Table S1*: Primer sequences used for RT-qPCR.*

Marker	Direction	Sequence
hGAPDH	Forward	5’-CTGACTTCAACAGCGACACC-3’
	Reverse	5’-AGGGGTCTACATGGCAACTG-3’
hTUJ1	Forward	5’-GGCCTTTGGACATCTCTTCA-3’
	Reverse	5’-ATACTCCTCACGCACCTTGC-3’
hMAP2	Forward	5’-GCATATGCGCTGATTCTTCA-3’
	Reverse	5’-CTTTCCGTTCATCTGCCATT-3’
hNestin	Forward	5’-GCTCAGGTCCTGGAAGGTC-3’
	Reverse	5’-TAAGAAAGGCTGGCACAGGT-3’
hSOX2	Forward	5’-CATGTCCCAGCACTACCAGA-3’
	Reverse	5’-AAGTTTTCTTGTCGGCATCG-3’
hPSD95	Forward	5’-GTGACGACCCATCCATTTTC-3’
	Reverse	5’-TGACATAGAGGCGAACGATG-3’
hSyn1	Forward	5’-GATGGGCAAGGTCAAGGTTG-3’
	Reverse	5’-ATTTGGCATCGATGAAGGGC-3’
hGFAP	Forward	5’-ACATCGAGATCGCCACCTAC-3’
	Reverse	5’-GAGGCTCACTCCCTGTCAAG-3’

~~***Figure S1:** RT-qPCR characterization of hNPCs at day 0 by means of mRNA expression of Nestin, SOX2, β-Tubulin III (TUJ1), glial fibrillary acidic protein (GFAP), microtubule-associated protein 2 (MAP2), Synapsin I (Syn1), and postsynaptic density protein 95 (PSD95). (A) Characterization of CTRL cells. (B) Characterization of RTT cell. Data represents the average from 3 scaffolds from one batch of cells (3 technical replicas), thus SE was not shown.*~~

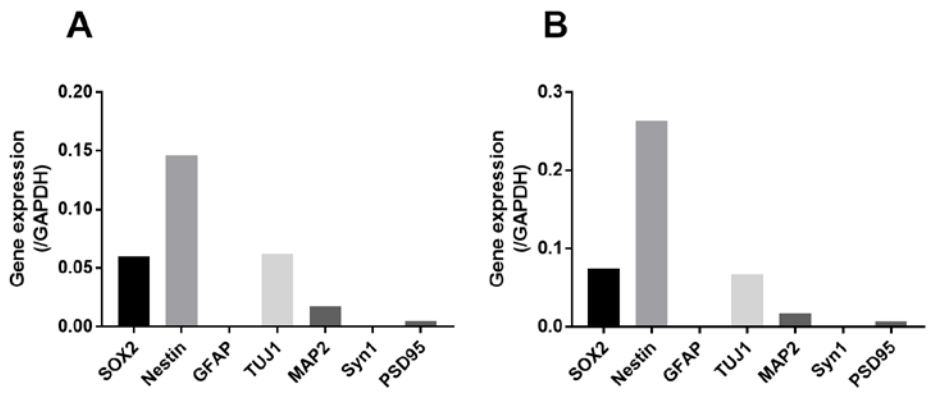

***Figure S1:** RT-qPCR characterization of hNPCs at day 0 by means of mRNA expression of Nestin, SOX2, β-Tubulin III (TUJ1), glial fibrillary acidic protein (GFAP), microtubule-associated protein 2 (MAP2), Synapsin I (Syn1), and postsynaptic density protein 95 (PSD95). (A) Characterization of CTRL cells. (B) Characterization of RTT cell. Data represents the average from 3 scaffolds from one batch of cells (3 technical replicas), thus SE was not shown.*

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~~***Figure S2:** Western Blot analysis of fold change in MAP2 and TUJ1 expression in CTRL cells that underwent different durations of electrical stimulation. Original Western Blots of optimization data (Figure 5 in the main text).*~~

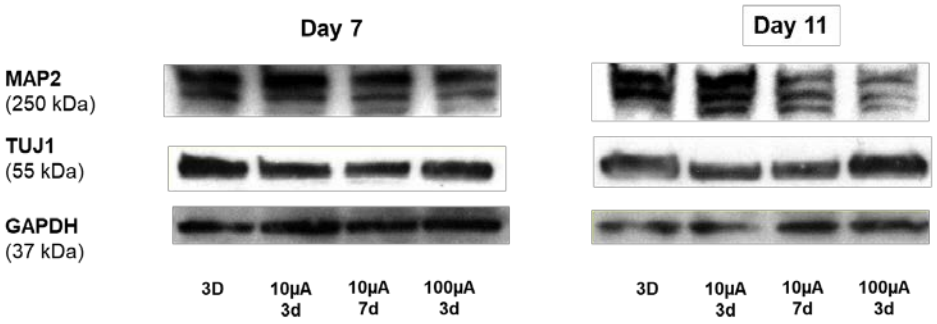

Figure S2: *Western Blot analysis of fold-change in MAP2 and TUJ1 expression in CTRL cells that underwent different durations of electrical stimulation. Original Western Blots of optimization data (Figure 5 in the main text).*

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~~***Figure S3:*** *Quantitative RT-qPCR analysis of Syn1 expression of cells grown on glass substrates and grown with/without stimulation on 3D-C at day 0, day 7 and day 11. (A) Syn1 mRNA expression in CTRL and RTT cells at day 7. (B) Syn1 mRNA expression in CTRL and RTT cells at day 11. Data represents the average from 3 scaffolds from one batch of cells (3 technical replicas), thus SE was not shown.*~~

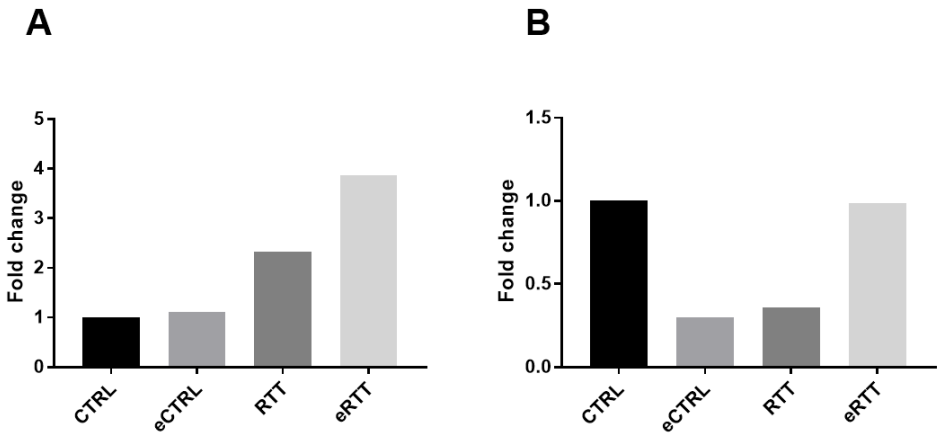

Figure S3: *Quantitative RT-qPCR analysis of Syn1 expression of cells grown on glass substrates and grown with/without stimulation on 3D-C at day 0, day 7 and day 11. (A) Syn1 mRNA expression in CTRL and RTT cells at day 7. (B) Syn1 mRNA expression in CTRL and RTT cells at day 11. Data represents the average from 3 scaffolds from one batch of cells (3 technical replicas), thus SE was not shown.*

~~***Figure S4:*** *Quantitative RT-qPCR analysis of Nestin expression of cells grown with and without stimulation on 3D-C at day 7, and day 11. (A) qPCR quantification of Nestin mRNA expression in CTRL and RTT cells at day 7. (B) qPCR quantification of Nestin mRNA expression CTRL and RTT cells at day 11. All data represents mean +/- SE, n=2.*~~

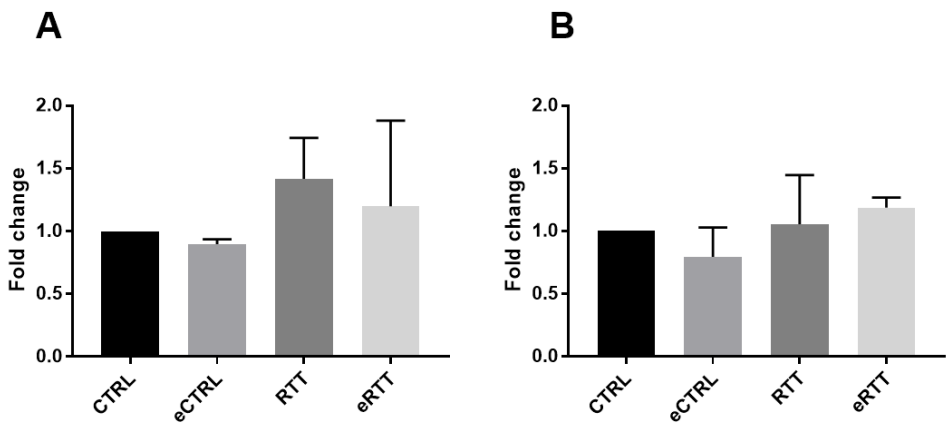

Figure S4: *Quantitative RT-qPCR analysis of Nestin expression of cells grown with and without stimulation on 3D-C at day 7, and day 11. (A) qPCR quantification of Nestin mRNA expression in CTRL and RTT cells at day 7. (B) qPCR quantification of Nestin mRNA expression CTRL and RTT cells at day 11. All data represents mean +/- SE, n=2.*

S1.1. Generation and Characterization of iPSC Lines

The following is a short summary of the generation of iPSCs from RTT patient fibroblasts (Coriell Institute for Medical Research, New Jersey, USA). This procedure was previously described in Chin et al. 2016 (Chin et al. 2016). Collected fibroblast cells were cultured using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS). Trypsinization was done using 0.25% trypsin-EDTA (Gibco) once cells reached 80% confluence and then cells were quenched with DMEM and 10% FBS. Centrifugation was then to isolate the supernatant which was then suspended in a transfection solution, Nucleofector™ kit (Lonza). Electroporation was done with episomal vectors containing Oct4, c-myc, Klf4, and Sox2 human cDNAs (Addgene). Cells were then plated in DMEM supplemented with 10 % FBS. After 48 h the medium was replaced with mTeSR™1 medium (StemCell Technologies). This culture medium was replenished for approximately 28–35 days at which point iPSC colonies were observed.

To detach iPSC colonies, dispase (1 U/ml) (StemCell Technologies) was added and then the colonies were then manually cut out. Most were reseeded onto Matrigel-coated plates (BD Biosciences) in mTeSR™1 medium with 10-μM Rho-associated protein kinase (ROCK) inhibitor (Y-27632). The remaining cells were used for further analysis and characterization. Karyotyping analysis was done by the cytogenetics lab in the Department of Pathology and Laboratory Medicine at KK Women's and Children's Hospital, Singapore. Teratoma formation assays were performed by injecting iPSCs subcutaneously into the dorsal flanks of SCID mice, to assess the in vivo pluripotency of the iPSC lines. Five-to-six weeks after injection, teratomas were dissected and fixed in 10 % buffered formalin phosphate solution. Sections were stained with hematoxylin and eosin for further analysis.

S1.2. Neural Induction of iPSCs

The following is a short summary of the neural induction of iPSCs and the subsequent differentiation of the neural progenitor cells to neurons. This procedure was previously described in Chin et al. 2016 (Chin et al. 2016). Once iPSCs cultures reached approximate confluence of 20 %, the mTeSR™1 medium was removed and replaced with neural induction media containing DMEM/F12 and neurobasal media in 1:1 ratio, 1× N2 supplement, 1× B27 supplement, 1× GlutaMAX, 1× penicillin/streptomycin, 5 μg/mL BSA, 4 μM CHIR99021 (Cellagentech), 3 μM SB431542 (Cellagentech), 0.1 μM Compound E (γ-Secretase Inhibitor XXI, EMD Chemicals Inc.), and 10 ng/mL human LIF (Millipore). Neural differentiation was then performed. The procedure for neural differentiation can be found in Section 2.2 of the main report.