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Supplemental Information

Controlling the Regional Identity of hPSC-Derived Neurons to Uncover Neuronal Subtype Specificity of Neurological Disease Phenotypes

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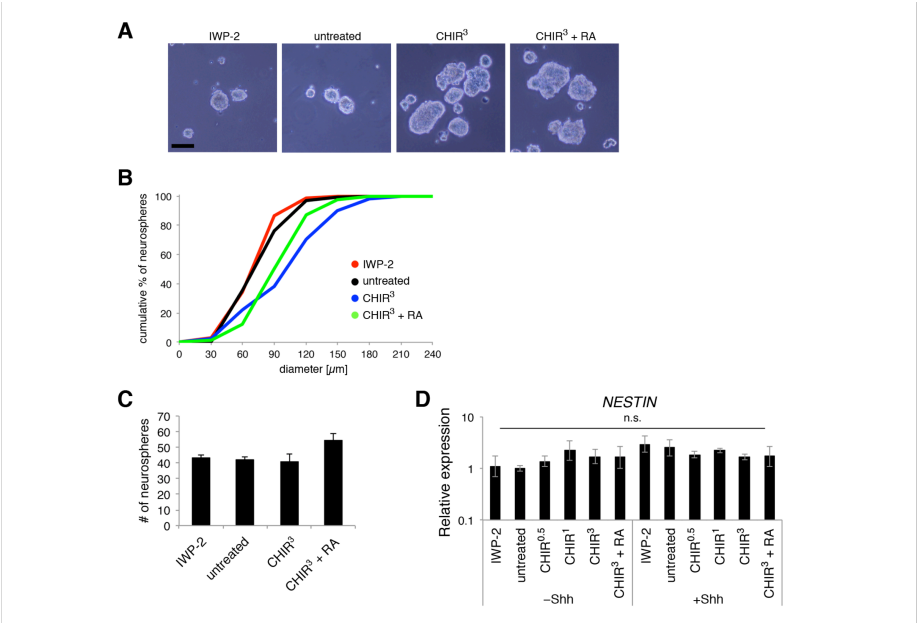

Figure S1. Patterning factors promoted expansion of neurospheres, but had no effect on neural induction

(A) Representative images of neurospheres (scale bar = 100 μm).

(B) Quantification of the size distribution of IWP-2, CHIR, and RA-treated neurospheres. While IWP-2 had no effect, the treatment of CHIR and RA promoted expansion of neurospheres.

(C) Quantification of the number of neurospheres (n = 3 technical replicates).

(D) qRT-PCR analysis of neurospheres for *NESTIN* expression (n = 3 independent experiments; mean ± SEM; n.s., not significant; ANOVA). Uniform expression of *NESTIN* confirmed that neural induction was unaffected by patterning factors.

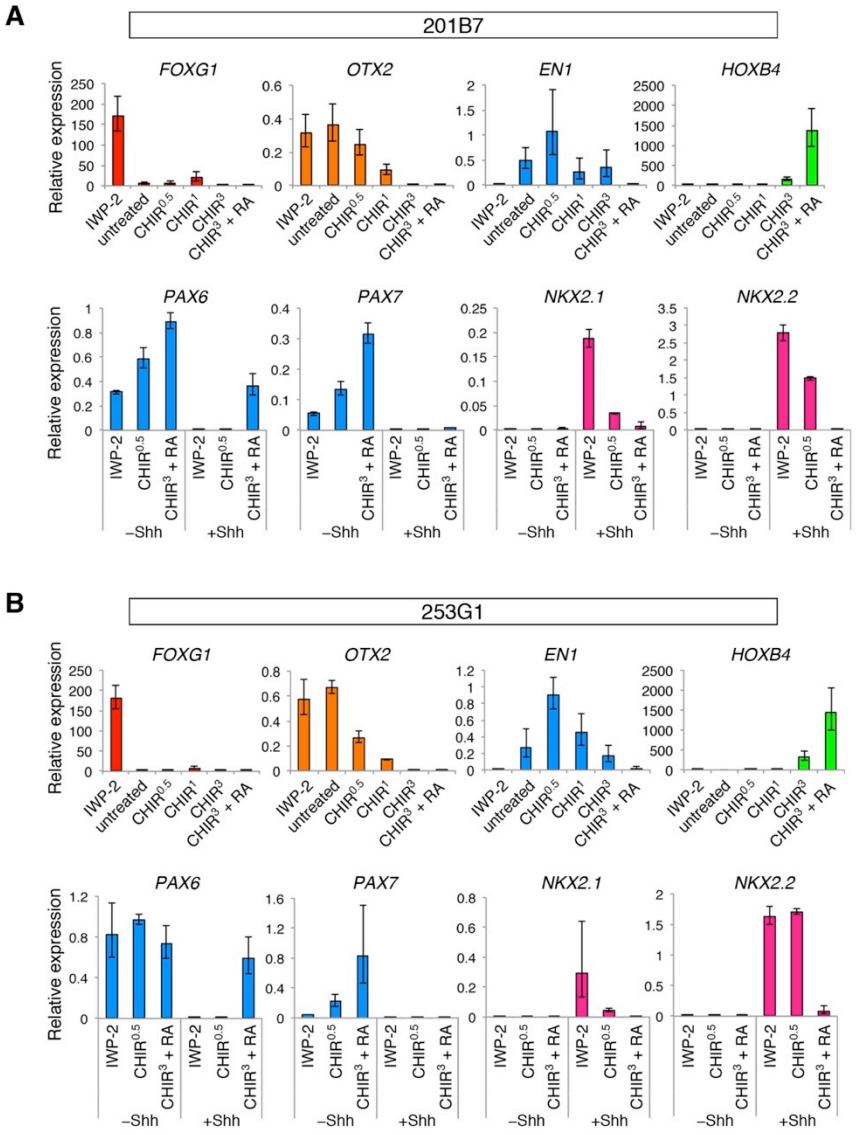

Figure S2. Region control was reproduced in iPSCs

(A) qRT-PCR analysis of 201B7-derived neurospheres for A-P and D-V markers expression relative to the untreated condition of KhES-1 (n = 3–4 independent experiments; mean ± SEM).

(B) qRT-PCR analysis of 253G1-derived neurospheres for A-P and D-V markers expression relative to the untreated condition of KhES-1 (n = 3 independent experiments; mean ± SEM).

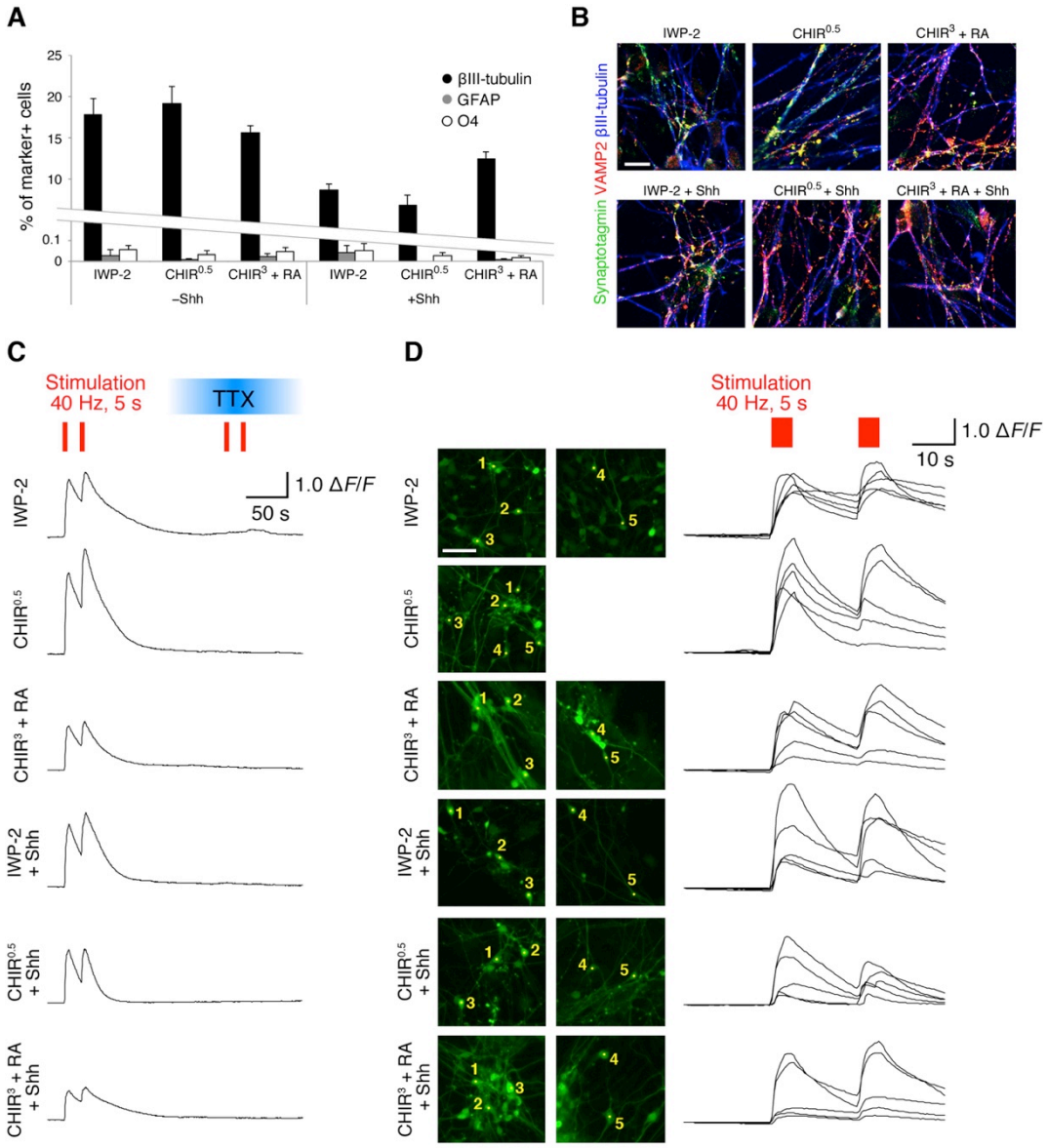

Figure S3. Differentiation capacity and neuronal functionality

(A) Quantification of the number of neurosphere-derived neurons, astrocytes, and oligodendrocytes (n = 3 independent experiments; mean ± SEM.). Neurospheres preferentially differentiated into neurons rather than astrocytes or oligodendrocytes.

(B) Immunocytochemical analysis of ESC-derived neurons for synapse markers (scale bar = 10 μm). The expression of synaptotagmin and VAMP2 was identified in each

condition.

(C) Traces of intracellular calcium signals. Calcium surges were induced by field stimulation and blocked by TTX.

(D) Traces of intracellular calcium signals in five cells. Cells 1–5 correspond to individual cells analyzed by calcium imaging (scale bar = 50 μm).

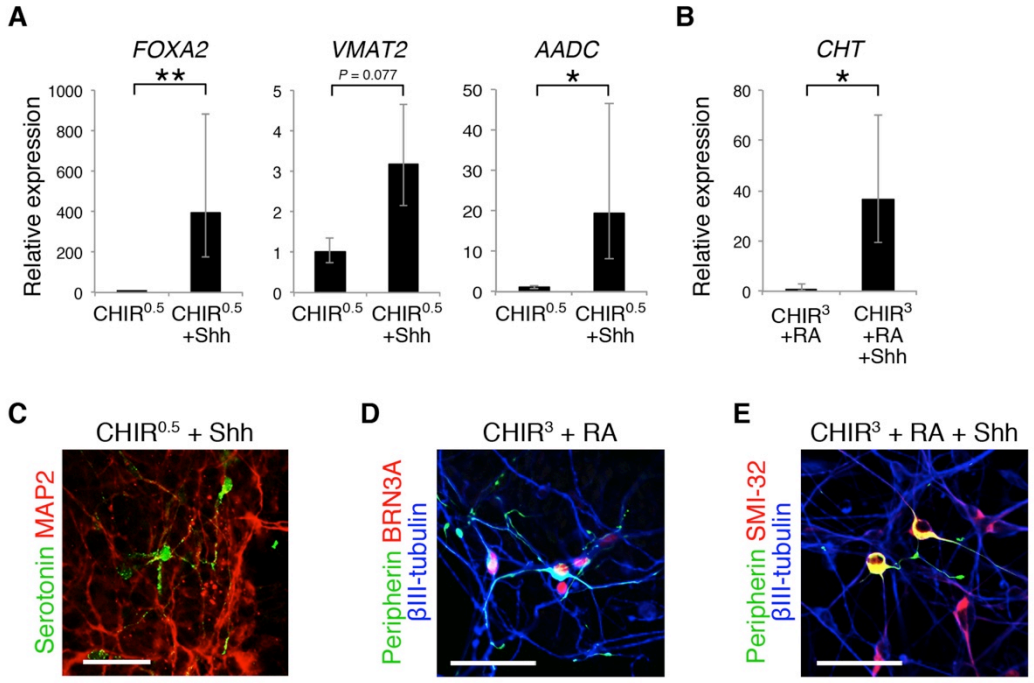

Figure S4. Further characterization of ESC-derived neurons

(A–B) qRT-PCR analysis of neurosphere-derived neurons for the expression of neuronal subtype markers (n = 3 independent experiments; mean ± SEM; *P < 0.05, **P < 0.01; Student's t test).

(C–E) Immunocytochemical analysis of neurosphere-derived neurons for neuronal subtype markers (scale bar = 50 μm).

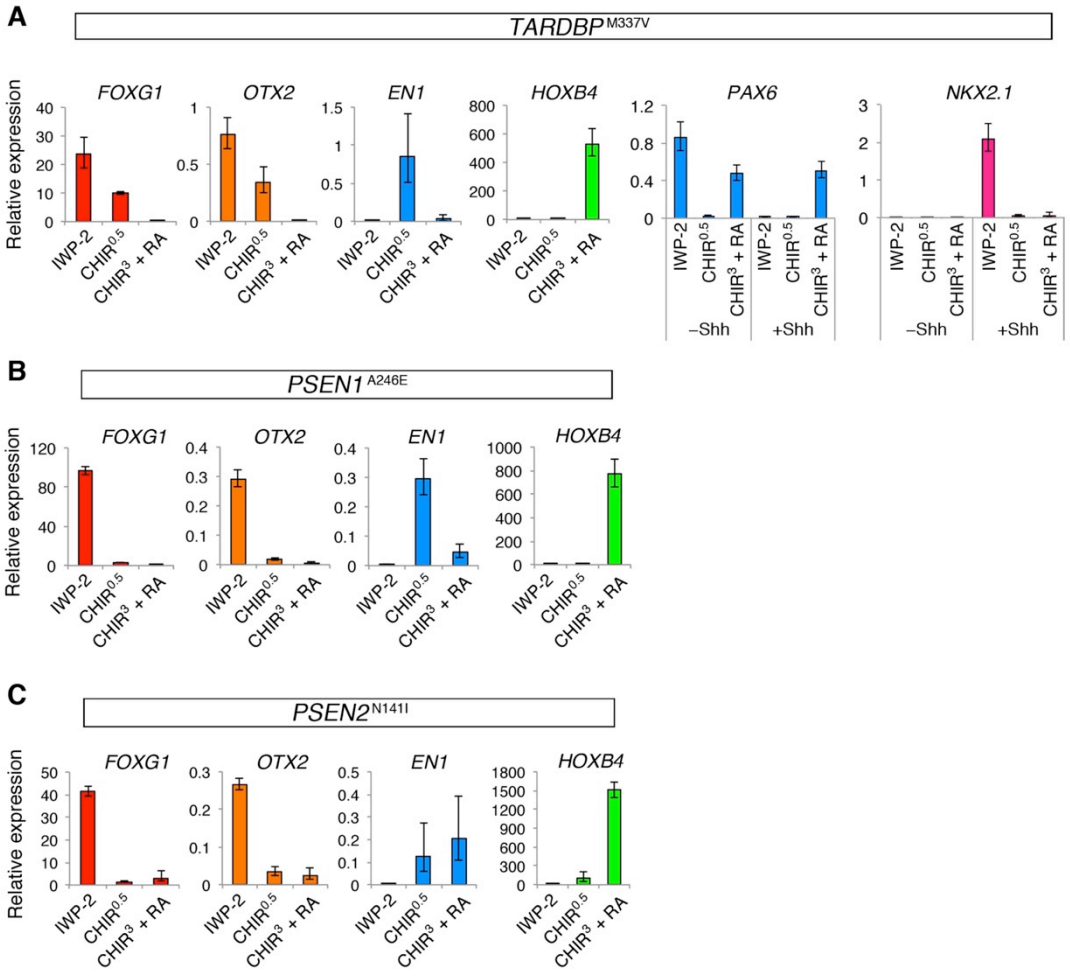

Figure S5. Control of regional identities of disease-specific iPSC-derived neural progenitors

(A) qRT-PCR analysis of *TARDBP* ^{M337V} iPSC-derived neurospheres for regional markers expression relative to the untreated condition of KhES-1 (n = 3 independent experiments; mean ± SEM).

(B) qRT-PCR analysis of *PSEN1* ^{A246E} iPSC-derived neurospheres for regional markers expression relative to the untreated condition of KhES-1 (n = 3 independent experiments; mean ± SEM).

(C) qRT-PCR analysis of *PSEN2* ^{N141I} iPSC-derived neurospheres for regional markers expression relative to the untreated condition of KhES-1 (n = 3 independent experiments; mean ± SEM).

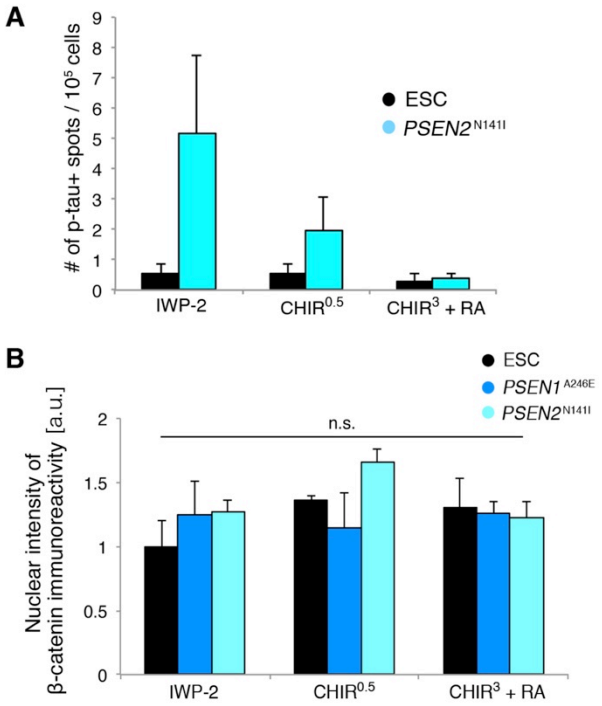

Figure S6. Region-specific phenotypes in the AD iPSC cultures

(A) Quantification of p-tau+ spots (n = 3 independent experiments; mean ± SEM). The data obtained from ESC cultures are same as shown in Fig.5.

(B) Quantification of nuclear intensity of β-catenin immunoreactivity (n = 3 independent experiments; mean ± SEM; n.s., not significant; ANOVA). See also Supplemental Experimental Procedures.

Table S1. Summary of immunocytochemical quantification

	Neurosphere[a]						Neuron[b]	
	FOXG1	OTX1	HOXB4	PAX6	NKX2.1	NKX2.2	TBR1	HB9
IWP-2	41 ± 1	8 ± 2	0 ± 0	81 ± 8	0 ± 0		23 ± 10	
IWP-2 + Shh				1 ± 0	40 ± 19		0 ± 0	
untreated	11 ± 2	61 ± 1	0 ± 0					
CHIR[3]	0 ± 0	0 ± 0	14 ± 4					
CHIR[3] + RA	0 ± 0	0 ± 0	63 ± 7	62 ± 21		1 ± 1		0 ± 0
CHIR[3] + RA + Shh				85 ± 4		20 ± 4		44 ± 3

[a]The frequency of neurospheres containing immunopositive cells is shown as the percentage of total neurospheres (n = 3 independent experiments; mean ± SEM).
[b]The frequency of immunopositive cells is shown as the percentage of MAP2+ neurons (n = 3 independent experiments; mean ± SEM).

Table S2. List of qRT-PCR primers

Gene	Forward	Reverse
AADC	AGGAAGCCCTGGAGAGAGAC	ATTGTCAAAGGAGCAGCATGT
ACTB	TGAAGTGTGACGTGGACATC	GGAGGAGCAATGATCTTGAT
ASCL1	GATGAGTAAGGTGGAGACACTGCG	CCGACGAGTAGGATGAGACCG
CHT	ATCCCAGCCATACTCATT	CAGAAACTGCACCAAGACCA
DLX2	ACGCTCCCTATGGAACCAGTT	TCCGAATTTCAGGCTCAAGGT
EMX1	AGGTGAAGGTGTGGTTCCAG	AGTCATTGGAGGTGACATCG
EMX2	GCTTCTAAGGCTGGAACACG	CCAGCTTCTGCCTTTTGAAC
EN1	CCGCGCACCAGGAAGCTGAA	CAGCGCCAGGCCGTTCTTGA
FOXA2	CCGTTCTCCATCAACAACCT	GGGGTAGTGCATCACCTGTT
FOXG1	CCCGTCAATGACTTCGCAGA	GTCCCGTCGTAAAACTTGGC
GATA3	CAGACCACCACAACCACACTCT	GGATGCCTTCCTTCTTCATAGTCA
HB9	GTCCACCGCGGGCATGATCC	TCTTCACCTGGGTCTCGGTGAGC
HOXB4	ACGTGAGCACGGTAAACCCCAA	ATTCCTTCTCCAGCTCCAAGACCT
HOXC4	TTCACGTTAGCACGGTGAAC	GACTTTGGTGTTGGGGAGTC
LBX1	CGCCAGCAAGACGTTTAAG	CTGCCCAAAGATGGTCATAC
LHX6	GCAGAACAGCTGCTACATCAAGAA	CAGTCGCTGGCGTAGATCTGTC
LHX8	GTGTCATACAGGTGTGGTTTC	GGGTACGTAGGCAGAATAAG
LHX9	GATGCCAAGGACCTCAAGC	CTCGTGCGTTTTGGAACC
LMX1A	GATCCCTTCCGACAGGGTCTC	GGTTTCCCACTCTGGACTGC
NESTIN	TTCCCTCAGCTTTCAGGACCCCAA	AAGGCTGGCACAGGTGTCTCAA
NGN2	CCTGGAAACCATCTCACTTCA	TACCCAAAGCCAAGAAATGC
NKX2.1	AGGGCGGGGCACAGATTGGA	GCTGGCAGAGTGTGCCCAGA
NKX2.2	AAACCATGTCACGCGCTCA	GGCGTTGTACTGCATGTGCT
OTX1	CACTAACTGGCGTGTTTCTGC	GGCGTGGAGCAAAATCG
OTX2	ACAAGTGGCCAATTCACTCC	GAGGTGGACAAGGGATCTGA
PAX6	ACCACACCGGTTTCCTCCTTCACA	TTGCCATGGTGAAGCTGGGCAT
PAX7	CTTCAGTGGGAGGTCAGGTT	CAAACACAGCATCGACGG
PET1	CGGCGGCGATGAGACAGAGCGGCGCCTCC	ACGCGATGCAGCCGGCGTTCGCGCGGTCA
SIX3	ACCGGCCTCACTCCCACACA	CGCTCGGTCCAATGGCCTGG
TBR1	ATGGGCAGATGGTGGTTTTA	GACGGCGATGAACTGAGTCT
TH	TCATCACCTGGTCACCAAGTT	GGTCGCCGTGCCTGTACT
VMAT2	CGGATGTGGCATTTTGTATGG	TTCTTCTTTGGCAGGTGGACTTC

Table S3. List of Antibodies and Dilutions

Protein	Species	Source (cat. #)	Dilution
βIII-tubulin	Mouse	Sigma (T8660)	1:500
β-catenin	Mouse	BD Transduction Laboratories (610154)	1:200
BRN3A	Mouse	Chemicon (MAB1585)	1:500
FOXG1	Rabbit	Abcam (ab18259)	1:500
GFAP	Rat	Invitrogen (13-0300)	1:500
HB9	Mouse	DSHB (81.5C10)	1:250
HOXB4	Rat	DSHB (I12)	1:100
MAP2	Mouse	Sigma (M4403)	1:500
MAP2	Rabbit	Chemicon (AB5622)	1:1000
NKX2.1	Mouse	Invitrogen (18-0221)	1:50
NKX2.2	Mouse	DSHB (74.5A5)	1:100
O4	Mouse	Chemicon (MAB345)	1:4000
OTX1	Mouse	DSHB (5F5)	1:5000
p-tau	Mouse	Innogenetics (90206)	1:500
PAX6	Rabbit	MBL (PD022)	1:200
Peripherin	Rabbit	Chemicon (AB1530)	1:1000
Serotonin	Goat	Immunostar (20079)	1:500
SMI-32	Mouse	Covance (SMI-32P)	1:2500
Synaptotagmin	Mouse	gift from Dr. Makoto Itakura	1:250
TBR1	Rabbit	Abcam (ab31940)	1:200
VAMP2	Rabbit	gift from Dr. Makoto Itakura	1:250

Supplemental Experimental Procedures

Neurosphere formation assay

Neurosphere size was assessed by measuring neurosphere diameters on differentiation day 7 by using the IN Cell Analyzer 1000 System (GE Healthcare Biosciences). Only neurospheres larger than 20 μm in diameter were analyzed.

Calcium imaging

Calcium imaging analyses were performed as described previously (Shimada et al., 2012; Zhou et al., 2014). For calcium dye loading, cells attached on glass coverslips were incubated at 37°C for 20 min with 4 μM Fluo-4 AM (Thermo Fisher Scientific) in imaging solution (117 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgSO_4$, 25 mM HEPES and 30 mM D-(+)-glucose), followed by washing for 30 minutes in imaging solution. Coverslips were then transferred into a custom-made field stimulation chamber and continuously perfused at 2 ml/min with imaging solution. Time-lapse image sequences (20× magnification) were acquired at 2 Hz using a Nikon Eclipse microscope (Nikon) equipped with a cooled CCD camera (iXon, Andor Technology). Extracellular field stimulation was performed with two parallel platinum wires at 25 V/cm. Each stimulation was a train of 500 microsecond pulses at 40 Hz for 5 seconds. The fluorescence change over time is defined as $\Delta F/F = (F - F_0)/F_0$, where F = the fluorescence at a given time point and F_0 = the fluorescence at time zero of each cell. To assess changes in calcium signaling in response to perturbation of neuronal activity, 0.2 μM the voltage-gated sodium channel blocker tetrodotoxin (TTX; Alomone Labs) was applied by bath application.

Quantification of nuclear β-catenin intensity

To assess GSK3β activity, the nuclear intensity of β-catenin immunoreactivity was quantified through automated image acquisition and analysis by using an IN Cell Analyzer 6000 System (GE Healthcare Biosciences) based on a protocol modified from Kirkeby and colleagues (Kirkeby et al., 2012).

Supplemental References

Kirkeby, A., Grealish, S., Wolf, D. a, Nelander, J., Wood, J., Lundblad, M., Lindvall, O., and Parmar, M. (2012). Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. Cell Rep. *1*, 703–714.

Shimada, H., Okada, Y., Ibata, K., Ebise, H., Ota, S.I., Tomioka, I., Nomura, T., Maeda, T., Kohda, K., Yuzaki, M., et al. (2012). Efficient Derivation of Multipotent Neural Stem/Progenitor Cells from Non-Human Primate Embryonic Stem Cells. PLoS One *7*.

Zhou, Z., Kohda, K., Ibata, K., Kohyama, J., Akamatsu, W., Yuzaki, M., Okano, H.J., Sasaki, E., and Okano, H. (2014). Reprogramming non-human primate somatic cells into functional neuronal cells by defined factors. Mol. Brain *7*, 24.