Supplementary Materials and Methods

Plasmids

miR-15b and TET3-expression constructs were inserted into the pCDH vector (System Biosciences). The inserted TET3-expression sequence was described previously [1]. TET3 shRNA oligonucleotide was inserted into pSicoR-GFP. The target TET3 sequence used was described previously in the same paper [1]. The miR-15b inhibitor used in this study was obtained from Guangzhou RiboBio.

Cell culture, transfection, and lentivirus infection

HEK 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), non-essential amino acids, and penicillin/streptomycin in a 37°C, 5% CO2 incubator. The cells were plated and cultured overnight before plasmid transfection. Transfection of plasmids was performed using GenEscort I (Nanjing Wisegen Biotechnology) according to the manufacturer's instructions. Lentivirus was packaged with one core vector, psPAX2, and pMD2.G in HEK 293T cells. Primary neural progenitor cells were isolated from E12 mouse embryonic cortex and cultured as follows. Briefly, cerebral cortices were dissected from mouse embryos at E12 and dissociated by incubation with Accutase (Life Technologies) for 5 minutes at 37˚C, followed by three washes in DMEM containing penicillin/streptomycin. The dissociated cells were cultured in neural stem cell basal medium (Millipore) supplemented with10 ng/ml basic fibroblast growth factor (bFGF) (Life Technologies) and 10 ng/ml epidermal growth factor (EGF) (Life Technologies) in untreated Petri dishes as neurospheres to expand NPCs. The neurospheres were passaged every 3 days. The dissociated cells from neurospheres in the

second passage were plated onto dishes coated with poly-ornithine and laminin in neural stem cell basal medium supplemented with 5ng/ml bFGF and 5ng/ml EGF, and they were then cultured for 24 hours before infection. To induce differentiation, the medium was replaced with DMEM supplemented with 2% B27 and 1% FBS, and the cells were further cultured for 3-5 days.

miRNA microarray analysis

Small RNA fractions were enriched from the mouse embryonic forebrains using the mirVana miRNA Isolation Kit (Ambion) according to the manufacturer's instructions. Purified RNA was labeled with fluorescein and hybridized on a miRNA microarray chip (Agilent Technologies) with 700 mature mouse miRNA probes. Finally, hybridization signals were detected, and the images were scanned and quantified. The data analysis was performed with GeneSpring 10.0.

Real-time polymerase chain reaction analysis

Total RNA was extracted from cultured cells using the RNAprep pure Micro Kit (Tiangen Biotech), according to the manufacturer's directions. Then, complementary DNA was reverse-transcribed from total RNA samples using the FastQuant RT Kit (Tiangen Biotech). The complementary DNA for mature miR-15b detection was reverse-transcribed from total RNA samples using the miRcute miRNA First-Strand cDNA synthesis Kit (Tiangen Biotech). Quantitative real-time PCR (qRT-PCR) was performed using the miRcute miRNA qPCR Detection Kit (SYBR Green I) for mature miR-15b and the SuperReal PreMix Plus (SYBR Green I) Kit (Tiangen Biotech) for other mRNAs in 20 μl of reaction mixture on an ABI PRISM 7500 sequence detector system (Applied Biosystems). The primer sequences for β-actin, TET3, Ki67, and cyclin D1 were from previous reports [2-5]. The

relative amount of each mRNA was determined by the $2^{-\Delta\Delta CT}$ method [6]. All qRT-PCR studies were repeated three times in triplicate for each sample.

Western blotting

Protein was isolated from cultured cells with RIPA lysis buffer (Beijing Solarbio Science and Technology) supplemented with protease inhibitor cocktail (Beijing Solarbio Science and Technology). The homogenates were centrifuged at 17, 000 *g* for 20 min at 4°C. The supernatants were collected, and protein concentration was measured witha BCA kit (Thermo Scientific). Protein samples (30 μg) were run on 12% SDS-PAGE gels (Life Technologies) and then transferred to nitrocellulose membranes (Whatman) with a semidry transfer system (BIO-RAD). The membranes were blocked with 5% nonfat dry milk (BD Bioscience) dissolved in Phosphate Buffered Saline (PBS) containing 0.05% Tween-20 for 1 hour at room temperature (RT). Membranes were incubated with primary antibody at 4°C overnight, followed by incubation with secondary antibodies for 1 hour at RT. The primary antibodies used in western blotting were rabbit anti-Tuj1 (1:2000, Sigma) and mouse anti-β-actin (1:3000, Proteintech Group). Secondary antibodies were donkey anti-rabbit IgG or donkey anti-mouse IgG (1:10000, Odyssey).

Luciferase assays

The TET3 3’-UTR fragments containing the predicted target sequence were generated and then cloned into the psiCHECK-2 dual-luciferase vector (Promega). The predicted miR-15b binding site was mutated using the Hieff Mut^{TM} Site-Directed Mutagenesis Kit (Shanghai Qcbio Science and Technology). HEK293T cells were seeded into 96-well plates and cotransfected with the luciferase

reporter vector together with miR-15b. Briefly, 48 h after transfection, cells were harvested for the luciferase assay using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

Dot blot

Dot blot was performed as previously described [7]. Briefly, genomic DNA was extracted from cultured cells using the TIANamp Genomic DNA Kit (Tiangen Biotech), according to the manufacturer's instructions. Genomic DNA was denatured at 99°C and spotted on nylon membrane (Millipore). The membrane was incubated at 80°C for 1 hour for crosslinking and then was blocked with 5% nonfat dry milk (BD Bioscience) dissolved in Phosphate Buffered Saline (PBS) containing 0.05% Tween-20 for 1 hour at RT. Membranes were incubated with primary antibodies against 5-hydroxymethylcytosine (5hmC) (1:10000, Active Motif) or 5-methylcytosine (5mC) (1:1000, Epigentek) at 4°C overnight, followed by incubation with secondary antibodies for 1 hour at RT. Secondary antibodies were donkey anti-rabbit IgG or donkey anti-mouse IgG (1:10000, Odyssey).

Chromatin Immunoprecipitation qPCR (ChIP-qPCR)

Chromatin immunoprecipitation (ChIP) was performed according to published work [8] following modification. Tissue was fixed in 1% formaldehyde and cross- linked cell lysates were sheared by sonication to generate chromatin fragments with an average length of 300bp. Complexes containing the target were then immunoprecipitated using the specific antibody (anti-TET3, GeneTex) overnight at 4°C, which had been incubated with protein A magnetic beads overnight at

4°C. After extensive washing, the precipitated protein-DNA complexes were eluted from the antibody and incubated overnight at 65℃ to reverse formaldehyde cross-link. The supernatant collected was used to the genomic DNA extraction with the TIANamp Genomic DNA Kit (Tiangen Biotech) according to the manufacturer's instructions. Samples were subjected to qPCR using specific primers on an ABI PRISM 7500 sequence detector system (Applied Biosystems). The primer sequence for β-actin (promoter) was from previous reports [9]. The following primer sequences for cyclin D1 are used: region1-F: CCAGCGAGGAGGAATAGATG, region1-R: AGCGTCCCTGTCTTCTTTCA [10]; region2-F: CGCAGCCCCATAAATCATGC, region2-R: CCCTTCGGGACAGTGATTCC.

Glucosylated Hydroxymethyl-sensitive qPCR (GlucMS-qPCR)

Genomic DNA was extracted from cultured cells using the TIANamp Genomic DNA Kit (Tiangen Biotech) according to the manufacturer's instructions. GlucMS-qPCR was performed using the EpiMark®5-hmC and 5-mC Analysis Kit (NEB) according to the manufacturer's instructions. The content of unmodified cytosine, 5hmC, and 5mC was calculated as described previously [9]. The primer sequences for cyclin D1 are listed as the following: cyclin D1-F: TCACCTTATCGGCTCACAAGT, cyclin D1-R: AGACACGATAGGCTCCTTCC.

In utero electroporation

Briefly, pregnant dams were deeply anesthetized with Nembutal. Then, 1-1.5 μl of plasmids (1-2 μg/μl) and 0.02% Fast Green (Sigma) were microinjected into the lateral ventricle of the forebrain in embryonic day 13 (E13) mouse embryos. Embryonic brains were electroporated using an

electroporator (BTX ECM830) with five 40-V pulses (50 ms duration; 950 ms interval). After electroporation, the brains from embryos were obtained at different times, depending on the experiments, and fixed with 4% PFA at 4˚C overnight. After dehydration with 30% sucrose for 48 h at 4°C, the brains were embedded in OCT compound (Sakura Finetek) and frozen. The frozen brains were cut coronally at 15-μm thickness. In the rescue experiments, the ratio of the concentration of TET3 plasmid to the miR-15b plasmid was 2:1. In the proliferation experiments, BrdU (100 mg/kg) was intraperitoneally injected into mice 2 h before sacrifice, and brains were harvested for BrdU staining [11]. In the cell cycle exit experiments, BrdU (100 mg/kg) was intraperitoneally injected 48 hours after electroporation, and brains were harvested at E16. In the BrdU birth-dating experiments, BrdU (100 mg/kg) was intraperitoneally injected into pregnant mice at E13 and the embryos were collected at E18 according to previous works [12, 13].

***In situ* hybridization**

miR-15b-5p fluorescence *in situ* hybridization (FISH) detection probe was generated with a 5'-FAM-labelled miRNA probe (Guangzhou RiboBio). Tissue *in situ* hybridization was performed according to the manufacturer's direction. Briefly, fresh-frozen 15 μm brains sections from E14 were fixed with 4% PFA for 20min. After that, we pre-hybridized the sections with pre-hybridization solution for 1h at 50°C, followed by hybridization with 15 nmol FAM-labelled miRNA probe for 2h at 22-25°C in the dark. The sections were then washed subsequently with wash solution I, II, III at 5°C higher than the hybridization temperature. For co-localization staining with Pax6 and Tbr2, the brain sections were first immunostained before in situ hybridization. Antibodies used here were rabbit anti-Pax6 (1:1000, Millipore) and rabbit anti-Tbr2 (1:1000,

Abcam). DAPI (2 µg/µl, Sigma) was used as nuclear counterstaining.

Immunostaining

Brain sections or cell cultures were fixed with 4% PFA for 30 min and blocked in 5% BSA containing 0.1% Triton X-100 (Sigma) for 1 h. Primary antibody incubation was subsequently performed overnight at 4˚C, followed by secondary antibody incubation at RT for 1h (cultured cells) or 2h (brain sections). For BrdU detection, slices were pretreated with 1 M HCl (10 minutes at 4˚C) and 2 M HCl (10 minutes at RT and 20 minutes at 37˚C), followed by subsequent washes in PBS containing 1% Triton X-100. Then, the brain sections were incubated with primary antibody overnight at 4˚C, followed by the secondary antibody for 2 h at RT. Immunostaining for 5hmC and 5mC detection was performed as described previously [14].

Antibodies used in immunohistochemistry and immunocytochemistry were rabbit anti-phospho-Histone H3 (pH3) (1:500, Cell Signaling Technology), rabbit anti-Ki67 (1:1000, Abcam), rabbit anti-Tuj1 (1:1000, Sigma), rabbit anti-Pax6 (1:1000, Millipore), rabbit anti-Tbr2 (1:1000, Abcam), rabbit anti-5hmC (1:500, Active Motif), rat anti-BrdU (1:1000, Abcam), and mouse anti-5mC (1:1000, Calbiochem). The secondary antibodies used were conjugates of Alexa Fluor 488, Cy3, or Cy5 (1:1000, Jackson ImmunoResearch). DAPI (2 µg/µl, Sigma) was used as nuclear counterstaining. All images were acquired using a Zeiss LSM 780 confocal microscope, and the signal intensity was analyzed using Axiovision software (Carl Zeiss).

Statistical analysis

Statistical analyses, including two-tailed Student's *t*-tests and one-way-ANOVAs followed by a

Tukey's test for post-hoc multiple comparisons, were performed using SPSS 16.0 for Windows. Differences were considered statistically significant at $P<0.05$(*) and at $P<0.01$(**). Data are presented as mean ± SEM.

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