Supplementary Methods

Production of recombinant lentivirus

High titer lentiviral particles were produced in 293LTV cells by transient transfection with calcium phosphate of transfer vector HS1-Cre (Suh et al., 2007), second-generation packaging construct pCMV-deltaR8.91, and vesicular stomatitis virus envelope-expressing construct pMD2.G. 72 h after transfection, the supernatant was collected and purified by ultracentrifugation. The pellet was resuspended in PBS and aliquots stored at -80°C until use. The amount of transducing units was determined by diluting serially concentrated lentivirus into cell medium, plating and growing for 3 days. Cells were immunocytochemically stained and clones per sample were counted. Measurement of p24 concentration was carried out with the HIV-1 p24 ELISA assay (BioCat, Heidelberg, Germany).

Lentivirus vector administration

Adult mice were anesthetized by an i.p. injection of a combination of fentanyl (0.05 mg/kg), midazolam (5 mg/kg) and medetomidin (0.5 mg/kg). Mice were positioned in a stereotactic apparatus and kept on an animal heating pad to control body temperature during surgery. The scalp was cut open in rostrocaudal direction and lidocaine spray was applied on the skull and the surrounding skin to reduce pain. 1 µl of the lentivirus ($1.4x10^{13}$ tu/ml) was injected bilaterally into the dentate gyrus at the following coordinates from bregma: -2.0 mm anteriorposterior, +/-2.0 mm mediolateral, -2.0 dorsoventral. The virus was applied using a microprocessor controlled mini-pump with a 34G beveled NanoFil needle at a flow rate of 200 nl/min. The injection needle was left in place for additional 5 min and then slowly removed. After sewing the scalp, anesthesia was antagonized by intraperitoneal injection of flumazenil (0.5 mg/kg), naloxon (1.2 mg/kg) and atipamezol (2.5 mg/kg). Additionally, mice received a subcutaneous injection of buprenorphine (0.05 mg/kg) to reduce pain and 1 ml of

0.9% saline to compensate fluid loss during and after surgery. The mice were returned to their home cages, which were kept on a heating plate at 37 °C overnight.

Generation of neurospheres

Neurosphere cultures were prepared from the DG of young adult (7-10 weeks) mice. Briefly, the tissue was enzymatically dissociated in Neural Tissue Dissociation Kit (Miltenyi Biotec) at 37°C for 35 min. Dissociation was stopped by adding 10 ml HBSS (Ca^{2+}, Mg^{2+}) (Invitrogen). Cells were then centrifuged at 300 x g for 10 min, resuspended in ice cold medium consisting of 0.9 M sucrose (Sigma-Aldrich,) in 0.5×HBSS, and centrifuged for 10 min at 750 x g. The cell pellet was resuspended in 10 ml HBSS (Ca^{2+}, Mg^{2+}), and centrifuged for 10 min at 300 x g. The cell pellet was finally resuspended in DMEM/F12 Glutamax (Invitrogen) supplemented with B27 (Invitrogen), 20 ng/ml EGF/FGF2 (Peprotech), 2 mM glutamine (Sigma-Aldrich), 100 units/ml penicillin (Invitrogen) and 100 µg/ml streptomycin (Invitrogen) and buffered with 8 mM HEPES, and cells were plated in 24-well plates. Neurospheres were used for experiments after 9-12 days *in vitro* (DIV).

Analysis of recombination efficiency

To analyze the number of recombination events, DG neurospheres were generated 4 days after the last tamoxifen injection of animals and kept in culture for 1-2 weeks. Recombination efficiency was then tested by PCR with genomic DNA isolated from single DG neurospheres by the use of the GenElute mammalian genomic DNA Miniprep Kit (Sigma-Aldrich). The DNA was tested in parallel on individual recombination events on the alleles specific for the CB1 and the Rosa26 locus. PCR conditions for the recombined CB1 allele resulting in a 600 bp fragment are as followed: 1x 95°C for 3 min, 42x (95°C for 1 min; 55°C for 1 min, 72°C for 1 min), 72°C for 5 min, using the forward primer 5′-GCTGTCTCTGGTCCTCTTAAA-3′ (G50) and the reverse primer 5′-CTCCTGTATGCCATAGCTCTT-3′ (G53). In case no

recombination event was detected, the same PCR conditions were performed but using another reverse primer 5′-GGTGT CACCT CTGAA AACAG A-3′ (G51) to validate the non-recombined allele by the occurrence of a 500 bp fragment. Recombination efficiency at the Rosa26 locus was detected by using the forward primer 5′-CTGGCTTCTGAGGACCG-3′ (21306) and the reverse primer 5′-CAGGACAACGCCCACACA-3′ (24500) with the following conditions: 1x 95°C for 3 min, 40x (95°C for 45 sec; 60°C for 45 sec, 72°C for 3 min), 72°C for 5 min resulting in a 1.4 kb fragment. Non-recombined alleles were detected with the same PCR conditions and the use of another reverse primer 5′-AAAGAACGGAGCCGGTTGGCGCCTA-3′ (Neo5), obtaining 450 bp.

Reverse transcriptase quantitative PCR

Total RNA from pooled DG Neurospheres (9 DIV) or FACS-sorted cells was extracted with RNeasy Mini or Micro kit (Qiagen), and reverse transcriptase reactions with random primers (High Capacity cDNA Reverse Transcription kit, Applied Biosystem) using 350 ng RNA, or 10-20 ng RNA in case of FACS-sorted cells, were performed. Taqman qPCR gene expression assays (Applied Biosystems) were carried out with Taqman probes (Assay ID) detecting mRNA of mouse CB1 (Mm00432621_s1), CB2 (Mm02620087_s), BDNF (Mm04230607_s1) or DAGLα (Mm00813830_m1). As reference gene, GAPDH (Mm99999915_g1) was used. qPCR reactions were performed with the 2x TaqMan gene expression master mix (Applied Biosystems) and analyzed with a ABI 7300 qPCR machine (Applied Biosystems).

Double Fluorescence *in situ* hybridization

Introduction and animals

We performed double fluorescence *in situ* hybridization experiments on coronal cortical sections and analyzed hippocampus, dorsal cortex and basolateral amygdala using FITC labeled riboprobe for GAD65 and digoxigenin labeled riboprobe for CB1 to detect CB1/GAD65 positive interneurons. This population was counted in 3 nes-CB1ko/ko mutants and corresponding 3 CB1wt/wt animals in order to determine whether the number of interneurons is altered in mutants versus wild-type. Three slides of each animal covering cortical region 1.3 – 2.1 mm posterior to bregma were analyzed, containing every 6th section from 3 different positions within the whole region. At least 200 cells each from hippocampus (dentate gyrus), cortex and amygdala were counted.

For determination of differentiation status in neurogenic cells in the hippocampal subgranular layer Dlx^{Cre} x CB1$^{fl/fl}$ (dlx-CB1ko/ko) animals and CB1$^{fl/fl}$ (CB1fl/fl) control (Monory et al., 2006) animals were used.

Tissue preparation

Adult mice (nes-CB1ko/ko and CB1wt/wt: 56 dptm; Dlx-^{Cre} x CB1$^{fl/fl}$ mutants and CB1fl/fl as control wt siblings: 2 months old) were killed by cervical dislocation. Brains were removed, snap-frozen on dry ice and stored at -80°C. After removing from -80°C, brains were mounted on Tissue Freezing Medium (Leica Biosystems) and 20 µm-thick coronal sections were cut from the forebrain on a cryostat Leica CM3050 S, dried on a 42°C warming plate and stored at -20°C until used.

Synthesis of probes

Both digoxigenin (DIG) and fluorescein isothiocyanate (FITC) labeled riboprobes were used. DNA templates were originally generated by RT-PCR from cDNA derived from total mouse brain, reported in Marsicano and Lutz (1999). For each probe, GenBank accession number, primer sequences and length of probe are listed therein.

PCR products were cloned into pBluescript KS^- and used as templates for riboprobe synthesis as described. Linearized template DNA was column purified (PCR purification kit, Invitrogen), resuspended in diethyl pyrocarbonate (DEPC)-treated H_2O at a concentration of 1 µg/µl, and stored at -20°C. For both probes *in vitro* transcription was carried out for 3 h at 37°C in a total volume of 20 µl containing 2 µg of linearized plasmid with inserts of desired marker genes (CB1, GAD65, calretinin, calbindin). In a double fluorescent *in situ* hybridization two genes can be detected simultaneously in the same section using two different RNA-probes, labelled with DIG or FITC and visualized with different fluorescence detection systems. The uridin-5'-triphosphates (UTPs) are available either coupled with digoxigenin or with FITC (DIG RNA labelling mix and Fluorescein isothiocyanate (FITC) RNA labelling mix, respectively, Roche) which also contains rATP, rCTP, rGTP. The independent reactions contained DNA (2 µg), 1 x transcription buffer, DIG RNA labelling mix or FITC RNA labelling mix, 40 units RNasin (Promega), 40 units of T3 or T7 RNA polymerase (Roche). Reactions were treated thereafter with 20 units of RNase-free DNaseI (Roche) for 20 min at 37°C, and labeled probes were purified by RNeasy Kit (Qiagen), and amounts determined by spectrophotometric measurement (NanoDrop 2000c, Thermo Scientific) and correct probe sizes verified on an agarose gel.

Restriction enzymes (New England Biolabs) used for linearization and RNA polymerases used for each probe were as follows (see Marsicano and Lutz, 1999): CB1 antisense: Bam HI, T3; CB1 sense: Eco RI, T7; GAD65 antisense: Bam HI, T3; GAD65 sense: Eco RI, T7; calretinin antisense: BamHI, T3; calretinin sense: Eco RI, T7; calbindin antisense: Eco RV, T7; calbindin sense: Bam HI, T3.

In situ hybridization

Slides were thawed for 30 min at RT, fixed in ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS, containing in mM: NaCl, 136; KCl, 2.7; Na_2HPO_4, 10; KH_2PO_4, 1.8, pH

7.4), rinsed twice in PBS for 5 min, incubated for 15 min in 1% H_2O_2 in Methanol, followed by two 2 min-washes in PBS. After 8 min incubation in 0.2 M HCl and another 2 min-wash in PBS, Proteinase K treatment (0.4 U/ml) was performed in 50 mM Tris/5 mM EDTA buffer. Sections were rinsed for 5 min in PBS and fixed again in ice-cold 4% paraformaldehyde in PBS, then incubated for 10 min in 0.1 M triethanolamine-HCl (pH 8.0) to which 600 µl of acetic anhydride was added (to 250 ml) twice, rinsed thereafter in PBS for 5 min and 0.9% NaCl in DEPC-H_2O for 5 min, then dehydrated in graded series of ethanol (30-50-70-80-95-100-100%) for 20 s each and air-dried. Hybridization was carried out overnight (16 h) in a humid chamber at 54°C in 100 µl of hybridization buffer/slide containing in ng/ml hybridization buffer: 400 ng DIG-UTP labeled CB1, 800 ng FITC-UTP labeled GAD65 for analysis/determination of interneuron number; 600 ng DIG-UTP labeled calretinin or calbindin, 600 ng FITC-UTP labelled CB1 (for analysis of marker gene expression on differentiation state). Hybridization buffer consisted of 50% formamide, 20 mM Tris-HCl, pH 8.0, 0.3 M NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0, 10% dextran sulfate (D8906, Sigma-Aldrich), 0.02% Ficoll 400 (F4375, Sigma-Aldrich), 0.02% polyvinylpyrrolidone (MW 40 000, PVP40, Sigma), 0.02% bovine serum albumin (BSA, A6793, Sigma-Aldrich), 0.5 mg/ml tRNA (Roche), 0.2 mg/ml fragmented herring sperm carrier DNA and 200 mM DTT.

Slides were rinsed in 5 x SSC (standard saline citrate) / 0.05% Tween 20 (9127.1, Roth) in 62°C (waterbath) to separate cover slips, (where 1x SSC contains 150 mM NaCl, 15 mm Na_3 citrate, pH 7.4), followed by one wash at 62°C in 50% formamide/2 x SSC/0.05% Tween 20, one wash at 62°C in 50% formamide/1x SSC/0.05% Tween 20, one wash in 0.1x SSC/0.05% Tween 20 at 62°C, each 30 min.

Visualization of probes

Slides were incubated for 1 h at 30°C for blocking in 4% heat inactivated sheep serum in TNT (Tris-NaCl-Tween buffer, i.e. 100 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20), then washes and incubations were continued in Shandon cassettes (Thermo Scientific Shandon Sequenza Immunostaining Racks, Thermo Scientific) at 30°C: 3 washes for 2 min each in TNT buffer, incubation at 30°C for 30 min Perkin Elmer (PE) blocking buffer (Perkin Elmer), incubation at 30°C for 2 h with anti FITC(Fab)-POD antibody (Roche Diagnostics) 1:1000 diluted in PE blocking buffer, followed by three washes in TNT buffer for 2 min each at 30°C. Immediately before use, fluorescent tyramin FITC (TSA Plus Tyramin FITC System, Perkin Elmer) was prepared by diluting 50 times into Amplification diluent, added to the slides and incubated for 15 min in the dark at 30°C. Slides were then washed in TNT, five times in 3% H_2O_2 in PBS, twice in TNT, each for 2 min, 30°C. For detection of DIG labeled probe, slides were incubated in PE blocking buffer for 30 min at 30°C, then over night at 4°C with anti- DIG(Fab)-POD antibody (Roche Diagnostics) 1:2000 diluted in PE blocking buffer. Slides were again washed in TNT buffer three times at 30°C, 2 min each, fluorescent tyramin CY3 (TSA Plus CY3 System, Perkin Elmer) was diluted 1:75 in Amplification diluent, incubated 15 min in the dark, 30°C, followed by five PBS washes, one containing 1:5000 DAPI to stain nuclei. Afterwards, slides were mounted in Mowiol mounting medium. Images were acquired using Leica DM5500B or a Zeiss Axiovert LSM710 laser scanning confocal microscope.

Cell isolation by fluorescence-activated cell sorting (FACS)

Animals

For FACS isolation of cells, CB1-floxed (Marsicano et al., 2003) and nestin-CreERT2 (Corsini et al., 2009) animals were bred with Gt(ROSA)26Sor$^{tm5(CAG-Sun1/sfGFP)Nat}$ mice (Jax strain 021039; Mo et al., 2015) as a reporter mouse line instead of ROSA-stop-YFP (Srinivas

et al., 2001) in order to achieve high GFP expression located in the nuclear membrane after tamoxifen treatment. Gt(ROSA)26Sor$^{tm5(CAG-Sun1/sfGFP)Nat}$ mice contain a loxP-flanked stop cassette upstream of the CAG promoter driving expression of coding sequences for the mouse nuclear membrane protein SUN1 (Sad1 and UNC84 domain containing 1) fused at its C-terminus to 2 copies of superfolder GFP (sfGFP) followed by 6 copies of myc, inserted in the Gt(ROSA)26Sor locus. Used male mice contained homozygous ROSA-stop-Sun1/sfGFP alleles, heterozygous nestin-CreERT2 allele and homozygous CB1-floxed/floxed alleles (named nes-CB1ko/ko$^{Sun1-sfGFP}$) or homozygous CB1-wt/wt alleles (named CB1wt/wt$^{Sun1-sfGFP}$) (in C57BL/6N background). Tamoxifen treatment occurred at an animal age of 8 weeks.

Tissue dissection and dissociation

On 28 or 56 dptm, mice were anesthetized, and the brain was quickly dissected. The hippocampus was transferred into ice-cold oxygenated artificial CSF (87 mM NaCl, 2.5mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 75 mM sucrose, 20 mM glucose, 1 mM $CaCl_2$, 2 mM $MgSO_4$). The tissue was digested by the Papain Dissociation System Kit protocol (Worthington) with some modifications. The papain buffer was supplemented with 10% trehalose (Sigma), as described by Campbell et al. (2017). After trituration with a fire-polished Pasteur pipette and centrifugation at 300 x g for 5 min, the gradient was made with Earle's Balanced Salt Solution (EBSS) with 10% FBS used in place of ovomucoid protease inhibitor solution. The final cell pellet was resuspended in PBS buffer. Cells were incubated with anti-CD133-APC (eBioscience) and anti-PSA-NCAM-PE (Miltenyi Biotec) antibodies for 30 min at 4°C. After two-rinse steps in cold-PBS, cells were filtered with a 30µm filter preseparation filter and kept at 4°C for the cell sorting.

Cell collection

The cells were sorted on a fluorescence-activated cell sorter BD FACSAria™ III, using FACSDiva™ Software (BD Biosciences). Forward scattering and side scattering excluded

debris, dead and doublet cells. Among the GFP^+ cells, the progenitor cells and neurons were isolated by using the following markers: CD133-APC^+ and PSA-NCAM-PE^+, respectively. Cells were sorted in RLT buffer for RNA extraction (Qiagen, RNeasy Micro kit), and RNA was extracted immediately after cell isolation.