

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, Related to Figure 1.

(A) Morphologies of hiPSCs and EBFM-derived day 9 and day 30 EBs. Scale bar, 500 μm ; P, passage number.

(B, C) Gene expression panels of hiPSCs (B) and EBFM-derived day 30 EBs (C). The delta Ct values for

Oct4, *NANOG*, *LIN28A*, *SOX17*, *T*, *SOX1*, and *PAX6* are shown. Red: high expression. (D) Hierarchical

clustering of clones in day 30 EBs based on the expression of *SOX1*, *PAX6*, *SOX17*, and *T*.

Figure S2, Related to Figure 1 and Figure 2.

Gene expression panels of the dSMADi-derived day 14 neural aggregates cultured under various conditions.

The delta Ct values for *Oct4*, *NANOG*, *LIN28A*, *SOX17*, *T*, *SOX1*, and *PAX6* are shown. Red: high gene

expression.

Figure S3, Related to Figure 2.

Morphologies of the day 7 and day 14 neural aggregates generated from the HDF-, CB-, and PBMN-derived

clones using dSMADi under various experimental conditions.

Table S1, Related to methods.

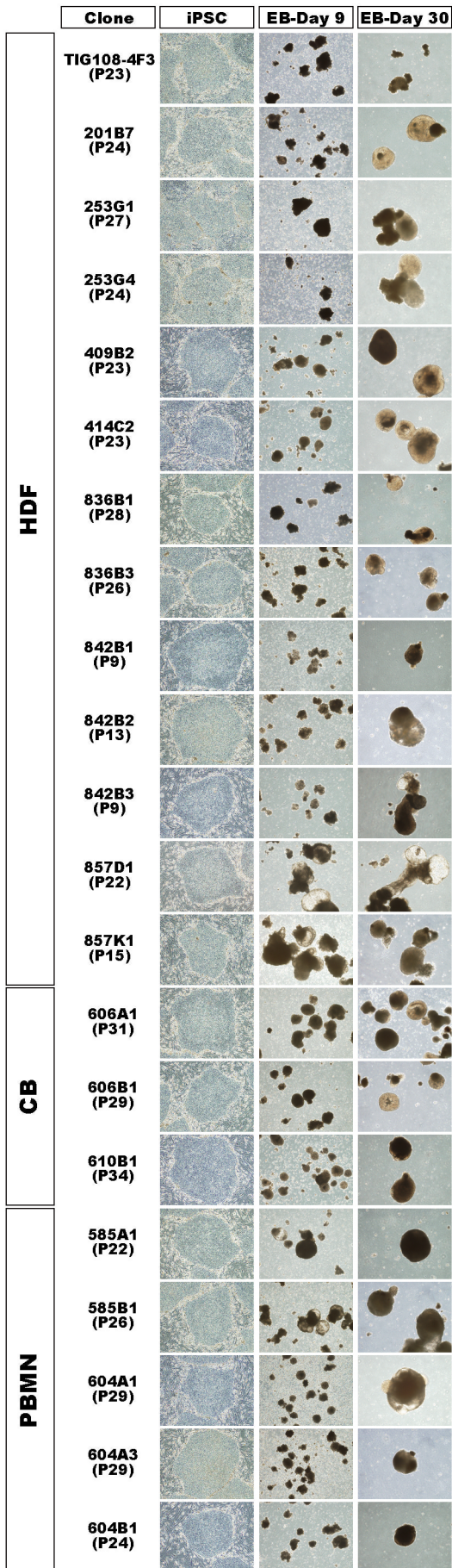
Table S2, Related to Figure 2.

Table S3, Related to Figure 3.

Table S4, Related to methods.

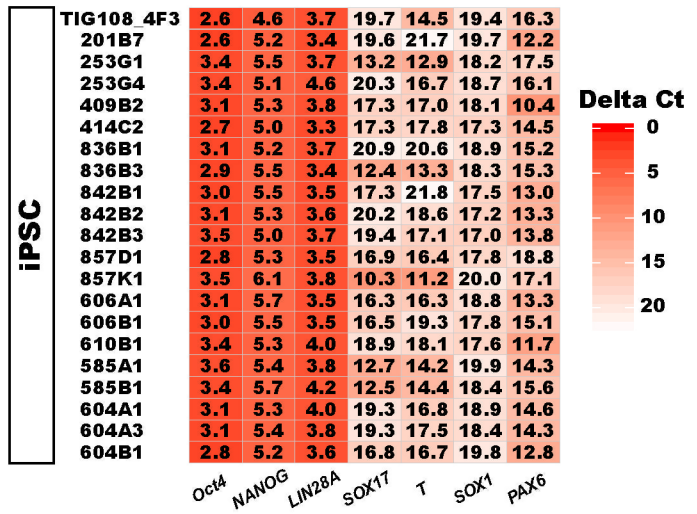
Figure S1

A

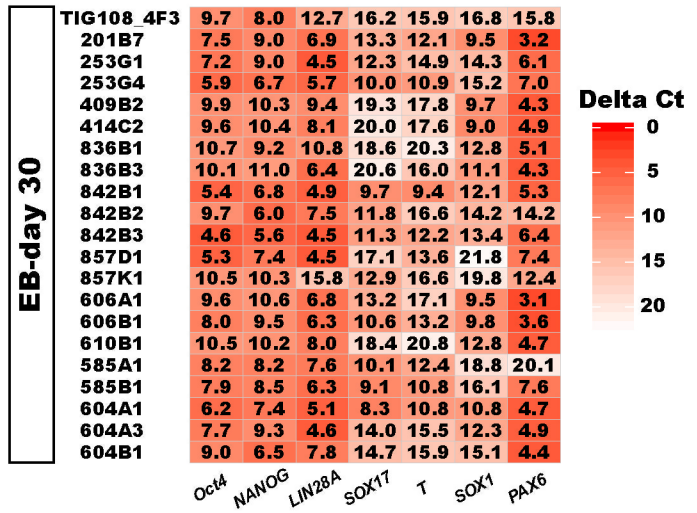


— 500 μ m

B



C



D

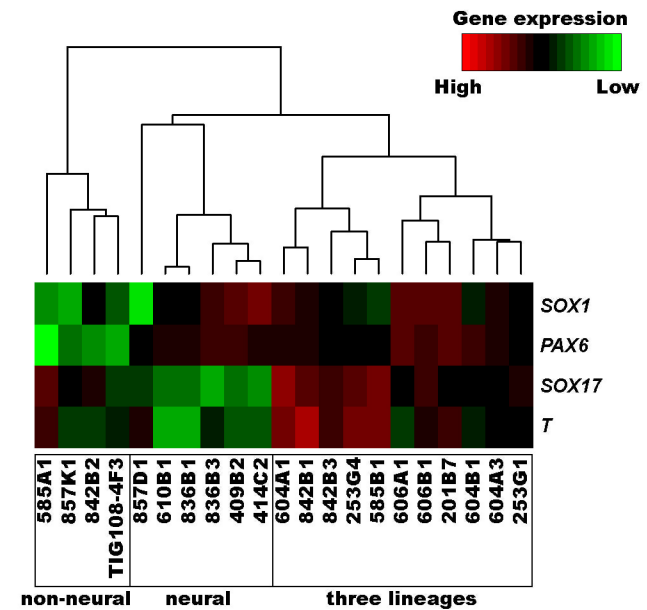


Figure S2

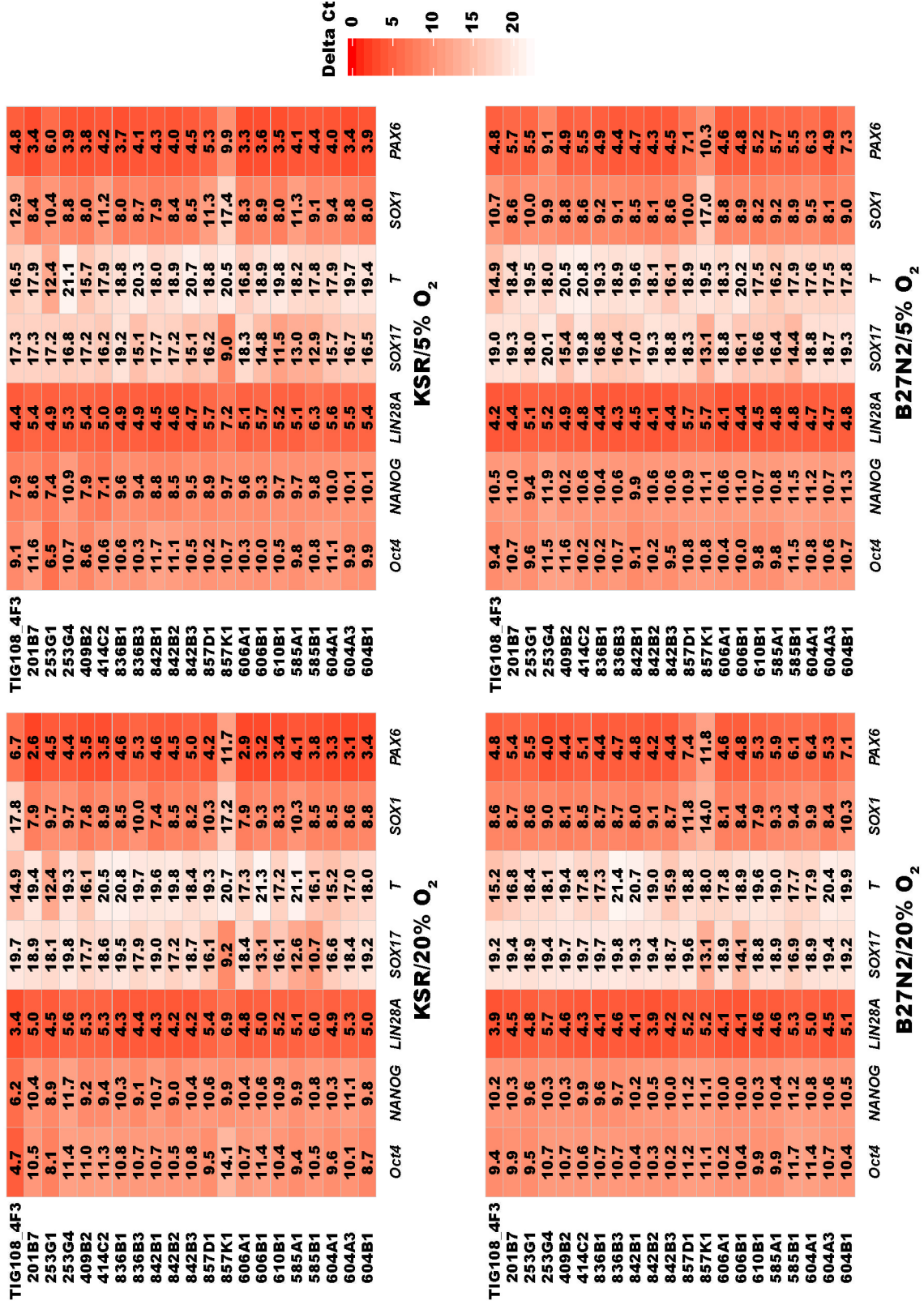
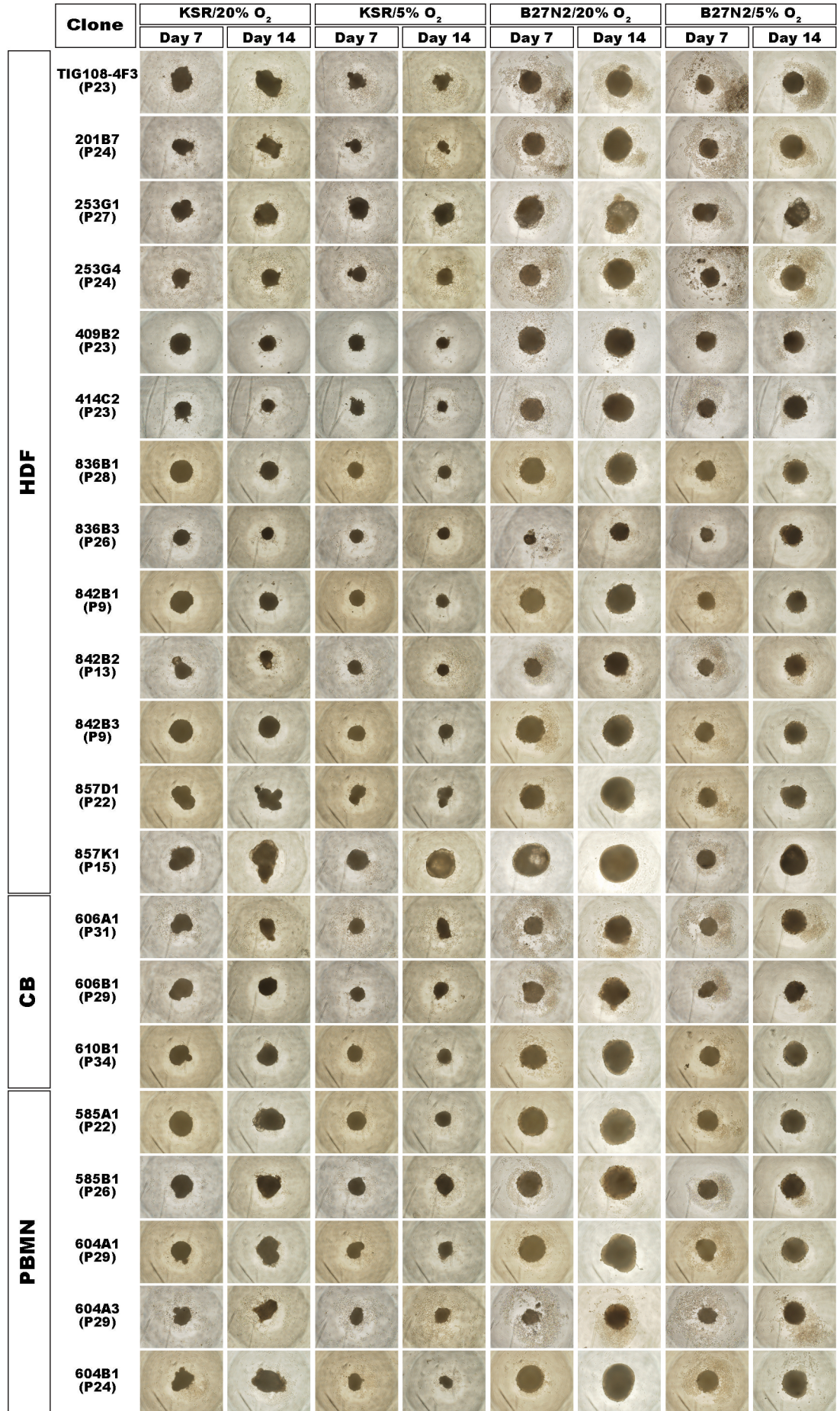


Figure S3



— 500 μm

Table S1, Related to Methods. Information of hiPSCs used in this study.

Clone	Passage no.	Vector	Factors	Feeder	Origin ^a	Age	Sex	Race ^b
TIG108-4F3	23	retrovirus	<i>Oct4, SOX2, KLF4, (M)</i>	SNL	HDF (TIG108)	40	F	J
201B7	24	retrovirus	<i>Oct4, SOX2, KLF4, c-Myc</i>	SNL	HDF (HDF1388)	36	F	C
253G1	27	retrovirus	<i>Oct4, SOX2, KLF4</i>	SNL	HDF (HDF1388)	36	F	C
253G4	24	retrovirus	<i>Oct4, SOX2, KLF4</i>	SNL	HDF (HDF1388)	36	F	C
409B2	23	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	HDF (HDF1388)	36	F	C
414C2	23	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	HDF (HDF1388)	36	F	C
836B1	28	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, GLIS1</i>	SNL	HDF (HDF1388)	36	F	C
836B3	26	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, GLIS1</i>	SNL	HDF (HDF1388)	36	F	C
842B1	9	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, GLIS1</i>	SNL	HDF (TIG114)	36	M	J
842B2	13	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, GLIS1</i>	SNL	HDF (TIG114)	36	M	J
842B3	9	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, GLIS1</i>	SNL	HDF (TIG114)	36	M	J
857D1	22	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, GLIS1</i>	SNL	HDF (TIG120)	6	F	J
857K1	15	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, GLIS1</i>	SNL	HDF (TIG120)	6	F	J
606A1	31	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	CB CD34+ (donor a)	0	F	UN
606B1	29	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	CB CD34+ (donor a)	0	F	UN
610B1	34	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	CB CD34+ (donor b)	0	M	UN
585A1	22	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	PBMN $\alpha\beta$ T (donor x)	30s	M	J
585B1	26	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	PBMN $\alpha\beta$ T (donor x)	30s	M	J
604A1	29	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	PBMN $\alpha\beta$ T (donor y)	30s	M	J
604A3	29	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	PBMN $\alpha\beta$ T (donor y)	30s	M	J
604B1	24	plasmid	<i>Oct4, SOX2, KLF4, MYCL, LIN28, p53-shRNA</i>	SNL	PBMN $\alpha\beta$ T (donor y)	30s	M	J

a: HDF, Human Dermal Fibroblast; CB, Cord Blood; PBMN, Peripheral Blood MonoNuclear cell.

b: J, Japanese; C, Caucasian; UN, Unknown.

Table S2, Related to Figure 2. Comparison of two types of media and oxygen levels on the size of day 7 and day 14 neural aggregates. Data were analyzed with the Steel-Dwass test. The resulting p-values are shown for the statistically significant differences (*; $p < 0.05$, **; $p < 0.01$). Differences that were not statistically significant are indicated by dashes (-).

			KSR				B27N2			
			20% O ₂		5% O ₂		20% O ₂		5% O ₂	
			Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
KSR	20% O ₂	Day 7	-	1.35E-04**	4.26E-04**	-	6.49E-06**	-	2.49E-02*	
		Day 14	-	4.99E-02*	3.16E-02*	-	3.10E-05**	-	-	
	5% O ₂	Day 7	-	-	-	1.86E-05**	8.10E-07**	9.15E-04**	8.10E-07**	
		Day 14	-	-	-	1.51E-04**	5.68E-06**	3.47E-03**	1.43E-05**	
B27N2	20% O ₂	Day 7	-	-	-	-	2.15E-04**	8.89E-03**	-	
		Day 14	-	-	-	-	-	8.10E-07**	8.48E-06**	
	5% O ₂	Day 7	-	-	-	-	-	-	2.73E-05**	
		Day 14	-	-	-	-	-	-	-	

Table S3, Related to Figure 3. The neural aggregates were clustered based on *FOXG1* and *SOX1* expression levels using the K-medoids clustering method. Clones selected for further expansion are checked in the column on the right.

Cell	Condition	Origin	<i>FOXG1</i> : <i>SOX1</i>	Selected clones for further expansion
TIG108_4F3	BN/20% O2	HDF	High : High	-
TIG108_4F3	BN/5% O2	HDF	High : High	-
201B7	BN/20% O2	HDF	High : High	-
253G1	BN/20% O2	HDF	High : High	✓
409B2	KSR/20% O2	HDF	High : High	-
409B2	KSR/5% O2	HDF	High : High	✓
409B2	BN/20% O2	HDF	High : High	-
409B2	BN/5% O2	HDF	High : High	-
414C2	BN/20% O2	HDF	High : High	-
836B1	KSR/20% O2	HDF	High : High	-
836B1	KSR/5% O2	HDF	High : High	-
836B1	BN/20% O2	HDF	High : High	-
836B3	KSR/20% O2	HDF	High : High	-
836B3	BN/20% O2	HDF	High : High	-
836B3	BN/5% O2	HDF	High : High	-
842B1	KSR/20% O2	HDF	High : High	-
842B1	KSR/5% O2	HDF	High : High	-
842B1	BN/20% O2	HDF	High : High	-
842B1	BN/5% O2	HDF	High : High	-
842B2	KSR/20% O2	HDF	High : High	-
842B2	KSR/5% O2	HDF	High : High	-
842B2	BN/20% O2	HDF	High : High	-
842B2	BN/5% O2	HDF	High : High	-
842B3	KSR/20% O2	HDF	High : High	-
842B3	KSR/5% O2	HDF	High : High	-
842B3	BN/20% O2	HDF	High : High	-
606A1	KSR/20% O2	CB	High : High	✓
606A1	BN/20% O2	CB	High : High	-
606B1	BN/20% O2	CB	High : High	-
610B1	KSR/20% O2	CB	High : High	-
610B1	KSR/5% O2	CB	High : High	-
610B1	BN/20% O2	CB	High : High	-
610B1	BN/5% O2	CB	High : High	-
604A3	BN/20% O2	PBMN	High : High	✓
TIG108_4F3	KSR/5% O2	HDF	Middle : High	-
201B7	KSR/5% O2	HDF	Middle : High	✓
201B7	BN/5% O2	HDF	Middle : High	-
253G1	KSR/5% O2	HDF	Middle : High	-
253G1	BN/5% O2	HDF	Middle : High	✓
414C2	KSR/20% O2	HDF	Middle : High	-
414C2	KSR/5% O2	HDF	Middle : High	-
836B3	KSR/5% O2	HDF	Middle : High	-
842B3	BN/5% O2	HDF	Middle : High	-
857D1	KSR/5% O2	HDF	Middle : High	-
606A1	KSR/5% O2	CB	Middle : High	-
606A1	BN/5% O2	CB	Middle : High	✓
606B1	KSR/20% O2	CB	Middle : High	✓
606B1	BN/5% O2	CB	Middle : High	-
585A1	KSR/5% O2	PBMN	Middle : High	✓
585B1	BN/5% O2	PBMN	Middle : High	-
604A3	BN/5% O2	PBMN	Middle : High	✓
604B1	KSR/5% O2	PBMN	Middle : High	-
201B7	KSR/20% O2	HDF	Low : High	-
253G1	KSR/20% O2	HDF	Low : High	✓
253G4	KSR/20% O2	HDF	Low : High	-
253G4	KSR/5% O2	HDF	Low : High	-
253G4	BN/20% O2	HDF	Low : High	-
253G4	BN/5% O2	HDF	Low : High	✓

414C2	BN/5% O2	HDF	Low : High	-
836B1	BN/5% O2	HDF	Low : High	-
857D1	KSR/20% O2	HDF	Low : High	-
857D1	BN/20% O2	HDF	Low : High	-
857D1	BN/5% O2	HDF	Low : High	-
606B1	KSR/5% O2	CB	Low : High	✓
585A1	KSR/20% O2	PBMN	Low : High	-
585A1	BN/20% O2	PBMN	Low : High	-
585A1	BN/5% O2	PBMN	Low : High	-
585B1	KSR/20% O2	PBMN	Low : High	-
585B1	KSR/5% O2	PBMN	Low : High	-
585B1	BN/20% O2	PBMN	Low : High	-
604A1	KSR/20% O2	PBMN	Low : High	-
604A1	KSR/5% O2	PBMN	Low : High	-
604A1	BN/20% O2	PBMN	Low : High	-
604A1	BN/5% O2	PBMN	Low : High	✓
604A3	KSR/20% O2	PBMN	Low : High	-
604A3	KSR/5% O2	PBMN	Low : High	✓
604B1	KSR/20% O2	PBMN	Low : High	-
604B1	BN/20% O2	PBMN	Low : High	-
604B1	BN/5% O2	PBMN	Low : High	-
TIG108_4F3	KSR/20% O2	HDF	Low : Low	-
857K1	KSR/20% O2	HDF	Low : Low	-
857K1	KSR/5% O2	HDF	Low : Low	-
857K1	BN/20% O2	HDF	Low : Low	-
857K1	BN/5% O2	HDF	Low : Low	-

Table S4, Related to Experimental Procedures. Primers for quantitative RT-PCR.

Gene Symbol	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>Oct4</i>	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCAAAC
<i>NANOG</i>	GCAGAAGGCCTCAGCACCTA	GGTCCCAGTCGGGTTCAC
<i>LIN28A</i>	CACGGTGCGGGCATCTG	CCTTCCATGTGCAGCTTACTC
<i>SOX17</i>	CCCATAGTTGGATTGTCAAAACC	CACACCCAGGACAACATTTCTTT
<i>T</i>	TGGAATGCCTGCCCATC	CCGTTGCTCACAGACCACA
<i>SOX1</i>	AGCAGTTGTTTCTGGAAGAGTCTGT	AGGCCCTTATCCCGGACTAA
<i>SOX2</i>	ATGCACCGCTACGACGTGA	CTTTTGCACCCCTCCCATTT
<i>PAX6</i>	ACCTGGCTAGCGAAAAGCAA	CCCGTTCAACATCCTTAGTTTATCA
<i>NES</i>	CCAAGACTGCCCTGGAAAC	CCTCCCTCTCCAAGGAAACA
<i>PROM1</i>	GCACTTACGGCACTCTTCACC	CTATTCCACAAGCAGCAAAAATCC
<i>CDH2</i>	GGACAGTTCCTGAGGGATCA	GGATTGCCTTCCATGTCTGT
<i>BLBP</i>	GGACTCTCAGCACATTCAAGAA	CCACATCACAAAAGTAAGGGT
<i>GFAP</i>	ACATCGAGATCGCCACCTAC	ACATCACATCCTTGTGCTCC
<i>S100B</i>	CTCATCGACGTTTTCCACCA	CACAACCTCCTGCTCTTTGATTT
<i>TUBA1A</i>	AGCTCGGCAGTCGCGAAGCAG	CAATGACTGTGGGTCCAAGTCTAC
<i>TUBB3</i>	GGGCCTTTGGACATCTCTTC	ACTCCTTCCGCACCACATC
<i>DLX2</i>	ACGCTCCCTATGGAACCAGTT	TCCGAATTTCAAGGCTCAAGGT
<i>FOXG1</i>	CAAGGAGGGCGAGAAGAAGA	GATGAACTCGTAGATGCCGTTG
<i>OTX1</i>	GTGGCGCTCAAGATCAACC	TCCCGCACTGGAGAGGACTT
<i>EN1</i>	TGGGTGTAAGTGCACACGTTATTC	TGTCCTCCTTCTCGTTCTTCTTCT
<i>GBX2</i>	CCAAGTGGAAACGGGTGAA	ATGCTGACTTCTGATAGCGAACC
<i>HOXC6</i>	CCCAGGACCAGAAAGCCAGT	GGTCTGGTACCGCGAGTAGAT
<i>GAPDH</i>	CCACTTTGTCAAGCTCATTTCCT	TCTCTTCCTCTTGTGCTCTTGCT