
iPSC 21.8 EZ p17

hESC H9 EZ p17

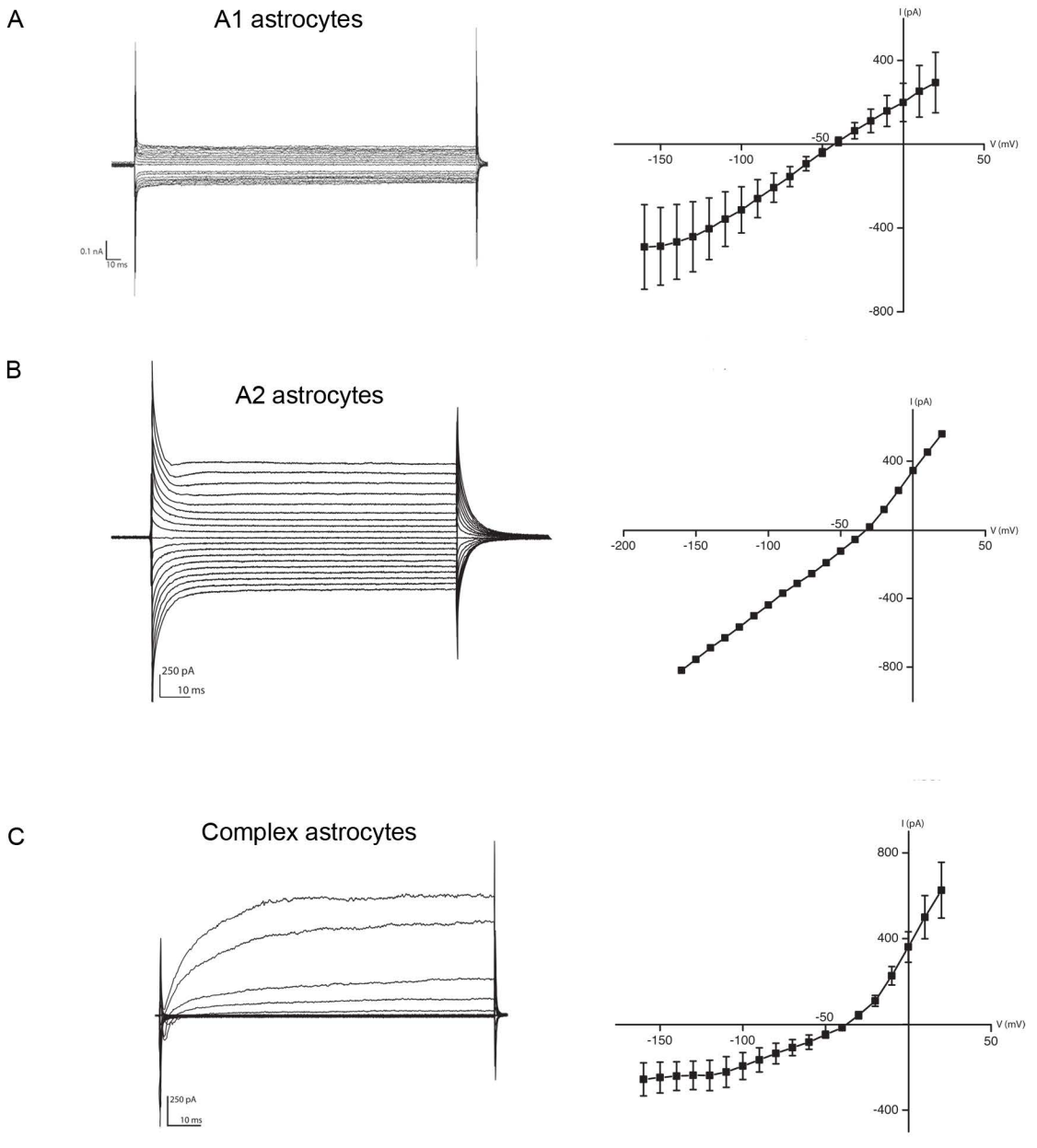
Supplementary Figure 2



Supplementary Figure 3

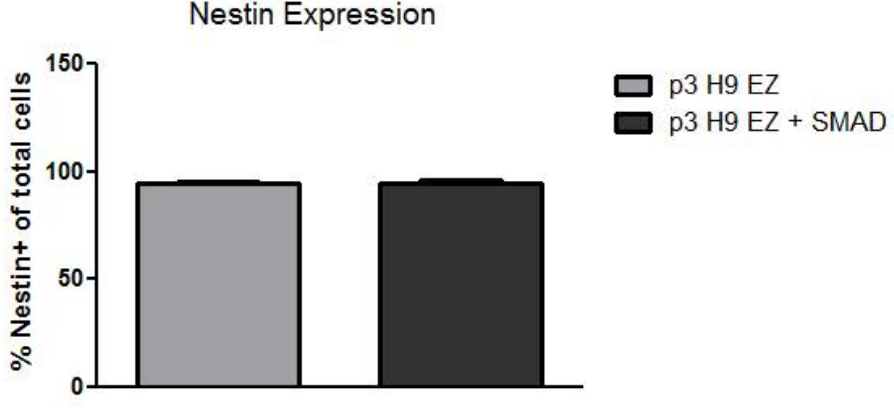


Supplementary Figure 4

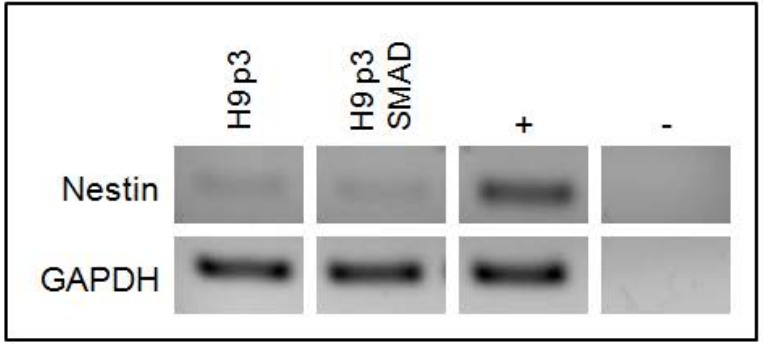


Supplementary Figure 5

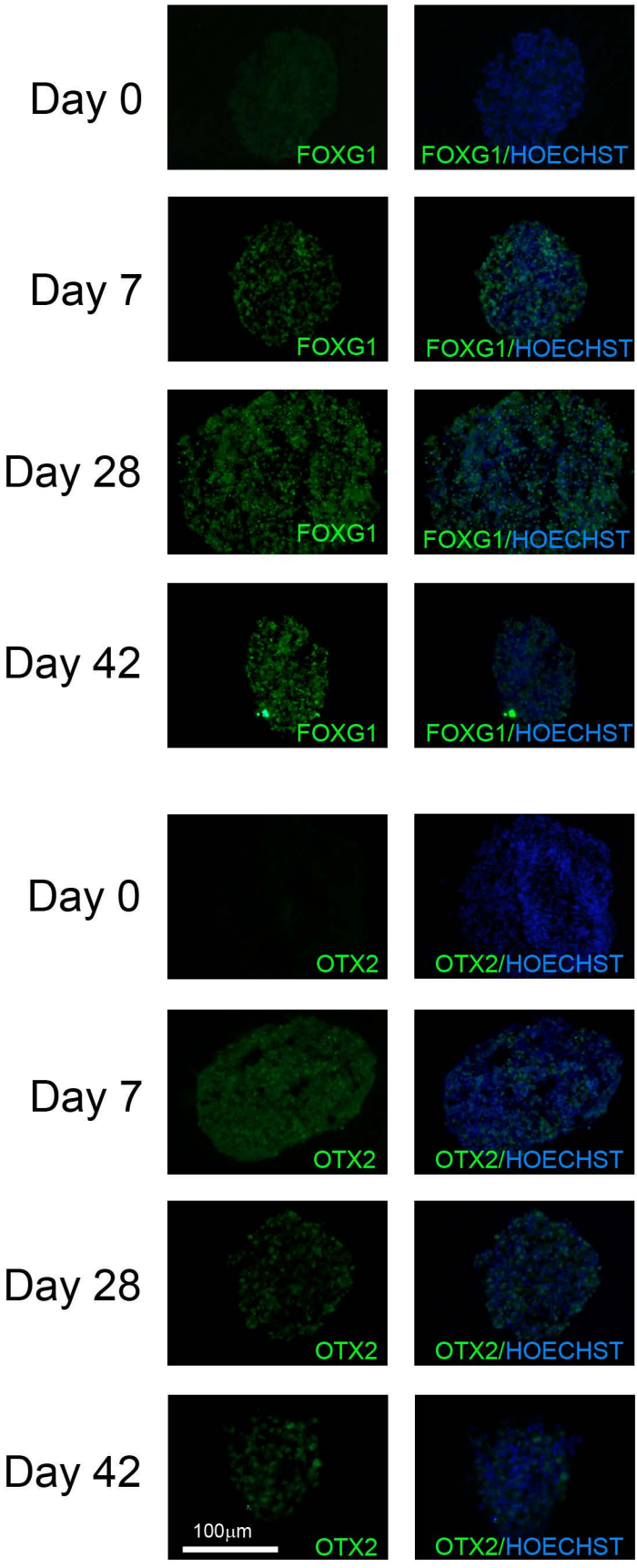
A



B



Supplementary Figure 6



Supplementary Table 1

Name	Fibroblast line	Reprogramming method	Healthy/Affected	Reference	Other
H9 (WA09) hESCs	N/A	N/A	Healthy	Thomson et al., 1998	
H1 (WA01) hESCs	N/A	N/A	Healthy	Thomson et al., 1998	
4.2 iPSCs	Coriell GM03814	Lentivirus O,S,N,L	Healthy	Ebert et al., 2009	
HD33i.8 (21.8) iPSCs	Coriell GM02183	Lentivirus O,S,N,L,K,M	Healthy	HD iPSC Consortium, 2012	multiple clones
FS1 iPSCs	ATCC CRL-2097	Lentivirus O,S,N,L	Healthy	Yu et al., 2007	multiple clones
DF-6-9	ATCC CRL-2097	oriP/EBNA episomal O,S,N,L,K,M	Healthy	Yu et al., 2009	multiple clones
DF-4-3	ATCC CRL-2097	oriP/EBNA episomal O,S,N,L,K,M	Healthy	Yu et al., 2009	
DF-19-9	ATCC CRL-2097	oriP/EBNA episomal O,S,N,L,K,M	Healthy	Yu et al., 2009	multiple clones
IPRN0019.6	NCA	Retrovirus O,S,K,M	Healthy	iPierian	
IPRN0008.2	NCA	Retrovirus O,S,K,M	SMA	iPierian	
IPRN0008.2S	NCA	Retrovirus O,S,K,M	SMA	iPierian	
3.6 iPSCs	Coriell GM03813	Lentivirus O,S,N,L	SMA	Ebert et al., 2009	multiple clones
7.12 iPSCs	Coriell GM009677	oriP/EBNA episomal O,S,N,L,K,M	SMA	Sareen et al., 2012	multiple clones
HD180i iPSCs	Coriell GM09197	Lentivirus O,S,N,L,M,K	HD	HD iPSC Consortium, 2012	multiple clones
HD60i iPSCs	Coriell GM03621	Lentivirus O,S,N,L,M,K	HD	HD iPSC Consortium, 2012	multiple clones
HD109i.1	NCA	Lentivirus O,S,K,M	HD	HD iPSC Consortium, 2012	
Coriell ND35658	NCA	Retrovirus O,S,K,M	ALS	http://ccr.coriell.org/Sections/Collections/NINDS/ipsc_list.aspx?PgId=711&coll=ND	
Coriell ND34391	NCA	Retrovirus O,S,K,M	PD	http://ccr.coriell.org/Sections/Search/Sample_Detail.aspx?Ref=ND34391&PgId=166	
57.4 iPSC	Coriell ND32157	Sendai O,S,K,M	PD	unpublished	multiple clones

N/A = not applicable

NCA = not commercially available

Supplementary Table 2

OCT4	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	+++	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	NA	-	-
4.2	+++	+	+	+	-	-	-	++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
21.8	+++	++	++	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	-

SOX2	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	+++	+++	+++	+	++	++	++	++	++	++	++	++	+++	++	++	++	++	++	++	+++	+++	++	+++	++	+	++	++	++	+++	++	NA	++	++
4.2	+++	++	++	+	++	++	++	++	++	++	++	++	+	++	+	++	++	++	++	++	-	-	-	-	-	-	-	-	-	-	-	-	NA
21.8	+++	+++	+++	++	++	++	+	++	++	++	++	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	NA	++	++

Nestin	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	+	++	++	+	++	++	++	++	++	++	+	++	+	+	+	++	++	++	++	+	+	+	++	++	++	++	++	++	++	++	++	NA	+	+
4.2	++	++	++	+	++	++	++	++	++	++	++	+	++	+	++	++	+	+	+	+	++	++	+	+	++	+	++	+	+	+	++	++	+	NA
21.8	++	++	++	++	+	+	+	++	++	++	++	+	++	++	+	+	++	++	++	++	+	++	++	++	++	++	++	++	++	++	++	NA	++	++

Pax6	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	-	++	++	+	+	-	-	-	+	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	++	-	NA	-	-
4.2	-	+	++	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	NA
21.8	-	++	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	NA	-	-

Foxg1	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	-	+++	+++	++	+	+	+	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	++	-	NA	++	+
4.2	-	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
21.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	-

HNK1	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	++	++	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	NA	+	-
4.2	++	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	NA
21.8	++	+	++	+	++	+	-	-	++	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	NA	-	+

GBX2	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	-	++	+	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	+	-	-	-	+	-	-	-	NA	-	-
4.2	-	+	+++	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
21.8	-	++	+++	++	++	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	NA	-	-

OTX2	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	++	+++	++	-	-	-	-	-	+	+	+	+	++	+	++	+	++	++	++	++	++	++	++	++	++	+	+	+	++	++	NA	++	++
4.2	++	++	++	+	+	+	++	++	+	++	+	+	+	+	+	+	++	+	+	+	+	++	++	++	++	+	+	+	+	+	+	+	NA
21.8	++	++	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	-

Pax7	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	NA	-	-
4.2	++	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA
21.8	++	+	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+	++	++	+++	+++	+++	+++	+++	++	+	++	+	++	NA	++	++

GAPDH	colony 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
H9	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	NA	+++	+++
4.2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	NA
21.8	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	NA	+++	+++

[+++] = high [++] = medium [+] = low [-] = not expressed [NA] = Not available

Supplementary Table 3

Target	Annealing Temperature (°C)	Amplicon Length (bp)	Cycles	Forward Primer Sequence (5' to 3')	Reverse Primer Sequence (5' to 3')
β-actin	60	198	30	GCCCATCTACGAGGGGTATG	GTGGCCATCTCTTGCTCGAAG
DACH1	57	208	30	GTGGAAAACACCCCTCAGAA	CTTGTTCCACATTGCACACC
FOXG1	57	204	30	CCCTCCCATTTCTGTACGTTT	CTGGCGGCTCTTAGAGAT
GAPDH	60	153	30	GTGGACCTGACCTGCCGTCT	GGAGGAGTGGGTGTCGCTGT
GBX2	62	173	35	CTCGCTGCTCGCCTTCTC	GCCAGTCAGTCAGATTGTCATCCG
HNK1	58	162	30	TCGCCTGGACTGGACTGGGG	TGGCCTTGGCCTCCCTCCTC
HOXB4	56	187	30	ACACCCGCTAACAAATGAGG	GCACGAAAGATGAGGGAGAG
Nestin	61	146	30	TCTCCAGAAACTCAAGCACCACTG	TCCTGATTCTCCTCTTCCAGGAGT
OCT4	60	168	30	GTACTCCTCGGTCCCTTTCC	CAAAAACCCTGGCACAAACT
OTX2	61	204	30	TCAACTTGCCCGAGTCGAGG	CAATGGTCGGGACTGAGGTG
PAX3	56	269	30	AAAGAGGAACAGCGCAGAA	GAGGTCTCCGACAGCTGGTA
PAX6	60	561	30	AACAGATGGGCGCAGACGGC	GGGCTGTGTCTGTTCGGCCC
PAX7	56	253	30	GGGAAGAAAGAGGAGGAGGA	CCTCGCGGGTGTATATGTCT
SOX1	61	210	35	CGCTCACTTTCCTCCGCGTT	GAATATAACTCCGCCGTCTG
SOX2	55	141	30	AGTCTCCAAGCGACGAAAAA	GCAAGAAGCCTCTCCTTG

Activation	94°C	10 min
PCR (x30 or 35 cycles)	94°C	60 sec
	annealing temp	60 sec
	72°C	90 sec
Hold	10°C	∞

Supplementary Table 4

Antigen	Species	Company
AP2	mouse	Developmental Studies Hybrodoma Bank
β-catenin	rabbit	Cell Signaling
BLBP	rabbit	Chemicon
Calbindin	rabbit	Sigma
Calretinin	goat	Abcam
ChAT	goat	Millipore
DARPP32	rabbit	Cell Signaling
FOXG1	rabbit	Neuracell
GABA	rabbit	Sigma
GFAP	rabbit	Dako
GLAST	Guinea pig	Chemicon
HB9 (MNR2)	mouse	Developmental Studies Hybrodoma Bank
Islet1	mouse	Developmental Studies Hybrodoma Bank
Ki67	mouse	Chemicon
Map2a/b	mouse	Millipore
Nanog	rabbit	Abcam
N-Cadherin	mouse	BD Biosciences
Nestin	rabbit	Millipore
NF160	mouse	Sigma
O4	mouse	Sigma
Olig2	rabbit	Chemicon
OTX2	rabbit	Chemicon
P75	rabbit	Promega
PAR3	rabbit	Millipore
Pax6	rabbit	Covance
Peripherin	rabbit	Millipore
PLZF	mouse	Calbiochem
SMI32R	mouse	Covance
Sox1	goat	Santa Cruz Biotechnology
Sox2	rabbit	Millipore
SSEA-3	mouse	gift from ESTOOLS Consortium
TH	mouse	ImmunoStar
Tra-1-81	mouse	gift from ESTOOLS Consortium
Tuj1 (βIII tubulin)	mouse	Sigma
Vimentin	mouse	Developmental Studies Hybrodoma Bank
ZO-1	rabbit	Invitrogen