Stem Cell Reports

Supplemental Information

Distinct Neurodegenerative Changes in an Induced Pluripotent Stem Cell Model of Frontotemporal Dementia Linked to Mutant TAU Protein

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Supplemental Figures

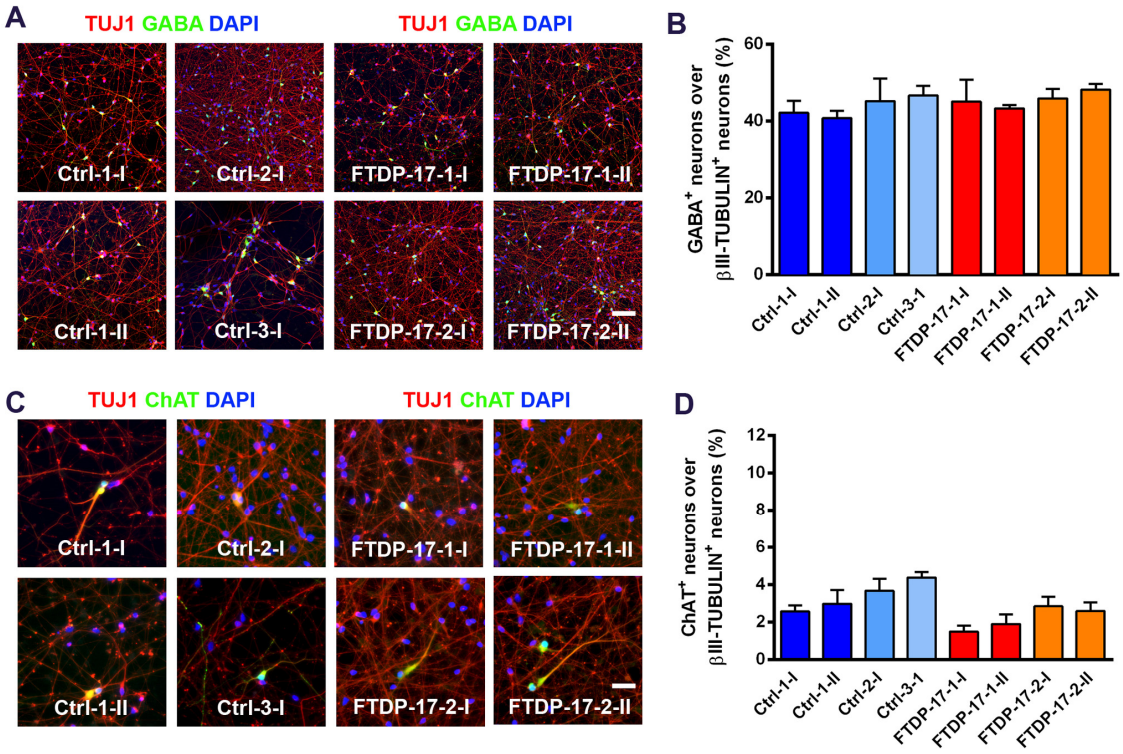

Figure S1: Differentiation of Ctrl and FTDP-17 iPS cells into mature neurons (related to Figure 2)

(**A**) Immunostainings for βIII-TUBULIN (TUJ1; red) and GABA (green) showing differentiation of Ctrl and FTDP-17 cells into GABAergic neurons. Nuclei were counterstained with DAPI (blue). Scale bar = 60 µm. **(B)** Quantification of immunostainings for βIII-TUBULIN and GABA in Ctrl and FTDP-17 neurons. Data are represented as mean of three independent differentiation experiments + SEM. **(C)** Immunostainings for βIII-TUBULIN (red) and ChAT (green) showing differentiation of Ctrl and FTDP-17 cells into cholinergic neurons. Nuclei were counterstained with DAPI (blue). Scale bar = 30 µm. **(D)** Quantification of immunostainings for βIII-TUBULIN and ChAT in Ctrl and FTDP-17 neurons. Data are represented as mean of three independent differentiation experiments + SEM.

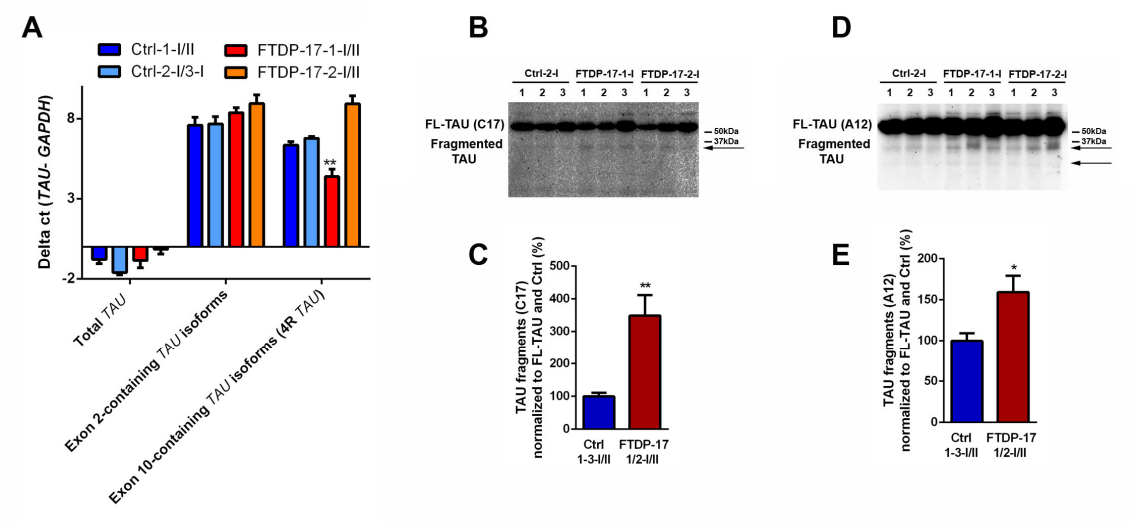

Figure S2: TAU pathology in FTDP-17 iPS cell-derived neurons (related to Figure 3).

(A) Delta ct values of qRT-PCRs on Ctrl- and FTDP-17 iPS cell-derived neurons for total *TAU*, exon 2-containing *TAU* isoforms and exon 10-containing *TAU* isoforms. Values were calculated as differences between the ct values of *TAU* (isoform) and the ct values of corresponding *GAPDH* as a housekeeping gene. Data are represented as mean of triplicates from independent differentiation experiments + SEM. One-way-ANOVA with posthoc-Tukey test was performed for statistical analysis ($^{**}p<0.01$). **(B+D)** Representative Western blot analysis on Ctrl and FTDP-17 neurons for TAU protein using the C17 (B) and A12 (D) antibodies. Independent replicates are shown for each line. **(C+E)** Quantification of TAU fragments normalized to FL-TAU and Ctrl in C17 (C) and A12 (E) Western blots. Data are presented as mean of replicates from three independent differentiation experiments per indicated lines (n=12 in C; n=11 in E) + SEM. Student's t-test was performed for statistical analysis ($^{*}p<0.05$; $^{**}p<0.01$).

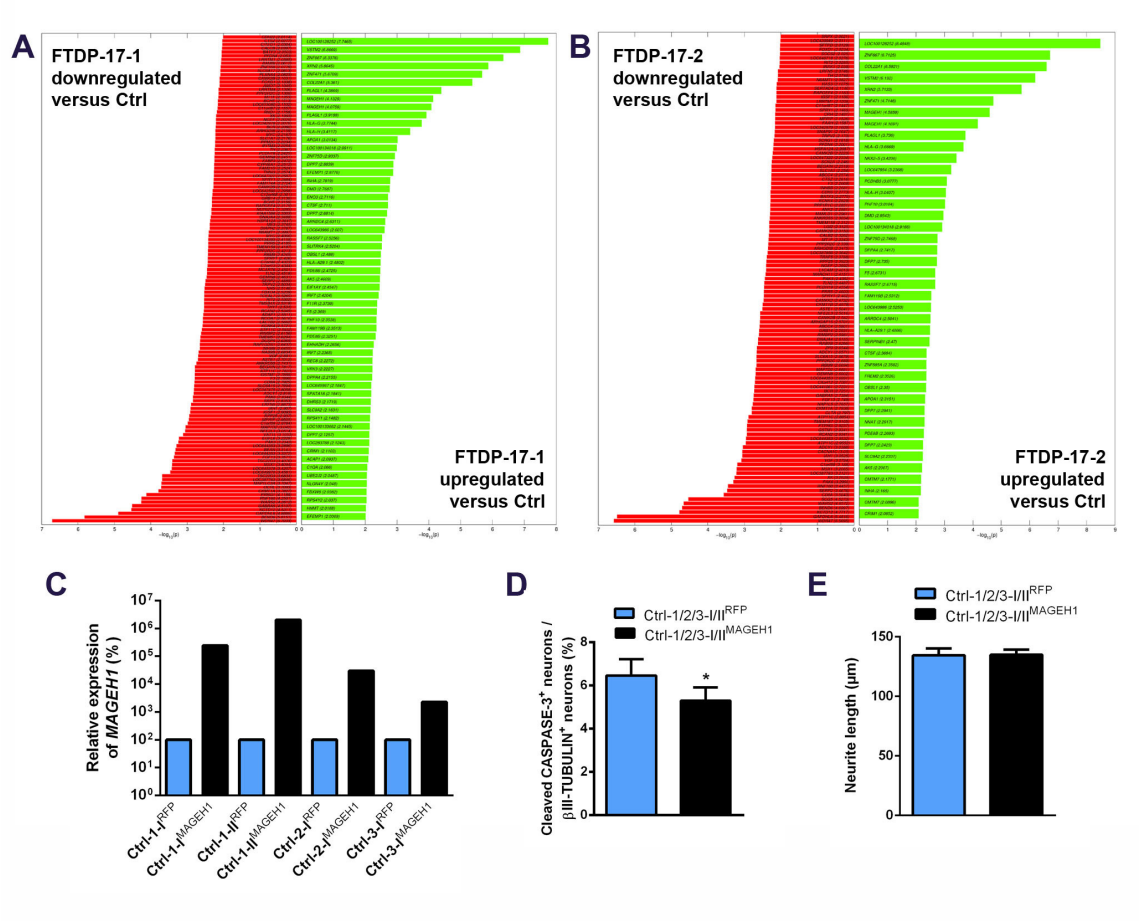

Figure S3: Differentially expressed genes in FTDP-17 neurons and *MAGEH1* overexpression in Ctrl neurons (related to Figure 5).

(A-B) Lists of differentially expressed genes (DEGs) with enhanced or reduced expression levels in FTDP-17-1 (A) and FTDP-17-2 (B) neurons and corresponding *p*-values. The bar lengths are proportional to the –log(*p*) of the DEGs. **(C)** Relative gene expression of *MAGEH1* in Ctrl-1/2/3-I/II^{MAGEH1} and Ctrl-1/2/3-I/II^{RFP} neurons. **(D)** Quantification of cleaved CASPASE-3^{+} neurons in Ctrl-1/2/3-I/II^{MAGEH1} and Ctrl-1/2/3-I/II^{RFP} neuronal cultures after 48h of rotenone treatment. At least 800 cells were counted per cell line and differentiation. **(E)** Quantification of neurite outgrowth in Ctrl-1/2/3-I/II^{MAGEH1} and Ctrl-1/2/3-I/II^{RFP} neurons 48h after plating. At least 100 neurons were measured per cell line and differentiation. Data in D and E are presented as mean of independent replicates from four different lines + SEM. Student's t-test was performed for statistical analysis ($^{*}p<0.05$).

Supplemental Tables

Table S1: Upregulated and downregulated genes in FTDP-17-1 and FTDP-17-2 neurons compared to Ctrl neurons with functional annotation (related to Figure 5)

Count	*p*-val	GSEA term link	Genes
		FTDP-17-1 upregulated	
5/152	0.00061727	EXTRACELLULAR_REGION_PART	*APOA1 C1QA DMD EFEMP1 INHA*
2/19	0.0021558	HYDRO_LYASE_ACTIVITY	*EHHADH ENO3*
2/23	0.0032029	REGULATION_OF_SECRETION	*APOA1 INHA*
3/102	0.0090825	EXTRACELLULAR_SPACE	*APOA1 C1QA INHA*
2/49	0.015058	CELL_CYCLE_ARREST_GO_0007050	*INHA PLAGL1*
2/52	0.017088	PROTEINACEOUS_EXTRACELLULAR_MATRIX	*DMD EFEMP1*
3/136	0.021052	RESPONSE_TO_EXTERNAL_STIMULUS	*F11R F5 INHA*
2/59	0.02258	GAMETE_GENERATION	*REC8 XRN2*
5/354	0.025771	BIOSYNTHETIC_PROCESS	*AK5 APOA1 DMD INHA RPS4Y1*
4/247	0.027406	CELLULAR_BIOSYNTHETIC_PROCESS	*AK5 DMD INHA RPS4Y1*
2/78	0.040793	RESPONSE_TO_WOUNDING	*F11R F5*
2/79	0.042332	SENSORY_PERCEPTION	*DHRS3 EFEMP1*
		FTDP-17-2 upregulated	
2/23	0.0025921	REGULATION_OF_SECRETION	*APOA1 INHA*
2/49	0.011854	CELL_CYCLE_ARREST_GO_0007050	*INHA PLAGL1*
3/152	0.020375	EXTRACELLULAR_REGION_PART	*APOA1 DMD INHA*
		FTDP-17-1 downregulated	
2/7	0.00080792	MONOOXYGENASE_ACTIVITY	*CYP46A1 TH*
3/29	0.001128	ANGIOGENESIS	*AMOT THY1 TNNI3*
2/11	0.0021326	CHANNEL_REGULATOR_ACTIVITY	*ADRA2A TNNI3*
4/74	0.002481	ACTIN_CYTOSKELETON_ORGANIZATION_AND_BIOGENESIS	*ADRA2A AMOT ARHGDIB RND1*
2/13	0.0030675	NEGATIVE_REGULATION_OF_CELL_ADHESION	*ARHGDIB RND1*
2/13	0.0030885	REGULATION_OF_ANGIOGENESIS	*AMOT TNNI3*
2/14	0.0036677	INTEGRIN_BINDING	*EGFL6 THY1*
3/43	0.0038277	CELLULAR_CATION_HOMEOSTASIS	*CALCB MYC THY1*
4/84	0.004137	ORGAN_MORPHOGENESIS	*AMOT MSX1 THY1 TNNI3*
2/16	0.0049455	REGULATION_OF_CELL_MIGRATION	*AMOT THY1*
4/90	0.0054186	GTPASE_REGULATOR_ACTIVITY	*ARHGDIB BCR RAPGEF4 THY1*
6/197	0.0058723	RECEPTOR_BINDING	*CALCB DIAPH2 EGFL6 FGF13 INHBB THY1*
5/155	0.0086715	CYTOSKELETON_ORGANIZATION_AND_BIOGENESIS	*ADRA2A AMOT ARHGDIB RND1 THY1*
2/22	0.0097675	HORMONE_ACTIVITY	*CALCB INHBB*
2/25	0.012662	RHO_PROTEIN_SIGNAL_TRANSDUCTION	*ADRA2A ARHGDIB*
3/81	0.024657	ENZYME_ACTIVATOR_ACTIVITY	*BCR FGF13 THY1*
2/39	0.031098	GTPASE_ACTIVATOR_ACTIVITY	*BCR THY1*
5/215	0.033959	ENZYME_REGULATOR_ACTIVITY	*ARHGDIB BCR FGF13 RAPGEF4 THY1*
3/93	0.036321	IMMUNE_RESPONSE	*ARHGDIB IFITM3 THY1*
2/42	0.036652	NEURON_DEVELOPMENT	*RND1 THY1*
2/43	0.038944	SMALL_GTPASE_REGULATOR_ACTIVITY	*ARHGDIB THY1*
5/223	0.04054	ANATOMICAL_STRUCTURE_MORPHOGENESIS	*AMOT MSX1 TH THY1 TNNI3*
3/96	0.041081	CYTOPLASMIC_VESICLE	*AMOT ARHGDIB OCRL*
2/44	0.041692	CELL_SURFACE	*SRPX THY1*

5/224	0.042267	TRANSCRIPTION_FACTOR_ACTIVITY	*BATF3 FOXD1 MYC NFE2L3 TSC22D3*

FTDP-17-2 downregulated

3/17	0.00022173	CALMODULIN_BINDING	*CACNA1C CAMKK2 RIT2*
2/5	0.00039141	NEGATIVE_REGULATION_OF_CYTOKINE_BIOSYNTHETIC_PROCESS	*INHBB SFTPD*
5/92	0.00080332	CYTOPLASMIC_MEMBRANE_BOUND_VESICLE	*ABCC4 ARHGDIB SCG5 SFTPD TMEM187*
2/7	0.00082743	HORMONE_SECRETION	*INHBB SCG5*
7/197	0.0012795	CELL_CELL_SIGNALING	*CRH FGF13 HCN2 INHBB RIT2 SCG5 SLC1A1*
4/64	0.0014954	ION_CHANNEL_ACTIVITY	*CACNA1C KCNK4 SCN2A TRPV2*
2/10	0.0018509	SECRETORY_GRANULE	*ABCC4 SCG5*
3/52	0.0069234	CATION_CHANNEL_ACTIVITY	*CACNA1C KCNK4 SCN2A*
2/22	0.0097429	REGULATION_OF_CELL_ADHESION	*ARHGDIB CDK6*
2/22	0.0098125	HORMONE_ACTIVITY	*CRH INHBB*
3/65	0.013357	CATION_TRANSPORT	*HCN2 KCNK4 SCN2A*
2/26	0.013933	VOLTAGE_GATED_CATION_CHANNEL_ACTIVITY	*CACNA1C SCN2A*
2/26	0.014035	SH3_SH2_ADAPTOR_ACTIVITY	*GRB14 SOCS2*
5/175	0.015232	SUBSTRATE_SPECIFIC_TRANSMEMBRANE_TRANSPORTER_ACTIVITY	*CACNA1C KCNK4 SCN2A SLC1A1 TRPV2*
2/35	0.026955	GTP_BINDING	*RIT2 SCG5*
2/36	0.028909	PROTEIN_PROCESSING	*CAMKK2 SCG5*

Table S2: Primary antibodies for immunocytochemistry

Antibody	Dilution	Company
mouse anti-NESTIN	1:300	R&D Systems (MAB1259)
goat anti-SOX1	1:150	R&D Systems (AF3369)
goat anti-FOXA2	1:150	Santa Cruz (sc-6554)
rabbit anti-TH	1:500	Pel Freez (P40101-150)
mouse anti-βIII-TUBULIN (TUJ1)	1:750	Covance (MMS-435P)
mouse anti-AT8	ICC 1:150 IHC 1:200	Thermo Scientific (MN1020)
rabbit anti-MAP2	1:1000	Santa Cruz (SC-20172)
rabbit anti-OCT4	1:200	Santa Cruz (SC-9081)
goat anti-NANOG	1:50	R&D Systems (AF1997)
mouse anti-SSEA-4	1:200	Millipore (SCR001/90231)
mouse anti-TRA 1-60	1:200	Millipore (SCR001/90232)
mouse anti-TRA 1-81	1:200	Millipore (SCR001/90233)
rabbit anti-HSPA5 (BiP)	1:100	Sigma (HPA038845)
rabbit anti-cleaved CASPASE-3 (Asp175)	1:1000	Cell Signaling (#9661)
goat anti-ChAT	1:300	Millipore (AB144)
rabbit anti-GABA	1:5000	Sigma (A2052)

Table S3: Primer for qRT-PCR.

*GAPDH*_for	CTG GTA AAG TGG ATA TTG TTG CCA T
*GAPDH*_rev	TGG AAT CAT ATT GGA ACA TGT AAA CC
*MAPT*_total_for	CTC GCA TGG TCA GTA AAA GCA A
*MAPT*_total_rev	GGG TTT TTG CTG GAA TCC TGG T
*MAPT*_Exon1_for	CGA AGT GAT GGA AGA TCA CG
*MAPT*_Exon2_rev	GTT CCT CAG ATC CGT CCT CA
*MAPT*_Exon2_for	TGA GGA CGG ATC TGA GGA AC
*MAPT*_Exon3_rev	TGT GGT TCC TTC TGG GAT CT
*MAPT*_Exon10_for	CCA AGT GTG GCT CAA AGG AT
*MAPT*_Exon12_rev	CCC AAT CTT CGA CTG GAC TC
*LOC100128252*_for	GCC ACT TCA TCT TGG ATG CT
*LOC100128252*_rev	GGC AAG AAT GCT GTG TCT CA
*MAGEH1*_for	GTG CCC CGG AGC AAT TTT C
*MAGEH1*_rev	CAG GCT GCA TTC CTA ACT TCC
*PAK3*_for	CAA GGG GCA TCA GGT ACT GT
*PAK3*_rev	AGA GAG CCA CCA GCC AAG TA
*SERP2*_for	GGA TGG CTA ACG AGA AGC AC
*SERP2*_rev	CAT GCC CAT CCT TAT GCT CT
*RIT2*_for	GCG GGT CCA GAG AGT ACA AG
*RIT2*_rev	GCA GTG TCC AAG ATG TCC AA
*VGF*_for	ACC ACC CTT TCC CCA ACT AC
*VGF*_rev	ATT CTC CAG CTC CTC CTG CT

Supplemental Experimental Procedures

Generation and culturing of iPS cells

Patient fibroblasts for iPS cell derivation were obtained from the Coriell Institute for Medical Research (Camden, New Jersey, USA) and had been derived from individuals carrying either the N279K mutation in exon 10 of the *MAPT* gene (FTDP-17-1; Cell repository code ND32945) or the V337M mutation in exon 12 of the *MAPT* gene (FTDP-17-2; Cell repository code ND32951). The generation of human iPS cells was performed using the lentiviral SF-OSKM-tomato vector (Warlich et al., 2011) expressing *OCT4*, *SOX2*, *KLF4* and *C-MYC* as one expression unit under the SFFV promoter as previously described (Dorn et al., 2015; Zaehres et al., 2010). Briefly, lentivirus was produced after co-transfection of packaging plasmids in 293T cells. Viral supernatant was concentrated by ultracentrifugation. Human patient-derived fibroblasts were transduced and passaged six days after transduction onto irradiated mouse embryonic fibroblast (MEF) feeders. iPS cell colonies were maintained on a layer of mitotically inactivated MEFs in human ES cell medium consisting of Knockout DMEM (Invitrogen) with 20% Knockout Serum Replacement (Invitrogen), 1 mM beta-mercaptoethanol (Invitrogen), 1% nonessential amino acids (NEAA, Invitrogen), 1% penicillin/streptomycin/glutamine (PAA), freshly supplemented with 5 ng/mL FGF2 (Peprotech). Pluripotent stem cells were split at ratios of 1:6 to 1:8 every seven days by mechanical disaggregation with 1 mg/mL collagenase IV (Invitrogen).

Characterization of iPS cells

Immunofluorescence staining of iPS cell colonies was performed using the primary antibodies OCT4 (Santa Cruz; SC-9081), NANOG (R&D Systems; AF1997), SSEA4 (Millipore; SCR001/90231), TRA 1-60 (Millipore; SCR001/90232) and TRA 1-81 (Millipore; SCR001/90233). For retroviral promoter silencing analysis, quantitative RT-PCR (qRT-PCR)

was performed using a vector-specific primer pair (*C-MYC* endo: CGT GAC CAG ATC CCG GAG TT, IRES exo: GCC TGC AAA GGG TCG CTA CA). Human iPS cells (1.5×10^6 cells/mice) were injected subcutaneously into the dorsal flank of severe combined immunodeficient mice. Eight weeks after the injection, teratomas were fixed overnight in 4% paraformaldehyde and embedded in paraffin. The sections were stained with hematoxylin and eosin dyes. For karyotype analysis, colcimid (KaryoMAX; Invitrogen) was added to a confluent culture of iPS cell-derived neural progenitor cells (NPCs) at a final concentration of 0.3 mg/mL. After three hours of incubation, cells were washed with PBS and singularized by treatment with prewarmed Trypsin-EDTA diluted in DMEM. Cells were subsequently centrifuged and the resulting cell pellet was resuspended in prewarmed KCl solution (75 mM) and incubated at room temperature for 7 minutes. Following centrifugation, cells were resuspended in ice-cold fixation solution (3:1 methanol/acetic acid) while shaking. Once fixed, cells were spun down and taken up in fresh fixative. Following 20 min at 4°C, different dilutions of cells were dropped onto glass slides (Menzel Gläser, Thermo Scientific) for analysis. Chromosomes were GTG-banded using standard procedures and metaphase spreads were analyzed on a Zeiss AxioScop and using the Cytovision software (Applied Imaging Corporation).

Generation and culturing of neural progenitor cells

NPCs were derived from iPS cells by treatment with small molecules as previously described (Hargus et al., 2014; Reinhardt et al., 2013). In short, iPS cell colonies from passages 10-15 were mechanically sectioned and enzymatically detached from MEFs. Pieces of iPS cell colonies were collected by sedimentation, resuspended in hES cell medium (without FGF) supplemented with 10 µM SB-431542 (Ascent Scientific), 1 µM dorsomorphin (Tocris), 3 µM CHIR99021 (CHIR; Axon Medchem) and 0.5 µM purmorphamine (PMA; Alexis) and subsequently cultured as embryoid bodies (EBs) in petri dishes. The medium was changed

after two days to N2B27 medium consisting in equal parts of DMEM-F12 (Invitrogen) and Neurobasal (Invitrogen) with 1:200 N2 supplement (Invitrogen), 1:100 B27 supplement lacking vitamin A (Invitrogen), 1% penicillin/streptomycin/glutamine and with the same small molecule supplements as mentioned before. On day 4, SB-431542 and dorsomorphin were withdrawn and 150 µM ascorbic acid (AA; Sigma) was added to the medium. On day 6, EBs were disintegrated into smaller pieces and plated on matrigel-coated (Matrigel, growth factor reduced, high concentration; BD Biosciences) 12-well plates (Nunc) in NPC expansion medium consisting of N2B27 supplemented with CHIR, PMA, and AA. Cells were split at ratios of 1:10 to 1:15 every five to six days.

Neuronal differentiation of neural progenitor cells

Generation of neurons from NPCs has been previously described (Hargus et al., 2014; Reinhardt et al., 2013). Briefly, singularized NPCs from passages 10 to 13 were plated on matrigel-coated plates in NPC expansion medium at a density of 2×10^5 cells per well. Two days after plating, the medium was changed to N2B27 medium supplemented with 10 ng/mL FGF8, 1 µM PMA and 200 µM AA. After six days of differentiation, the neuronal induction medium was replaced by neuronal maturation medium consisting of N2B27 with 10 ng/mL BDNF, 10 ng/mL GDNF, 10 ng/mL TGF-β3 (all from Peprotech), 200 µM AA and 500 µM dbcAMP (Sigma Aldrich). Cultures of neurons were split at ratios of 1:3 to 1:5 when confluent by detachment with a cell spatula and disintegration of the neuronal network into smaller pieces using a 1,000 µL pipette. Medium was changed every other day and cultures were analyzed after 20 days in maturation medium unless indicated otherwise.

Immunocytochemistry

For confocal microscopy, cells were cultured on matrigel-coated glass coverslips. After fixing the cells for 20 min at 4°C with 4% paraformaldehyde in PBS (Invitrogen), cells were washed

three times with 0.1% Triton X-100 (Sigma) in PBS (PBS-TX). Blocking was performed using 10% normal goat serum (NGS) in PBS-TX for 45 min at room temperature. Subsequent to blocking, coverslips were washed three times with PBS and primary antibodies were applied overnight at 4°C in 10% NGS in PBS. The next day, following three washing steps with PBS, secondary antibodies were applied for one hour at room temperature. Cells were subsequently washed three times with PBS-T (0.05% Tween-20), including a DAPI counterstaining for nuclei in the second washing step. Cells were mounted in Shandon Immu-Mount mounting medium (Fisher Scientific) and imaged on a Zeiss LSM700 confocal microscope. For quantification purposes, differentiated cells were disaggregated and seeded as single cells at a density of 6 x 10^4 cells per well in maturation medium on matrigel-coated 24-well plates. Two days after replating, cells were fixed and stained as mentioned above. Primary antibodies and their corresponding dilutions used in this study are listed in Table S2. All secondary antibodies were obtained from Invitrogen and were conjugated to AlexaFluor fluorochromes.

Quantitative RT-PCR

Total RNA was extracted from NPC-derived neurons with the RNeasy mini kit (QIAGEN) according to the manufacturer´s protocol and including an on-column DNase digest. Isolated RNA was reverse-transcribed using the High Capacity cDNA reverse Transcription Kit (Applied Biosystems). qRT-PCR was performed on an Applied Biosystems StepOne Plus real time cycler with the Power SYBR Green PCR master mix (Applied Biosystems). The melting curve of each sample was determined to ensure the specificity of the products. PCR conditions were as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 sec at 95°C and 1 min at 60°C. Relative expression levels were calculated using the $2^{-2\Delta}$ method and normalized to biological reference samples and using *GAPDH* as the housekeeping gene unless otherwise noted. The primer sequences used in this study are listed in Table S3.

Electrophysiology

All measurements were conducted at room temperature in whole-cell configuration of the patch-clamp technique. Recording pipettes with resistance from 6-7 MΩ were made from borosilicate glass (GT150TF-10, Clark Electromedical Instruments, Pangbourne, UK) and filled with a solution containing (in mM): K-gluconate, 88; K_3-citrate, 20; phosphocreatine, 15; NaCl, 10; HEPES, 10; $MgCl_2$, 1; $CaCl_2$, 0.5; BAPTA, 3; Mg-ATP, 3; and Na_3-GTP, 0.5 to maintain cytosolic energy sources, buffer conditions and physiological ion concentrations. The pH was set to 7.25 with KOH and osmolality was set to 295 mOsm/kg. Cells were continuously superfused with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 125; KCl, 2.5; NaH_2PO_4, 1.25; $NaHCO_3$, 24; $MgSO_4$, 2; $CaCl_2$, 2; glucose, 10. The pH was adjusted to 7.35 by aeration with 95% O_2/5% CO_2 and osmolality was set to 305 mOsm/kg. Recordings were done with an EPC-10 amplifier and the Patchmaster Software (HEKA Elektronik, Lamprecht, Germany). Mean ± SD of resting membrane potential [mV] / input resistance [MΩ] / capacitance [pF] / series resistance [MΩ] were as follows: Ctrl-1-I: -65.86±3.87 / 826.57±311.38 / 14.41±7.37 / 11.1±2.59; Ctrl-2-1: -57.29±3.01 / 898.57±122.77 / 14.35±4.34 / 11.94±2.41; FTDP-17-1-I: -58.43±7.67 / 1012.0±232.03 / 17.15±5.92 / 12.11±1.54; FTDP-17-2-I: -65.75±11.64 / 710.25±200.53 / 17.52±4.87 / 12.71±3.62 . Voltage clamp mode: from a holding potential of −70 mV cells were subjected to a step protocol from -140 to 80 mV with an increment of 10 mV (duration 500 ms). Current clamp mode: action potentials were elicited with for each cell determined current steps to induce hyperpolarization in 10 mV steps from -100 to -10 mV. Tetrodotoxin (TTX, 0.5 µM, Tocris) was used to block voltage gated sodium channels. Currents under control conditions and TTX application were recorded successively from identical cells. A liquid junction potential of 12 mV was measured and taken into account when analyzing the data. Recordings were digitally analyzed using the Fitmaster software (HEKA Elektronik) and illustrated with Origin 8.5G (Origin Lab, Northampton, MA, USA).

Whole genome expression analysis

RNA samples for microarray analysis were prepared using RNeasy columns (Qiagen) with on-column DNA digestion. 300 ng of total RNA per sample was used as the input in the linear amplification protocol (Ambion), which involved the synthesis of T7-linked double-stranded cDNAs and 12 hours of *in vitro* transcription incorporating the biotin-labeled nucleotides. Purified and labeled cRNA was then hybridized for 18 hours onto HumanHT-12 v4 expression BeadChips (Illumina) following the manufacturer's instructions. After the recommended washing, the chips were stained with streptavidin-Cy3 (GE Healthcare) and scanned using the iScan reader (Illumina) and the accompanying software. The samples were exclusively hybridized as biological replicates.

Microarray data processing

The bead intensities were mapped to the corresponding gene information using BeadStudio 3.2 (Illumina) and background correction was performed using the Affymetrix Robust Multi-array Analysis (RMA) background correction model (Irizarry et al., 2003). Variance stabilization was performed using the log2 scaling, and gene expression normalization was calculated with the quantile method implemented in the lumi package of R-Bioconductor. Data post-processing and graphics were performed with in-house developed functions in MATLAB. Hierarchical clusters of genes and samples were performed with the one minus the sample correlation metric and the Unweighted Pair-Group Method using Average (UPGMA) linkage method as previously described (Kim et al., 2009)**.**

Protein analysis

Cell pellets of differentiated neurons were extracted on ice with RIPA-Buffer (Tris pH 8, NaCl, natriumdesoxycholate, NP40, SDS) containing protease inhibitors (Mini complete, Roche). Protein lysates were mixed with 6x Laemmli buffer and incubated at 95°C for 5

minutes. Denatured proteins were loaded on a 4-12% polyacrylamide gel (NuPAGE, Invitrogen) and electrophoretically separated before being transferred on a PVDF membrane. The membrane was blocked and incubated with antibodies against TAU (clone HT7, Pierce, MN1000, mouse, 1:1000; clone TAU-5, Invitrogen, AHB0042, mouse, 1:1000; clone A12, Santa Cruz, sc-166062, mouse, 1:100; clone C17, Santa Cruz, sc-1995, goat, 1:150; clone C3, Millipore, 36-017, mouse, 1:1500), BiP (Cell Signaling, C50B12, rabbit, 1:1000), p-PERK (Santa Cruz, sc-32577, rabbit, 1:1000) or PUMA (Abcam, ab9643, rabbit, 1:1000). After three washing steps, the blot was incubated with an HRP-coupled secondary antibody. The membrane was washed and then incubated with chemiluminescent HRP substrate solution (Millipore, GE). Protein expression levels were quantified by densitometric analysis using the Image Lab software (BioRad) and standardized on GAPDH (Sigma, G9545, rabbit, 1:200000) or β-ACTIN (Sigma, A5441, mouse, 1:200000).

Analysis of post-mortem tissue from FTDP-17 patients by immunohistochemistry, qRT-PCR and Western blot

Formalin fixed and paraffin-embedded sections and frozen post-mortem tissue from FTDP-17 patients (FTDP-17 A: 62 years, female, P301L; FTDP-17 B: 55 years, female, P301L; FTDP-17 C: male, N279K; FTDP-17 D: 47 years, female, N279K) and control individuals (Ctrl A: 49 years, male; Ctrl B: 80 years, female) were obtained from the Department of Pathology and Laboratory Medicine (Dr. Bernardino Ghetti´s Lab) at Indiana University. Immunohistochemistry was performed on de-paraffinized and rehydrated sections on an automated staining system (Auto Stainer Link 48, DAKO). Sections were counterstained with haematoxylin before being dehydrated and covered in Eukitt. Antibodies employed for immunohistochemistry were anti-AT8 (no pretreatment; 1:200) and anti-BiP (pretreatment with target retrieval solution, pH 6.1; 1:100).

For qRT-PCR, RNA was isolated from frozen midbrain tissue specimen using Maxwells 16 LEV simplyRNA Tissue Kit on Maxwell 16 Instrument (Promega). Isolated RNA was transcribed into cDNA using the High Capacity cDNA reverse Transcription Kit (Applied Biosystems) and qRT-PCR was performed on an Applied Biosystems StepOne Plus real time cycler with the Power SYBR Green PCR master mix (Applied Biosystems).

For Western blot analysis, cell lysates were generated from frozen midbrain tissue specimen by mechanical grinding of the tissue followed by homogenization with an ultra turrax. SDS sample buffer was added to samples holding 40 µg of total protein followed by incubation at 95°C for 5 min. Protein samples were separated in 10% polyacrylamide/SDS gels and subsequently blotted onto PVDF membranes. Primary antibodies employed for Western blotting were anti-BiP (Cell Signaling, rabbit, 1:1000) and anti-GAPDH (Sigma, rabbit, 1:200000). The HRP-labeled antibody anti-rabbit (Cell Signaling, 1:5000) was used as secondary antibody. Visualization of the proteins was achieved by incubation with chemiluminescent HRP substrate solution (Millipore, GE).

Knockdown of *MAGEH1*

Gene knockdown of *MAGEH1* was performed using lentiviral particles containing a mix of 5 different Mission® shRNA (Sigma) constructs encoding *MAGEH1*-specific shRNAs. Control cell populations were prepared using scrambled Mission® shRNA (Sigma) lentiviral particles. Virus particle production and transduction of NPCs was done as previously described (Hargus et al., 2014). Cells that had not been transduced were removed prior to neuronal differentiation through puromycin (5 µg/ml) selection for one week.

Lentiviral expression of *MAGEH1*

MAGEH1 was cloned from cDNA derived from FTDP-17-1 neurons using gene specific primers with attb gateway cloning sites attached (for: 5´-

GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGCCTCGGGGACGAAAGAGTCGG- 3′, rev: 5′-GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAAGGGGCGGAATAACCCCTAGCA-3′). PCR was performed using Phusion polymerase (NEB) and PCR conditions were set according to the manufacturer's recommendations. *MAGEH1* sequence integrity was verified by sequencing. For overexpression in NPCs, the PCR product was introduced into the pLEX_307 plasmid using the Gateway cloning technique. The expression vector pLEX_307 was a gift from David Root (Addgene plasmid # 41392). Production of the lentiviral pLEX_307 vector expressing either MAGEH1 or dtTomato (red fluorescent protein) and transduction of NPCs was performed as previously described (Hargus et al., 2014). Cells that had not been transduced were removed prior to neuronal differentiation through puromycin (5 μg/ml) selection for one week.

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