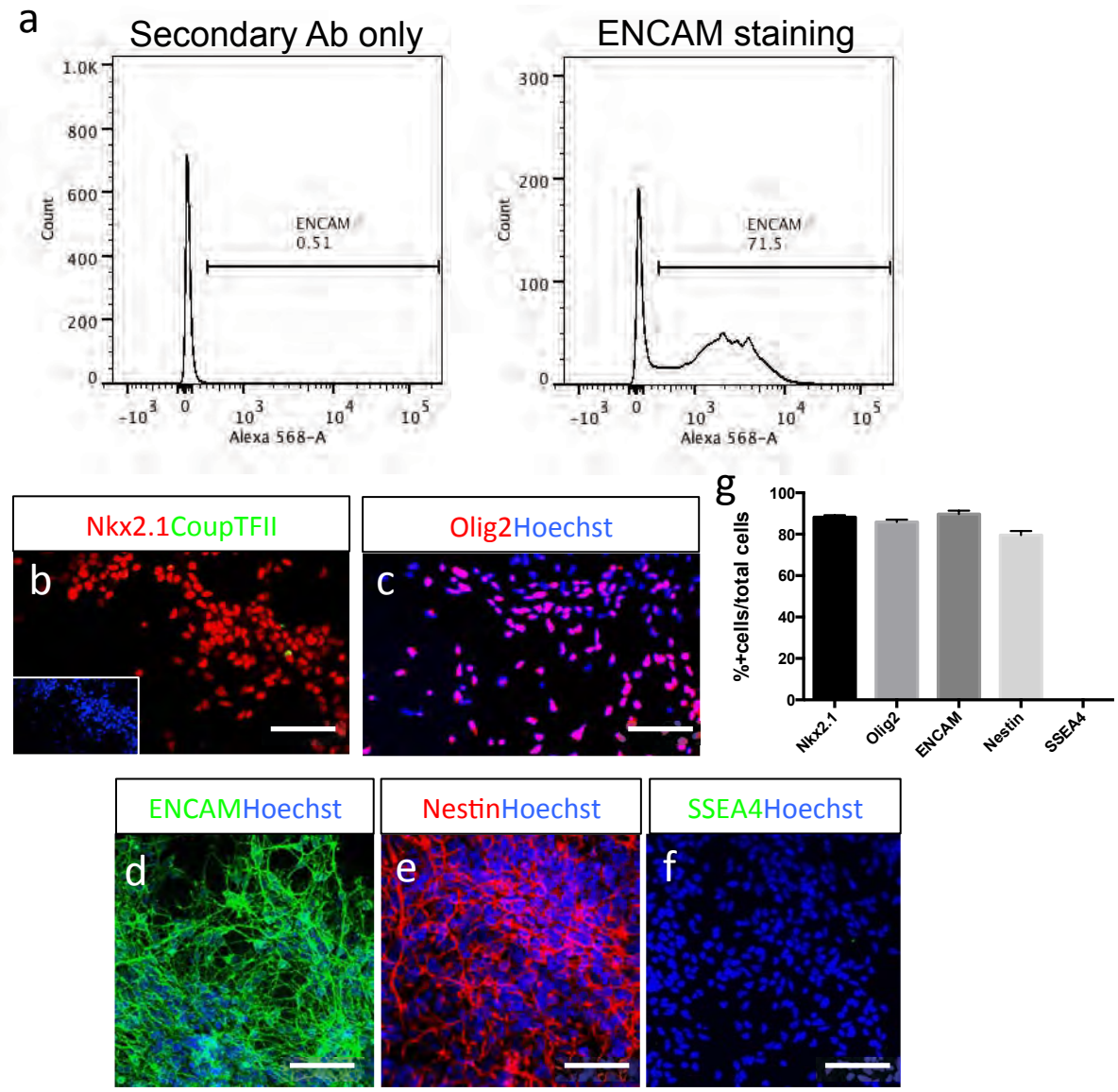
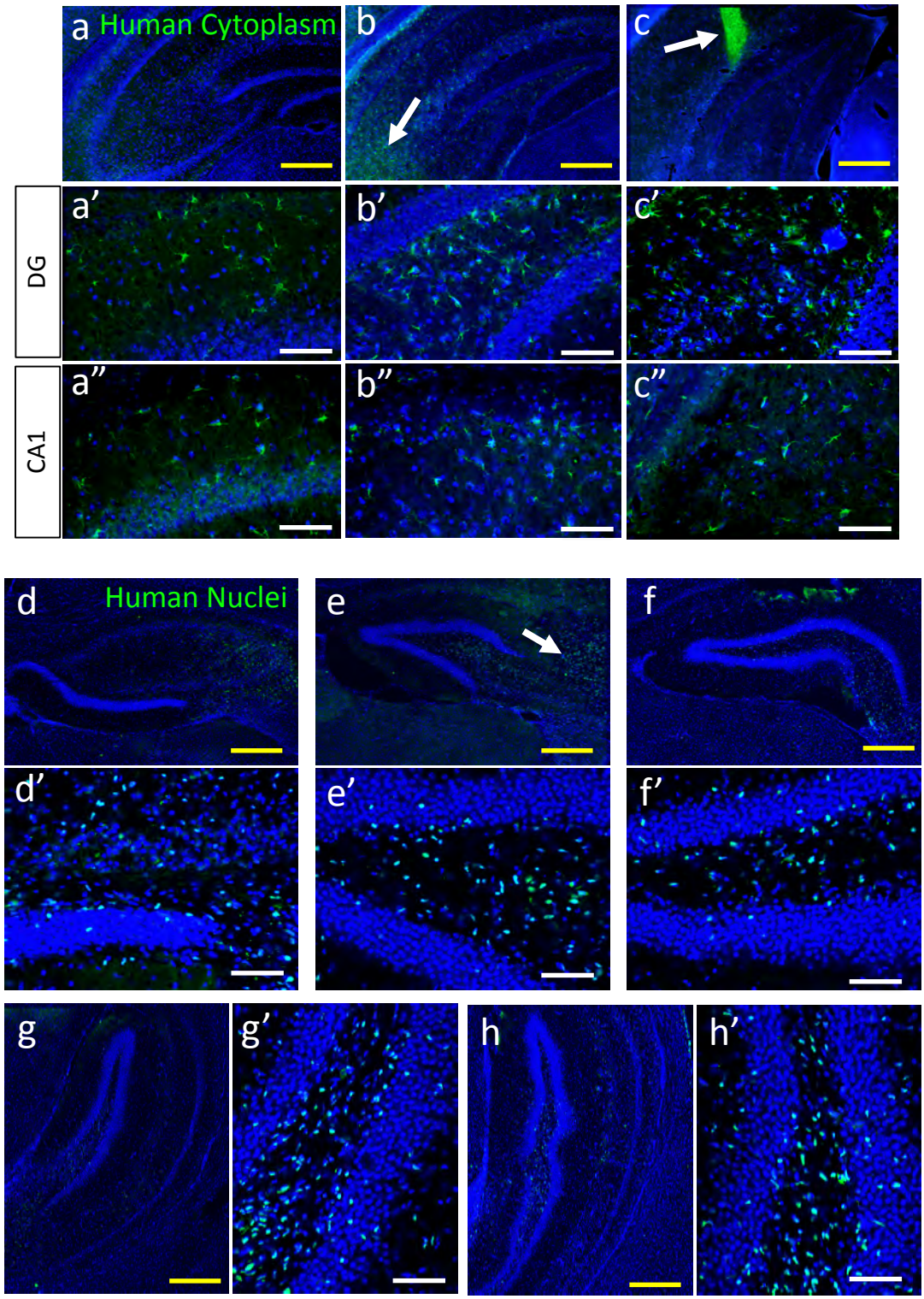


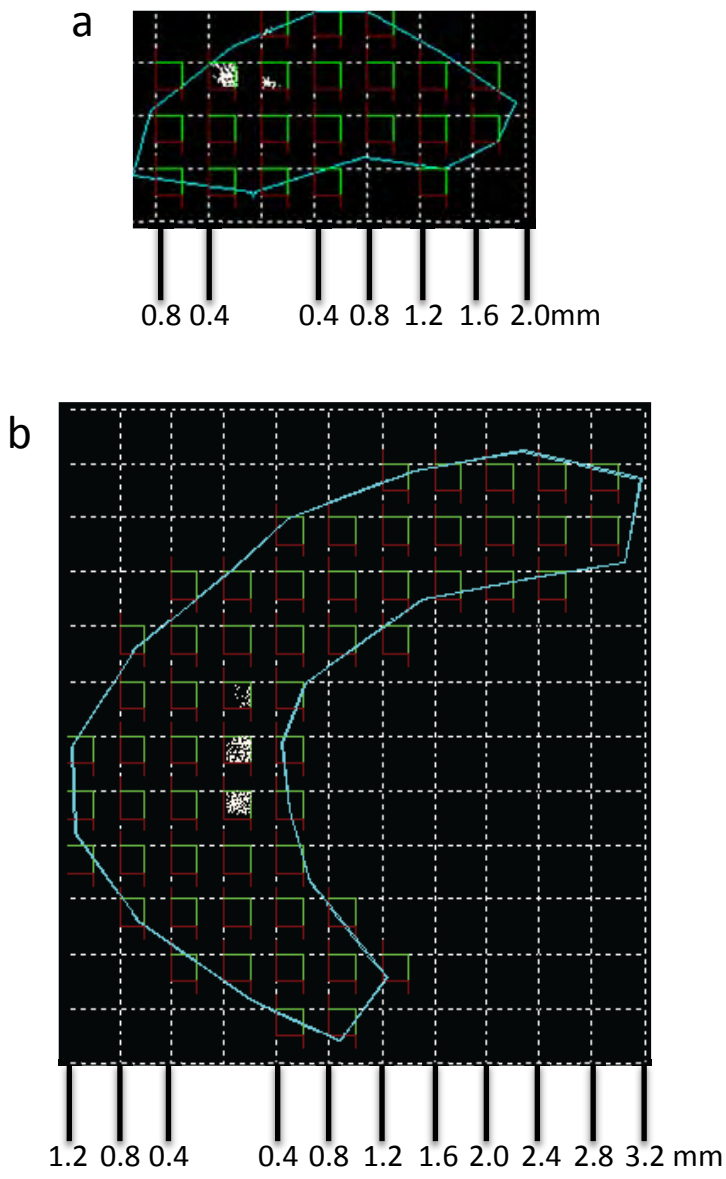
Supplementary Figure 1. FACS isolation of ENCAM+ cells after MGE differentiation of human pluripotent stem cells.

(a) Representative FACS graph for isolating ENCAM^{+} cells. Left panel shows secondary antibody only control, and right panel show cells with ENCAM staining. (b-f) Immunocytochemistry analysis of FACS isolated cells. White scale bar: 100 μm. (g) Cell counting analysis of FACS sorted cells (n=3). Related to Fig. 1.



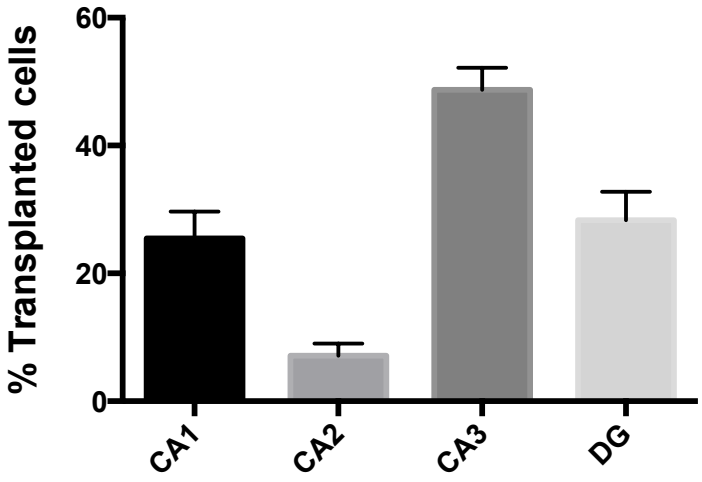
Supplementary Figure 2. Transplanted human MGE cells migrate robustly and integrate in adult epileptic brains.

Four month PT, transplanted cells display robust migration and integration into the host brain, as shown by human cytoplasm-specific antibodies (a-c) and human Nuclei-specific antibodies (d-h). a'-h' show enlarged picture of dentate gyrus regions and a"-c" show enlarged picture of CA1 regions from corresponding figures, depicting migration from the injection site. White arrows indicate injection sites. Yellow scale bar: 500 μm. White scale bar: 100 μm. Related to Fig. 1.

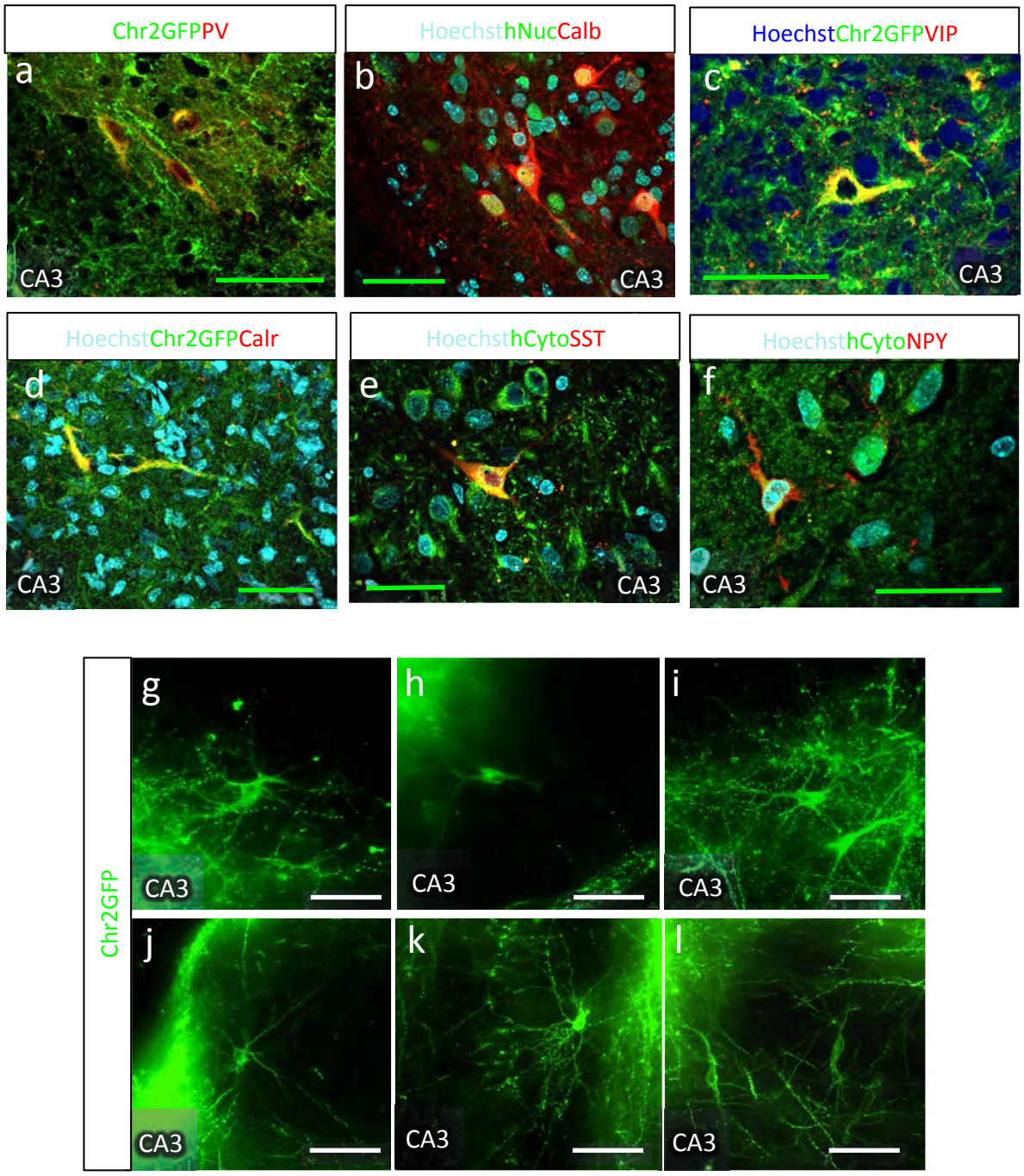


Supplementary Figure 3. Representative cell counting trace along with counting grid and counting frame used.

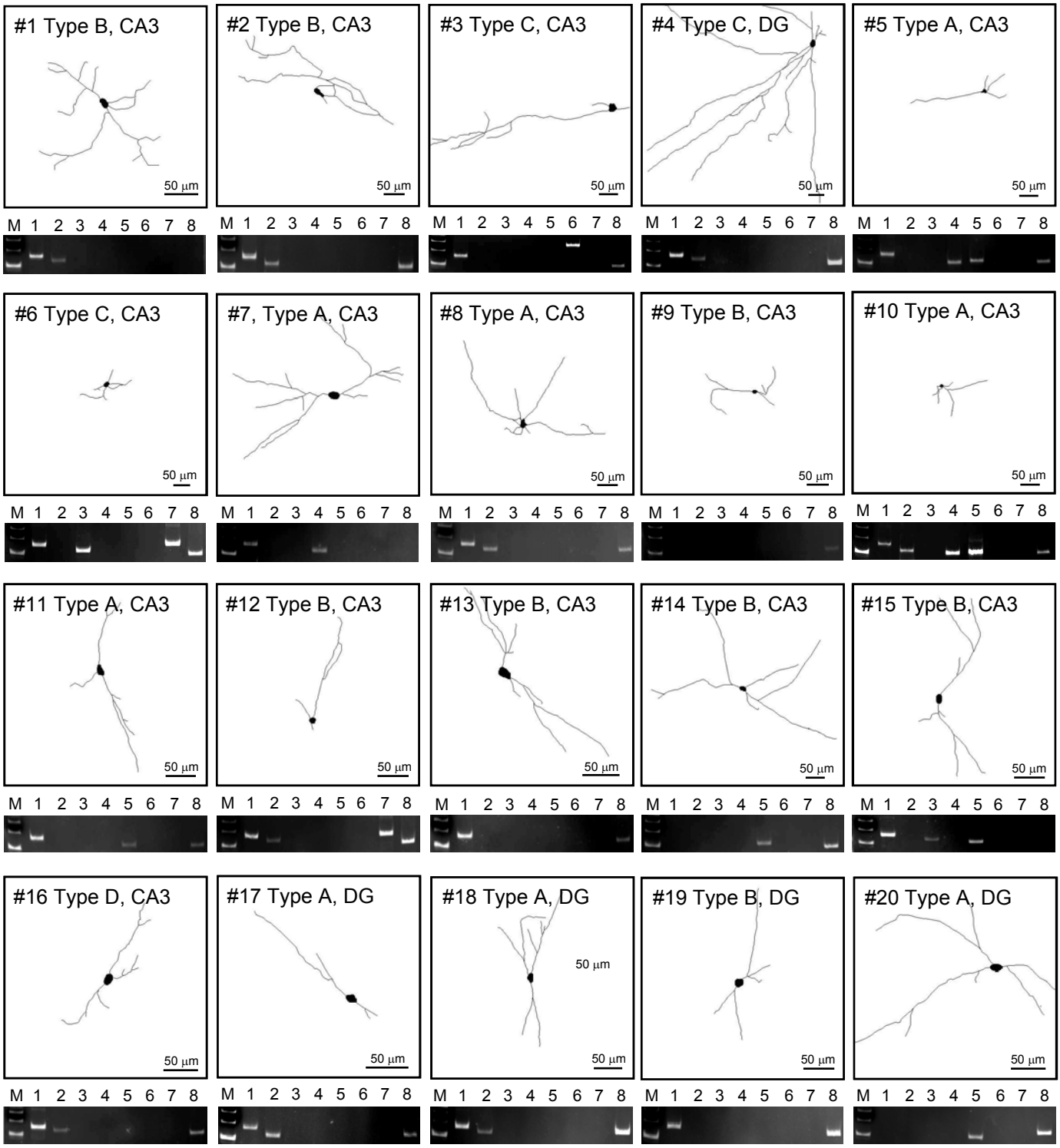
Two of the cell counting traces from 2 week PT brain sections. White dotted lines are counting grid (400 μm X 400 μm), and red and green squares (200 μm X 200 μm) are counting frame where actual counting took place. Green contour was drawn along the hippocampus and white dots are cell counting traces. Related to Fig. 1.



Supplementary Figure 4. quantification of transplanted cells in different areas of hippocampus. Related to Fig. 1.

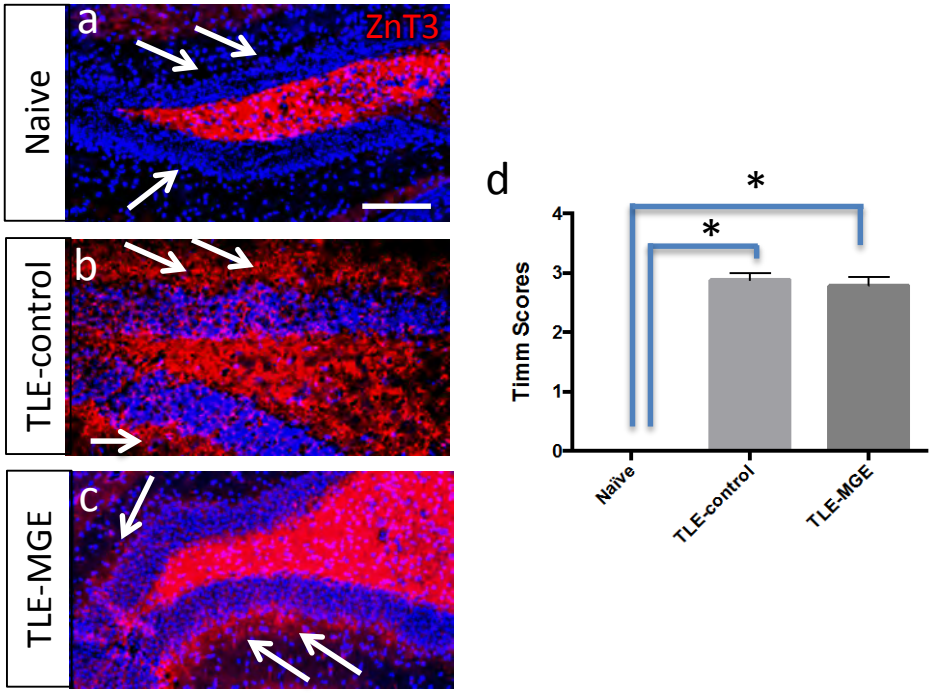


Supplementary Figure 5. Immunohistochemitry analysis of transplanted neurons. Green scale bar: 50μm. White scale bar: 100 μm. Related to Fig. 2.



Supplementary Figure 6. Morphology and RNA profile of human MGE-derived cells transplanted into the hippocampus

Neurolucida tracing shows the cell body and processes of individual grafted cells in the hippocampus. CA3, cornu ammonis region 3; DG, dentate gyrus. Action potential firing pattern of each cell is also indicated as type A (repetitive, see Figure 4c), type B (single), type C (delayed), and type D (burst). Gel images below the traces indicate the RNA profile of the corresponding cells. M, size marker (300, 200, and 100 bp from top to bottom); lane 1, GAD67; lane 2, GAD65; lane 3, parvalbumin; lane 4, calreticulin; lane 5, somatostatin; lane 6, vasoactive intestinal peptide; lane 7, neuropeptide Y; lane 8, Sox6. Related to Fig. 4.



Supplementary Figure 7. MGE transfection does not affect Mossy fiber sprouting.

Hippocampal brain sections from naïve Nod-Scid mice, TLE control mice and transplanted TLE mice were stained using anti-ZnT3 antibody, to analyze mossy fiber sprouting. White arrows in b & c indicate mossy fiber sprouting, whereas in a points absence of mossy fiber sprouting. White scale bar: 100 μm. (d) Quantification of Mossy fiber sprouting (n=7). Related to Fig. 7.

Supplemental Experimental Procedures

PSC culture and differentiation into MGE cells

Human H7 ESC (WA07, WiCell, passage 41-51) was maintained on Matrigel (BD, San Hose, CA) in mTeSR media (Invitrogen, Calsbad, CA) with 10 ng/ml bFGF (Peprotech, Rocky Hill, NJ), and passaged using Dispase (Stem Cell Technologies, Vancouver, BC, Canada). For differentiation, PSCs were trypsinized and grown as floating aggregates in low adherent flasks in KSR media (20 % knockout serum replacement, DMEM, 2 mM L-glutamine and 10 μM β-mercaptoethanol (all from Invitrogen)). Rock inhibitor (Y-27632, 10 μM, Tocris, Bristol, United Kingdom) was added on the first day of differentiation. After two weeks of floating culture, cells were transferred to polyornithine- (PLO; 15 mg/ml; Sigma, St. Louis, MO) and fibronectin- (FN; 1 mg/ml; Sigma, St. Louis, MO) coated surfaces in N3 media. For MGE induction, cells were treated with LDN193189 (100 nM, Stemgent, Cambridge, MA) from d0 to d14, with SB431542 (10 μM, Tocris) from d0 to d7, with IWP2 (5 μM, EMD Millipore) from d0 to d7, with SAG (0.1 μM, EMD Millipore) from d0 to d21, and with FGF8 (100 ng/ml, Peprotech) from d8 to d21 (Kim et al., 2014). After 3 weeks of differentiation, cells were transferred to differentiation media (N3 media (Chung et al.) with 10 ng/ml GDNF (Peprotech), 10 ng/ml BDNF (Peprotech) and 2.5 μM DAPT (Tocris). At day 25 of differentiation, cells were subject to a fluorescence-activated cell sorting (FACS). Cells were trypsinized and incubated with anti-ENCAM antibody (BD) in FACS media (phenol-free, Ca^{++}, Mg^{++} -free Hank's buffered saline solution (HBSS; Invitrogen, Carlsbad, CA) containing Penicillin-Streptomycin, 20 mM D-Glucose and 2% fetal

bovine serum) for 20 min, followed by incubation for 10 min with Alexafluor-568-conjugated anti-rat IgM antibodies (Invitrogen, Carlsbad, CA). All washing steps were performed in FACS media. Cells were filtered through cell strainer caps (35 μm mesh) to obtain a single cell suspension (5 x 10^6 cells/ ml for sorting), followed by FACS using FACSAria (BD Biosciences, San Jose, CA) and FACSDiva software (BD Biosciences, San Jose, CA). Debris, dead cells and doublets were excluded by forward and side scatter gating. ENCAM positivity was determined compared to negative controls lacking the primary antibody and lacking primary and secondary antibodies. A portion of sorted cells were plated on PLO/FN-coated coverslips for immunocytochemical analysis and the rest were resuspended in transplantation media (HBSS with 10 ng/ml GDNF, 10 ng/ml BDNF and 20 μM Boc-Asp(OMe) fluoromethyl ketone (BAF; Sigma-Aldrich)) and used for transplantation.

Induction of temporal lobe epilepsy (TLE) in Nod-Scid mice

The Animal Care and Use Committee at McLean Hospital approved all animal procedures. The mice were housed with 12 hr light/dark cycles with free access to food and water. For induction of TLE, 7-week old male and female Nod-Scid mice (Charles River Laboratory) were injected with 400mg/kg Pilocarpine i.p., 30 minutes after N-methylscopolamine bromide (1 mg/kg, ip) administration to reduce peripheral cholinergic effects (Mazzuferi et al., 2012). To limit the duration of status epilepticus (SE) and extent of damage in the hippocampus, diazepam (10 mg/kg) was injected ip 90 min after seizure induction. The severity of convulsive responses was monitored and classified according to the modified Racine scale (Shibley and Smith, 2002). Ten days after pilocarpine

injection, mice that showed stage 3, 4 or 5 seizure were subject to 7 days of continuous video monitoring for spontaneous recurrent seizures (SRS) using an Eco Black Box security camera system (Lorex Technology). Mice showing SRS with stage 3, 4 or 5 during the 7-day recording period were designated as "TLE mice" in this study, and they were randomly assigned for subsequent transplantation and behavioral analysis.

Transplantation of hMGE cells into hippocampus of TLE model mice.

FACS-sorted hESC-derived MGE cells or the same volume of transplantation media as described above were injected into hippocampus of TLE mice using a Leica Angle Two digital stereotaxic instrument (Leica Biosystems) fitted with a Cunningham Mouse Adaptor (Stoelting, Inc, Downers Grove, IL). TLE mice were anesthetized using an induction chamber supplied with 4-5 % isoflurane (Sigma) mixed with 0.8-1 L/min oxygen using a calibrated vaporizer. Animals were then administered continuous isoflurane (1-2%) mixed with oxygen (0.8-1.0 L/min) via snout mask for the duration of anesthesia. Body temperature was maintained using air-activated iron oxide heat packets. Cell suspensions or control vehicle injections were disseminated throughout the hippocampus with one rostral injection and three caudal injections (Hunt et al., 2013) at the following coordinates: AP 1.75 mm, L $\pm$2.3 mm, V -1.7 mm for rostral CA3 site; AP 3.25 mm, L $\pm$3.0 mm, V -3.65 mm, -2.9 mm and -2.0 mm for the three caudal sites along the dorso-ventral axis of the hippocampus in this coronal plane. Injection coordinates were verified by ink injections as well as cell injection followed by histological analysis 1-2 weeks after transplantation. A total of 5 x 10^4 MGE cells in a 0.5 µl volume were delivered at each target coordinate. Animals received a sterile, stainless steel bone screw

recording electrode (diameter 0.5mm, length 1.1mm; Plastics One), soldered with a recording lead wire, positioned epidurally through a rostral burr hole in the skull (AP 1.75 mm, L +2.3 mm), and a reference electrode was positioned caudal to lambda. Electrodes were cemented in place with a rapid-curing dental cement (DenMat Holdings, Lompoc, CA).

Behavioral analysis

Mice were maintained under a 12-hours light/dark cycle with water and food available ad libitum. All behavioral tests were done during the light phase of the light/dark cycle.

Continuous Video-EEG recording of transplanted mice

Three months after transplantation, seizure activity of control or MGE-transplanted TLE mice was recorded using a MP150 Biopac data acquisition System, EEG100C EEG amplifier module and AcqKnowledge 4.0 EEG Acquisition and Reader Software (BIOPAC Systems Inc.) along with Eco Black Box security camera system (Lorex Technology). EEG seizures with high-frequency, high-voltage synchronized polyspike profiles with amplitudes greater than 2-fold background and a duration of greater than 15 sec (Hunt et al., 2013) were analyzed using AcqKnowledge 4.0 EEG Acquisition and Reader Software (BIOPAC Systems Inc.) by investigators who were blind to treatment conditions. This was followed by confirmation of EEG seizure activity by video recording. Each animal was recorded over 5-10 days, totaling 42 days for naïve mice (n=6), 79 days for control TLE mice (n=11), and 83 days for MGE-transplanted TLE mice (n=9). The mice with more than 15,000 surviving human nucleus^{+} cells in each

hippocampi were included in the behavioral analysis. (One mouse from MGE-transplanted group did not meet this criterion and was excluded. It showed mild seizure activity with 0.7 seizure per day).

Y maze

We used a three-arm Y maze for this study: each arm 3 cm wide, 40 cm in length, and with a wall height of 12 cm. Mice were initially placed within one arm, and the sequence and number of entries was recorded for each mouse over a 10 min period. The percentage of triads in which all three arms are represented (i.e., ABC, CAB or BCA, but not BAB for example), was recorded as a spontaneous alternation, and used to estimate short-term memory. The total number of arm entries was used as an indicator of locomotor activity. Arms were cleaned between tests to remove odors and residues. Y maze test was done under normal ambient room lighting.

Novel object recognition test

For a training session, each mouse was placed into an open field box (42 x 42 x 31 cm) containing two identical objects and allowed to freely explore for 3 min. One hour after the training session, one of the familiar objects was replaced with a novel object (defined as the test session). The time that each animal spent exploring the novel object compared to the familiar object was recorded and traced using Ethovision software (Noldus, Wageningen, The Netherlands), with a 3 cm radius around each object as the "interaction zone". The test box and objects were cleaned between sessions. Results are expressed as recognition index (% Novel object exploration duration = time spent near

novel object/[time spent near novel object + time spent near familiar object] x 100). The percentage visit frequency is calculated as follows (% Novel object exploration frequency = number of visit to novel object/[number of visit to novel object + number of visit to familiar object] x 100).

Locomotion test

The home cage (7 1⁄2" x 11 1⁄2" x 5") containing an individual mouse was placed in the center of a photobeam activity system (PAS) monitoring frame (San Diego Instruments) with 4x8 photobeam configuration for 15 min under standard overhead lighting conditions. Total photobeam break numbers were detected by PAS software.

Handling test

Aggressiveness of the mice was assessed as described previously (Hunt et al., 2013) with some modifications. Each of the following three tasks was performed for 15 sec: 1) nonstressful handling (stroking slowly along the back of the mouse in the direction of the grain of fur), 2) stressful handling (vigorous stroking against the grain of the fur), 3) pinching at the tail base with a rubber-ended forceps (Fine Science tools). Reaction to each handling was scored by investigators blinded to treatment conditions using the following rating scale: 1- initial struggle, but calmed within 15 sec, 2- struggle for more than 15 sec, 3- struggle for more than 15 sec and exhibiting one or more defensive reactions (piloerection, flattening of the ears against the head, attempt to bite or back away from the experimenter), and 4- struggled for more than 15 sec and exhibited

flight behavior (loud vocalization or wild running). Summation of these three scores provided a total aggressiveness score for each mouse.

Immunohistochemistry, Cell counting and statistical analysis

Transplanted mice were terminally anesthetized with an ip overdose of pentobarbital (150 mg/kg, Sigma) and perfused transcardially with heparin saline (0.1 % heparin in saline) followed by paraformaldehyde (4 %) 2 weeks or 4 months post grafting. Brains were removed, postfixed in 4 % paraformaldehyde for 12 hours, equilibrated in 20 % sucrose/PBS solution, and then sectioned coronally at 40-μm using a freezing microtome. For immunofluorescence staining, tissue sections were incubated with blocking buffer (PBS, 10 % normal donkey serum (NDS)) containing 0.1 % Triton for 10 minutes. Cells were then incubated overnight at 4°C with primary antibodies diluted in PBS containing 2 % NDS. The primary antibody list can be found at the end of Supplemental Experimental Procedures. After rinsing with PBS, samples were incubated with fluorescent dye-labeled secondary antibodies (Alexa 488- Alexa 568- or Alexa 647-labeled IgG; Invitrogen, Carlsbad, CA) in PBS containing 2 % NDS for 30 minutes at room temperature. After rinsing with PBS, Hoechst 33342 (4 mg/ml) was used for counterstaining, and tissue sections were mounted onto slides in Mowiol 4–88 (Calbiochem, Gibbstown, NJ). Confocal analysis was performed using an Olympus DSU Spinning Disc Confocal on an IX81 inverted microscope (Center Valley, PA), installed with MetaMorph software. StereoInvestigator image-capture equipment and software (Microbright Field, Williston, VT) were used for cell counting and estimation of total cell number in the graft using the optical fractionator workflow from every 12th sections. A

400 μm x 400 μm grid was used along with 200 μm x 200 μm counting frame as shown in Figure S3. For migration analysis, human Nuclei+ cells within 400 μm, between 400-800 μm, 800-1200 μm or1200-1600 μm from injection tract were counted using separate markers.

To assess mossy fiber sprouting, Timm scores were determined as previously described (Shibley and Smith, 2002), with 0 for no ZnT3 staining in granule cell layer, 1 for patchy staining in granule cell layer, 2 for punctate staining in molecular layer and 3 for continuous staining in molecular layer.

Transmission Electron Microscopy (TEM)

For TEM analysis, mice were perfused in 4 % PFA/0.5 % glutaldehyde solution, and brains were removed, postfixed in the same fixative, and 40 μm coronal slices obtained using a vibrating microtome. Immunohistochemistry was performed as described above, using anti-human cytoplasm antibody and biotinylated anti-mouse antibody, followed by an ABC kit (Vector) and a diaminobenzidine (DAB) substrate kit (Vector) with Nickel intensification according to manufacturer's instruction. DAB-stained brain slices were post-fixed in an aqueous solution of 1 % osmium tetroxide (OsO_4, Electron Microscopy Sciences) and 1 % potassium ferrocyanide (Electron Microscopy Sciences) for one hour, followed by embedding in Embed 812 epoxy resin (Electron Microscopy Sciences) using standard ethanol gradient dehydration and propylene oxide:resin gradient infiltration and polymerization protocols. Ultrathin (70-80 nm) sections were cut from graft core region and collected on 200 mesh copper grids

(Electron Microscopy Sciences) previously cleaned ultrasonically in acetone. Images were acquired on a JEOL 1200EX TEM operating at 80 kV accelerating voltage.

Single cell reverse transcription-polymerase chain reaction (scRT-PCR)

After whole-cell patch-clamp recording for more than 20 minutes, the recording pipettes were withdrawn slowly for the formation of outside-out patch. Intracellular contents (~ 6 μl) were expelled from the pipettes and collected in Eppendorf tubes containing 1.0 μl RNaseOUT (40 U/ μl, Invitrogen) and 1.5 μl nuclease-free water and stored at –20 °C. Samples were first treated with TurboDNase (Invitrogen) that is compatible with high-salt recording solutions to remove genomic DNA contamination, followed by reverse transcription using SuperScript III (Invitrogen) with pool of gene-specific outside primers in 20 μl reaction according to manufacturer's instruction. cDNAs were subject to 25 cycles (95°C, 2 min; 25 cycles of (95°C, 20 s; 55°C, 20s; 72°C, 20s); 72°C, 5min) of pre-amplification using pool of gene-specific outside primers and GoTaq DNA polymerase (Promega) in 50 μl reaction. In the second round of PCR, 1 μl each cDNA was amplified using nested primers and GoTaq DNA polymerase (Promega) with 30 cycles (95°C, 2 min; 30 cycles of (95°C, 20 s; 55°C, 20s; 72°C, 20s); 72°C, 5min) in 25 μl reaction. To check the possibility of mRNA contamination from surrounding tissues, recording patch pipettes were inserted into the same brain slices containing the hippocampus without the formation of tight giga-seal and its contents was analyzed likewise and did not show any signal above background level (Fig. 4f, n = 3). As a positive control, we used 1 ng of total human brain RNA and all primers successfully

amplified cDNA with correct size. Primer list can be found at the end of Supplemental Experimental Procedures.

Antibody list used in the experiments.

Antibody	Species	Dilution	Source
ß-tubulin	Rabbit	1/2000	Covance
ß-tubulin	Mouse	1/2000	Covance
Calbindin	Rabbit	1/10000	Swant
Calretinin	Goat	1/5000	Swant
GABA	Rabbit	1/1000	Sigma
Lhx6	Rabbit	1/1000	Gift from Dr. Pachnis
NCAM	Mouse	1/1000	SCBT
Nestin	Mouse	1/1000	Millipore
Neuropeptide Y	Sheep	1/5000	Millipore
Nkx2.1 (TTF1)	Rabbit	1/2000	Epitomics
Human Nucleus	Mouse	1/1000	Millipore
Olig2	Rabbit	1/500	Millipore
Parvalbumin	Mouse	1/5000	Millipore
PSD-95	Rabbit	1/1000	Cell Signaling
PSD-95	Mouse	1/1000	Neuro Mab
Somatostatin	Rat	1/5000	Millipore
Synaptophysin	Rabbit	1/2000	Pierce
Synaptophysin	Mouse	1/500	Abcam
ENCAM	Rat	1/1000	BD
NeuN	Mouse	1/500	Chemicon
VGAT	Rabbit	1/1000	Synaptic Systems
Gephyrin	Mouse	1/1000	Synaptic Systems
Human Cytoplasm	Mouse	1/500	Stem Cells Inc
Ki67	Mouse	1/1000	Millipore
Sox6	Rabbit	1/1000	Millipore
Somatostatin	Goat	1/1000	SCBT
GFAP	Rabbit	1/1000	Dako
CoupTFII	Mouse	1/1000	Persus Proteomics
SSEA4	Mouse	1/1000	SCBT
Cy3-Human Nucleus	Mouse	1/1000	Millipore
ZnT3	Rabbit	1/1000	Gift from Dr. Palmiter
VIP	Rabbit	1/1000	ImmunoStar

Primer list.

Outside primers		
GAD67	Forward	ATACCTCTTCCAGCCAGAC
	Reverse	GCTCGCCATTGAAAACCATC
Parvalbumin	Forward	ACCTGTCTGCTAAAGAAACC
	Reverse	GGGGATGGGGGAGTAAAAAATAAC
Somatostatin	Forward	CAACCAGACGGAGAATGATG
	Reverse	GCTGAAGACTTGGAGGATTAG
Neuropeptide Y	Forward	TAGGTAACAAGCGACTGGGG
	Reverse	GGGCTGAAAATAGGAAAAGGC
Calretinin	Forward	AAGGCAAGGAAAGGCTCTGG
	Reverse	CCGTTCAAGTCAAACATCCG
Vasoactive intestinal peptide	Forward	TCTTCTCACAGACTTCGGC
	Reverse	CATTTGTTTTCTAAGGCGGG
Sox6	Forward	ATACAAACCCCGACCGAAACGCAC
	Reverse	CCGCCATCTGTCTTCATAC
GAD65	Forward	AACACCACTTTGTCTCTGAG
	Reverse	GGTAGTTTGGCACACCTAAC

Nested primers		
GAD67	Forward	GCAGTATGATGTCTCCTACG
	Reverse	GTATTCAGCCAGTTCCAGG
Parvalbumin	Forward	TGCTGGAGACAAAGATGGGGAC
	Reverse	ATTGGGTGTTCAGGGCAGAGAG
Somatostatin	Forward	GCAGGATGAAATGAGGCTTG
	Reverse	CAGGATGTGAAAGTCTTCCAG
Neuropeptide Y	Forward	GCTGCGACACTACATCAAC
	Reverse	CTCATTTCCCATCACCACATTG
Calretinin	Forward	TCCTGCCAACCGAAGAGAAC
	Reverse	TAGCCACTCCTGTCTGTGTC
Vasoactive intestinal peptide	Forward	CTCTTTACAGGGCACCTTC
	Reverse	GGTCTTCTGAGATGTTACTGC
Sox6	Forward	AGCAACTGATGAGGTCTCG
	Reverse	CACCAGGATACACAACACC
GAD65	Forward	AAATCGTAGGTGTTGGCTC
	Reverse	GATACCCAGTTTGAGGTTCC

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Supplemental Video 1. Seizure activity of TLE mice. Related to Fig. 7.
Supplemental Video 2. Handling test. Related to Fig. 7.