SUPPLEMENTAL DATA

Human iPSC generation and characterization: Three oriP/EBNA—based episomal vectors (obtained from Addgene), pEP4EO2SEN2K, pEP4EO2SET2K, and pCEP4-M2L were co-transfected into 2X10^6 human newborn fibroblasts (ATCC CRL-2703) via nucleofection using Amaxa 4D-nucleofector system. The transfected fibroblasts were placed on Matrigel-coated dishes in human fibroblast medium (DMEM, 10% FBS, 2mM L-glutamine, 50U/ml penicillin + 50 mg/ml streptomycin). On day one post-transfection, the fibroblast medium was replaced with human embryonic stem cell (hESC) medium (GlobalStem, Molecular Transfer, Inc.), supplemented with 20% KnockOut™ serum (Invitrogen) and 0.5mM sodium butyrate (Invitrogen).{Choi, 2011 #18} Culture medium was refreshed every other day. Around 18 days, the visible ESC-like morphology colonies were picked and transferred to MEF feeder layers in hESC medium supplemented with 20ng/ml of basic fibroblast growth factor (bFGF, Invitrogen).

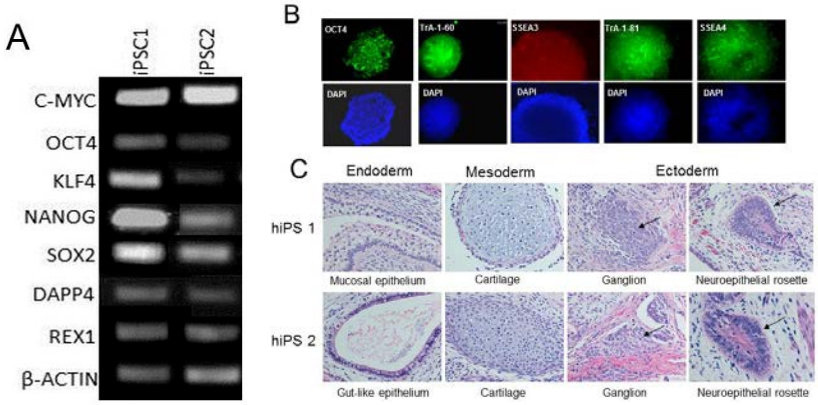

Figure S1. Characterization of iPSC

A) RT-PCR of hES-marker genes in 2 iPSC colonies. B) immunofluorescent labeling of iPSC colony 1 with hESC markers Oct4, TrA-1-60, SSEA3, TrA-1-81, SSEA4. C) Teratoma formation after in vivo implantation of iPS cells from 2 colonies. 3 embryonic germ layers (endoderm - mesothelial and gut-like epithelium; mesoderm – cartilage, and ectoderm – ganglion and neuroepithelial rosettes) were observed in each teratoma. 40X

Verification and characterization of human iPS colonies: 1) Cytogenetic analysis/karyotyping: G-banded chromosomal analyses was performed by the UTHSCSA IIMS Clinical and Molecular Cytogenetics Lab. Figure S1 shows the results for passage 7 of iPSC B1. 2) AP staining. iPS cells were washed twice with cold PBS and fixed by 4% paraformaldehyde (Fisher Scientific) for 2 minutes at RT. Fixed cells were washed 3X with PBS and stained using the Leukocyte Alkaline phosphatase kit (Sigma) according to the manufacturer's instructions. 3) RT-PCR: Expression of the hES-cell marker genes State-Specific Embryonic Antigen 3 and 4 (SSEA3 and 4), Tumor-Related Antigens (TrA1-60, TrA-1-81), Oct3/4, Nanog, and Sox2, and Developmental Pluripotency-associated Protein 4 (DPPA4) is assessed using the primer information provided in Seki et al.{Seki, 2010 #73} Total RNA was isolated with Ambion TaqMan Gene Expression Cells-to-CT Kit (Life Technologies) and PCR performed with high fidelity Platinum PCR Supermix (Invitrogen). 4) Immunostaining:

Primary antibodies used are: anti-SSEA-1, anti-TRA 1-60, anti-TRA 1-80(Millipore) at 1:50, Anti-SSEA-3 (Millipore) at 1:50, Anti-Oct 3/4 (Millipore) at 1:100. Secondary antibodies used were Anti-mouse IgG Alexa Fluor 488 (Invitrogen), Anti-Rat IgM Dylight 650(Abcam), and Anti-mouse IgM Alexa Fluor 488 (Invitrogen). 5) Demonstration of pluripotency potential: Human iPSCs at passage 12 were collected, and 2x10^6 cells were injected into hind tibialis muscles of 15-month old RAG2-/- immunodeficient mice (by Stem Cell Core of Barshop Institute, UTHSCSA). At 6-8 weeks post-injection, teratomas were obtained and fixed in 4% paraformaldehyde, paraffin embedded and processed for histology (UTHSCSA/CTRC Histology Core Lab). The sections were stained with hematoxylin and eosin. Representative tissues of three embryonic germ layers were observed indicating that the tumor was teratoma and the iPS cells have *in vivo* capability of differentiation into the three embryonic germ layers.

qRT-PCR: cDNA was synthesized from 2ug DNaseI-treated total RNA using High-Capacity cDNA Reverse Transcription Kit (A&B Applied Biosystems™ #CAT: 4368814). Real time PCR was run in triplicate with RT² SYBR Green FAST Mastermix kit (Qiagen) using Applied Biosystems 7900 HT, sequence detection system. Cycle threshold value was generated and the -ΔΔCt method was used to determine fold-change of gene expression between samples normalized to either 18S, or TATA-box binding protein (TBP). TBP was used as internal control when comparing across developmental time points as mRNA transcript abundance is stable for this gene across these time points. Results from a representative set of genes is shown in Figure S2.

qRT-PCR primer list

GENE	FORWARD PRIMER	REVERSE PRIMER
ASCL1	CAACGCCACTGACAAGAAAG	GGAGCTTCTCGACTTCACCA
NPY	CAGGCAGAGATATGGAAAAC	TTACACGATGAAATATGGGC
AGRP	GAAGAAGACAACTGCAGAAC	CAGGTCTAGTACCTCTGC
POMC	GACACTGGCTGCTCTCCAG	AGCAGCCTCCCGAGACA
MC4R	CTTATGATGATCCCAACCCG	GTAGCTCCTTGCTTGCATCC
LEPR	ATGTTCCGAACCCCAAGAAT	GGACCACATGTCACTGATGC
FTO	CTGGCCAGTGAAAGGGTCTAAT	GGCAGCAAGTTCTTCCAAAGC
ADIPOR2	CCCAAGAAGTCCGAGACACG	TGTTGGCTCGTTCATGGGAT
SLC17A7 (VGLUT1)	TCAATAACAGCACGACCCAC	TCCTGGAATCTGAGTGACAATG
TCF7L2	AAGAGCAAGCGAAATACTAC	CTTCTTTCCATAGTTATCCCG
LINGO2	TCCAACGACACAAGTTCTAATG	TATTCAAGGTCAATGCTGGTT

FOXP2	CCACGAAGACCTCAATGGTT	TCACGCTGAGGTTTCACAAG
IRX3	CTCTCCCTGCTGGGCTCT	CAAGGCACTACAGCGATCTG
18S	ATCGGGGATTGCAATTATTC	CTCACTAAACCATCCAATCG
GAPDH	ACAGTTGCCATGTAGACC	TTTTTGGTTGAGCACAGG
TBP	AACAACAGCCTGCCACCTTA	GCCATAAGGCATCATTGGAC

Western blotting: Neuronal cultures were collected and lysed in protein extraction buffer and incubated on ice for 30 minutes with vortexing briefly. Protein concentration was assessed using Bio-Rad DC Protein Assay. 30 ug protein were separated on 4- 15% mini-protein TGX Precast Gels (Bio Rad #Cat:456-1084) and transferred to PVDF membranes. Primary antibodies used were: rabbit-anti-TCF7L2 (Cell Signaling Technology, CAT#:2569S,1:1000), anti-GLP1R (Abcam, CAT#:ab39072, 1:1000) Anti-AGRP (Abcam, CAT#:ab89114, 1:800). Secondary antibody was anti-rabbit (cell signaling technology, CAT#: 7074S). The membrane was developed with ECL substrate (Fisher Scientific, #CAT: PI-34087) mixed with SuperSignal West Femto Substrate (PI-34094, Fisher scientific #CAT: PI-34094).

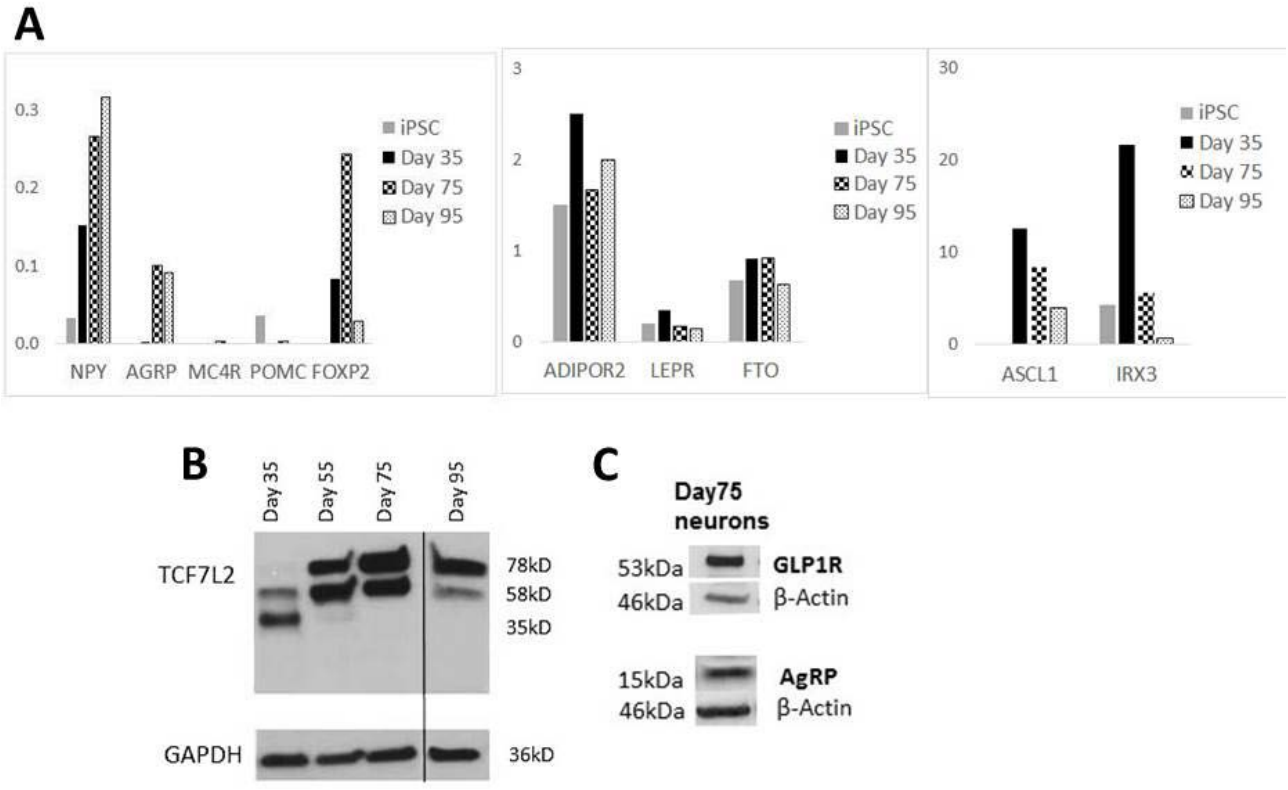

Figure S2. A) mRNA levels of representative genes assayed by qRT-PCR in iPSC and neuronal cultures at Days 35, 75 and 95. The y-axis shows expression relative to TBP. Missing bars indicate no detectable expression at that time point. B) Western blot of TCF7L2 protein in neurons. Neuronal cultures were assayed at Days 35, 55, 75 and 95 for TCF7L2 expression. C) Western blot of GLP1R and AgRP in Day 75 neurons.

FACS: As described in the methods of the main document, flow cytometric analyses of neurons was conducted using indirect immunofluorescent staining for NPY and AGRP on 3 independent neuronal cultures under standard non-fasting conditions similar to that used for transcriptomic profiling. The standard workflow and results for an unstained control sample are shown in Figure S3.

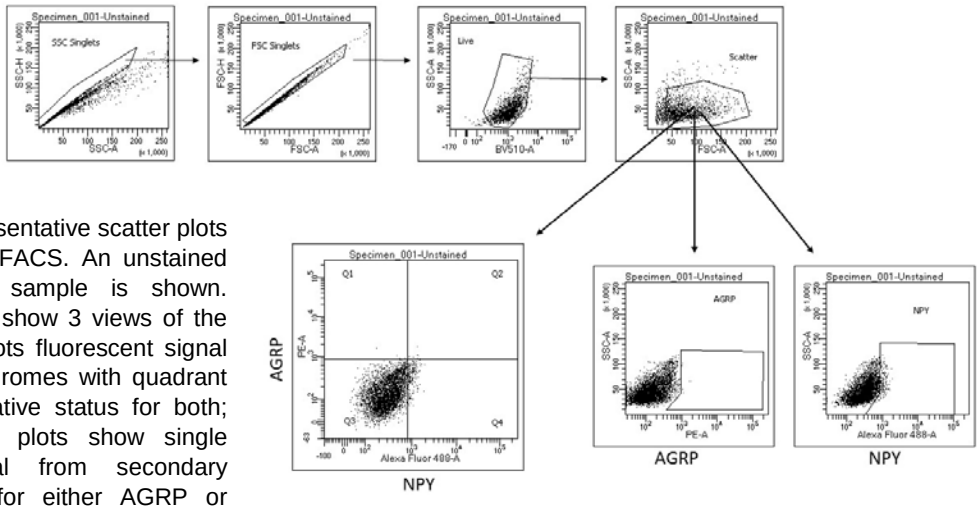

Figure S3. Representative scatter plots generated during FACS. An unstained neuronal culture sample is shown. Lower three plots show 3 views of the live cells – left plots fluorescent signal from both fluorochromes with quadrant Q3 denoting negative status for both; middle and right plots show single fluorescent signal from secondary antibodies used for either AGRP or NPY.

***Immunostaining*:** To determine presence of astrocytes in cultures, differentiated neurons were harvested and transferred to PLO/laminin-coated glass coverslips for immunostaining with glial fibrillary associated protein (GFAP). Two antibodies for GFAP were used: Millipore, cat#MAB360 mouse monoclonal, and Abcam cat#7260 rabbit polyclonal. Mouse C57Bl6 primary astrocytes were used as a positive control for GFAP (derived in lab of James Lechleiter, UT Health San Antonio). Figure S3 shows representative images of 3 separate cultures. No GFAP positive cells were observed in the neuronal cultures with either antibody as shown in Figure S4.

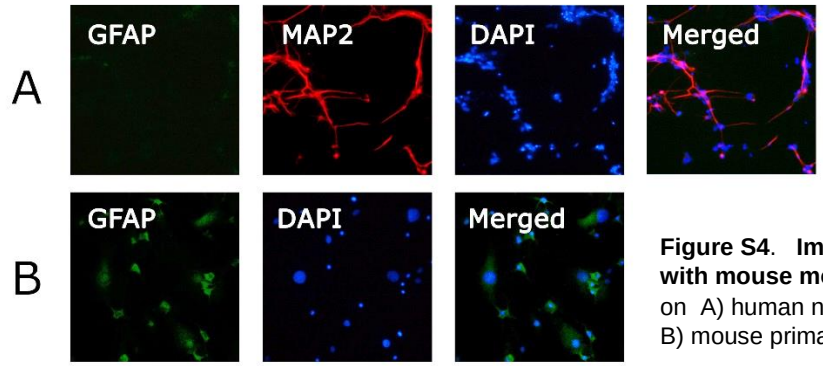

Figure S4. **Immunostaining with mouse monoclonal GFAP** on A) human neuronal culture B) mouse primary astrocytes

***Patch-clamp procedure for mature neurons**:* Whole-cell recordings of 75-day neurons were made with patch pipettes. Currents were recorded using an Axopatch-200A amplified and digitized (5-10 kHz) using Patch Master (Heka Electronik, Lambrecht, Germany). Analysis was carried out with Igor (Wavemetrics Inc.,OR). The external recording solution contained (mM): NaCl, 150; KCl, 4; MgCl2, 1; glucose, 10; CaCl2, 2; TES, 5 (pH 7·2). The recording borosilicate glass pipettes had resistances of 3-5 MΩ when filled with the internal solution containing (mM): KCl, 150; EGTA, 0.5; TES, 5 (pH 7·2), 4 mM Mg-ATP. The holding potential was −50 mV in all experiments. Glutamate was dissolved in external recording solution and applied to the cells through a double-barrel fast piezoelectric perfusion system via a Burleigh PZ150M.

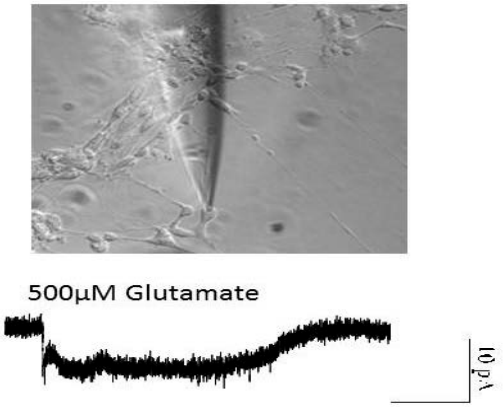

Figure S5 Patch clamp recording of human iPSC-derived neuron

K-means cluster analysis of miRNAs identified through RNAseq: For the 1206 miRNA identified using RNAseq, K-means cluster algorithm was applied to identify miRNA clusters that follow similar expression patterns during progression from Day 35-115. Calinski and Silhouette index were evaluated to identify optimum number of clusters for k-means algorithm. Six clusters best represented the 299 miRNAs that were significantly differentially expressed at absolute fold change>2 between at least two time points.

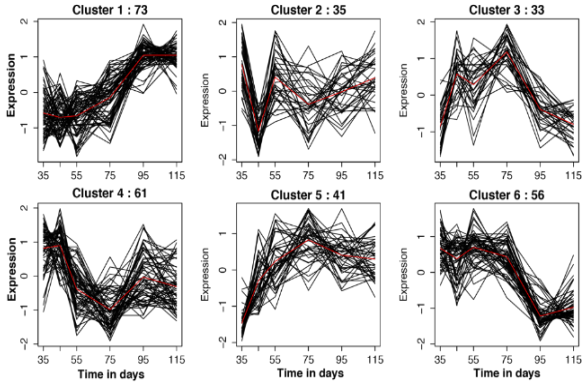

Figure S6. K-means cluster analysis of miRNA expression profiles across neuronal maturation Days 35-115. The number of genes belonging to each cluster are shown at the top of each plot. The y-axis plots the expression of each transcript centered and scaled so that each gene will have mean 0 and sd 1. Neuronal culture age in days is shown on the x-axis.

Benchmarking *in vitro* neuronal development to *in vivo* development: Isoform switching of developmentally regulated genes as assessed by mRNA expression profiles: increase in SLC17A7 encoding VGLUT1 and decrease in SLC17A6 encoding VGLUT2 at Day 95; decrease in GLRA2 encoding glycine receptor alpha 2 subunit and increase in GLRB encoding glycine receptor beta subunit at Day 95; moderate increase in GLRA1 encoding glycine receptor alpha 1 subunit at Day 115.

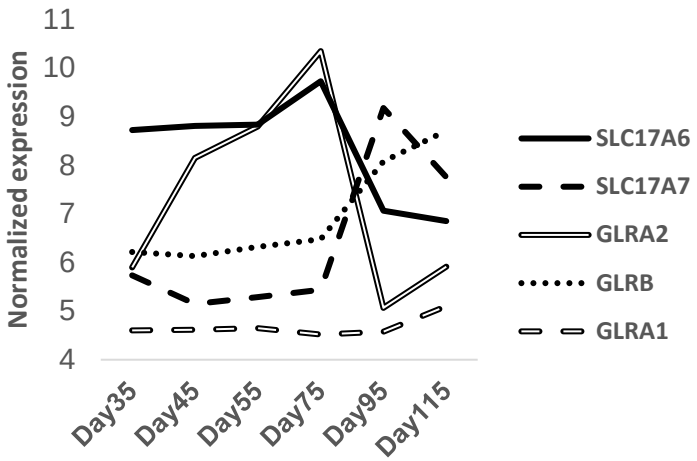

Figure S7. Temporal mRNA expression of genes undergoing isoform switching.