SUPPLEMENTARY FIGURES, TABLES and METHODS

Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome

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SUPPLEMENTARY MATERIALS AND METHODS

iPSC generation and characterization

We collected dermal fibroblasts from two individuals with Timothy syndrome (TS) and three unaffected controls and derived thirty-six iPSC lines using infection with retroviruses expressing *SOX2*, *OCT3/4*, *KLF4* and *C-MYC*. The iPSC lines were extensively characterized using a set of assays that included immunostaining with NANOG and TRA 2-49/6E specific antibodies, karyotyping, generation of teratomas in nude mice, and mapping of viral integration sites (**Supplementary Fig 1**; see ref. 1 for a complete description of iPSC generation and characterization, and **Supplementary Table 1** for the list of lines used in each assay). In addition, we verified that all iPSC lines derived from individuals with TS were heterozygous for the TS mutation (c.1216G>A). We also characterized the iPSC lines with whole-genome microarrays. Hierarchical clustering and principal component analysis of the iPSC lines based on differentially expressed genes revealed that the iPSC lines were transcriptionally similar to each other and to human embryonic stem cells (ESC), and significantly different from primary fibroblasts and other cell types (**Supplementary Fig. 2** and **Fig. 2a**).

iPSC maintenance

Human iPSCs and the embryonic H9 line were cultured on irradiated DR4 mouse embryonic fibroblast feeders in the following iPSC media: DMEM/F12 (1:1) medium (Invitrogen) containing 20% KnockOut^{TM} SR (Invitrogen), 1 mM non-essential amino acids (Invitrogen), 3 mM L-glutamine (Invitrogen), 0.1 mM β-mercaptoethanol (Sigma-Aldrich), 100 units ml^{-1} penicillin and 100 µg ml^{-1} streptomycin (Invitrogen), and 10–15 ng ml^{-1} bFGF (R&D Systems).

Neural differentiation protocol (detailed)

To generate Embryoid Bodies (EBs), iPSC colonies were detached by incubation for 25-30 min at 37°C with 0.35 mg ml^{-1} dispase (Invitrogen). EBs were kept in suspension in iPSC media without FGF2 in low attachment plates (Corning) for four days. For neural induction, day five EBs were plated in Neural Media (NM) on polyornithine/laminin coated plates with FGF2 (20 ng μl^{-1}) and EGF (20 ng μl^{-1}). NM media contained: Neurobasal (Invitrogen, cat. no. 10888), B-27® Supplement Minus Vitamin A (50×, cat. no. 12587-010), GlutaMAX (Invitrogen, 100×), 100 units ml^{-1} penicillin and 100 µg ml^{-1} streptomycin (Invitrogen). Attachment of the EBs was facilitated by a 12 hours pulse with 10% FBS. During the 10 days of induction, the NM media was changed every day. Rosettes were picked mechanically and transferred to low attachment wells (Corning) in NM with FGF2 (20 ng μl^{-1}) and EGF (20 ng μl^{-1}), and kept in suspension for seven days. Media changes on the neurospheres were performed every other day, and mechanical trituration was performed on the third and the fifth day. For neural differentiation, ~10 neurospheres were plated on 12 or 15 mm polyornithine/laminin coated coverslips in NM media supplemented with FGF2 (20 ng μl^{-1}) and EGF (20 ng μl^{-1}) for the first 24 hours, and then media was changed to NM supplemented with BDNF (20 ng μl^{-1}) and NT3 (20 ng μl^{-1}). Half of the media was changed every other day for 21–22 days. In the second week after plating, all cells on the coverslips were detached by incubation in HBSS (Invitrogen) for 20 min, and gently mixed and replated on 12 or 15 mm polyornithine/laminin coated coverslips.

Proliferation assay

Neuropsheres that were kept in suspension for 7 days were plated on polyornithine/laminin coated coverslips in NM media supplemented with FGF2 (20 ng μl^{-1}) and EGF (20 ng μl^{-1}). After

migration of the neural progenitors, the cells were passaged twice with accutase, FAC-sorted using a mouse antibody to FORSE-1 (1:75), and replated at a density of 1×10^5 cells/cm^2 in NM media supplemented with FGF2 (20 ng μl^{-1}) and EGF (20 ng μl^{-1}). After two more passages (1:2), the coverslips were fixed and stained for the mitotic marker PH3. PH3$^+$ cells were counted and normalized to the total number of cells (as estimated by nuclear staining with Hoescht).

Migration assay

Neurospheres with a diameter of approximately 400 μm that were kept in suspension for 7 days were plated on polyornithine/laminin coated coverslips in NM media without growth factors. Phase contrast images were taken with an EVOS microscope (10× objective) after 12, 24, 48 and 72 hours. At each time point, the distance (in μm) for the three most distant cells in each neurosphere (seven per line, two control lines and three TS lines) was measured using the *Image J* software.

Infection with the Synapsin-1 reporter

A serotype-5 adeno-associated virus (AAV5) expressing YFP under the human Synapsin-1 promoter was used to selectively label neurons that have reached a certain level of maturation *in vitro*. The virus was raised in 293 cells, purified and titrated. Neuronal cell cultures were infected around day 30 of differentiation during half media changes by adding purified virus at a determined titer.

Electrophysiological recordings

Electrophysiological experiments were performed at room temperature on 30- to 43-day old neurons grown on glass coverslips coated with polyornithine/laminin. After one wash, coverslips were transferred to the recording chamber containing: 129 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 30 mM Glucose, 25 mM HEPES, pH 7.4 (NaOH). Cells were visualized with an inverted Nikon microscope (TE2000) and recorded using an EPC 10 amplifier (HEKA). Patch pipettes (5-7 MΩ) were manufactured from borosilicate glass tubes (BF150, Sutter) and filled with the following intracellular solution: 120 mM KGlu, 20 mM KCl, 4 mM NaCl, 4 mM Mg2ATP, 0.3 mM NaGTP, 10 mM Na2PCr, 0.5 mM EGTA, 10 mM HEPES, pH 7.25 (KOH). Analog signals were filtered at 2 kHz via a 4-pole Bessel low pass filter and digitized at 5 kHz. Data was collected and initially analyzed with the *Patchmaster* software (HEKA). Further analysis and statistics were performed with *Igor Po* and *Microsoft Excel*. Recorded cells were classified based on the value of the speed of the AP rise (see **Supplementary Fig. 7** and **Supplementary Table 3**).

Immunocytochemistry

NPCs or neurons plated on 12 or 15 mm coverslips (Warner Instruments) and grown in 24-well plates were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. The coverslips were washed twice in PBS and incubated in blocking solution (3% BSA in PBS, 0.25% Triton X-100, 10% goat serum). The samples were incubated with the indicated primary antibodies for 3 h at room temperature or overnight at 4°C and then washed three times in PBS at room temperature before incubation with secondary antibodies (Alexa Fluor 488 anti-mouse/rabbit IgG [1:1000], and/or Alexa Fluor 594 anti-mouse/rabbit IgG [1:1000]) for 1 h at room temperature in blocking solution). The samples were then washed five times with PBS and incubated for 5 min with Hoechst 33258 (Molecular Probes, 1:10.000). The coverslips were mounted with AquaMount on glass microscope slides and imaged on a Zeiss M1 Axioscope with a 10×, 20×, 40× or 63× objective. The following primary antibodies were used: MAP2-specific antibody raised in mouse (Chemicon, 1:250), TH-specific antibody raised in rabbit

(Peel-Freeze, 1:300), FOXA2-specific antibody raised in rabbit (Millipore, 1:400), GABA-specific antibody raised in mouse (Sigma, 1:1000), PAX6-specific antibody raised in rabbit (Covance, 1:300), N-Cadherin-specific antibody raised in mouse (Santa Cruz Antibodies, 1:50), FORSE-1-specific antibody raised in mouse (Developmental Studies Hybridoma Bank, 1:75), PH3-specific antibody raised in rabbit (Chemicon, 1:250), Doublecortin-specific antibody raised in guinea pig (Millipore, 1:1000). See ref. 1 for details on immunostaining of iPSC lines.

Analysis of single cell qPCR data

Data was collected and analyzed using the *Fluidigm Real-Time PCR Analysis* software (v2.1.3 and v3.0.2) and a heatmap was generated (**Supplementary Fig. 4a**). Further data analysis was performed using *Microsoft Excel*. Every experiment contained samples for five standard dilutions of a mixed human cDNA library, a negative control and cDNA from 90 single cells (**Supplementary Fig. 4b,c**). Cells were identified based on *RPS18* expression, the gene encoding the 18S small ribosomal subunit. Results from conventional qPCR reactions and Fluidigm Dynamic Arrays performed on the same sample of cells showed high correlation ($R^2 >$ 0.99) (**Supplementary Fig. 4d,e**) suggesting no difference in the reaction performance between the two technologies. As expected, single neurons from mouse cortical cultures displayed high correlation between housekeeping genes (*Gapdh* versus *Rps18*, **Supplementary Fig. 4f**), exons of the same transcript (exon 44 versus 8a of *Cacna1c*, **Supplementary Fig. 4g**) confirming that the technology provides reliable results with single cell samples. The accuracy of Fluidigm Dynamic Arrays was also assessed by comparing gene expression levels in the population of human cells as determined by Illumina microarrays with gene expression levels measured using Fluidigm Dynamic Arrays (**Supplementary Fig. 4h–j**). Fluidigm data was converted into a population measurement by multiplying the fraction of cells in a population expressing a gene by the average expression level of the gene. For *NESTIN*, *SATB2* and *FOXP1* (shown), as well as for most genes expressed by more than 20% of the cells, we observed excellent correlation between the two measurements. This indicates that Fluidigm arrays are a reliable way of measuring single cell gene expression.

We also attempted to validate the Fluidigm measurements by comparing the fraction of cells expressing specific marker genes with the expression of the corresponding proteins in the same population of cells as determined by immunocytochemistry. While the expression of genes and proteins was always strongly correlated **(Supplementary Fig. 4k)**, the absolute number of cells that expressed a gene was always greater than the numbers of cells that expressed the corresponding protein. For instance, between 3–5 times more cells expressed *MAP2* and *DCX* mRNAs than expressed the proteins (**Supplementary Fig. 5**). This difference could arise from at least two sources. First, the two kinds of experiments have very different signal to noise characteristics and were therefore quantified in different ways. Cells stained with antibodies showed a wide range of staining intensities and significant levels of background fluorescence and reliable counting by multiple observers required a high threshold for inclusion. This likely excluded many cells that expressed low levels of a protein. In contrast, Fluidigm arrays indicated that expression of most genes was largely binary at the single cell level making it simple to distinguish cells expressing or not expressing a marker gene. A second potential reason for the discrepancy between the two methods is that the translation of many mRNAs is regulated and therefore mRNA expression does not always correlate well with the expression of a protein. This is particularly true when there are multiple splice isoforms of a particular gene and the antibody only recognizes a subset of the proteins. In the case of *MAP2*, our PCR primers amplify all the isoforms, while the antibody only recognizes the two most mature forms of the protein (MAP2-A and B) but not the form that is found in less mature cells (MAP2-C).

Real time quantitative PCR

For expression analysis, template cDNA was prepared from 200 ng of total RNA by reverse transcription. Gene expression was quantified using real time quantitative PCR in combination with gene specific primers and the SYBR GREEN system (Roche). The reactions were performed on an Eppendorf Realplex4 cycler (Eppendorf). All samples were run in duplicates or triplicates. Values were normalized on *GAPDH* expression. Relative gene expression after 9 hours of KCl stimulation was calculated using the $2^{(-_{\Delta\Delta}ct)}$ method.

High-performance liquid chromatography measurements

Media from neuronal cultures at day 43 of differentiation was collected and stored with 7M $HClO_4$ (7 µl for 0.5 ml of media) at –80°C until further analysis. Norepinephrine (NE) and dopamine (DA) levels in the samples were determined by HPLC with electrochemical detection. Media was centrifuged, and the filtered supernatant was injected into the column. The HPLC mobile phase (0.1 M sodium phosphate buffer, 0.13 mM EDTA, 2.3 mM 1-octanesulfonic acid, 20% MeOH, pH 6.0) was pumped through a chromatography column (SC-5ODS, Eicom, San Diego, CA) and a 50 µl sample loop at a flow rate of 0.23 ml min^{-1}. A graphite electrode (WE-3G, Eicom) set at a potential of 450 mV was used for electrochemical detection. Quantification was achieved with a PowerChrom analysis system (ADInstruments, Sydney, Australia) using external NE and DA standards (Sigma, St. Louis, MO). NE and DA concentrations were calculated by comparing the HPLC peak of NE and DA in samples with the peak area of known concentrations of the standards analyzed on the same day. The levels of catecholamines were normalized to the estimated number of neurons per well. The number of neurons per well was estimated by extrapolating the number of neurons ($MAP2^+$ cells out of all $Hoechst^+$ cells) counted on at least six randomly imaged fields (10×) per coverslip.

Generation of the conditional TS mouse:

Construction of the targeting vector

Flag- and myc-tagged rat $Ca_V1.2$ channel cDNAs was produced using PCR with Pfu turbo polymerase (Stratagene) with primers containing AscI sites at both ends of fragment containing Kozak, start and stop sequences using the pGW-Flag/myc-$Ca_V1.2$ plasmid as a PCR template[2]. The PCR fragment was digested with AscI and purified using gel extraction. The STOP-eGFP-Rosa26TV vector (Addgene #11739) was digested using AscI restriction enzyme and treated with alkaline phosphatase, calf intestinal (CIP). The digested fragment and vector were ligated using T4 ligase and transformed in bacteria. The $Ca_V1.2$ channel cDNA with the T1039Y mutation (FMTY) or with the T1039Y and G406R mutations (FMTYGR or TS) were sequenced and verified using 14 primers that detect the channel gene.

Generation of mutant mice

Gene targeting in mouse embryonic stem (mES) cells was performed in the R1 (129sv/svj) mES cell line by electroporating 50 µg of an SgfI-cut targeting vectors and selecting recombinant colonies with G418. Targeting was verified by Southern blotting of genomic DNA and by using PCR with two primer sets (5` region, W2 and lox-R; 3` region, M1 and R2) to confirm homologous recombination.

The following primers were used:

W1 (5`-GGTGCAAGCACGTTTCCGACTTGAGTTGCC-3`),

W2 (5`-GAGGGCGGCTTGGTGCGTTTGCGG-3`),

M1 (5`-caggacagcaaggggaggattgggaag-3`),
R1 (5`-CTTTTGATAAGGCTGCAGAAGGAGCGGGAGAAATG-3`),
R2 (5`-CTAGGTCAGAGAGTCTTGCCTGCAAACCAC-3`), and
lox-R (5`- caaggaaaccctggactactgcgccctac-3`)
A positive clone was injected into C57BL/6 blastocysts to obtain chimeric mice. Germ-line transmission of the channel containing the TS allele was confirmed by PCR as described above. The identified germ-line male was subsequently crossed with Foxg1-Cre mice (129(Cg)-*Foxg1*$^{tm1(cre)Skm}$*/J* , the Jackson laboratory)[3] to generate mice that express the TS channel only in the forebrain.
Forebrain mRNA was prepared using the RNeasy Mini kit and RNase-Free DNase set (Qiagen). cDNA was synthesized from 1 µg RNA using the SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen). The cDNA (21 µl) was diluted (1:5) with DNase-free water (Invitrogen) and 1 µl of the samples was used for conventional RT-PCR with AccuPower PCR PreMix (Bioneer, K-2016) to confirm gene expression of TS mutant channels *in vivo* using a primer set of FM-F (5`-ccaccatggactacaaggacgatgacg-3`) and FM-R2 (5`-caatgctgatgcatgccctcctgatgg-3`). There were no differences in body and brain size between control and TS mice.

Immunohistochemistry
P0-P0.5 brains were fixed with 4% PFA and 10-30% sucrose, frozen in isopentane on dry ice and stored at –80^{o}C. The samples were mounted on a microtome (Leica SM2000R) and brain slices (30-40 µm) were prepared. The sections were permeabilized, blocked and incubated at 4^{o}C for 24 hours with the following antibodies: Satb2 specific antibody raised in mouse (Abcam, 1:50), Foxp1 specific antibody raised in rabbit (Abcam, 1:2000), Ctip2 specific antibody raised in rat (Abcam, 1:500), NeuN specific antibody raised in mouse (Millipore, 1:100). The samples were then washed four times (4 × 20 min) using cold PBS and incubated with secondary antibodies: Alexa Fluor 594, Alexa 555 and/or Alexa Fluor 488 (Molecular Probes, 2 µg ml^{-1}) at room temperature for 30 min. After staining with Hoechst 33285 (Molecular Probes, 1:10 000 dilution in PBS), the slices were washed four times and mounted on Superfrost Plus glass slides (VWR) using Aqua Poly/Mount (Polysciences). Slices were then imaged on a fluorescent microscope (Zeiss, Axio Imager M1).

References

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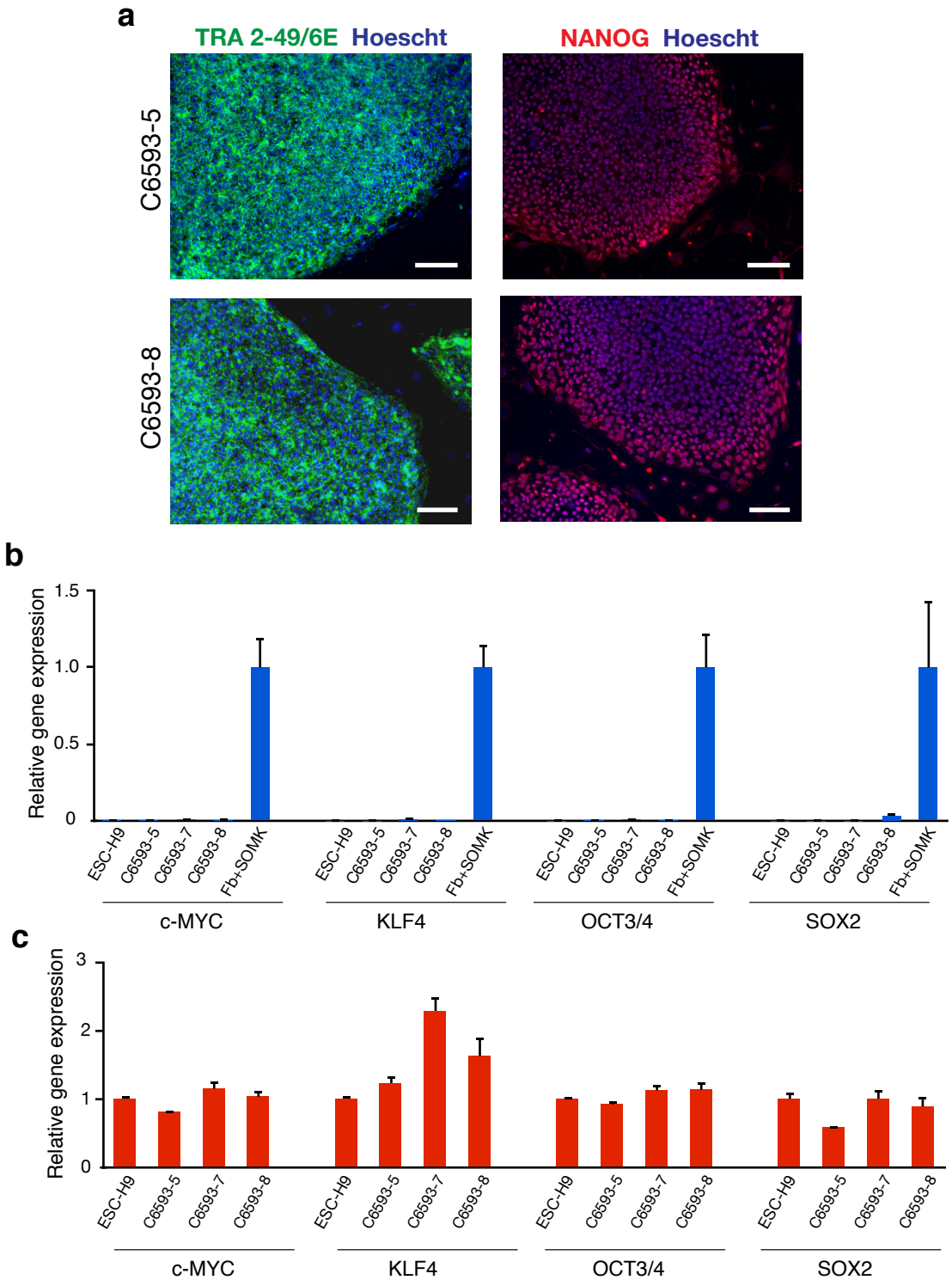
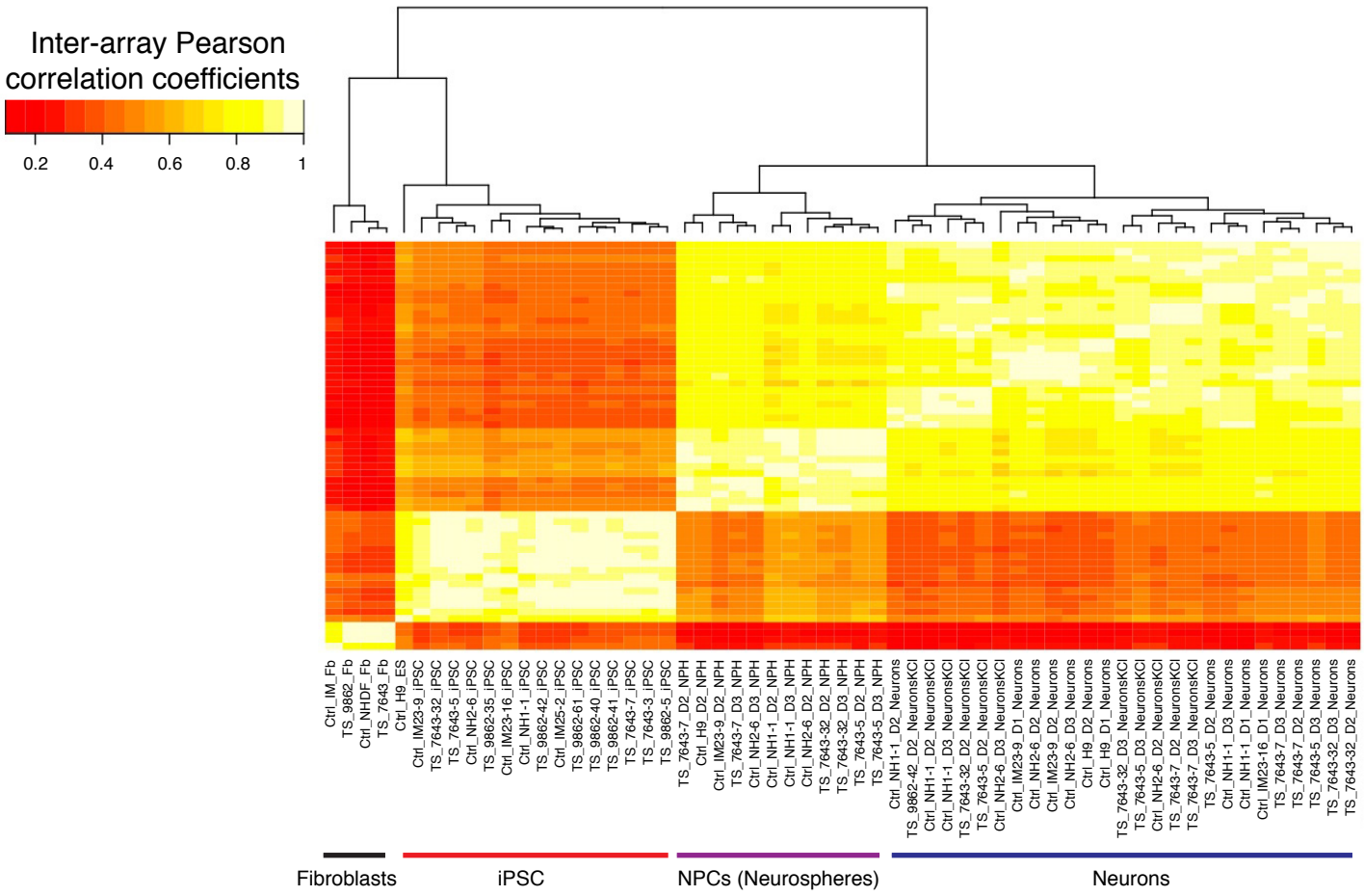
3. Hebert, J.M. & McConnell, S.K. Targeting of cre to the Foxg1 (BF-1) locus mediates loxP recombination in the telencephalon and other developing head structures. *Dev Biol* **222**, 296-306 (2000).



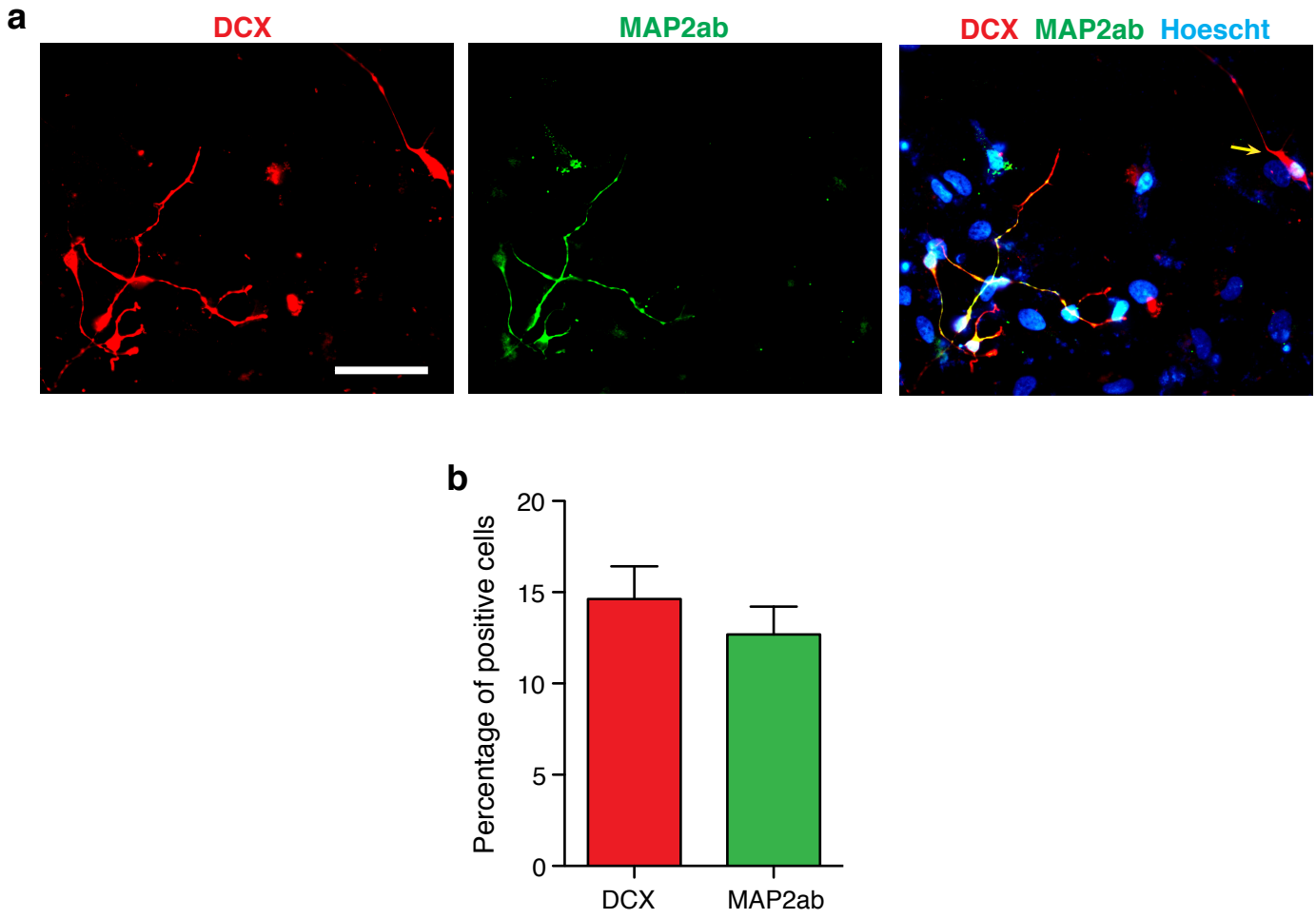
Supplementary Figure 1. Characterization of iPSC lines from a control subject (C6593)

(**a**) Immunocytochemistry of iPSC lines generated from one control subject (C6593) using TRA-2-49/6E (green) and NANOG (red) specific antibodies. Hoescht staining is in blue. Scale bars are 200 μm. (**b**) Quantitative RT-PCR for the expression of exogenous reprogramming genes. The human ESC line H9 (ESC-H9) and fibroblasts infected with the four retroviruses (Fb + SOMK) were used as a negative and positive control, respectively (mean ± s.d). The values for Fb + SOMK were normalized to 1. (**c**) Quantitative RT-PCR for the expression of exogenous reprogramming genes (mean ± s.d). Values for ESC-H9 were set to 1. For characterization of all the additional lines see ref. 12 in the main text.



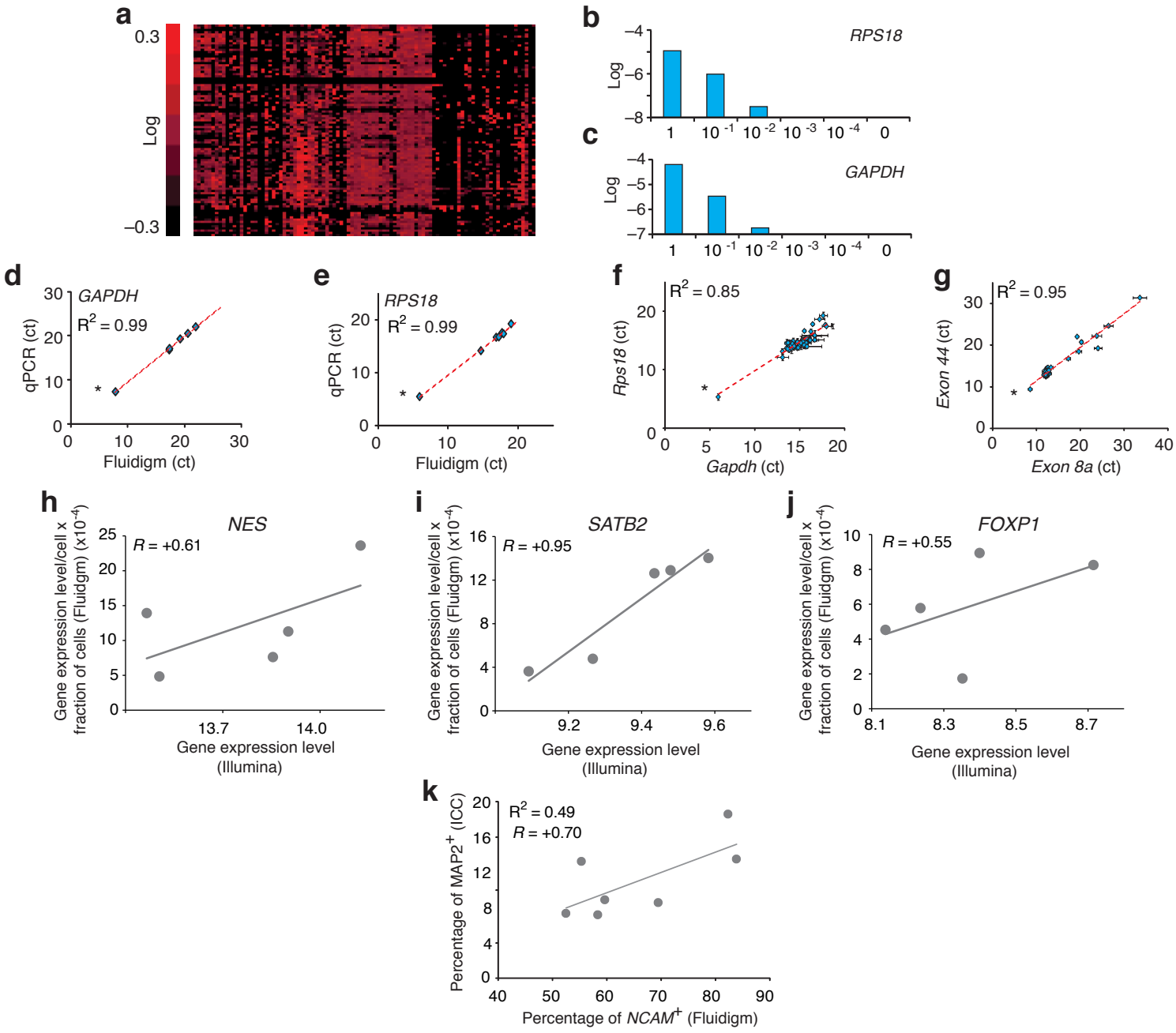
Supplementary Figure 2. Heatmap and hierarchical clustering based on inter-array Pearson correlation coefficients (PCC)

The heatmap depicts inter-array PCCs color-coded according to the legend. Inter-array PCCs were calculated using the top 3222 most variable genes (coefficient of variance > 0.05). Hierarchical clustering shows distinct clusters corresponding to cell types (fibroblasts, iPSC, NPCs and neurons). Note the high correlation among iPSCs from the same individual as well as those from different individuals, and the low correlation between iPSCs and primary fibroblasts. Fb: fibroblasts, NPH: neurospheres, NeuronsKCl: neurons after nine hours of 67 mM KCl stimulation. D1, D2 and D3 indicate independent neural differentiation experiments of the same iPS line.



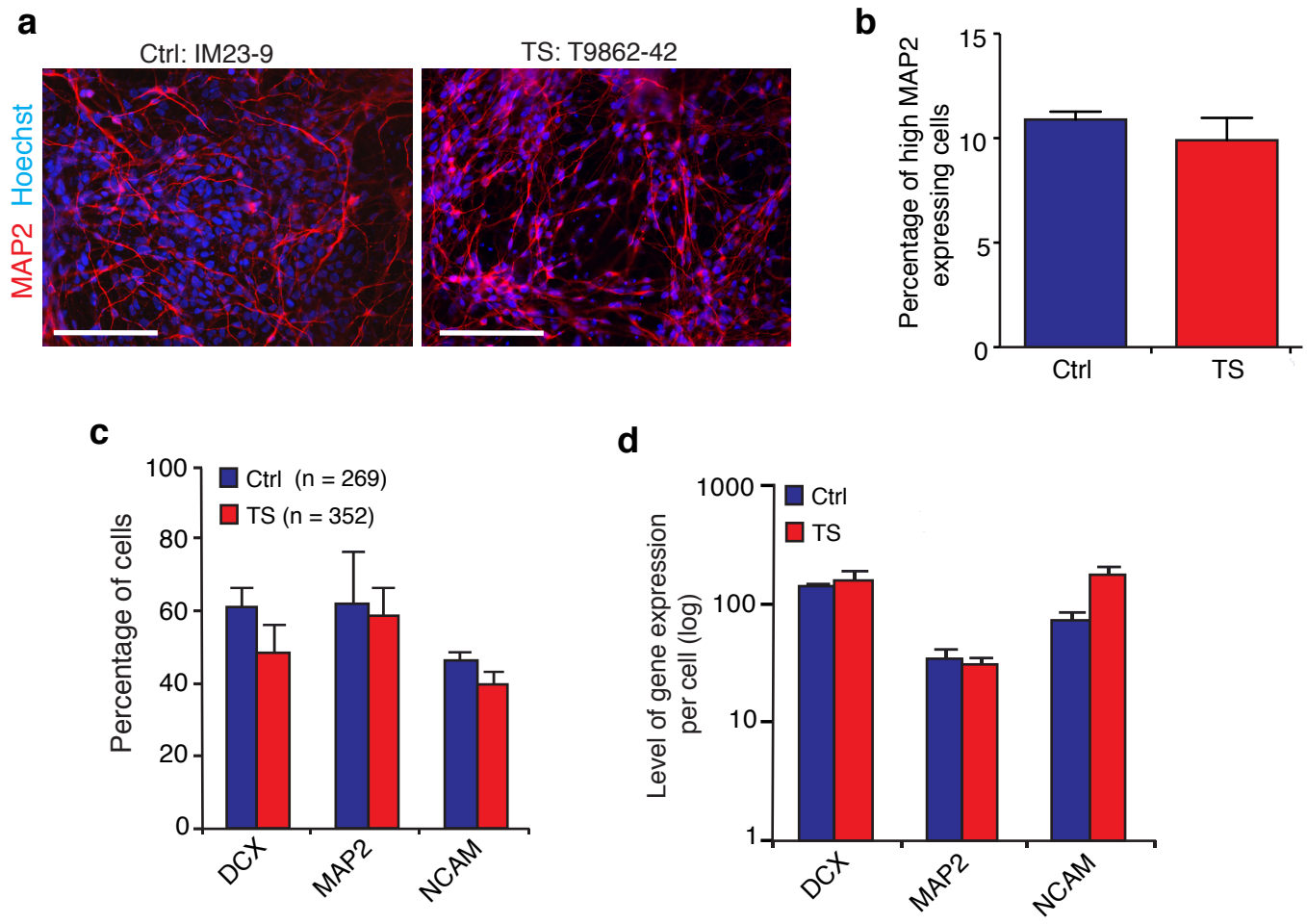
Supplementary Figure 3. Expression of MAP2ab and DCX in iPSC-derived neurons.

(**a**) Representative immunocytochemistry images of iPSC-derived neuronal cultures stained with DCX (red) and MAP2ab (green) specific antibodies (scale bar is 50 µm). Nuclear staining (Hoechst 33258) is shown in blue. (**b**) Quantification of the proportion of cells expressing MAP2 or DCX.



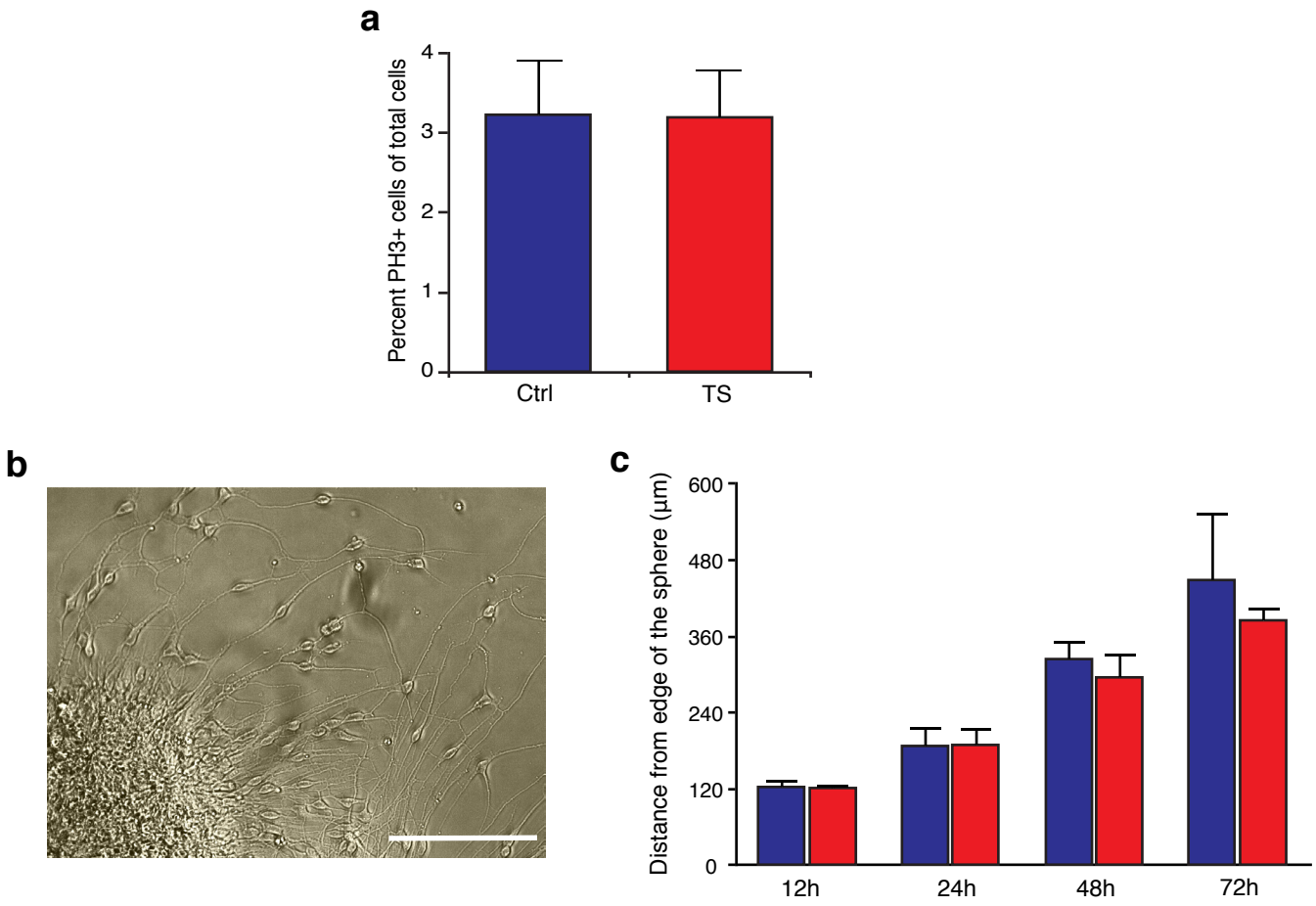
Supplementary Figure 4. Validation of Fluidigm Dynamic Arrays

(**a**) Representative heat-map of 96.96 Fluidigm Dynamic Array data showing the expression of 96 genes (horizontal axis) in 96 human cells (vertical axis) generated from one TS-iPSC line. (**b**) and (**c**) Fluidigm amplification of a dilution series (horizontal axis) of mixed human cDNA shows linear amplification. (**d**) and (**e**) Comparison of data from Fluidigm 96.96 dynamic arrays and conventional qPCR reactions from iPSC-derived neuronal cultures. Shown are the correlations of Ct values from seven single cells and a small cell cluster indicated by the asterisk. (**f**) Correlation of the single cell gene expression of two housekeeping genes, *Gapdh* and *Rps18,* measured using Fluidigm Dynamic Arrays. Forty-seven single cells and one cell cluster (asterisk) were manually collected and assayed on a 48.48 array. Error bars represent s.d. for technical replicates. (**g**) Correlation between the expression levels of two different exons from the *Cacna1c* gene in single neurons. (**h, i, j**) Correlation between population gene expression levels as determined by Illumina microarrays and single cell gene expression as measured by Fluidigm Dynamic Arrays. Single cell data was converted into population data by multiplying the fraction of cells expressing a gene by the average gene expression per cell. Each data point represents the analysis of cells from a single differentiated iPSC line plotted against the average gene expression as determined for that line using Illumina arrays. (**k**) Correlation between the proportion of cells expressing MAP2 measured using a MAP2 specific antibody and the proportion of cells expressing *NCAM* measured using Fluidigm Dynamic Arrays.



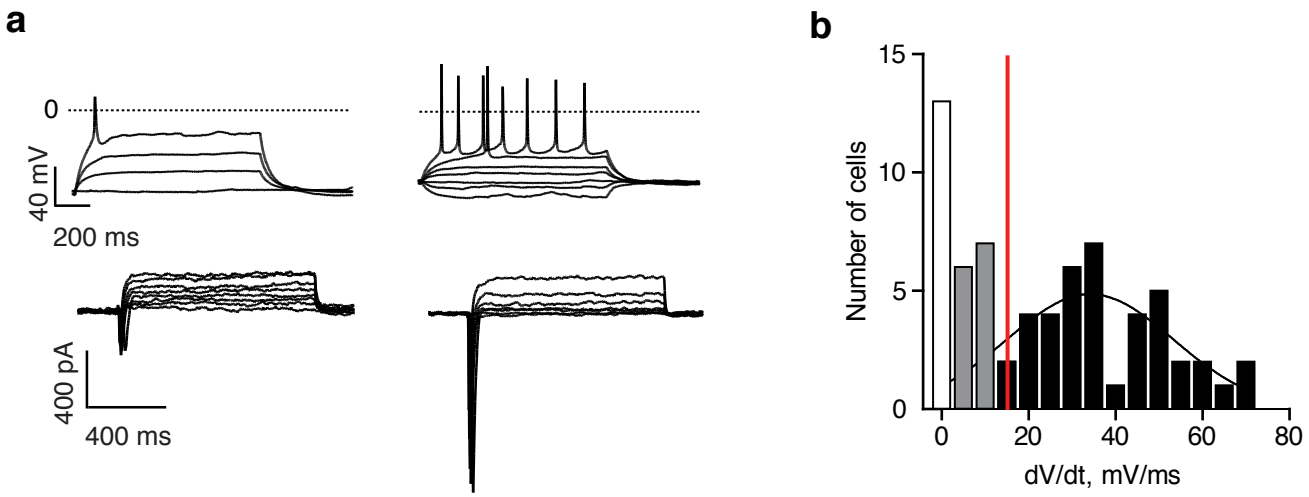
Supplementary Figure 5. **Expression of MAP2, NCAM and DCX in TS individuals and controls measured by immunocytochemistry and single cell qPCR**

(**a**) Representative images of neurons derived from control (left, line IM23-9) and TS (right, line T9862-42) at day 43 of neural differentiation showing immunostaining for the postmitotic neuronal marker MAP2 (red) and the nuclear marker Hoechst 33258 (blue). Scale bars is 200 µm. (**b**) Measurement of the fraction of cells staining positive for high levels of MAP2 determined from immunocytochemistry experiments (mean ± s.e.m.; Ctrl: 10.89% ± 0.27 versus TS: 9.90% ± 0.75, t-test, $P > 0.05$, data from four TS iPSC lines and three control iPSC lines differentiated at least twice; the proportion of MAP2 in the ES line H9 was 10.93% ± 1.47). (**c**) Fraction of cells expressing *DCX*, *MAP2* and *NCAM* in TS ($n = 352$ cells) and control cultures ($n = 269$ cells) at day 45 of differentiation (ANOVA, $P > 0.05$). (**d**) Levels of *DCX*, *MAP2*, and *NCAM* gene expression levels per cell in TS and control cultures. There was no statistical difference between levels of expression per cell in TS versus control cells.



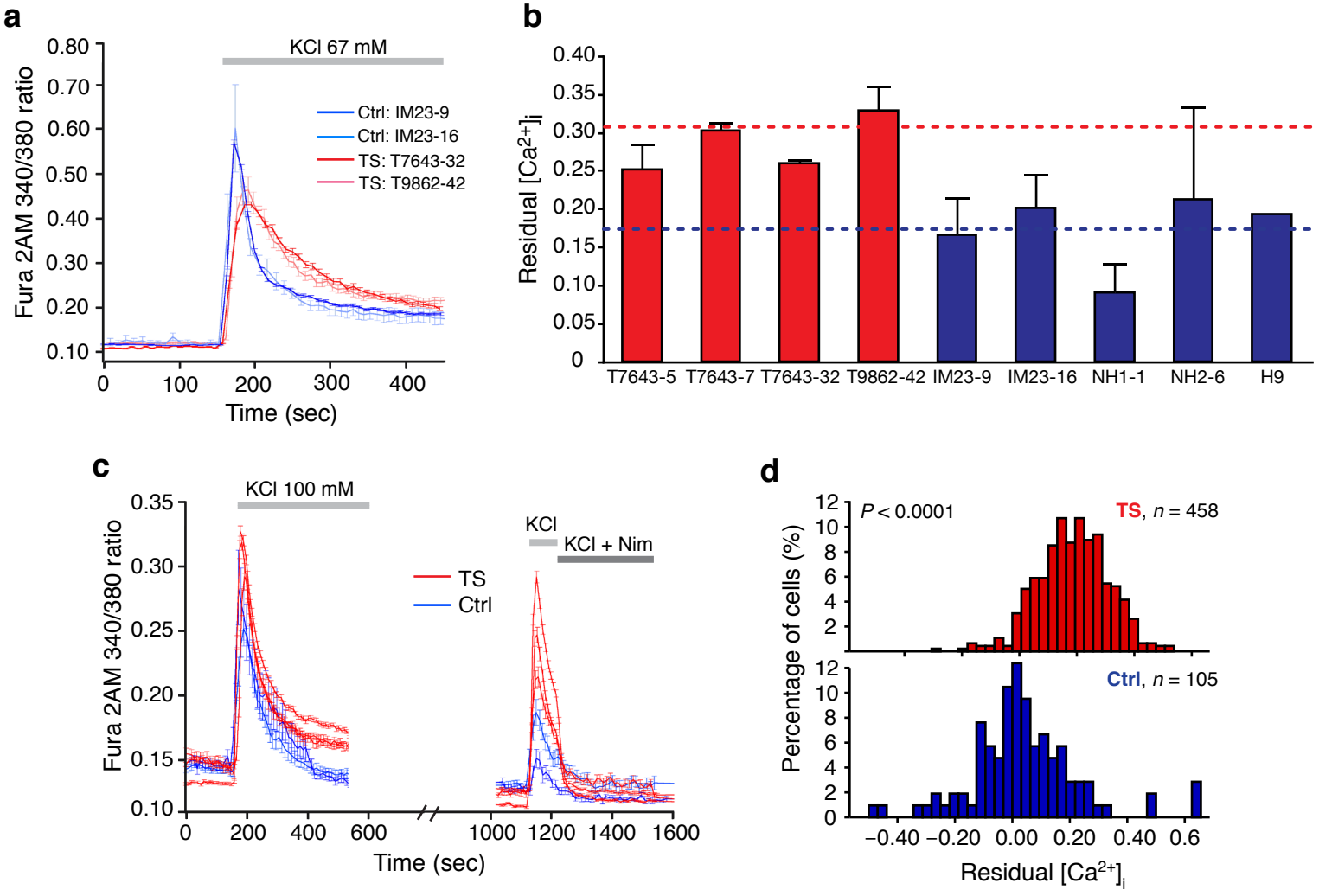
Supplementary Figure 6. Characterization of proliferation and migration of TS and control NPCs

(**a**) Percentage of FORSE-1^{+} NPCs derived from controls or TS individuals stained with a PH3-specific antibody (mean ± s.e.m.; TS: 3.67% ± 0.70 versus Ctrl: 3.70% ± 0.80; t-test, $P > 0.05$). (**b)** Phase contrast image of neural progenitors migrating out of a neurosphere 48 hours after plating (scale bar is 200 µm). (**c)** Migration of NPCs from TS (three lines, $n = 105$ cells) or control (two lines, $n = 45$ cells) subjects measured 12, 24, 48 or 72 hours after plating ($P > 0.05$, ANOVA). In addition, no difference could be observed between TS and control lines in the number of cells separated from the neurosphere cluster at 12, 24, 48 or 72 hours (ANOVA, $P > 0.05$, data not shown).



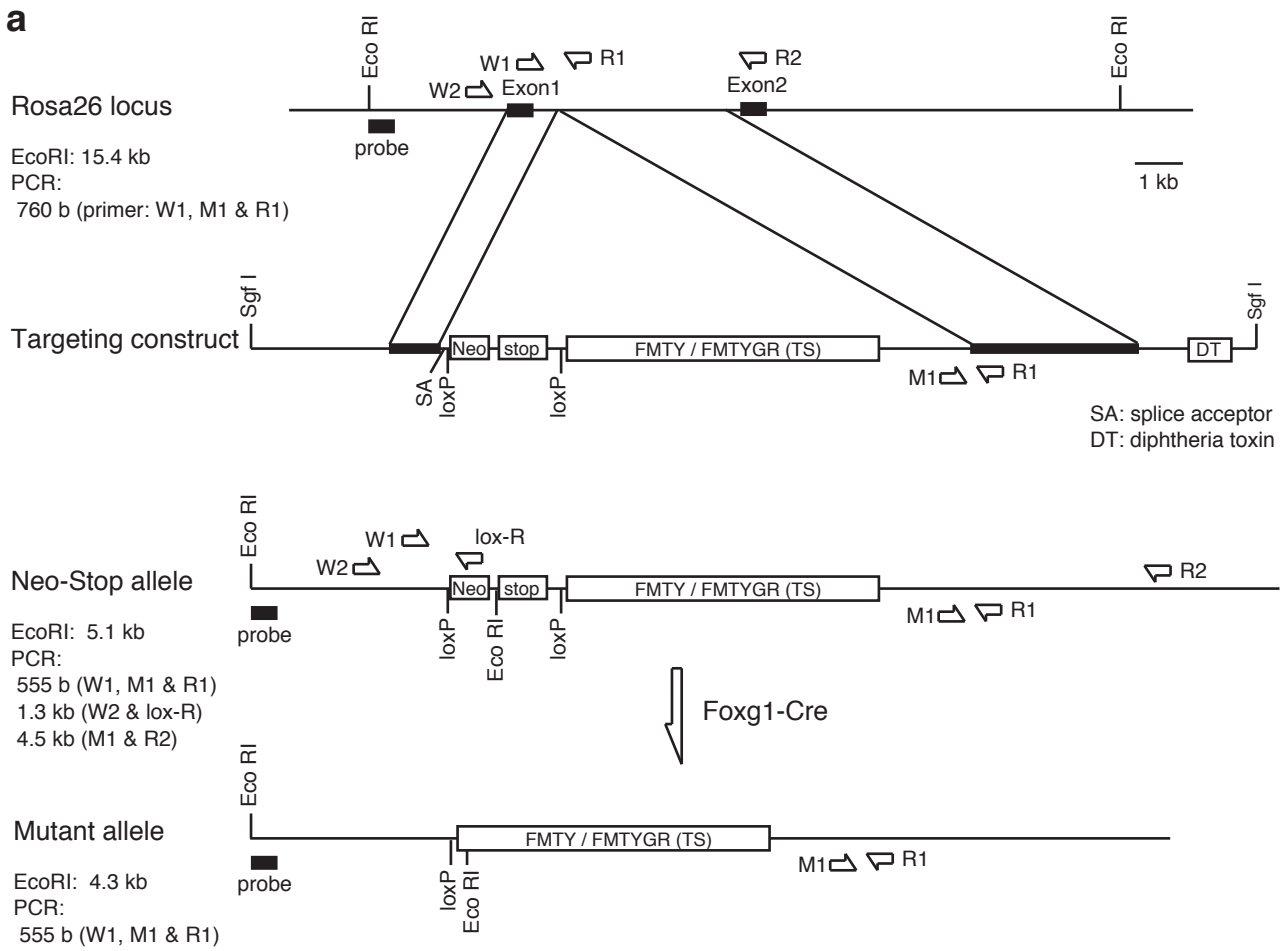
Supplementary Figure 7. Electrical properties of iPSC-derived neurons.

(**a**) Representative current (upper; ΔI_{inj} = 5 pA) and voltage (lower; from –80 mV, ΔV = 10 mV, V_{hold}= –90 mV) clamp recordings from immature (left) and mature neurons (right). (**b**) Histogram showing the distribution of dV/dt following current injection for all recorded cells (*n* = 66). Non-responding cells are shown in white. The distribution of the responding cells (dV/dt = [15–70] mV/ms, black columns) was fitted with a Gaussian function. A threshold (red line) to define immature (left, grey columns) and mature (right, black columns) neurons was set at one standard deviation left of the mean. The electrical properties for the classified cells are presented in **Supplementary Table 3**.



Supplementary Figure 8. Calcium imaging in control and TS-derived neurons and NPCs.

(**a**) Average $[Ca^{2+}]_i$ measured in control (blue) or TS (red) derived neurons at 43 days of differentiation. Each trace represents averages for cells derived from different iPSC lines. The histogram summarizing the data is shown in Fig. 1j**.** (**b**) Residual $[Ca^{2+}]_i$ in neurons across multiple experiments and multiple differentiated iPSC lines. For each TS and control line the residual $[Ca^{2+}]_i$ following depolarization with 67 mM KCl was averaged for all responding cells, and the average and s.e.m across experiments are presented. TS lines are shown in red, control lines are shown in blue. Dashed lines represent the average for TS lines (in red) and control lines (in blue). (**c**) Average Fura-2 $[Ca^{2+}]_i$ measurements of NPCs stimulated with two depolarizing pulses of 100 mM KCl. The second pulse is followed by addition of 5 μM of the L-type channel blocker nimodipine. NPCs from two control iPSC lines (blue) and three TS iPSC lines (red) are shown. (**d**) Histograms of the residual $[Ca^{2+}]_i$) in single NPCs derived from TS (red; $n = 458$ cells from three lines) or control (blue; $n = 105$ cells from three lines) subjects (*t*-test, $P < 0.0001$).



Supplementary Figure 9. Generation of the conditional TS mouse

(**a**) Targeting strategy for the generation of the conditional mutant mice expressing Flag- and myc-tagged $Ca_V1.2$ channels with the T1036Y (dihydropyridine insensitive mutation) and the G406R (TS mutation, FMTYGR) mutations. Channel expression is driven by the endogenous Rosa26 promoter and is controlled by a lox-STOP-lox recombination site. (**b**) Genotyping to distinguish wild-type (*Rosa26*$^{+}$/*Rosa26*$^{+}$) and Neo-stop/mutant alleles (*Rosa26*^{FMTYGR}/*Rosa26*$^{+}$) using three primer pairs (upper: W1, M1 and R1). Bottom shows an image of a gel electrophoresis for Foxg1-Cre genotyping. (**c**) Genotyping to confirm homologous recombination in the 5` and 3` regions of the targeting cassette (upper: 5` region, W2 and lox-R; bottom: 3` region, M1 and R2). (**d**) RT-PCR showing expression of TS mutant channel in the F1 progeny of TS lox-stop-lox mice crossed to Foxg1 CRE mice.

SUPPLEMENTARY TABLE 1

INDIVIDUAL	TS: T7643			TS: T9862		Ctrl: NH		Ctrl: IM			Ctrl: C6593		22q11del	huES
CLONES/ LINES	T7643-5	T7643-7	T7643-32	T9862-42	T9862-61	NH1-1	NH2-6	IM23-9	IM23-16	IM25-2	C6593-5	C6593-8	1728-10	H9
Teratoma assay (iPSC)	x	x	x	x	x	x	x	x	x	x			x	x
Karyotyping (iPSC)	x	x	x	x	x	x	x	x	x				x	x
Investigation of viral integration sites in iPSC	x	x	x	x	x	x	x	x	x	x				n/a
Calcium Imaging of neurons	x	x	x	x			x	x	x					x
Calcium Imaging of progenitors	x	x	x			x	x	x						
Electrophysiology			x	x				x	x					x
Proliferation assay		x	x						x					x
Migration assay	x	x	x				x	x						
Catecholamine measurements (HPLC)	x		x				x	x	x					x
Single cell qPCR	x		x	x			x	x	x					
Immunocytochemistry (MAP2, TH)	x	x	x	x			x	x	x				x	x
Rescue of TH phenotype	x		x		x					x	x	x		

SUPPLEMENTARY TABLE 2

Symbol	Gene[1]	Forward primer	Reverse primer	Primerbank[2] accession
18S	*RPS18*	GATGGGCGGCGGAAAATAG	GCGTGGATTCTGCATAATGGT	11968182a1
5HT2-2C	*HTR2C*	TCTTAATGTCCCTAGCCATTGCT	TACCGATCCAGCGATATAGCG	4504541a3
ASCL1	*ASCL1*	TCTTCGCCCGAACTGATGC	CAAAGCCCAGGTTGACCAACT	4757788a1
CALB1	*CALB1*	AGGGAATCAAAATGTGTGGGAAA	TCCTTCAGTAAAGCATCCAGTTC	4826655a2
CALB2	*CALB2*	TCAGAGATGTCCCGACTCCTG	GCCGCTTCTATCCTTGTCGTAA	4502543a1
CAMK2	*CAMK2A*	AAACTGAAGGGAGCCATTCTCA	GAGGATTCCATTAACTGAACGCT	25952114a1
CTIP2	*BCL11B*	TGGGTGCCTGCTATGACAAG	GGCTCGGACACTTTCCTGAG	12597635a1
CUX1	*CUX1*	GCTCTCATCGGCCAATCACT	TCTATGGCCTGCTCCACGT	
DCX	*DCX*	CCTTGGCTAGCAGCAACAGT	CCACTGCGGATGATGGTAA	
DDC	*DDC*	ACTGGCTCGGGAAGATGCT	CCGATGGATCACTTTGGTCC	4503281a1
DLX1	*DLX1*	CCATGCCAGAAAGTCTCAACA	GGCCCAAACTCCATAAACACC	31418473a3
EN1	*EN1*	GAGCGCAGGGCACCAAATA	AATAACGTGTGCAGTACACCC	7710119a2
ETV1	*ETV1*	CTGGATGACCCGGCAAATTCT	CCTCTTCAGGCTCAATCAGTTT	1045061a3
FOXG1	*FOXG1*	GCCACAATCTGTCCCTCAACA	CGGGTCCAGCATCCAGTAG	32307177a3
FOXP1	*FOXP1*	AGACAAAAAGTAACGGTTCAGCC	CGCACTCTAGTAAGTGGTTGC	21750965a3
GAD65	*GAD2*	GGCTTTTGGTCTTTCGGGTC	GCACAGTTTGTTTCCGATGCC	4503875a1
GAD67	*GAD1*	GCCAGACAAGCAGTATGATGT	CCAGTTCCAGGCATTTGTTGAT	4503873a2
GAPDH	*GAPDH*	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT	7669492a3
GCH1	*GCH1*	ACAAGTTCAGGAGCGCCTTAC	AGTAGAGGGCTCAACCCTTTATT	5058993a1
GSX2	*GSX2*	ATGTCGCGCTCCTTCTATGTC	ATGCCAAGCGGGATGAAGAAA	18959212a1
HNK1	*B3GAT1*	CCTGGCGTGGTCTACTTCG	GCAGGTTGACGGCAAATCC	12408652a1
LHX6	*LHX6*	TGAGAGTCAGGTACAGTGCG	GCCCATCCATATCGGCTTTGA	7657305a1
MAP2	*MAP2*	CTGCTTTACAGGGTAGCACAA	TTGAGTATGGCAAACGGTCTG	24416560a1
MSX2	*MSX2*	CACCCTGAGGAAACACAAGAC	AACTCTGCACGCTCTGCAAT	27886557a2
NCAM	*NCAM1*	ACATCACCTGCTACTTCCTGA	CTTGGACTCATCTTTCGAGAAGG	10834990a1
NES	*NES*	GGCGCACCTCAAGATGTCC	CTTGGGGTCCTGAAAGCTG	35019a1
NKX2.1	*NKX2-1*	AGCACACGACTCCGTTCTC	GCCCACTTTCTTGTAGCTTTCC	4507715a1
NURR1	*NR4A2*	TGTGTTCAGGCGCAGTATGG	TCCCGAAGAGTGGTAACTGTAG	27894349a1
OLIG2	*OLIG2*	GGACAAGCTAGGAGGCAGTG	ATGGCGATGTTGAGGTCGTG	7341207a2
OTX2	*OTX2*	CAACCGCCTTACGCAGTCAA	GGGGTGCAGCAAGTCCATAC	11119420a1
PAX6	*PAX6*	ATGTGTGAGTAAAATTCTGGGCA	GCTTACAACTTCTGGAGTCGCTA	4505615a1
PCP2	*PCP2*	AGAGGCCAGCAGAAAAGTGACT	GTGGCTCAGCAGATTGAAGAA	
PLZF	*ZBTB16*	GGGACTTTGTGCGATGTGGT	ATTGCGGTGGAAGAGGATCTC	21359888a1
PVALB	*PVALB*	GCTGAACGCTGAGGACATCAA	TCACATCATCCGCACTCTTTTTC	4506335a1
REELIN	*RELN*	TCCGGGACAAGAATACCATGT	CCAAATCCGAAAGCACTGGAA	27436938a2
SATB2	*SATB2*	TCTCCCCCTCAGTTATGTGAC	AGGCAAGTCTTCCAACTTTGAA	5689405a3
TBR2	*EOMES*	CCGGGCACCTATCAGTACAG	GGTTGCACAGGTAGACGTG	22538470a1

TH	*TH*	GCCCTACCAAGACCAGACGTA	CGTGAGGCATAGCTCCTGA	37127a2
VGAT	*SLC32A1*	CCGAGTGGTGAACGTAGCG	GTGGCGATAATGGACCAGGAC	17999520a3
VGLUT1	*SLC17A7*	CGACGACAGCCTTTTGTGGT	GCCGTAGACGTAGAAAACAGAG	9945322a2
VGLUT2	*SLC17A6*	GGGAGACAATCGAGCTGACG	CAGCGGATACCGAAGGAGATG	9966811a1
VGLUT3	*SLC17A8*	AAACCGGAAATTCAGACAGCA	CCAAAGACCCTGTTAGCAGCA	21322234a2
ZIC1	*ZIC1*	GTTCGGAGCACTATGCTGC	TTGCACGACTTTTTGGGGTTG	22547182a2
ZO1	*TJP1*	AGTCCCTTACCTTTCGCCTGA	TCTCTTAGCATTATGTGAGCTGC	28416400a1

[1] According to the HUGO Gene Nomenclature Committee

[2] Athanasia Spandidos, Xiaowei Wang, Huajun Wang and Brian Seed: PrimerBank: a resource of human and mouse PCR primer pairs for gene expression detection and quantification. Nucl. Acids Res. 2010 38:D792-9. http://pga.mgh.harvard.edu/primerbank

SUPPLEMENTARY TABLE 3

Group/Parameter		dV/dt (mV/ms)	$AP_{threshold}$ (mV)	AP_{amp} (mV)	R_{in} (GΩ)	C_m (pF)	RMP (mV)	I_{Na} max (pA)	*n*
No AP		n/a	n/a	n/a	2.4 ± 0.5	25.2 ± 2.1	-36 ± 3	-33 ± 14	13
dV/dt < 15.2		6.2 ± 1.0	-13.3 ± 2.8	17.9 ± 3.7	3.4 ± 0.4**	16.0 ± 2.0	-26 ± 2	-281 ± 30**	15
dV/dt >15.2	**TS**	39.1 ± 2.6	-24.4 ± 1.7	54.5 ± 2.2	1.9 ± 0.2	26.6 ± 3.0	-30 ± 2	-692 ± 189	26
	Ctrl	33.8 ± 4.4	-24.4 ± 1.6	51.6 ± 3.0	2.3 ± 0.4	19.4 ± 3.1	-28 ± 4	-768 ± 126	12

**, $P < 0.001$, ANOVA.

Abbreviations: AP, action potential; dV/dt, the rate of the AP rise; AP_{amp}, AP amplitude; $R_{in,}$, input resistance; C_m, membrane capacitance; RMP, resting membrane potential; I_{Na} (max), maximum amplitude of the sodium current; *n*, number of cells.

The dV/dt ratio was calculated for the first AP measured in response to increasing current injections. The $AP_{threshold}$ was measured from the phase plane plot as the voltage at which dV/dt increased abruptly. The AP_{amp} was calculated as a difference between the threshold and the peak of the AP. R_{in} was measured from the linear voltage deflections in response to negative and positive current injections around V_{hold}=[-60, -65] mV. C_m was calculated as the ratio of τ_{on} and R_{in}.

SUPPLEMENTARY TABLE 5

	Probe ID	Gene	Fold changes (TS *vs.* control)			Fold changes (depolarization *vs.* resting state)			Disease Association
			Resting neurons	*Depolarized neurons*	*Progenitors*	*TS-specific upregulation*	*TS-specific lack of upregulation*	*TS-specific downregulation*	
1	ILMN_1657451	***SRPK2***	0.70	-	-	-	-	-	ASD susceptibility gene*
2	ILMN_1752294	***PCDH9***	0.65	-	-	-	-	-	ASD susceptibility gene*
3	ILMN_1746137	***ST7***	1.36	-	-	-	-	-	ASD susceptibility gene*
4	ILMN_1814790	***SHANK2***	-	-	-	1.22	-	-	ASD susceptibility gene*
5	ILMN_1659753	***LAMP2***	0.62	0.54	0.60	-	-	-	ID susceptibility gene*
	ILMN_2243687	***LAMP2***	0.70	0.59	-	-	0.82	-	
	ILMN_2279961	***LAMP2***	-	0.68	-	-	-	-	
6	ILMN_1711596	***PQBP1***	0.68	-	-	-	-	-	ID susceptibility gene*
	ILMN_2325234	***PQBP1***	0.60	-	0.62	1.14	-	-	
7	ILMN_2252136	***YWHAE***	0.43	0.43	0.50	-	-	-	ID susceptibility gene*
8	ILMN_2361862	***VLDLR***	0.71	-	-	-	-	-	ID susceptibility gene*
9	ILMN_1673352	***IFITM2***	2.37	3.30	1.74	-	-	-	Upregulated in ASD postmortem cortex#
10	ILMN_1805750	***IFITM3***	2.46	3.24	1.57	-	-	-	Upregulated in ASD postmortem cortex#
11	ILMN_1784553	***SDC2***	1.54	-	-	-	-	0.66	Upregulated in ASD postmortem cortex#

***ASD**, autism spectrum disorders; **ID**, intellectual disability*

** The ASD susceptibility gene list was compiled by Pinto et al., Nature 466: 368–372 (2010)*

 # Garbett et al., Neurobiology of Disease 30:303–311 (2008)