Stem Cell Reports, Volume *13*

Supplemental Information

Human Neural Stem Cells Reinforce Hippocampal Synaptic Network and Rescue Cognitive Deficits in a Mouse Model of Alzheimer's Disease

Ting Zhang, Wei Ke, Xuan Zhou, Yun Qian, Su Feng, Ran Wang, Guizhong Cui, Ran Tao, Wenke Guo, Yanhong Duan, Xiaobing Zhang, Xiaohua Cao, Yousheng Shu, Chunmei Yue, and Naihe Jing

Figure S1. Related to Figure 1

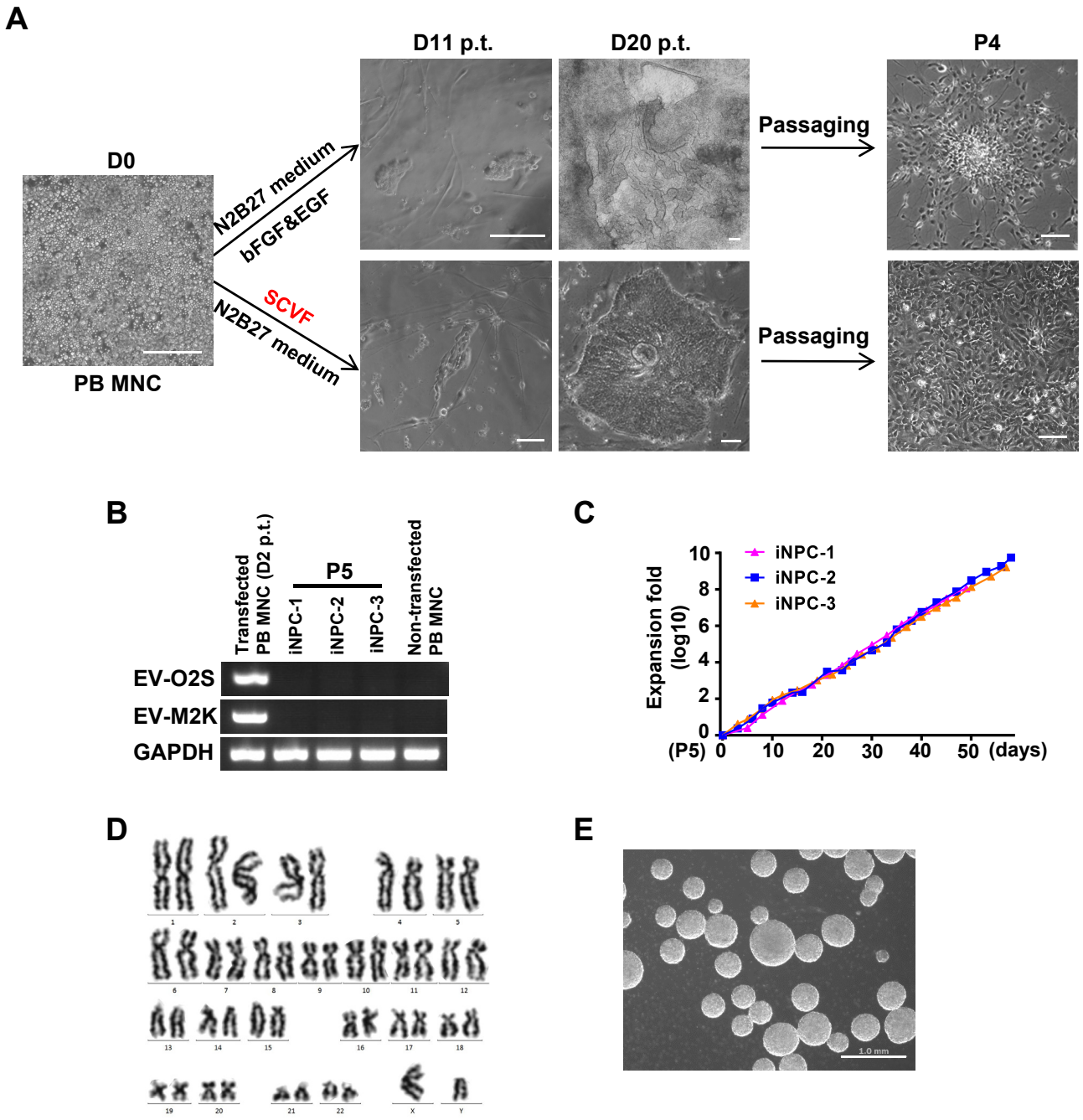

Figure S1. Direct reprogramming of peripheral blood cells into human iNPCs

A. Morphology of early human iNPC clusters at day 11 and 20 after transfection, respectively, and colonies at passage 4 generated without 4 chemicals in N2B27 containing bFGF and EGF, or with 4 chemicals in N2B27 only.

B. PCR analysis of the episomal vector-harboring iPS genes in PB MNCs 2 days after transfection, in three human iNPC lines at passage 5 and in PB MNCs without factor transfection.

C. Growth-curve showing the stable maintenance of human iNPCs up to 25 passages.

D. The karyotype analysis of human iNPCs at passage 25.

E. Neurospheres formed by human iNPCs at passage 15.

Scale bars, 200 μm (A) and 1 mm (E).

Figure S2. Related to Figure 1

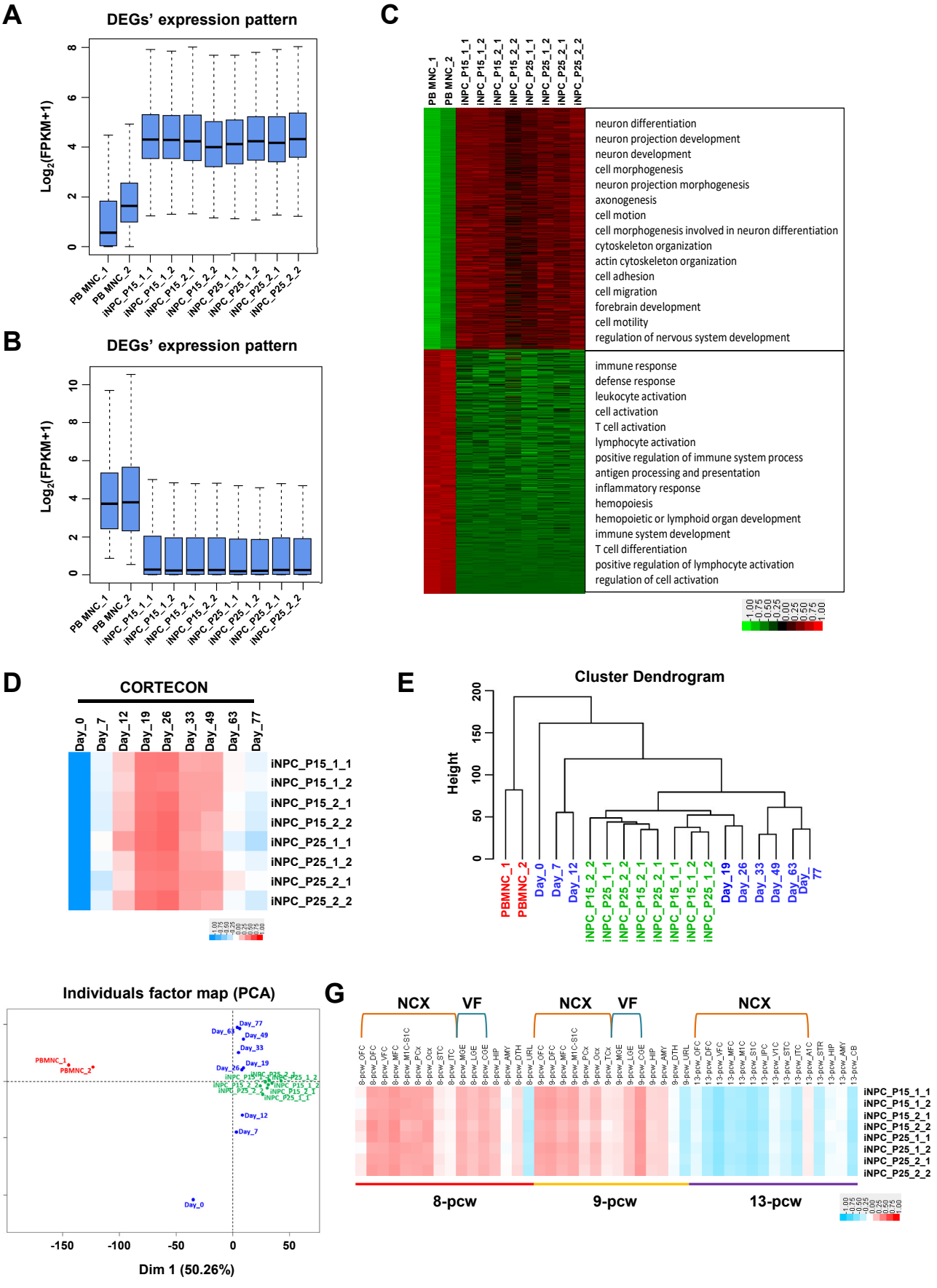

Figure S2. Whole transcriptome profiling of human iNPCs

A. The expression pattern of top 1000 upregulated differential expressed genes (DEGs) from RNA sequencing of PB MNCs from two individuals, and two PB MNC-derived iNPCs at passage 15 and 25 with two representative lines, respectively.

B. The expression pattern of top 1000 downregulated DEGs from RNA sequencing of PB MNCs from two individuals, and two PB MNC-derived iNPCs at passage 15 and 25 with two representative lines, respectively.

C. Heat map based on bulk RNA-sequencing data demonstrating global gene expression pattern of PB MNCs from two individuals, and two PB MNC-derived iNPCs at passage 15 and 25 with two representative lines, respectively. The enrichments of biological relevance in right panel highlighted by Gene Ontology (GO) analysis. Red and green colors represent higher and lower gene expression levels, respectively.

D. Comparative analysis of whole transcriptome of human iNPCs to a published temporal transcriptome dataset of hESC-derived NPCs with prefrontal cortex identity.

E. Hierarchical cluster analysis of the gene expression profiles of PB MNCs and human iNPCs shown in 1F, and a published temporal transcriptome dataset of hESC-derived NPCs.

F. Scatter plot analysis of the global gene expression profiles of human iNSCs at passage 15 and 25 versus the PB MNCs and hESC-derived NPCs.

G. Comparative analysis of whole transcriptome of human iNPCs to database from BrainSpan Atlas of the Developing Human Brain.

Figure S3. Related to Figure 2

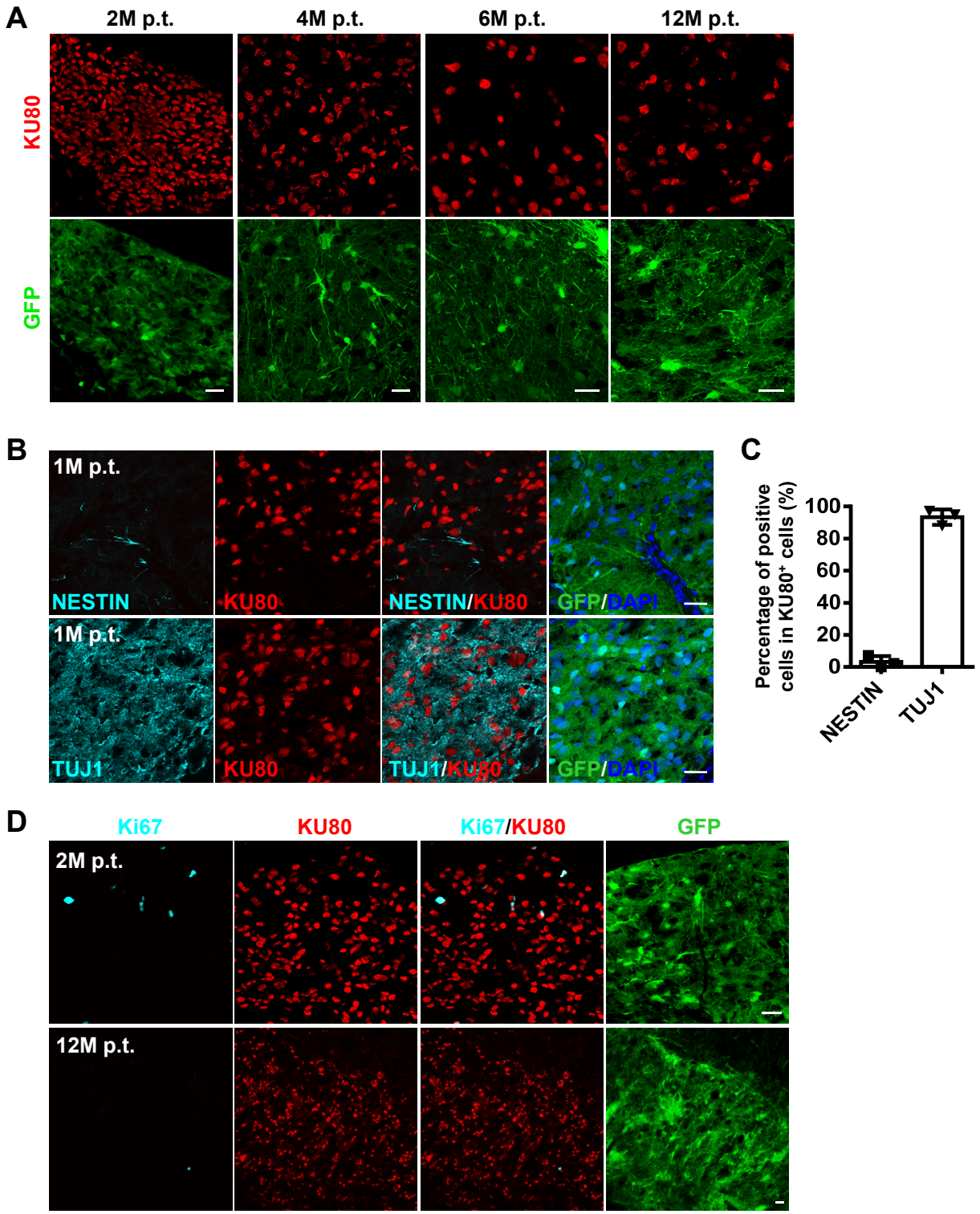

Figure S3. Human iNPCs gave rise to neurons and astrocytes in mice hippocampus after transplantation

A. Immunofluorescence analysis of $KU80^+$ among GFP^+ grafted human cells at 2, 4, 6 and 12 months post transplantation (p.t.).
B. Immunofluorescence analysis of NESTIN (top) or TUJ1(bottom) expression among $KU80^+$ human cells at 1 month p.t.
C. Quantification of the results shown in B. Note the small percentage of $NESTIN^+$ neural progenitor cells among $KU80^+$ human cells.
D. Immunofluorescence analysis of Ki67 expression among $KU80^+$ human cells at 2 and 12 months p.t.

Scale bars, 25 μm (A, B and D). n = 3 mice per time point. Data are represented as scatter plots with mean ± SD.

Figure S4. Related to Figure 5

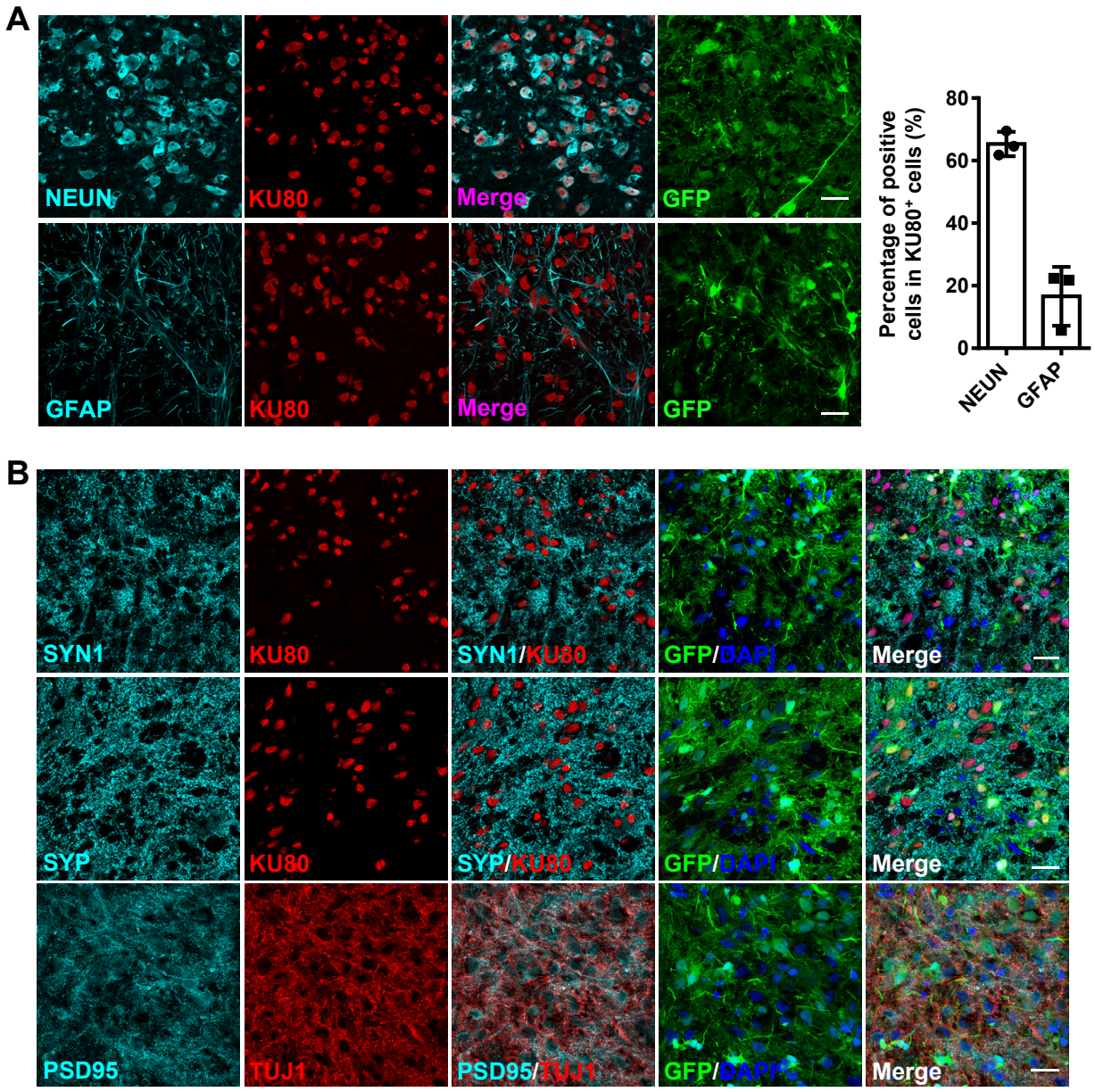

Figure S4. Differentiation of human iNPCs in hippocampus of AD mice after transplantation

A. Immunofluorescence analysis of NEUN or GFAP expression among KU80^{+} human cells in the hippocampus of Rag2$^{-/-}$/AD mice at 4 months p.t. (left panel) and the quantitative analysis shown in the right panel. n = 3 mice. Data are represented as scatter plots with mean ± SD.

B. Double immunofluorescence analysis of presynaptic markers SYNAPSIN1 or SYNAPTOPHYSIN with KU80 expression as well as postsynaptic marker PSD95 with TUJ1 expression among GFP^{+} human cells in the hippocampus of Rag2$^{-/-}$/AD mice at 4 months p.t.

Scale bars, 25 μm (A, B).

Figure S5. Related to Figure 6

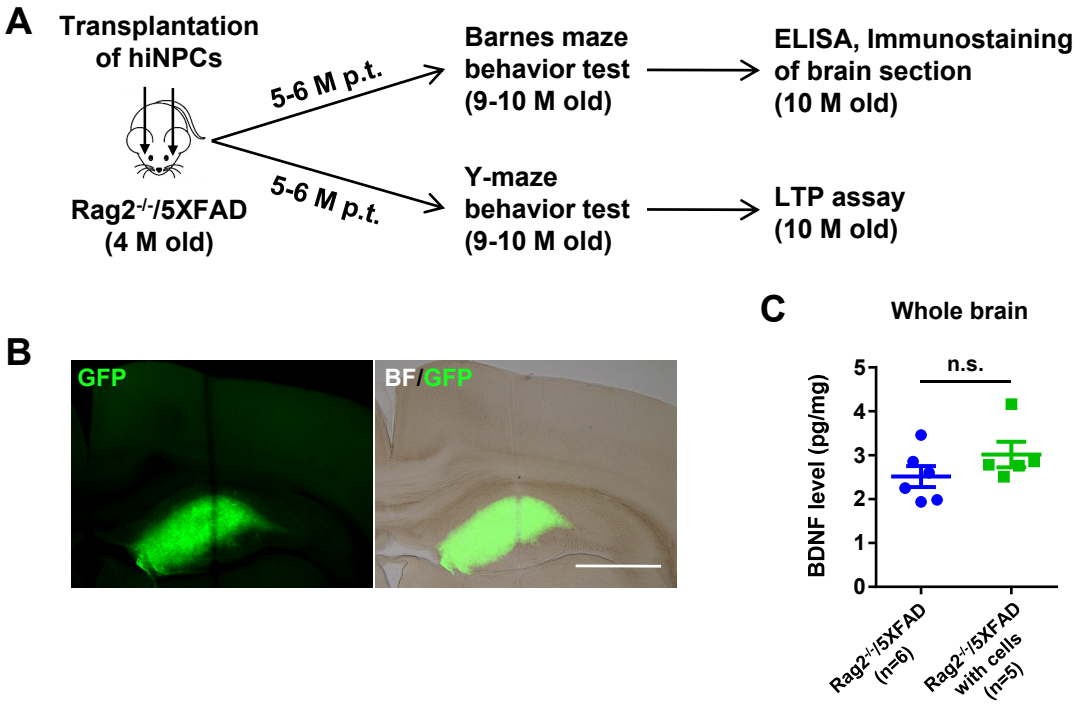

Figure S5. The grafted green human iNPCs in hippocampus of host mice

A. Schematic representation of timeline for human iNPCs transplantation and subsequent measurements in AD mice.

B. The GFP^+ human cells in hippocampus on the coronal brain slice from transplanted mice at 6 months post transplantation and after LTP measurement. Scale bar, 1 mm.

The concentration of secreted BDNF in whole brain of $Rag2^{-/-}$/5XFAD with and without human iNPCs at 6 months p.t. Data are presented as scatter plots with mean ± SEM. Student's t test (two-tailed), P=0.212.

Table S1. Primary antibody list.

Primary antibodies					
Name	**Host**	**Company**	**Cat.No.**	**Dillution**	**RRID**
PAX6	Rabbit	Chemicon	AB2237	1:200	AB_1587367
SOX1	Rabbit	Self-produced	/	1:200	/
SOX2	Rabbit	Abcam	ab59776	1:200	AB_945584
NESTIN	Rabbit	Self-produced	/	1:200	/
Ki67	Rabbit	Abcam	ab16667	1:50	AB_302459
FABP7	Rabbit	Abclonal	A3246	1:50	/
NANOG	Goat	R&D	AF1997	1:200	AB_355097
OCT4	Mouse	Santa cruz	SC-5279	1:200	AB_628051
TUJ1	Mouse	Biolegend	MMS-435P	1:500	AB_2313773
TUJ1	Rabbit	Biolegend	MRB-435P	1:500	AB_663339
MAP2	Mouse	Sigma	M4403	1:200	AB_477193
NEUN	Rabbit	Millipore	ABN78	1:200	AB_10807945
GFAP	Rabbit	Abcam	ab16997	1:200	AB_443592
O4	Mouse (IgM)	Chemicon	MAB345	1:50	AB_94872
TBR1	Rabbit	Abcam	ab31940	1:100	AB_2200219
VGLUT1	Rabbit	SYSY	135302	1:200	/
GAD67	Mouse	Millipore	MAB5406	1:200	AB_2278725
Tyrosine Hydroxylase (TH)	Rabbit	Millipore	AB152	1:500	AB_390204
ChAT	Goat	Millipore	AB144P	1:100	AB_2079751
KU80 (STEM101)	Mouse	Takara	Y40400	1:100	/
Synapsin 1 (SYN1)	Rabbit	Millipore	574777	1:200	AB_2200124
Synaptophysin (SYP)	Rabbit	Abcam	ab32127	1:200	AB_2286949
Synaptophysin (SYP)	Mouse	SYSY	101011	1:200	AB_887824
Gephyrin (GPHN)	Mouse	SYSY	147011	1:500	AB_887717
PSD95	Rabbit	Abcam	ab18258	1:1000	AB_444362
6E10	Mouse	Covance	SIG-39300	1:200	AB_662803
Iba1	Rabbit	Wako	1919741	1:1000	AB_2665520

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Isolation and expansion of human adult peripheral blood mononuclear cells (PB MNC)

The use of human adult peripheral blood was approved by the Biomedical Research Ethics Committee, SIBS, CAS, and Ruijin Hospital Ethics Committee, Shanghai JiaoTong University School of Medicine, with written informed consent from the donors. 3 participants were recruited in the study. They were 30-, 61- and 56-year old healthy male adults, and designated as donor 1, 2 and 3, respectively. Isolation and expansion of peripheral blood mononuclear cells (PB MNCs) was performed as previously described (Dowey et al., 2012). Briefly, 3-8 ml of adult peripheral blood was collected, and mononuclear cells (MNCs) were isolated using Ficoll-Paque Premium (Sigma) by density gradient centrifugation. The MNCs were then cultured for 8 to 12 days in MNC medium (IMDM: Ham's F12 (1: 1) supplemented with 1% ITS-X, 1% chemically defined lipid concentrate, 1×Glutamax, 50 μg/ml L-ascorbic acid (Sigma), 5 mg/ml BSA (Sigma), 200 μM 1-thioglycerol (Sigma), 100 ng/ml SCF (PeproTech), 10 ng/ml IL-3 (PeproTech), 2 U/ml EPO (R&D system), 40 ng/ml IGF-1 (PeproTech), 1 μM dexamethasone (MP Biomedicals) and 100 μg/ml holo-transferrin (Sigma)), which favors the expansion of erythroblast but does not support lymphocytes growth. Cell numbers were counted and medium was refreshed every two days. When the cell number became approximately same or greater over the starting cell number, the MNCs were suitable for reprogramming. All reagents were purchased from Thermo Fisher Scientific if not otherwise specified.

Generation of iNPCs from human adult PB MNCs by episomal vector transfection

The oriP/EBNA1-based episomal vectors EV SFFV-OCT4-2A-SOX2 (SFFV-OS), EV SFFV-MYC-2A-KLF4 (SFFV-MK) and EV SFFV-BCL-XL have been described previously (Su et al., 2013). The process for human iNPC generation is schematically summarized in Figure 1A. Two million PB MNCs were nucleofected with a mixture of the episomal vectors (4 μg SFFV-OS, 4 μg SFFV-MK and 2 μg SFFV-BCL-XL) following the Amaxa 4D-Nucleofector Protocols for Unstimulated Human $CD34^+$ Cells

(Lonza, P3 primary cell 4D-Nucleofector X Kit). After nucleofection, the PB MNCs were recovered in MNC medium for 2 days and plated onto irradiated MEFs on Matrigel-coated 12-well-plate at a density of 2×10^5/well in MEF medium. The medium was replaced the next day with KSR medium (20% KSR in DMEM/F12 supplemented with 1×Glutamax, 1×NEAA, 0.1 mM β-ME, 10 ng/ml bFGF (Pufei) and 0.25 mM NaB (Sigma)). After 5 days, the medium was changed to N2B27 medium (DMEM/F12: Neurobasal (1:1) supplemented with 1×N2, 1×B27, 1× Glutamax and 0.1 mM β-ME) with small molecule chemicals cocktail including 10 μM SB431542 (Selleck), 3 μM CHIR99021 (Selleck), 0.5 mM VPA (Selleck) and 10 μM Forskolin (Selleck). Culture medium was partially changed every two days. Around 20 days after nucleofection, the NPC-like colonies were picked manually and mechanically triturated into small clusters and reseeded on Matrigel-coated 24-well-plates for further expansion and evaluation. For the first two passages, the human iNPCs were cultured in the N2B27 medium supplemented with 10 ng/ml hLIF (R&D system), 3 μM SB431542 and 3 μM CHIR99021. Then the cells were transferred to the NSC medium (N2B27 medium supplemented with 20 ng/ml bFGF and 20 ng/ml EGF (Peprotech)) to achieve a homogeneous morphology. Cells were purified at passage 4 as neurospheres if they failed to show homogeneous morphology. From passage 5, the established human iNPCs were continuously maintained in NSC medium. For all the passages, human iNPCs were detached using Accutase and cultured on Matrigel-coated dishes. For each donor, 6 lines of iNPCs were generated and stably maintained. All reagents were purchased from Thermo Fisher Scientific if not otherwise specified.

RNA-seq and analysis

PB MNCs from donor 1 and 2 and two lines of iNPCs from each donor were randomly chosen for RNA-seq and analysis. Total RNA was extracted using Trizol reagent (Pufei) and RNA-seq libraries were prepared following the method published previously (Chen et al., 2017). Briefly, the RNA was reversed transcribed by SuperScript II reverse transcriptase (Thermo Fisher Scientific). The double-stranded cDNA was amplified using KAPA HiFi HotStart ReadyMix (KAPA Biosystems) and purified using 1: 0.75

ratio of AMPure XP beads (Bechman Coulter). After quantification by Qubit, cDNA was applied to Bioanalyzer 2100 on a High-Sensitive DNA chip (Agilent Bioanalyzer) to check the library size distribution. Amplified cDNA (~5 ng) was then used to construct Illumina sequencing libraries using Illumina's Nextera DNA sample preparation kit following the manufacturer's instructions. All sample libraries were sequenced on HiSeq2500 instrument (Illumina).

Raw reads were mapped to the hg19 version of human genome using TopHat2 version 2.0.4 program (Trapnell et al., 2009). We calculated fragment per kilobase per million (FPKM) as expression level using Cufflinks version 2.0.2 software (Kim et al., 2013). Genes with the FPKM>1.0 in at least one sample across all samples were retained for further analysis and the expression levels were transformed to log-space by using the $\log_2$ (FPKM+1). Differentially expressed genes (DEGs) among different samples were identified using Rankprod (Hong et al., 2006) with P value < 0.05 and fold change > 2. Functional enrichment of gene sets with different expression patterns was performed using the Database for Annotation, Visualization and Integrated Discovery v6.8 (DAVID v6.8) (Huang da et al., 2009). DEG heat maps were clustered by hierarchical clustering and visualized using Java Tree View software (Saldanha, 2004). PCA analysis was performed using R (http://www.r-project.org). Pearson correlation was used to compare the human iNPC lines with published database CORTECON (van de Leemput et al., 2014) and BrainSpan (http://brainspan.org).

In vitro differentiation of human iNPCs

For spontaneous differentiation of human iNPCs into neurons and astrocytes, human iNPCs were dissociated with Accutase and seeded on PDL-Laminin coated 35 mm dish at a density of 2×10^5 cells/dish and differentiated in the neural differentiation medium consisting of B27 medium (Neurobasal medium with 1×B27, 2 mM Glutamax and 0.1 mM β-ME) supplemented with 1 μg/ml Laminin (Sigma), 1 μg/ml FN (Sigma), 10 ng/ml BDNF (Peprotech), 10 ng/ml NT3 (Peprotech), 10 ng/ml IGF-1 (Peprotech), 10 ng/ml CNTF (Peprotech) and 1 μM cAMP (Sigma). At differentiation day 7, the cells were dissociated into single cells by Accutase and reseeded on PDL-Laminin coated 35

mm dish at a density of 2×10^5 cells/dish and further differentiated in the neural differentiation medium with the addition of 0.2 μM Compound E (Millipore) from differentiation day 9 to 19. For oligodendrocyte differentiation, human iNPCs were first treated with SAG (300 nM) (Millipore) for 10 days and then seeded on PDL-Laminin coated 35 mm dish at a density of 1×10^5 cells/dish in the oligodendrocyte differentiation medium (DMEM/F12 with 1×N2, 1×Glutamax, 0.1 mM β-ME, 30 ng/ml T3 (Sigma), 100 ng/ml Biotin (Sigma), 10 ng/ml PDGF-AA (R&D System), 1 μM cAMP, 10 ng/ml IGF-1, 10 ng/ml NT3 and 10 ng/ml CNTF). Half of the medium was changed every two days during differentiation. Differentiated cells were harvested for evaluation at differentiation day 28 (for neuronal or astrocyte differentiation) or day 35 (for oligodendrocyte differentiation). All reagents were purchased from Thermo Fisher Scientific if not otherwise specified.

Immunofluorescence staining

To prepare brain slices for immunofluorescence staining, mice were anesthetized and perfused transcardially with PBS followed by fixative (4% paraformaldehyde in PBS). Brains were removed from the skull, postfixed overnight, and then transferred into a 20% sucrose solution in PBS for 24 hrs for cryoprotection. The brains were then frozen in -80°C for 2 hrs, cryosectioned into 15 μm thick coronal sections and mounted on gelatinized glass slides. Immunofluorescence staining of brain slices and cultured cells was performed as previously described (Gao et al., 2001; Xia et al., 2007). Primary antibodies and dilutions used in this study are shown in Table S1. Alexa Fluro 488, 546, or 633 secondary antibodies (donkey anti-mouse, donkey anti-rabbit, or donkey anti-goat secondary antibodies, Thermo Fisher Scientific) were used as secondary antibodies. DAPI was used to counterstain nuclei. The images were captured with Olympus BX50 or Leica TCS SP8 confocal laser scanning microscope. Images were collected at 1024×1024-pixel in resolution. For quantification of immunofluorescence staining, 5 fields were randomly chosen for each experiment (*in vitro*) or each mouse brain (*in vivo*).

Generation of human iNPCs lines expressing GFP or ChR2-mCherry

The plasmid FUGW containing GFP (a gift from Dr. David Baltimore (Addgene plasmid # 14883)) was used to generate GFP^+ iNPCs (Lois et al., 2002). To generate iNPCs expressing ChR2-mCherry, the ChR2-mCherry fragment was amplified by PCR from the pAAV-EF1a-ChR2-mCherry (a gift from Dr. Minmin Luo, NIBS, China) and subcloned into the lentiviral vector pFuw-TRE to construct the pFuw-TRE-ChR2-mCherry plasmid. The plasmid FUGW or pFuw-TRE-ChR2-mCherry was packaged into lentivirus that was transfected into iNPCs (< passage 10) as previously described (Tiscornia, 2006). After lentiviral transfection, GFP or mCherry positive iNPC cells were sorted using a FACS Arial cell sorter (BD Biosciences) and propagated for at least 5 passages in NSC medium. The GFP^+ or ChR2-$mCherry^+$ iNPCs were then transplanted into mice brain and analyzed at the indicated time.

Mice

In order to avoid the immune suppression treatment on the host mice and make grafted human neural progenitors survive better, the immunodeficient mice, including wild-type (WT) and AD mice, were recruited in this study. The immunodeficient $Foxn1^{-/-}$ mice were purchased from Shanghai SLAC Laboratory Animal Company (Shanghai, China), which served as the WT control. The strain of heterozygous transgenic AD-model mice 5XFAD (Jackson No. 006554) was purchased from Jackson Lab. The immunodeficient $Rag2^{-/-}$ mice were a gift from Dr. Lijian Hui (SIBCB, China). 5XFAD mice were crossed with $Rag2^{-/-}$ mice to obtain $Rag2^{+/-}$/5XFAD mice, which were then self-crossed to generate the $Rag2^{-/-}$/5XFAD immunodeficient AD model mice. Male mice were used in the study. The cell transplantation was performed in immunodeficient WT mice at 2 months of age and $Rag2^{-/-}$/5XFAD mice at 4 months of age. The behavioral tests were performed in $Rag2^{-/-}$/5XFAD with or without grafted human iNPCs at 9-10 months of age. All of the above mice were housed in a pathogen-free facility and all of the animal experiments were carried out following protocols approved by the Animal Ethics Committee of the Shanghai Institutes for Biological Sciences.

Cell transplantation

For transplantation, human iNPCs at passage 15 were dissociated into single cells using Accutase and suspended in neural differentiation medium supplemented with 10% Matrigel at a density of 5×10^4/μl. Bilateral injections of 2 μl single-cell suspension containing 1×10^5 GFP^+ or ChR2-$mCherry^+$ human iNPCs were then targeted to the hippocampus dentate gyrus (DG) region following coordinates relative to Bregma: AP: -1.06 mm; ML: ±1.0 mm; DV: -2.5 mm. The cells were injected into immunodeficient mice $Foxn1^{-/-}$ (2 months old) or $Rag2^{-/-}$/5XFAD (4 months old) using a 5μl Hamilton micro-syringe (33-gauge) at a rate of 0.4 μl/min. Bilateral injections of 2μl neural differentiation medium containing growth factors into hippocampus of $Rag2^{-/-}$/5XFAD mice as the sham/vehicle controls for behavioral task. Mice were anesthetized with Avertin (0.6 ml/25 g body weight), and the surgery was performed on the stereotaxic apparatus (RWD life science).

Acute brain slice preparation

The use and care of laboratory animals complied with the guidelines of the Animal Advisory Committee at the State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University. Immunodeficient WT or AD mice with grafted human iNPCs 2, 4 and 6 months post transplantation were anesthetized with sodium pentobarbital (50 mg/kg) and sacrificed by decapitation. The brain tissues were immediately dissected out and immersed in ice-cold slicing solution (see below). For mice of 6 months post transplantation, we perfused the mice transcardially with ice-cold sucrose-based slicing solution before decapitation to improve the quality of slices. In ice-cold sucrose-based slicing solution (normal aerated artificial cerebrospinal fluid, i.e. ACSF, listed below but with NaCl replaced with equimolar sucrose) that had been bubbled with 95% O_2 and 5% CO_2, tissue blocks containing hippocampus were sliced coronally with a vibratome (Leica VT1000S). Slices (300 μm thick) were collected and incubated at 35 °C in ACSF containing (in mM): NaCl 126, KCl 2.5, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, NaH_2PO_4 1.25, and dextrose 25 (315 mOsm, pH 7.4). After 60-min incubation, slices were then incubated at room temperature until use.

Electrophysiological recording

Whole-cell patch-clamp recording was performed in human iNPC-derived neurons on differentiating day 50 (*in vitro*) and in $EGFP^+$ or $mCherry^+$ human iNPC-derived neurons in the coronal brain slices of immunodeficient mice (*in vivo*). The whole-cell recordings were performed with a Multiclamp 700B (Molecular Devices). The cultured cells were patched as previously described (Yue et al., 2015). For the grafted cells, acute slices were transferred to the recording chamber and perfused with aerated ACSF at a rate of 1.2 ml/min. Slices were visualized under upright infrared differential interference contrast microscope (BX51WI, Olympus). We performed recordings from grafted cells, which were identified by their expression of GFP, with patch pipettes filled with Alexa Fluor-594-containing internal solution (in mM): K-gluconate 145, $MgCl_2$ 2, Na_2ATP 2, HEPES 10, and EGTA 0.2 (286 mOsm, pH 7.2). The impedance of patch pipettes for somatic recording was 3-5 MΩ. Some cells showed spontaneous firing after membrane rupture because of their depolarized membrane potential. For these cells, we hyperpolarized them to -70 mV to examine membrane properties, but they were not included in data analysis of resting membrane potential. Recordings with series resistance >30 MΩ were discarded. In current clamp mode, step currents (with steps of 10 pA, 500 ms in duration) were applied to evoke action potentials (APs). Hyperpolarizing current pulses (-10 pA, 500 ms) were injected to test the input resistance. In voltage clamp mode, spontaneous excitatory postsynaptic currents (sEPSCs) were recorded when membrane potentials were clamped at -70 mV (i.e. the reversal potential of IPSCs), while spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded at EPSC reversal potential, 0 mV. The kinetics of PSCs were analyzed with MiniAnalysis 6.03 (SynaptoSoft Inc., NJ, USA).

Optogenetic experiment

For the immunodeficient mice transplanted with ChR2-expressing grafted cells, we used single-photon setup to examine whether there were synaptic connections forming between host and grafted cells. We used a homemade AOD-based rapid laser

stimulation system equipped with a 473-nm blue laser (50 mW; Cobolt Inc., Sweden). The laser beam was coupled to the light path of an Olympus upright microscope and delivered to the slices through a water-immersion 40X objective (0.8 N.A.). Firstly, we patched a grafted cell with ChR2-mCherry expression on the cell membrane and tested whether light stimulation at the perisomatic region could reliably induce APs. Then we recorded a neighboring host cell identified by their firing pattern and morphology (filled with Alexa Fluor-594 through patch pipettes). Whole field random light stimulation was delivered to activate grafted cells. We clamped the recorded cell at -70 mV and 0 mV to examine the excitatory or inhibitory synaptic inputs induced by light stimulation, respectively.

Spontaneous alternation Y-maze task

Spontaneous alternation performance was tested as described previously (Ohno et al., 2006). Each mouse was placed in the center of the symmetrical Y-maze and was allowed to explore freely through the maze during an 8-min session. The sequence and total number of arms entered was recorded. Experiments were done blind with respect to the genotype and experimental conditions of the mice. Percentage alternation is as follows: number of triads containing entries into all three arms/maximum possible alternations (the total number of arms entered minus 2) × 100.

Barnes maze

Barnes maze behavior test was performed as previously described (Sunyer et al., 2007). The maze was 90 cm in diameter with 20 holes equally spaced along the perimeter. One of the holes was designated as the target hole, which led into a darkened escape box. An electric metronome (AROMA, Shenzhen, China) was used as a buzzer to make a noise of 85 dB, which acted as an aversive stimulus that motivate the mice to find the target hole. Visual cues were placed surrounding the maze, which served as the reference points for the mice to locate the target hole. For training trial, all mice received 4 trials per day with an inter-trial interval of 15 min during the first 4 days.

Each trial consisted of 10 s in the disorientation box in the center of the maze, followed by 3-min exploration time to locate the target hole. Mice that failed to escape were gently guided to the target hole. After escaping, they were left in the escape box for 1 min. The probe trail was performed on day 5, when the escape box was covered and mice were allowed to explore the maze for 90 s. The latency to the first encounter of the target hole (primary latency) and the latency to enter the escape box (total latency) were recorded during training trial. The primary latency, duration in target quadrant and the numbers of nose pokes above each hole were recorded in the probe trial.

Field potential recording

Hippocampal recordings procedures were the same as previously described (Cao et al., 2007; Tang et al., 1999; Wang et al., 2008). Briefly, Whole-brain coronal slices (370 μm thickness) containing the hippocampus were cut using a vibroslicer (Vibratome 3000, Vibratome, St Louis, MO, USA) with cold (4 °C) and oxygenated (95 % O_2, 5 % CO_2) modified artificial cerebrospinal fluid (ACSF) containing (in mM): choline chloride 110, KCl 2.5, $CaCl_2$ 0.5, $MgSO_4$ 7, $NaHCO_3$ 25, NaH_2PO_4 1.25 and D-glucose 25 (pH 7.4). The slices were recovered in an incubation chamber with normal ACSF containing (in mM): NaCl 119, $CaCl_2$ 2.5, KCl 2.5, $MgSO_4$ 1.3, $NaHCO_3$ 26.2, Na_2HPO_4 1.0 and D-glucose 11 (pH 7.4, 95 % O_2 and 5 % CO_2) for 60 min at 31 °C. A unipolar tungsten stimulating electrode (FHC, USA) was placed in CA3 region to deliver electro-stimuli. A glass microelectrode (3 MΩ, 0.5 M CH_3COONa) was positioned in the CA1 region to record extracellular field potentials. Test responses were elicited at 0.033 Hz. After obtaining a stable baseline response for at least 15 min, LTP was induced by three trains of theta burst stimulation (TBS) (10 bursts of four pulses at 100 Hz separated by 200 ms).

Measurement of BDNF

The BDNF level was measured in the whole brain and the hippocampus of $Rag2^{-/-}$ /5XFAD with and without grafted human iNPCs following the manufacturer's protocol (Promega). After sacrifice of mice with Nembutal overdose, half brains (without the

cerebellum and olfactory bulb) were taken and hippocampal tissues were dissected from the other half brain. The tissues were quickly frozen on dry ice, then weighed and homogenized on ice in 150 mg/ml T-PER (Pierce) with protease and phosphatase inhibitor cocktails (Sigma). The homogenate was spun at 16,000 g for 1 hour at 4 °C, and the fresh supernatant was used for ELISA analysis.

Statistical analysis

All statistical analyses were performed in GraphPad Prism software (GraphPad 7.0). Cell counting and electrophysiological data were presented as mean ± SD, while BDNF ELISA, behavior test and LTP data were presented as mean ± SEM. Student's t test (two-tailed) was performed for statistical analysis between two groups. One-way or two-way ANOVA with Tukey's multiple comparison post hoc test was used when three or more groups were compared. Sample size (n) values were provided in the relevant text, figures and figure legends. The statistical analyses were obtained from three independent experiments. Statistical significance was set at $*P < 0.05$.

Data availability

All RNA-seq data are available at the Gene Expression Omnibus (GEO) under accession number GSE107806.

REFERENCES:

Cao, X., Cui, Z., Feng, R., Tang, Y.P., Qin, Z., Mei, B., and Tsien, J.Z. (2007). Maintenance of superior learning and memory function in NR2B transgenic mice during ageing. The European journal of neuroscience *25*, 1815-1822.

Chen, J., Suo, S., Tam, P.P., Han, J.J., Peng, G., and Jing, N. (2017). Spatial transcriptomic analysis of cryosectioned tissue samples with Geo-seq. Nature protocols *12*, 566-580.

Dowey, S.N., Huang, X., Chou, B.K., Ye, Z., and Cheng, L. (2012). Generation of integration-free human induced pluripotent stem cells from postnatal blood mononuclear cells by plasmid vector expression. Nature protocols *7*, 2013-2021.

Gao, X., Bian, W., Yang, J., Tang, K., Kitani, H., Atsumi, T., and Jing, N. (2001). A role of N-cadherin in neuronal differentiation of embryonic carcinoma P19 cells. Biochem Biophys Res Commun *284*, 1098-1103.

Hong, F., Breitling, R., McEntee, C.W., Wittner, B.S., Nemhauser, J.L., and Chory, J. (2006). RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis.

Bioinformatics *22*, 2825-2827.
Huang da, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols *4*, 44-57.
Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome biology *14*, R36.
Lois, C., Hong, E.J., Pease, S., Brown, E.J., and Baltimore, D. (2002). Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. Science *295*, 868-872.
Ohno, M., Chang, L., Tseng, W., Oakley, H., Citron, M., Klein, W.L., Vassar, R., and Disterhoft, J.F. (2006). Temporal memory deficits in Alzheimer's mouse models: rescue by genetic deletion of BACE1. The European journal of neuroscience *23*, 251-260.
Saldanha, A.J. (2004). Java Treeview-extensible visualization of microarray data. Bioinformatics *20*, 3246-3248.
Su, R.J., Baylink, D.J., Neises, A., Kiroyan, J.B., Meng, X., Payne, K.J., Tschudy-Seney, B., Duan, Y., Appleby, N., Kearns-Jonker, M.*, et al.* (2013). Efficient generation of integration-free ips cells from human adult peripheral blood using BCL-XL together with Yamanaka factors. PloS one *8*, e64496.
Sunyer, B., Patil, S., Höger, H., and Lubec, G. (2007). Barnes maze, a useful task to assess spatial reference memory in the mice.
Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., and Tsien, J.Z. (1999). Genetic enhancement of learning and memory in mice. Nature *401*, 63-69.
Tiscornia, G., Singer, O. and Verma, I. M. (2006). Production and purification of lentiviral vectors. Nat Protoc *1*, 241-245.
Trapnell, C., Pachter, L., and Salzberg, S.L. (2009). TopHat: discovering splice junctions with RNA-Seq. Bioinformatics *25*, 1105-1111.
van de Leemput, J., Boles, N.C., Kiehl, T.R., Corneo, B., Lederman, P., Menon, V., Lee, C., Martinez, R.A., Levi, B.P., Thompson, C.L.*, et al.* (2014). CORTECON: a temporal transcriptome analysis of in vitro human cerebral cortex development from human embryonic stem cells. Neuron *83*, 51-68.
Wang, H., Feng, R., Phillip Wang, L., Li, F., Cao, X., and Tsien, J.Z. (2008). CaMKII activation state underlies synaptic labile phase of LTP and short-term memory formation. Current biology : CB *18*, 1546-1554.
Xia, C., Wang, C., Zhang, K., Qian, C., and Jing, N. (2007). Induction of a high population of neural stem cells with anterior neuroectoderm characters from epiblast-like P19 embryonic carcinoma cells. Differentiation *75*, 912-927.
Yue, W., Li, Y., Zhang, T., Jiang, M., Qian, Y., Zhang, M., Sheng, N., Feng, S., Tang, K., Yu, X.*, et al.* (2015). ESC-Derived Basal Forebrain Cholinergic Neurons Ameliorate the Cognitive Symptoms Associated with Alzheimer's Disease in Mouse Models. Stem cell reports *5*, 776-790.