Supplemental Document

Methods

Animals

This study employed young adult (2 months old) male F344 rats (n=52) obtained from Harlan. Animals were housed in an environmentally controlled room with a 12:12-hr light-dark cycle and with ad libitum food and water. All experiments were performed as per the animal protocol, approved by the institutional animal care and use committee of the Texas A&M Health Sciences Center and Central Texas Veterans Health Care System.

Induction of status epilepticus and study group assignment

Following 7-10 days of acclimatization, status epilepticus (SE) was induced in 42 rats through graded intraperitoneal injections of kainic acid (KA, 5.0 mg/Kg) every hour until the onset of seizure activity. The time at first occurrence of stage IV or stage V seizure was considered as 0 h. After the occurrence of first seizure, all rats developed SE, which was typified by either continuous stage IV seizures or stage IV seizures with intermittent stage V seizures within 1h. Following 2h of seizure activity, behavioral seizures were terminated through an injection of diazepam (5 mg/Kg, i.p.). Four rats died during the procedure, and 33 rats were assigned to either SE alone group (n=16) or SE + Grafts group (n=17). In SE + Grafts group, 12 rats were grafted with human induced pluripotent stem cell (hiPSC) derived medial ganglionic eminence-like cells (hMGE cells) and 5 rats were grafted with hiMGE cells transduced with AAV 5 vectors carrying hSyn-hM4Di-mCherry DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). Additional 5 rats were used as clozapine-N-oxide (CNO) controls in SE alone group. Furthermore, 10 age-matched naïve control rats were used for comparing cognitive and mood function and histological changes. A supplemental figure illustrates the sequence of various experiments (Fig. S1).

hiPSC culture, generation of NKX2.1+ MGE progenitors and transplantation

hiPSCs (iPS (IMR90)-4 from Wisconsin International Stem Cell Bank) were grown on a Matrigel layer (BD Matrigel™, cat. no. 354230) and cultured in TeSR™-E8™ medium (StemCell

Technologies, cat. no. 5940). For the generation of hMGE-progenitors, a previously developed protocol was employed with minor modifications (1, 2). Briefly, hiPSCs were chemically induced to generate primitive neuroepithelial cells. Neuroepithelial cells were treated with 1000ng/ml sonic hedgehog (Shh) for two weeks to induce MGE-like progenitors. Cells were transplanted ~35 days following the initiation of neuroepithelial induction. Before transplantation, single cell suspensions were prepared from the neurospheres and tested for the viability. For transduction experiments, neurospheres were dissociated on day 30 into small cell aggregates of approximately 10-20 cells by TrypLE treatment and repeated mild trituration. These aggregates were allowed to recover in the neural induction medium containing 100ng/ml Shh for 24 hours and transduced with AAV5- hSyn-hM4Di-mCherry (Roth Lab, Vector Core, University of North Carolina, Chapel Hill) for 48 hours. Single cells were centrifuged thrice at 600rpm for 10 minutes to remove dead cells. Over 85% viability was observed in all preparations. For all transplantation experiments, cell suspensions were primed with neural differentiation medium containing 1μM cAMP, 10ng/ml each of BDNF, GDNF, and IGF-1 before transplantation.

For transplantation experiments, rats were anesthetized with an intramuscular injection of the anesthetic cocktail (a mixture of ketamine 50 mg/ml, xylazine at 4.5 mg/ml and acepromazine at 0.4 mg/ml) at a dose of 0.7 ml/kg body weight. Using a stereotaxic device, 100,000 viable cells in 1.0 μl of differentiation medium were grafted into 3 sites in each hippocampus using the following coordinates. (i) anteroposterior (AP) - 3.1mm posterior to the bregma, lateral (L) - 1.6 mm lateral to the midline, ventral (V) - 3.5 mm from the brain surface; (ii) AP - 4.0 mm, L - 2.6 mm, V - 3.5 mm; (iii) AP - 4.5 mm, L- 3.2 mm, V - 3.5 mm. These coordinates were chosen using the rat brain atlas. Cells were transplanted in 0.2 μl spurts over a period of 5-8 minutes. Daily cyclosporine A injections (10mg/kg) were given starting 2 days before transplantation and until experimental endpoint to avoid transplant rejection.

EEG electrode implantation surgery and recordings of EEG

We employed a time-locked video-digital EEG monitoring system (AS40 from Grass-Telefactor). The methods for implantation of electrodes were similar to that described in our previous report (3). The surgery for fixing EEG electrodes involved aseptic survival surgery using a stereotaxic apparatus. Each rat was deeply anesthetized and fixed to a stereotactic device. Burr holes were made in the skull using appropriate coordinates to implant EEG recording electrodes and screws. Three sterile metal EEG recording electrodes with mounting

screws (Plastics one) were placed epidurally, over the right fronto-parietal cortex (recording electrode #1), the left fronto-parietal cortex (recording electrode #2) and the left cerebellum (reference electrode) respectively. Also, 1-2 screws without electrodes were placed over the frontal cortex. The screws and electrodes were cemented in place, and electrode leads were attached to a micro plug, which was cemented to the animal's head. Two weeks later, each rat was placed in a Plexiglas cage, the connector cable of the video-EEG system was fixed into the electrode pedestal on the rat's head. The video-EEG system monitored simultaneously occurring behavior and EEG activity in awake, freely behaving rats with ad libitum access to food and water. The EEG recordings were done continuously for 21 days with low-frequency filter (LF) set at 0.3Hz, high frequency (HF) set at 35 Hz, and data rate at 200 Hz. After the completion of EEG recordings, EEG tracings were analyzed for the frequency of all SRS, the frequency of stage V-SRS (the most severe form of SRS), the average duration of individual SRS and the total time spent in seizure activity for the recording period. Furthermore, a detailed spectral analysis was performed in a blinded fashion for randomly chosen SRS (20 SRS/animal, n=5/group) and interictal segments (4-10 segments/animal, n=5/group). For the study involving activation of DREADDs with CNO, EEG recordings were collected from animals receiving grafts of hiMGE cells tranduced with AAV5- hSyn-hM4Di-mCherry. The recordings were made in the pre-CNO period (4 days), CNO administration period (4 days, 3 mg/Kg once every 8 hours) and the post-CNO period (4 days, commencing two days after the last CNO injection).

Behavioral tests

The behavioral tests for measuring cognition and mood comprised an object location test (OLT), a novel object recognition test (NORT), a pattern separation test (PST), an eating-related depression test (ERDT) and a sucrose preference test (SPT). All object based tests (OLT, NORT, and PST) were performed using an open field apparatus measuring 100 cm x 100 cm.

Object location test (OLT)

Each rat was observed in an open field with three successive trials separated by 15-minute intervals. A detailed description of this test is available in our previous report (4). In brief, the rat was placed in an open field for 5 minutes in the first trial for acclimatization to the testing apparatus (habituation phase) whereas, in trial 2, the rat was allowed to explore two identical objects placed in distant areas of the open field (sample phase). In trial 3 (testing phase), one of

the objects was moved to a new area (novel place object, NPO) while the other object remained in the previous place (familiar place object, FPO). Both trials 2 and 3 were video recorded using Noldus-Ethovision video-tracking system to measure the amount of time spent with each of the two objects. Exploration of the object was defined as the length of time a rat's nose was 1 cm away from the marked object area. The results such as the percentage of object exploration time spent in exploring the NPO and FPO as well as the total object exploration time in trial 3 were computed. The percentage of time spent with the NPO and FPO was calculated by using the following formula: the time spent with the NPO / the total object exploration time × 100.

Pattern separation test (PST)

This test comprised four successive trials separated by 30-minute intervals. In the trial 1, rats were acclimatized for a period of 5 minutes in the open field apparatus. The trial 2 comprised exploration of a pair of identical objects (type 1 objects) placed in distant areas on a floor pattern (pattern 1 or P1) for 5 minutes. The trial 3 involved exploration of the second pair of identical objects (type 2 objects) placed in distant areas on a different floor pattern (pattern 2 or P2) for 5 minutes. In trial 4, one of the objects from trial 3 was replaced with an object from trial 2, which became a novel object on pattern 2 (NO on P2) whereas the object retained from trial 3 became a familiar object on P2 (FO on P2). The rat could explore objects for 5 minutes. Both trials 3 and 4 were video recorded using Noldus-Ethovision video-tracking system. Exploration of objects was defined as the length of time a rat's nose was 1 cm away from the object area. The results such as the time spent in exploring the NO on P2 and the FO on P2 and the total object exploration time were computed from trial 4. Furthermore, NO and FO discrimination index was calculated by using the following formula: the time spent with the NO on P2 / the total object exploration time × 100.

Novel Object Recognition test (NORT)

The first two trials of this test were similar to the OLT described above. However, in trial 3 (testing phase), one of the objects was replaced with a new object (novel object, NO) while the other object remained in its location (familiar object, FO). Trials 2 and 3 were video recorded to quantify the amount of time spent with NO and FO. The results such as the percentage of object exploration time spent in exploring the NO and FO, the total object exploration time in trial

3 were collected. The percentage of time spent with the NO vis-à-vis FO was calculated by using the following formula: the time spent with the NO / the total object exploration time × 100.

Eating related depression test (ERDT)

This test was adapted from a novelty suppressed feeding test (NSFT, 2, 5, 6). Animals were first deprived of food for 24 hours, but provided ad libitum access to water. After 24 hours, each rat was removed from its home cage and placed temporarily in an identical new cage. Regular chow food pellets were placed in one corner of animal's home cage over a white paper. The rat was removed from the temporary cage and placed back in its home cage diagonally opposite to the position of the food. Timer commenced as soon as the release of rat into its home cage. The movement of the rat was observed carefully for 5 min. The results such as the latency to smell food and the latency to the first bite of food were recorded. Commencement of eating was considered when the rat started nibbling pellets with the use of its forepaws for the first time. For rats that failed to smell or eat food in the ceiling period of 5 min, latency value was taken as 300 seconds. Once the test was done, rats were provided with ad libitum access to food and water.

Sucrose preference test (SPT)

This test measures mood dysfunction in rodents by examining decreases in the preference for sweet fluids such as sucrose or saccharin solution. This test was adapted from the assessment of anhedonia in psychiatric patients (7, 8). The test comprised four days of monitoring. On day 1, rats were housed individually and given free access to two identical bottles containing 1% sucrose solution and were provided ad libitum access to food. Rats were trained to adapt to sucrose solution for 24 hours. On day 2, one bottle was replaced with a new bottle containing regular water for 24 hours, again with ad libitum access to food. On day 3, rats were deprived of water and food for 22 hours. On day 4, rats were tested for their preference towards consuming sucrose-containing water over regular water. Following fasting, rats were given free access to two bottles: one containing 100 ml of 1% sucrose solution and another containing 100 ml of regular water. Two hours later, the consumed volume in both bottles was recorded using a graduated measuring cylinder. Following the completion of this test, all rats were placed back to their previous housing conditions and provided ad libitum access to water

and food. Sucrose preference rate was calculated using the formula, sucrose consumption / (water consumption + sucrose consumption) × 100.

Tissue processing and immunohistochemistry

Following completion of EEG recordings and behavioral tests, rats belonging to SE alone group and SE+ Grafts group and age-matched naïve controls underwent intracardiac perfusions with 4% paraformaldehyde. The brains were dissected, post-fixed in 4% paraformaldehyde overnight, and processed for sectioning using a cryostat. Thirty- micrometer-thick coronal sections were cut through the entire hippocampus and collected serially in 24-well plates containing the phosphate buffer. Several sets of serial sections (every 15th or 20th) through the entire hippocampus were selected and processed for immunohistochemistry. Briefly, the sections were etched with PBS solution containing 20% methanol and 3% hydrogen peroxide for 20 minutes, rinsed thrice in PBS, treated for 30 minutes in PBS containing 0.1% Triton-X 100 and an appropriate serum (10%) selected based on the species in which the chosen secondary antibody was raised. After overnight incubation with the respective primary antibody solution, sections were washed thrice in PBS and incubated in an appropriate secondary antibody solution for an hour. The list of primary and secondary antibodies used are provided in Table 1. The sections were washed thrice in PBS and treated with an avidin-biotin complex reagent (Vector Lab, PK-6100) for an hour. The peroxidase reaction was developed using diaminobenzidine (Vector Lab, SK-4100) or vector SG (Vector Lab, SK-4700) as chromogens, and the sections were mounted on gelatin-coated slides, dehydrated, cleared and cover slipped with permount.

Stereological quantification of cells and neurons

The optical fractionator method in the StereoInvestigator system (Microbrightfield Inc., Williston, VT) interfaced with a Nikon E600 microscope through a color digital video camera (Optronics Inc., Muskogee, OK) was employed for all absolute cell counts performed in the study. This comprised quantification of numbers of: (i) HNA positive grafted cells in the entire host hippocampus, ii) DCX positive cells in the granule cell layer and sub granular zone (GCL-SGZ), and the dentate hilus (DH), iii) Reelin+ cells in the dentate hilus, iv) interneurons positive for parvalbumin (PV), neuropeptide Y (NPY) or somatostatin (SS) in the DH +GCL. A detailed methodology employed in these counts was described in our previous report (3, 9, 10). In brief,

using a 100X lens, cells were measured in every 15th or 20th section from 50-500 counting frames (each measuring 40 × 40 μm) selected through a systematic random sampling scheme. By entering parameters such as grid size, the thickness of the top guard zone (4 μm) and the optical dissector height (i.e., 8 μm), numbers and locations of counting frames and the depth for counting were determined. A computer steered motor-powered stage then permitted the section to be evaluated at every counting frame sites. A choice in the Stereo Investigator program let the experimenter to remain oblivious to the running cell counts until all sections for each animal were completed. The StereoInvestigator program later calculated the total number of cells in each chosen region by utilizing the optical fractionator formula, as described in our earlier reports (3).

Dual immunofluorescence staining and confocal microscopic analyses

The procedures employed for dual immunofluorescence are described in our previous reports (11-15). The primary and secondary antibodies used in these analyses are listed in Table 1. Optical Z-sections of 1.0 or 1.5 μm were sampled from different regions of the grafted cell populations in various subfields of the hippocampus using a confocal microscope (Nikon), and the images were analyzed using NIS image browser. Immunostaining spanning over three successive Z-sections were considered as positive staining.

Analysis of aberrant mossy fiber sprouting

A set of serial sections (every 20^{th}) through the hippocampus were processed for immunostaining using a rabbit ZnT3 antibody (1:250; Synaptic Systems). The extent of abnormal mossy fiber sprouting in the dentate supragranular layer was quantified separately for upper and lower blades of the DG using image J, as described in our previous report (16).

Statistical analysis

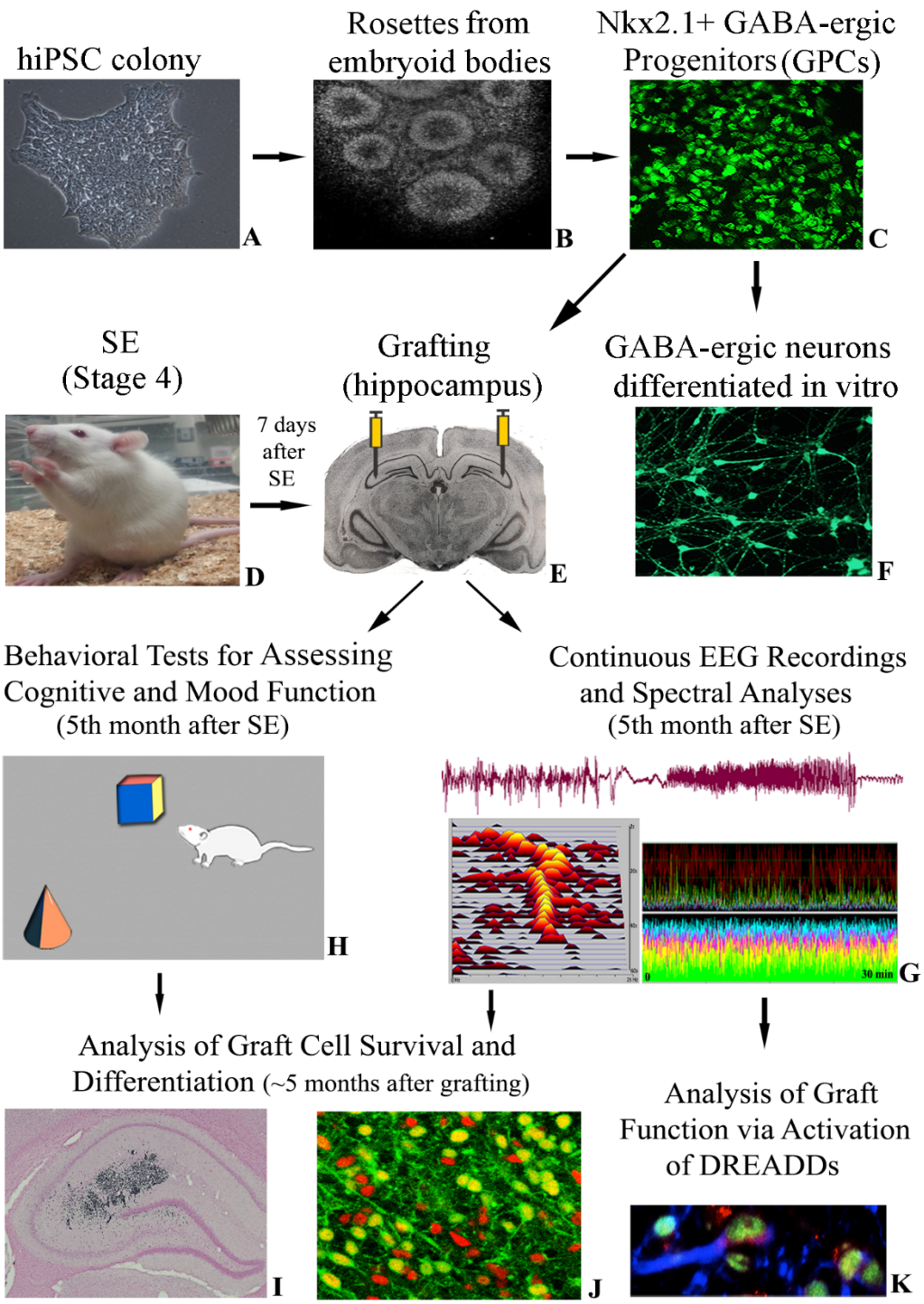
Statistical analyses were performed using Prism software. One-way analyses of variance (one-way ANOVA) with Newman-Keuls multiple comparison post hoc tests were employed when 3 or more groups were compared. Comparison within groups in the behavioral tests or comparison between the two groups (e.g., DCX counts in DG) employed unpaired, two-tailed t-test.

Numerical data were presented as mean ± SEM and $p < 0.05$ was considered as statistically significant.

Table 1

Primary antibody	Source	Dilution
Mouse anti-Human Nuclei	Millipore, MAB1281	1:1000
Mouse anti-NeuN	Millipore, MAB377	1: 1000
Rabbit anti-GABA	Sigma, A2052	1: 5000
Mouse anti-Parvalbumin	Sigma, P3088	1:2000
Rabbit anti-Neuropeptide Y	Peninsula Laboratories, T-4070	1:10000
Rabbit anti-Somatostatin	Peninsula Laboratories, T-4546	1:5000
Rabbit anti-Calretinin	Millipore, AB5054	1:1000
Mouse anti -Reelin	Millipore, MAB5364	1:1000
Rabbit anti-GFAP	Millipore, AB5804	1: 1000
Rabbit anti-NG2	Millipore, AB5320	1: 500
Rabbit anti-ChAT	Millipore, AB143	1:3000
Goat anti-Doublecortin	Santa Cruz, sc-8066	1:300
Mouse anti-ZnT3	Synaptic Systems, 197 011	1:1000
Rabbit anti-MAP2	Millipore, AB5622	1:1000
Mouse anti-NKX2.1	Millipore, MAB5460	1: 100
Rabbit anti-Ki67	Vector Labs, VP-RM04	1: 200
Rabbit anti-Sox 2	Millipore, AB5603	1: 500
Mouse anti-Nestin	Santa Cruz, sc-33677	1:200
Rabbit anti Oct 4	Millipore, AB3209	1: 500
Rat anti SSEA3	Abcam, ab16286	1: 500
Mouse anti-TRA-1-81	Abcam, ab 16289	1: 500
Mouse anti human Synaptophysin	ThermoFisher, 14-6525-80	1: 500
Goat anti-PSD95	Abcam, ab12093	1:500
Rabbit anti Tuj-1	Millipore, AB9354	1:1000
Secondary antibody		
Biotinylated anti-goat (H+L)	Vector Lab, BA-9500	1: 500
Biotinylated anti-mouse (H+L)	Vector Lab, BA-2001	1:500
Biotinylated anti-rabbit (H+L)	Vector Lab, BA-1000	1:500
Donkey anti mouse AF 594	Invitrogen, A-21203	1:200
Donkey anti goat AF 488	Invitrogen, A11055	1:200
Donkey anti rabbit AF 488	Invitrogen, A21206	1:200
Donkey anti rat AF 488	Invitrogen, A 21208	1:200
Donkey anti-rabbit AF 350	Invitrogen, A10039	1:200
Goat anti mouse AF 488	Invitrogen, A 32723	1:200
Goat anti-mouse AF594	Invitrogen, A 11032	1:200

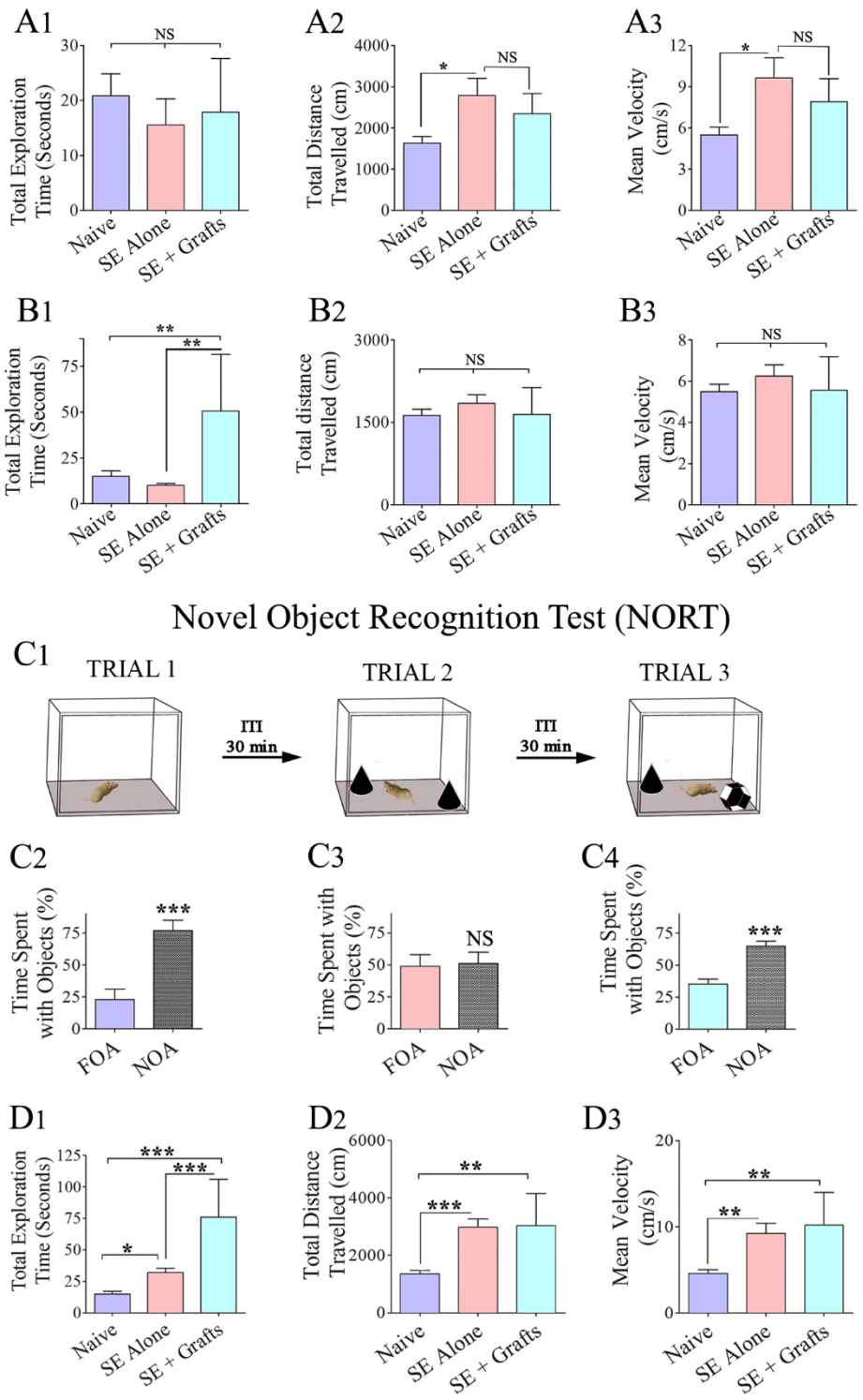
Supplementary Figure 1



Supplementary Figure 1 (S1): *Graphical illustration of the research design.* Human iPSC-derived median ganglionic eminence-like cells (hiPSC-MGE cells) were generated using a directed differentiation protocol. This process sequentially comprised the expansion of hiPSCs (A) into embryoid bodies, induction of neural rosettes from embryoid bodies (B), and expansion of neural rosette cells into NKX2.1+ gamma-amino butyric acid-ergic (GABA-ergic) progenitor

cells (C; Liu et al., 2013). Immunostaining showed that >92% of the cells expanded were positive for NKX2.1. Status epilepticus (SE) was induced in rats with graded intraperitoneal injections of kainic acid (D). Rats that developed continues stage IV and/or stage V seizures for 2 h were randomly assigned to two groups. Animals in one group served as SE alone controls (SE alone group, n= 16) while animals in the other group received grafts of hiPSC-MGE cells (SE + Grafts group, n=12) or hMGE cells transduced with AAV hsyn-hMDi4-mCherry (SE + DREADDs graft group, n=5) into the hippocampus at 7 days post-SE (E). Epidural electrodes were implanted to subgroups of animals belonging to SE alone and SE + grafts groups (n=6/group) in the 4th month after SE and electroencephalographic (EEG) recordings were made continuously for 3 weeks in the 5th month after SE (G). In the 5th month after SE, additional subgroups of animals from SE alone (n=10) and SE + Grafts groups (n=6) were subjected to multiple behavioral tests along with age-matched naïve control rats (n=10), to assess cognitive, memory, pattern separation and mood function (H). Animals were euthanized 5 months after SE or SE and grafting via intracardiac perfusions, brain tissues were harvested, and several single immunohistochemical and multiple dual immunofluorescence staining were performed for analyses of graft cell survival (I), differentiation (J) and proliferation using various antibody markers. In animals receiving hiPSC-MGE cells transduced with AAV hsyn-hMDi4-mCherry (K), EEG recordings were made in three phases to evaluate the function of graft derived cells in suppressing spontaneous recurrent seizures. The phases comprised prior to clozapine-N-oxide (CNO) injections (pre-CNO period), immediately after CNO injections (CNO period) and after CNO washout (post-CNO period).

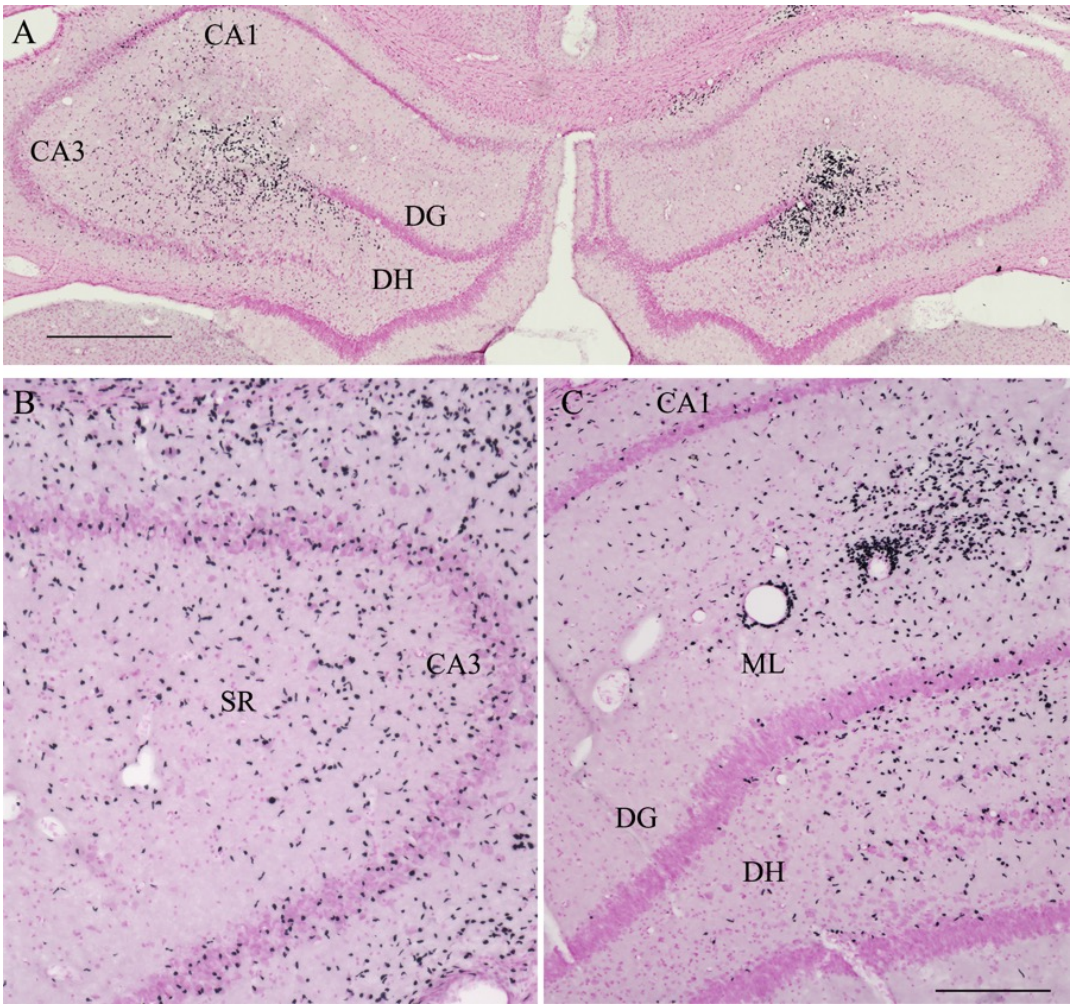
Supplementary Figure 2



Supplementary Figure 2 (S2): *Additional parameters analyzed in the object location and pattern separation tests (OLT and PST, top two panels), and the results of a novel object recognition test (NORT, lower 3 panels).* Bar charts in A1-B3 compare the total exploration time

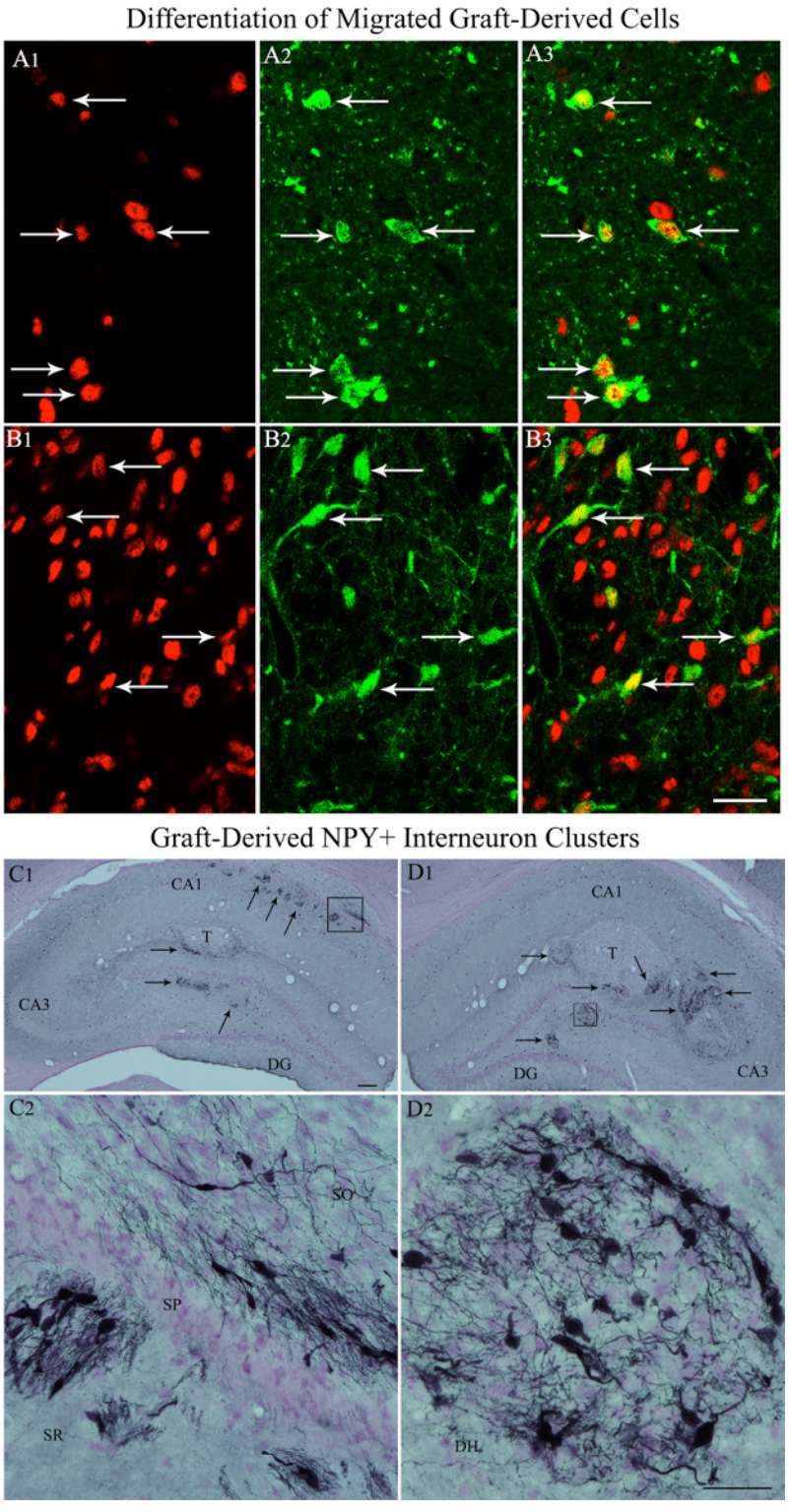
(A1, B1), the total distance travelled (A2, B2) and mean velocity (A3, B3) observed during an OLT (A1-A3) and a PST (B1-B3) between rats belonging to naïve, SE alone, and SE plus human iPSC-derived median ganglionic eminence-like cell (hiPSC-MGE cell) grafts (SE + grafts) groups. *, $p<0.05$, **, $p<0.01$. Animals in SE alone group traveled more distance at a greater velocity than animals in other groups in an OLT, and animals in SE + grafts group explored objects for a greater amount of time than animals in naïve and SE alone groups in a PST. *Figures C1-D3 illustrate the experimental design and results of a NORT.* The various trials involved in this test and the inter-trial interval (ITI) between trials are graphically showed in C1. Bar charts in C2-C4 compare percentages of time spent with different objects. Naive control animals (C2) showed a greater affinity for the novel object area (NOA) over the familiar object area (FOA), implying normal object recognition memory function (A2). Animals in SE alone group (C3) however showed impaired memory, which was apparent from their object exploration time spent nearly equivalently with the FOA and NOA (C3). In contrast, animals receiving hiPSC-MGE cell grafts (C4) showed a greater affinity for exploring the NOA than the FOA, suggesting preserved object recognition memory function, akin to naïve control animals. Bar charts in D1-D3 compare the total exploration time, total distance travelled and the mean velocity during a NORT between rats belong to the three groups. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$. Animals in SE alone and SE + grafts groups explored objects for a greater amount of time and traveled greater distance at a higher velocity than naïve control animals.

Supplementary Figure 3



Supplementary Figure 3 (S3): *Migration of graft-derived cells away from the core of human iPSC-derived median ganglionic eminence-like cell (hiPSC-MGE cell) grafts.* Figure A illustrates an example of graft core and migrated graft-derived cells bilaterally in the dorsal hippocampus, with human nuclear antigen (HNA) immunostaining. Figures B and C show extensive migration of graft-derived cells into different layers of the CA3 subfield (B), and CA1 subfield and dentate gyrus (C). DG, dentate gyrus; DH, dentate hilus; ML, molecular layer; SR, stratum radiatum. Scale bar, A = 400 µm. B, C = 100 µm.

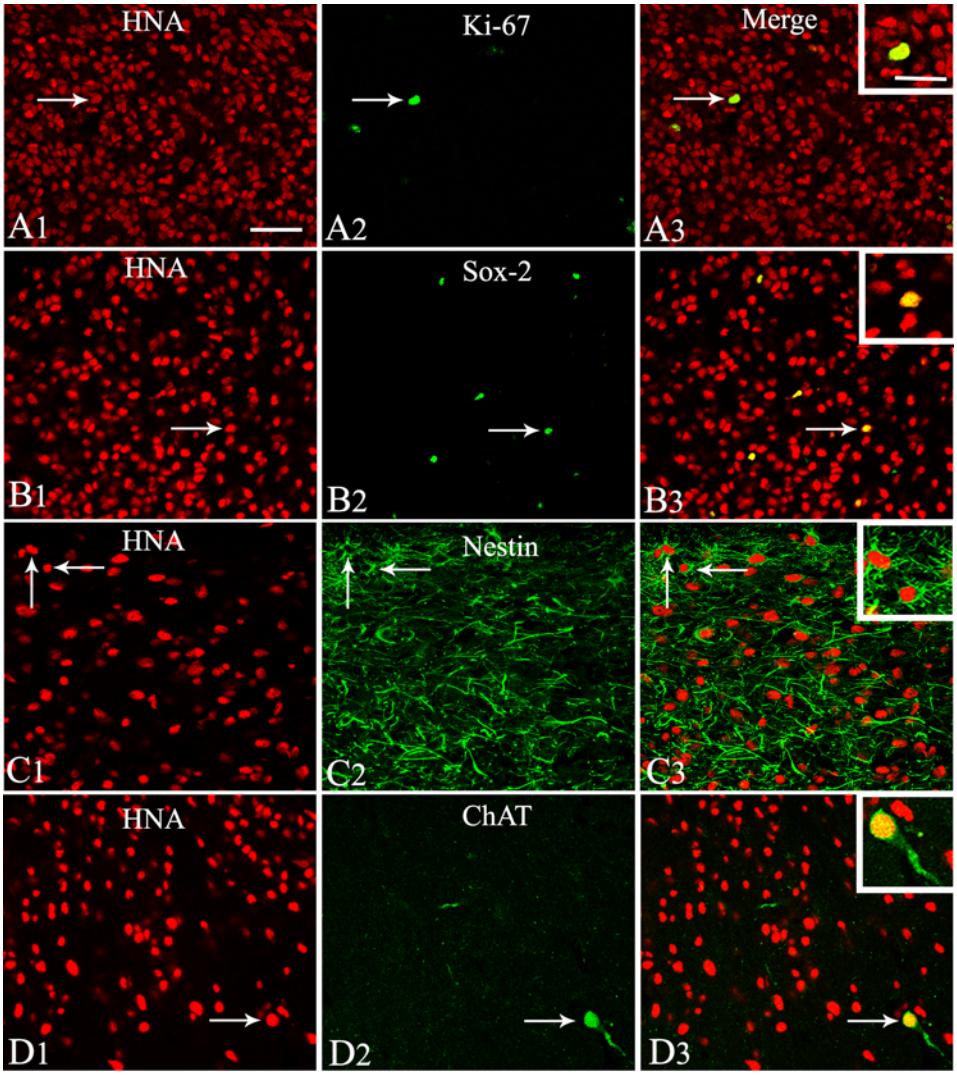
Supplementary Figure 4



Supplementary Figure 4 (S4): *Graft-derived cells located away from the core of human iPSC-derived median ganglionic eminence-like cell (hiPSC-MGE cell) grafts also differentiate into interneurons.* Figures A1-B3 illustrate the differentiation of migrated graft-derived cells into gamma-amino butyric acid (GABA) positive interneurons (A1-A3) and neuropeptide Y (NPY) positive interneurons (B1-B3). Scale bar, A1-B3 = 20 µm.

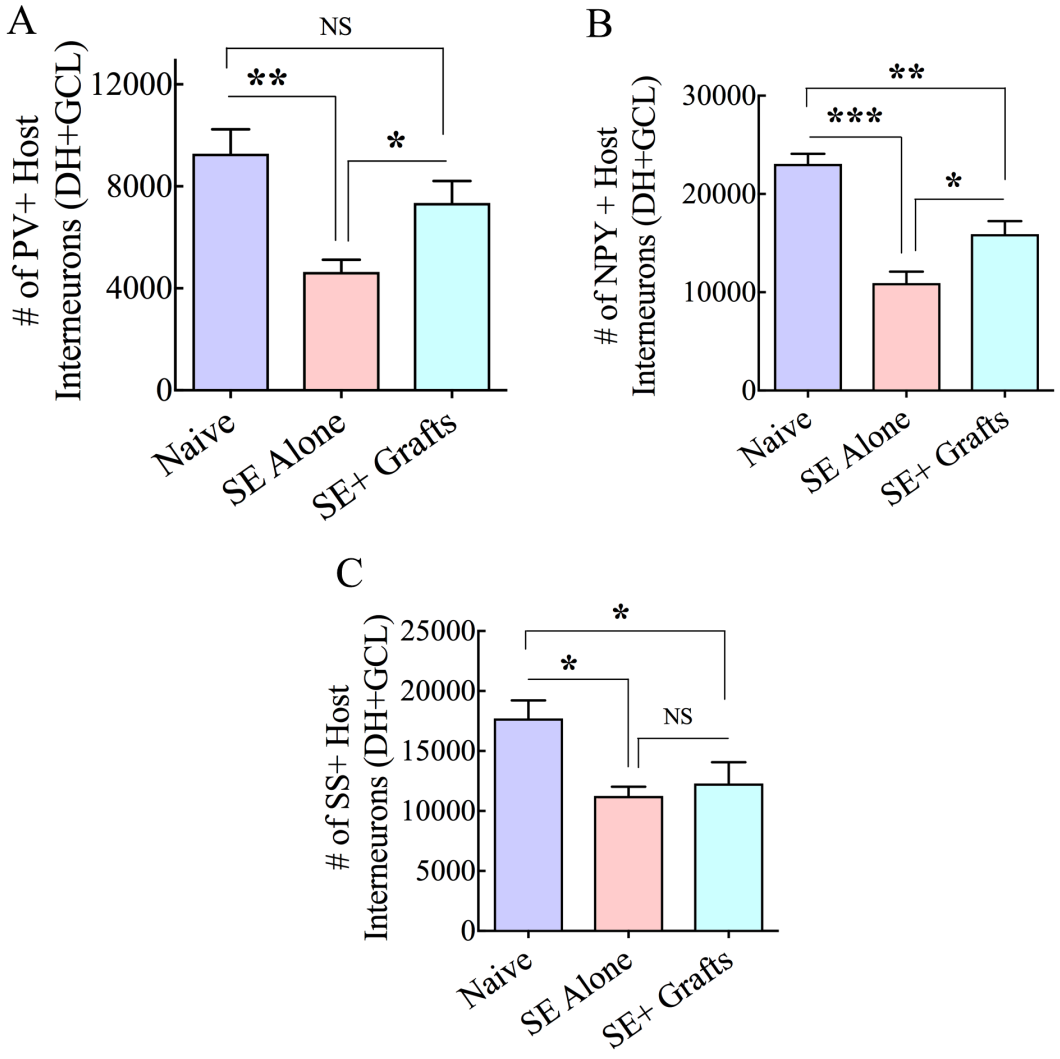
NPY expression is seen in clusters of graft-derived cells located both adjacent to and away from the main core of hiPSC-MGE cell grafts. Figures C1 and D1 illustrate multiple clusters of NPY+ interneurons in the CA1 subfield, the hippocampal fissure, dentate hilus, dentate molecular layer (arrows in C1, D1) and the CA3 stratum radiatum (D1). Figures C2 and D2 are magnified views of boxed regions from C1 and D1 illustrating NPY+ interneurons with thick dendrites and fine axonal processes. Scale bars, C1 and D1= 100 µm; C2 and D2 = 20 µm.

Supplementary Figure 5



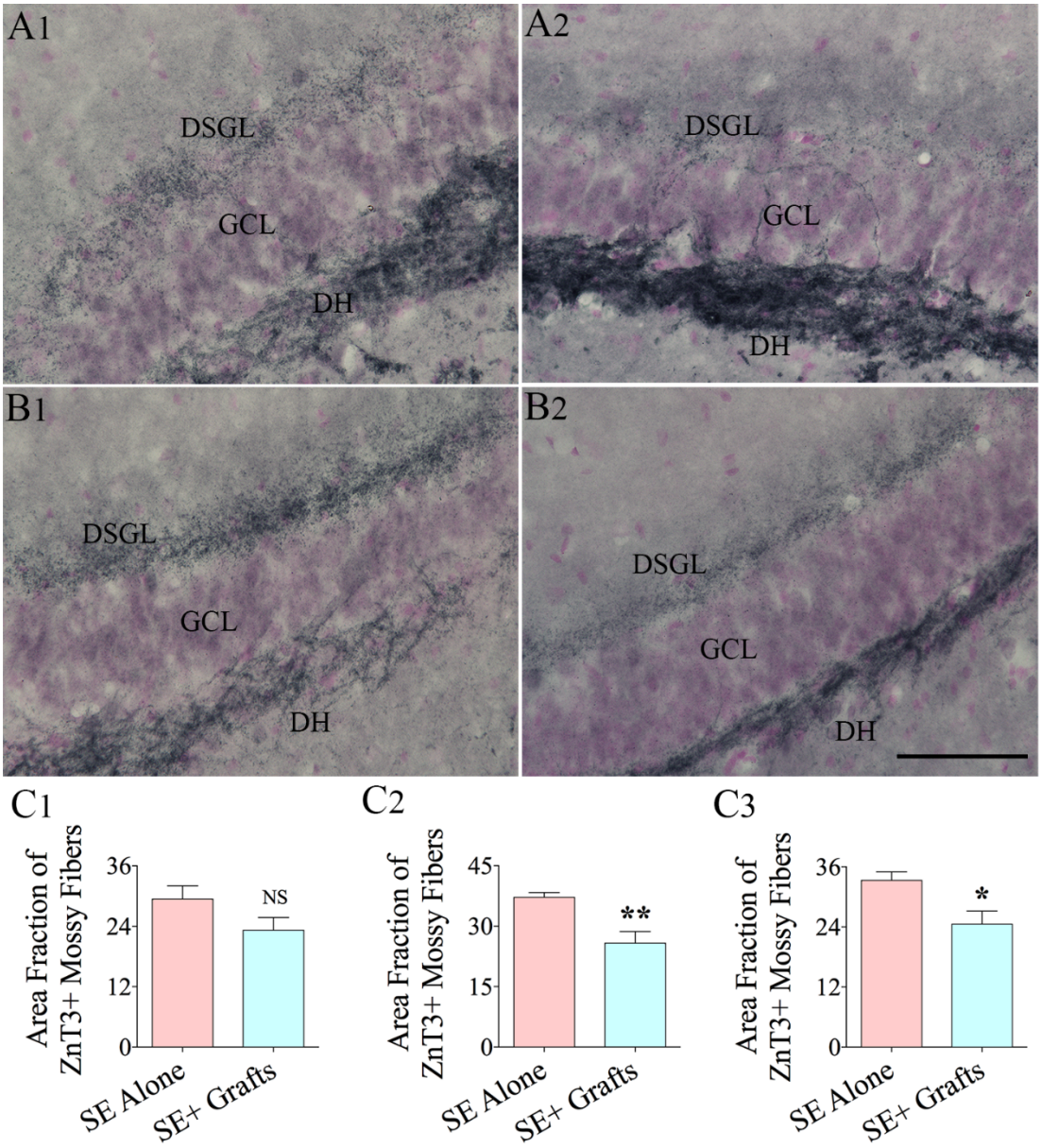
Supplementary Figure 5 (S5): *Human iPSC-derived median ganglionic eminence-like cell grafts display minimal proliferation, comprise a small fraction of neural progenitor cells, and give rise to negligible numbers of cholinergic neurons.* Figures A1-D3 illustrate dual immunofluorescence staining of graft-derived cells for human nuclear antigen (HNA) with markers of dividing cells (Ki-67; A1-A3), neural stem/progenitor cells (Sox-2; B1-B3), primitive intermediate protein (nestin; C1-C3) and cholinergic neurons (choline acetyltransferase [ChAT]; D1-D3). Ki-67 was expressed in <1% of graft-derived cells (A1-A3) whereas Sox-2 was expressed in ~8% of graft-derived cells (B1-B3). Nestin expression in the soma was restricted to ~4% of graft-derived cells but many processes within grafts expressed nestin (C1-C3). Furthermore, <1% of graft-derived cells expressed the cholinergic neuronal marker ChAT (D1-D3). Scale bars, A1-D3 = 50 μm; Insets = 25 μm.

Supplementary Figure 6



Supplementary Figure 6 (S6): The bar charts in A, B, and C compare numbers of host PV+, NPY+ and SS+ interneurons in DH+GCL between different groups (n=5/group). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Supplementary Figure 7



Supplementary Figure 7 (S7): **Human iPSC-derived median ganglionic eminence-like cell (hiPSC-MGE cell) grafting into the hippocampus after status epilepticus (SE) diminishes aberrant mossy fiber sprouting into the dentate supragranular layer (DSGL).** Figures A1-B2 illustrate ZnT3 immunohistochemical staining of the upper and lower blades of the dentate gyrus (DG) from a rat that received no grafts after SE (A1, B1, SE alone) and a rat that received grafts after SE (A2, B2, SE + grafts). Note that hiPSC-MGE cell grafting after SE diminished the extent of mossy fiber sprouting in the DSGL (A2, B2), in comparison to dense sprouting with SE alone (A1, B1). DH, dentate hilus; GCL, granule cell layer. Scale bar, A1-B2 = 100 µm. Bar charts in C1, C2 and C3 compare the area fraction of ZnT3+ mossy fiber density between SE

alone and SE + grafts groups for the upper and lower blades of the DG (C1, C2), and the entire DG (C3). *, $p<0.05$; **, $p<0.01$.

References

1. Liu Y, et al. (2013) Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. *Nat Protoc* 8, 1670-1679.
2. Upadhya D, et al. (2016) Neural stem cell or human induced pluripotent stem cell-derived GABA-ergic progenitor cell grafting in an animal model of chronic temporal lobe epilepsy. *Curr Protoc Stem Cell Biol* 38: 2D.7.1-2D.7.47.
3. Rao MS, Hattiangady B, Reddy DS, Shetty AK (2006). Hippocampal neurodegeneration, spontaneous seizures, and mossy fiber sprouting in the F344 rat model of temporal lobe epilepsy. *J Neurosci Res* 83, 1088-1105.
4. Hattiangady B, et al. (2014) Object location and object recognition memory impairments, motivation deficits and depression in a model of Gulf War illness. *Front Behav Neurosci* 8, 78.
5. Powell TR, Fernandes C, Schalkwyk LC (2012) Depression-related behavioral tests. *Curr Protoc Mouse Biol* 2, 119-127.
6. Samuels BA, Hen R (2011) Neurogenesis and affective disorders. *Eur J Neurosci* 33*,* 1152-1159.
7. Fawcett J, Clark D C, Scheftner W A, Gibbons RD (1983) Assessing anhedonia in psychiatric patients: the pleasure scale. *Arch Gen Psychiatry* 40, 79-84.
8. Willner P, Muscat R, Papp M (1993) Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neurosci Biobehav Rev* 6, 525-534.
9. Hattiangady B, Rao MS, Shetty AK (2004) Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. *Neurobiol Dis* 17, 473-490.
10. Rao MS, Shetty AK (2004) Efficacy of doublecortin as a marker to analyze the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus. *Eur J Neurosci* 19. 234-246.
11. Hattiangady B, Rao MS, Shetty AK (2008). Grafting of striatal precursor cells into hippocampus shortly after status epilepticus restrains chronic temporal lobe epilepsy. *Exp Neurol* 212, 468-481
12. Waldau B, Hattiangady B, Kuruba R, Shetty AK (2010) Medial ganglionic eminence-derived neural stem cell grafts ease spontaneous seizures and restore GDNF expression in a rat model of chronic temporal lobe epilepsy. *Stem Cells* 28:1153-1164.
13. Hattiangady B, Shetty AK (2012) Neural stem cell grafting counteracts hippocampal injury-mediated impairments in mood, memory, and neurogenesis. *Stem Cells Transl Med* 1, 696-708.
14. Shetty AK, Hattiangady B (2016) Grafted subventricular zone neural stem cells display robust engraftment and similar differentiation properties and form new neurogenic niches in the young and aged hippocampus. *Stem Cells Transl Med* 5, 1204-1215.
15. Mishra V, et al. (2015) Resveratrol treatment after status epilepticus restrains neurodegeneration and abnormal neurogenesis with suppression of oxidative stress and inflammation. *Sci Rep 5*, 7807.
16. Shetty AK, Zaman V, Hattiangady B (2005) Repair of the injured adult hippocampus through graft-mediated modulation of the plasticity of the dentate gyrus in a rat model of temporal lobe epilepsy. *J Neurosci* 25*,* 8391-8401.