Supplemental materials

Table S1- Target Sequences of lentiviral *shRNAs*

Gene name	shRNA target sequence
tet1-1	ccggtctaaccagtgtgctaatatactcgagtatattagcacactggttagatttttg
tet1-2	ccggtttcaactccgacgtaaatatctcgagatatttacgtcggagttgaaatttttg
tet2-1	ccgggagcgttcctcagtatcatttctcgagaaatgatactgaggaacgctcttttttg
tet2-2	ccggatgtccttgtaggactataatctcgagattatagtcctacaaggacatttttttg
tet3-1	ccgggaaagatgaaggcccatattactcgagtaatatgggccttcatctttctttttg
tet3-2	ccggctgataccctccggaagtatgctcgagcatacttccggagggtatcagtttttg
gapdh	ccggtccgggaaactgtggcgtgatctcgagatcacgccacagtttcccggatttttt

Table S2 - Sequences of quantitative real time PCR primers

Gene	*Sense primer*	*Antisense primer*
human tet1	5'-tagctatgtctcgatcccgc-3'	5'-aggttaaggactctgggttc-3'
human tet2	5'-aagcctgatggaacaggata-3'	5'-cagagagagaaggttcacta-3'
human tet3	5'-gtcaatggtgctagagagcc-3'	5'-agggccagggccgtctgcag-3'
pcp2	5'-taggagagatacacaatggac-3'	5'-ctctggagcagggccaccctg-3'
engrailed-1	5'-ccgagcatggaagaacagcag-3'	5'-ggcggcagacagggtgccgca-3'
hsp105	5'-gggccagccatgtcggtggtt-3'	5'-tgaaatctcttaaagctagag-3'
cerebellin1	5'-cgccggaggcgcgatgctggg-3'	5'-gtgctcctgatggcagagaaa-3'
tubulin	5'-tggagccgggaataactg-3'	5'-gcctcgtcctcgccctcctc-3'

Figure S1

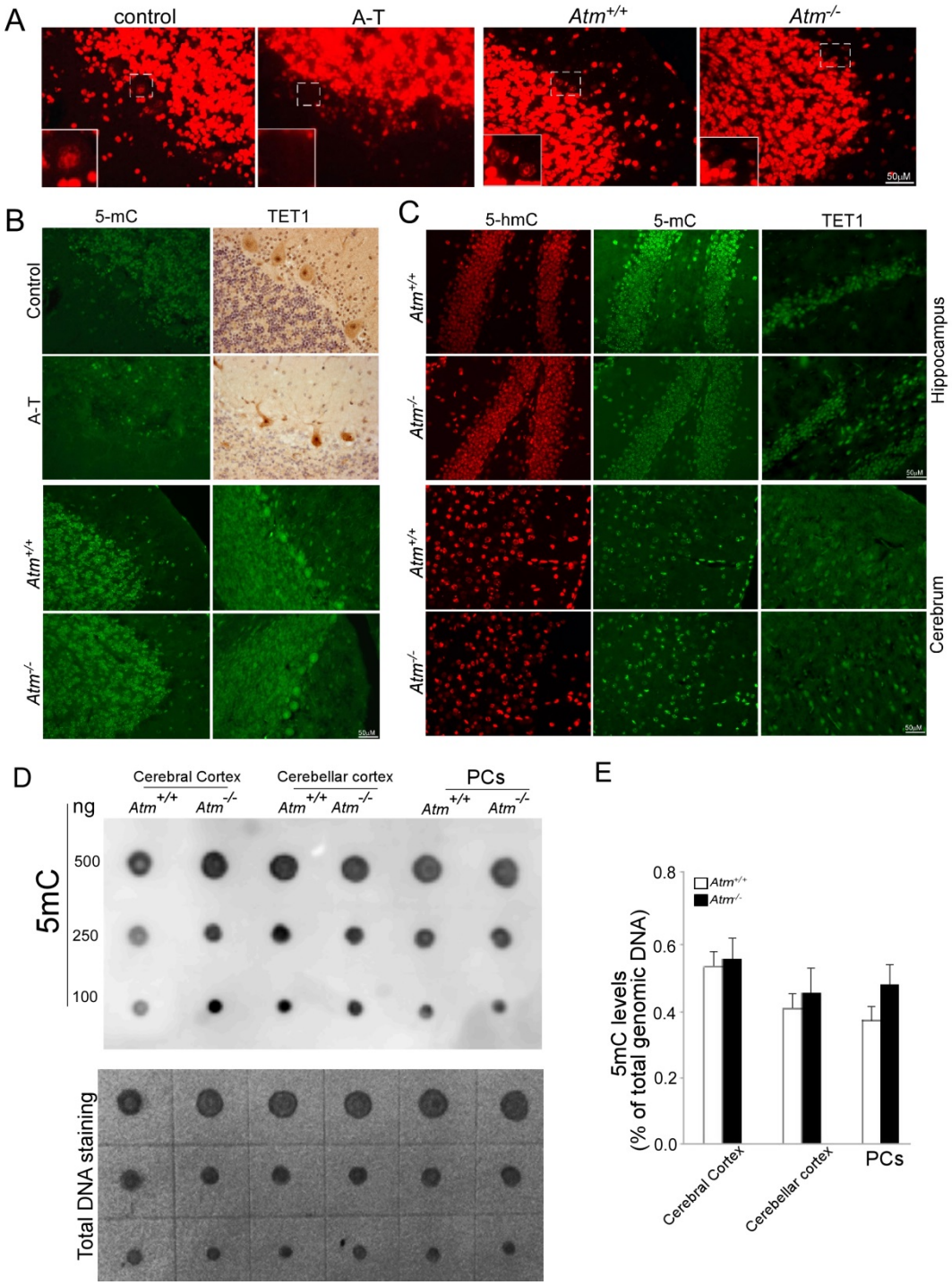

Figure S1. 5mC and TET1 show few alterations in ATM-deficiency. (**A**) Paraffin sections of control and A-T human cerebellum as well as 10 µm cryostat sections of wild type and *Atm*$^{-/-}$ mice brains were immunostained with 5hmC. 3-5 images per slide, 3 slides per patient or mouse. Scale bar, 50 µm. (**B**)Upper panel, paraffin sections of control and A-T human cerebellum were immunostained with 5mC (green) and TET1 (Brown) antibodies. Lower panel, ten micron cryostat sections of wild type

and *Atm*$^{-/-}$ mice brains were immunostained with 5mC and TET1. 4-5 images per slide, 3 slides per patient. Scale bar, 50μm. (**C**) Ten micron cryostat sections of wild type and *Atm*$^{-/-}$ mice brains were immunostained with5hmC, 5mC and TET1. 3-5 images per slide, 4 slides per mouse Scale bar, 50μm. (**D-E**) 5mC-specific dot-blot intensities of genomic DNA isolated from the cortex, cerebellum as well as isolated PCs of *Atm*$^{+/+}$ and *Atm*$^{-/-}$ adult mice. Total genomic DNA was stained as the quality of loading control. Amounts of 5mC are shown as a percentage of total nucleotides in the genome, which are quantified from 5mC-specific dot-blot intensities normalized to positive controls. (*, $p < 0.05$, Student's t-test; mean ± S.E.M.; n = 3-5 per each type of mouse).

Figure S2

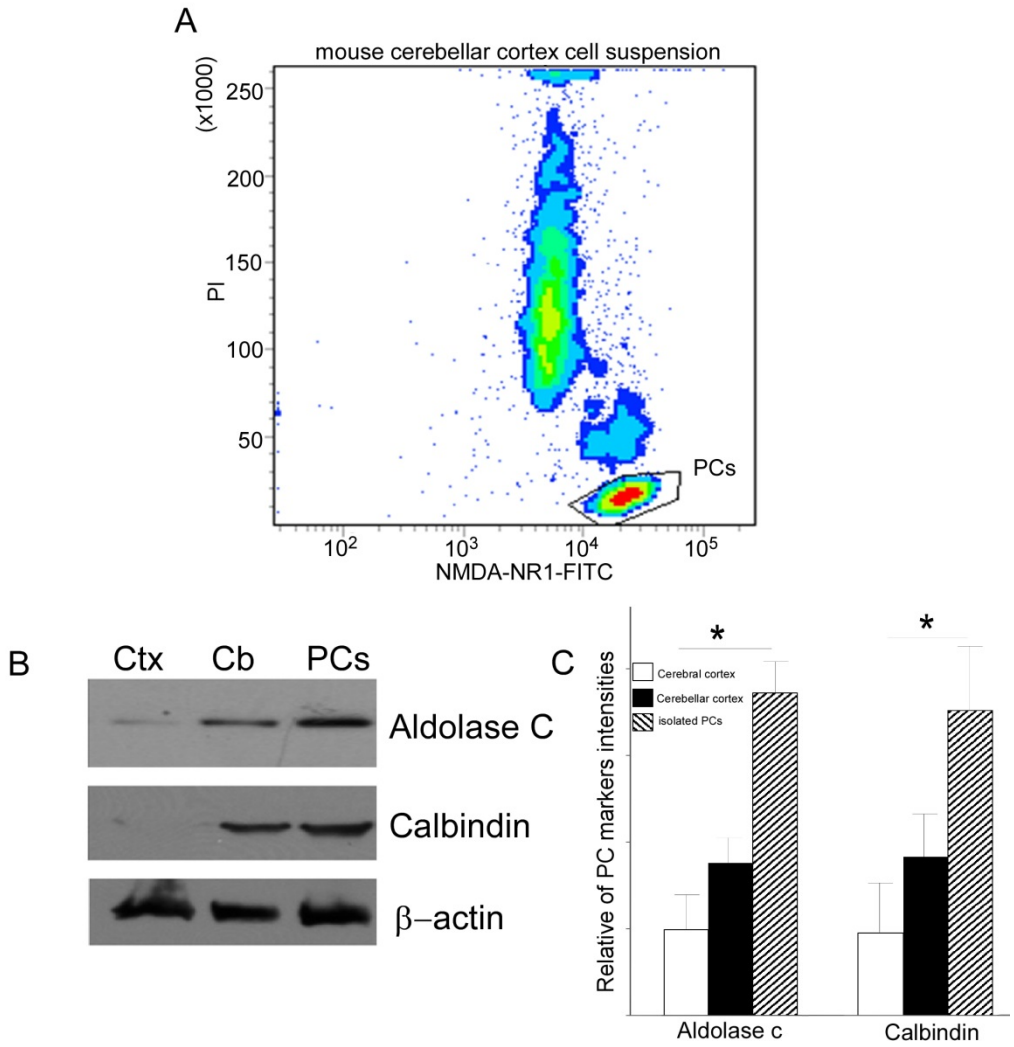

Figure S2. Sorting Purkinje cells (PCs) by FACS from mouse cerebellum. (**A**) A typical dot plot illustration of cell suspension. The sort region containing the PC fraction is shown. The *x* axis represents the relative fluorescence intensity of FITC-Labelled NMDA-NR1, and the y axis represents the relative fluorescence intensity of PI. (**B**) Protein extracts from adult mouse cortex, cerebellum and isolated PCs were probed on western blots with Aldolase C and Calbindin antibodies, β–actin was as a control. (**C**) The normalized intensity of Aldolase C and Calbindin illustrated in panel B). (n=3-5 experiments). * = $p < 0.05$ by Student's t-test. Error bars represent S.E.M.

Figure S3

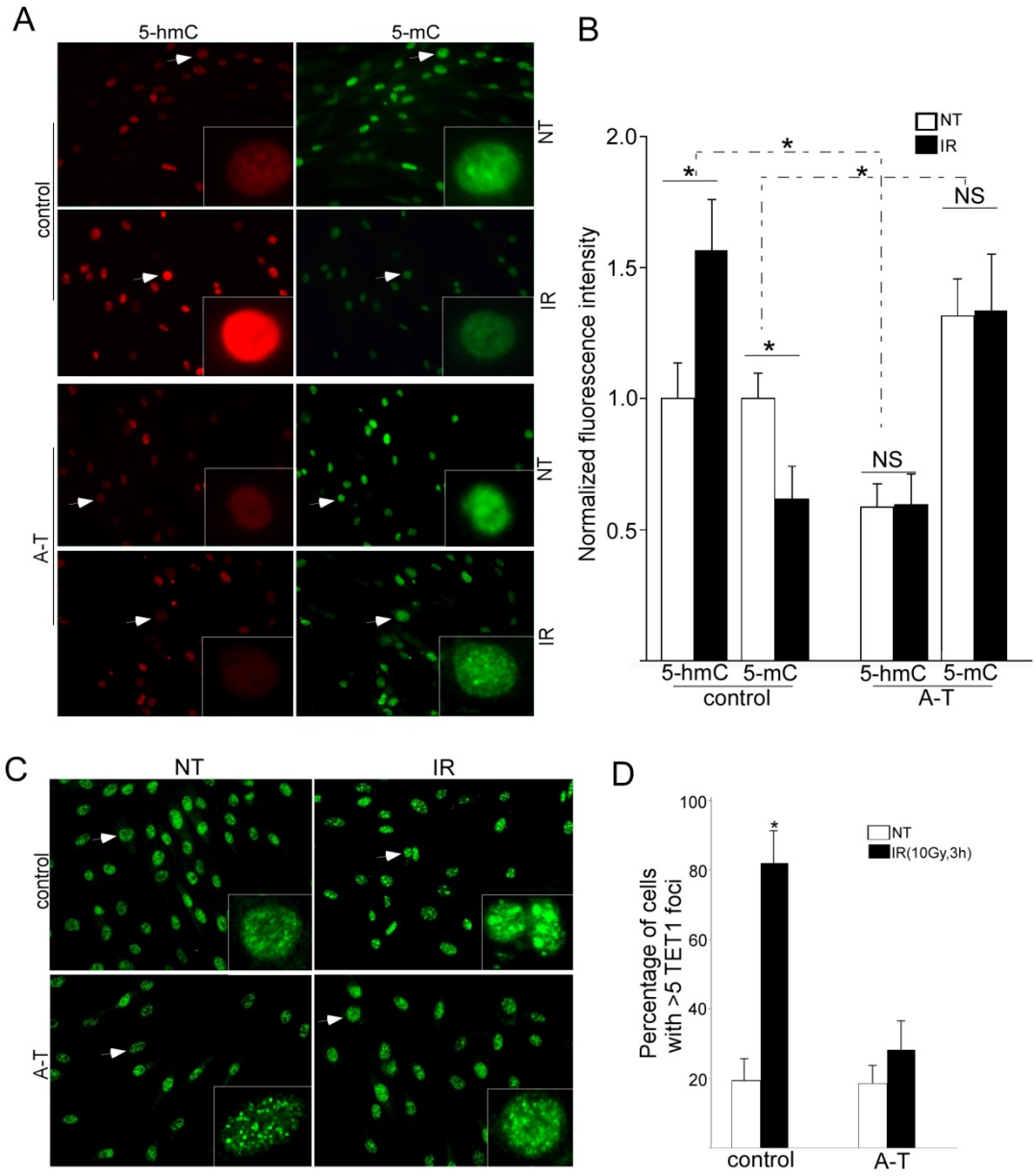

Figure S3. TET1-mediated conversion from 5mC to 5hmC responds to DNA damage. (**A**) Human skin fibroblasts were treated with or without 10Gy IR. 6 hours late, cells were fixed and stained with 5mC and 5hmC. (**B**) Quantification of normalized intensities of 5mC and 5hmC such as those illustrated in **A**). Data are presented as mean ± s.e.m. (n = 60–80 cells). *P < 0.01; NS: no significant difference, P > 0.05; unpaired t test. (**C**) Human skin fibroblasts were treated with or without 10Gy IR. 6 hours late, cells were fixed and stained with TET1. White arrows represent nuclear foci. (**D**) Quantification of percentage of ＞5 TET1 foci cell

number such as those illustrated in **C**). Data are presented as mean ± s.e.m. (n = 30-50 cells). *P < 0.01.

Figure S4

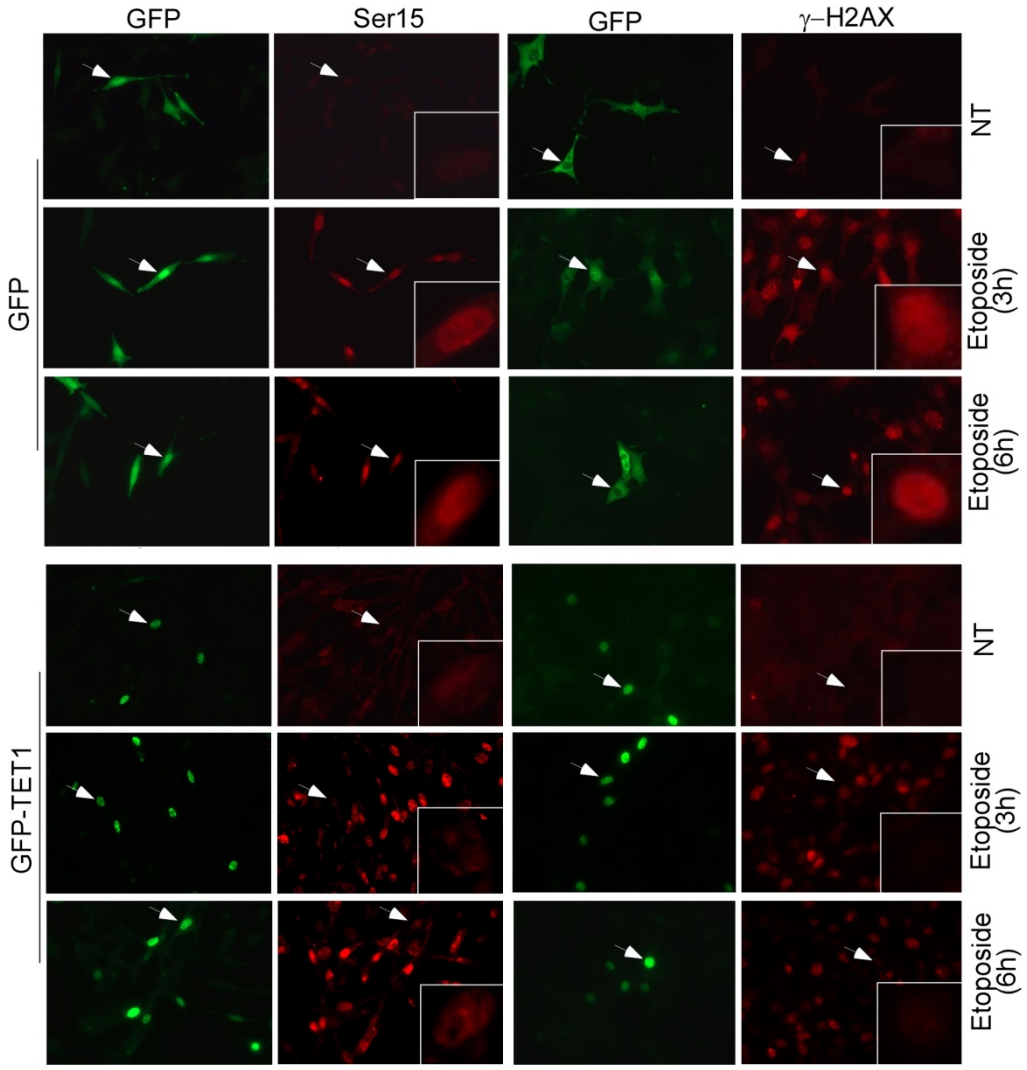

Figure S4.TET1-mediated 5hmC regulates DNA damage response (DDR). (**A-B**) HT22 cells were transfected with GFP or GFP-TET1 at day 1. Differentiated media were added at day3. Cells were treated with etoposide for different time at Day 5 and stained with γ-H2AX and P-Ser15 antibodies.White arrow indicates DDR positive cells. Data are presented as mean ± s.e.m. (n = 50-80 cells).

Figure S5

Figure S5. MeDIP and hMeDIP sequencing read density. The number of aligned reads is displayed by chromosome, normalized by total reads per sample and by chromosome length. Results are grouped by treatment (Control/A-T), tag (Input/5mC/5hmC) and region (Cerebellum/Cortex). Each sample is identified with a different color. It is apparent that for the Cerebellum Input samples, there is likely one male and one female sample per treatment group, based on the disparity in Y

chromosome alignments. Without a similar contrast it is difficult to judge the gender of the immunoprecipitated samples.

Figure S6

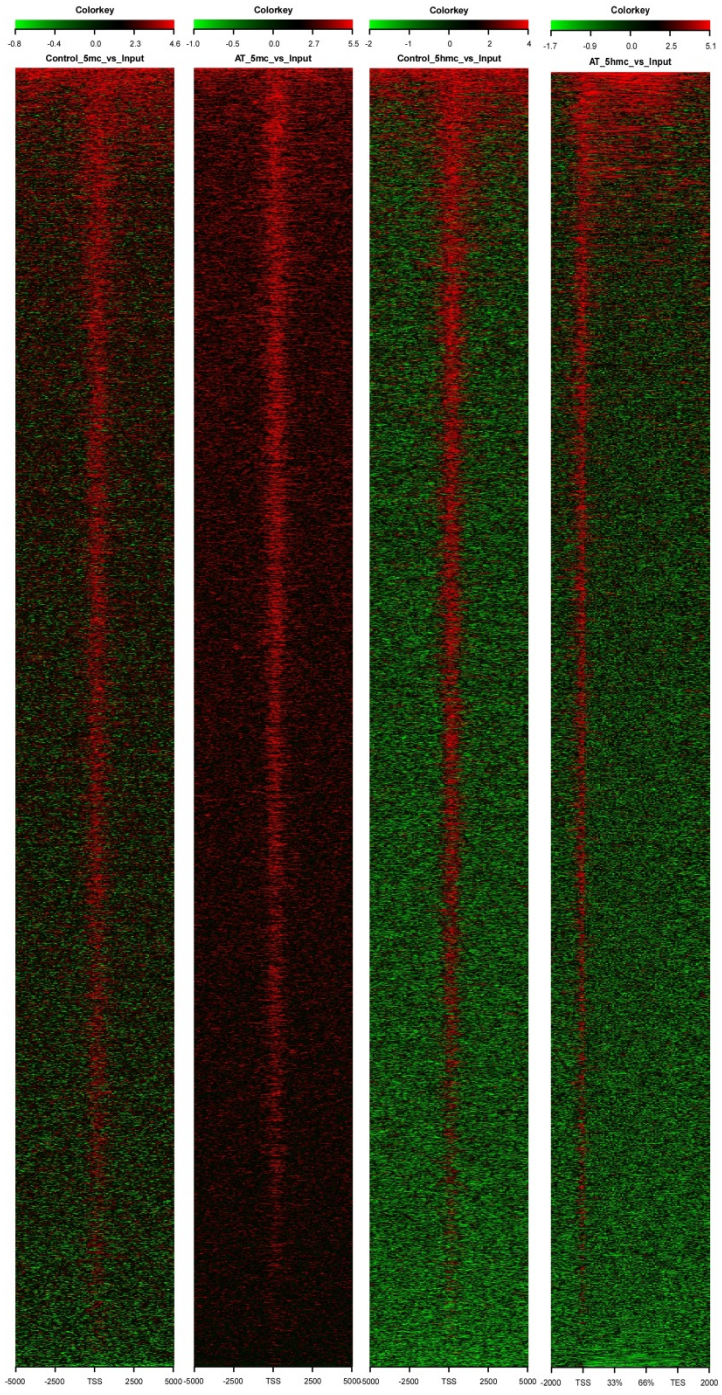

Figure S6. A heat-map analysis of changes (control vs. A-T) in the levels of 5mC and 5hmC in gene loci is shown for four patient sample pairs. 5hmC changes

negatively correlate with changes in 5mC. Each row represents gene TSS regions. Red indicates a gain and green represents a loss.

Figure S7

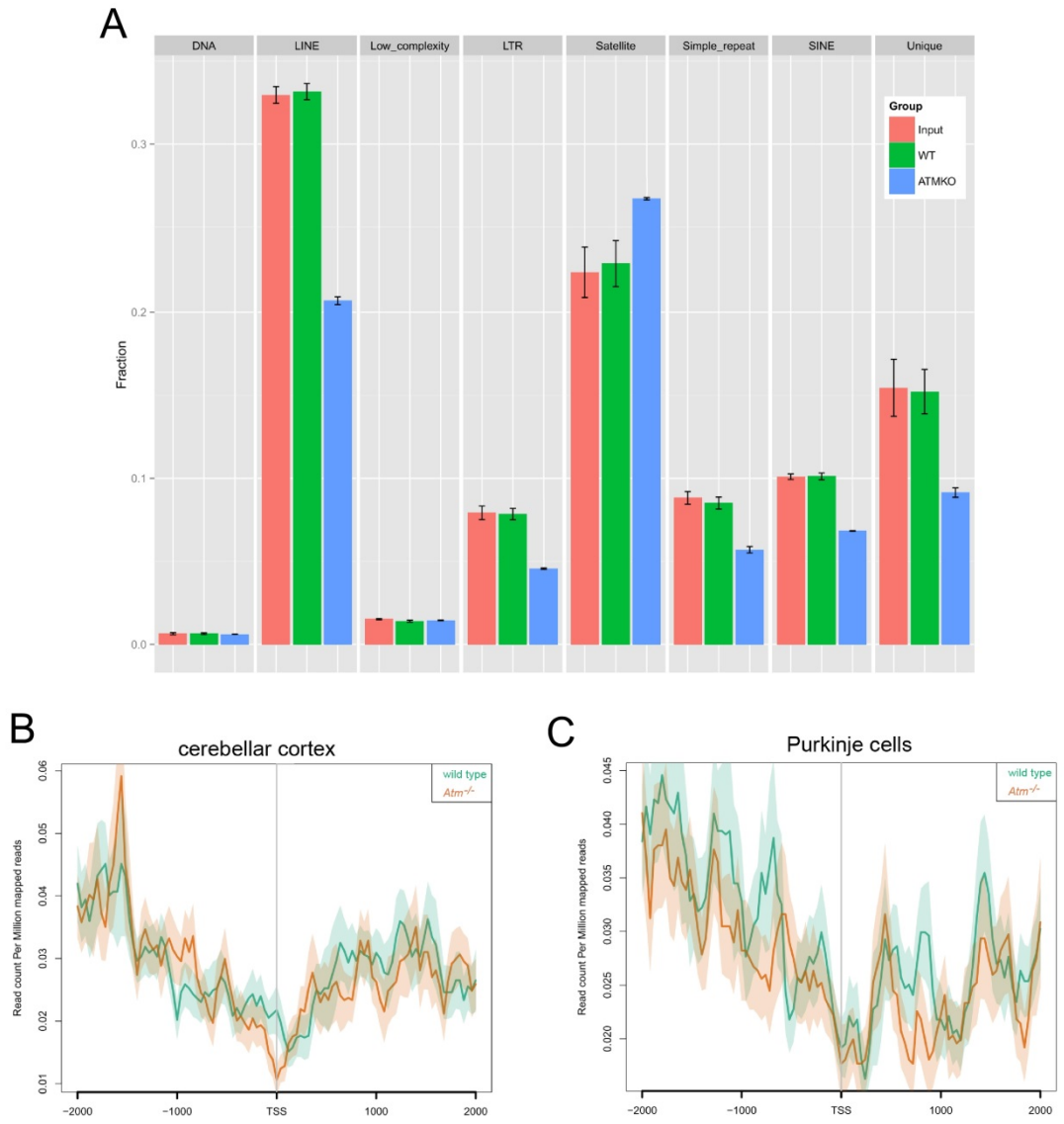

Figure S7. Mouse hMeDIP sequencing reads aligned to major classes of repeated genomic regions. **(A)**Mouse PCs were isolated by FACS and used to prepare a 5hmC-immunoprecipated sample for hMeDIP-seq. The resulting sequencing reads were aligned with genome and classified according to their alignment with classes defined by the UCSC RepeatMasker track. The y-axis shows the fraction of total reads aligned per sample (n=3 for WT, ATMKO [*Atm*$^{-/-}$]; n=2 for input). The decrease in LINE and increase in Satellite alignments for the ATMKO group were different

from Input and WT (ANOVA, p<0.001). (**B-C**)Metagene analysis read densities around all RefSeq transcripts centered on either TSS or TTS in mice cerebellar cortex and isolated PCs. Brown indicates decreased 5hmC in *Atm*$^{-/-}$ mice isolated PCs (P < 0.05, unpaired t test, 50-bp windows, 10-bp increments).

Figure S8

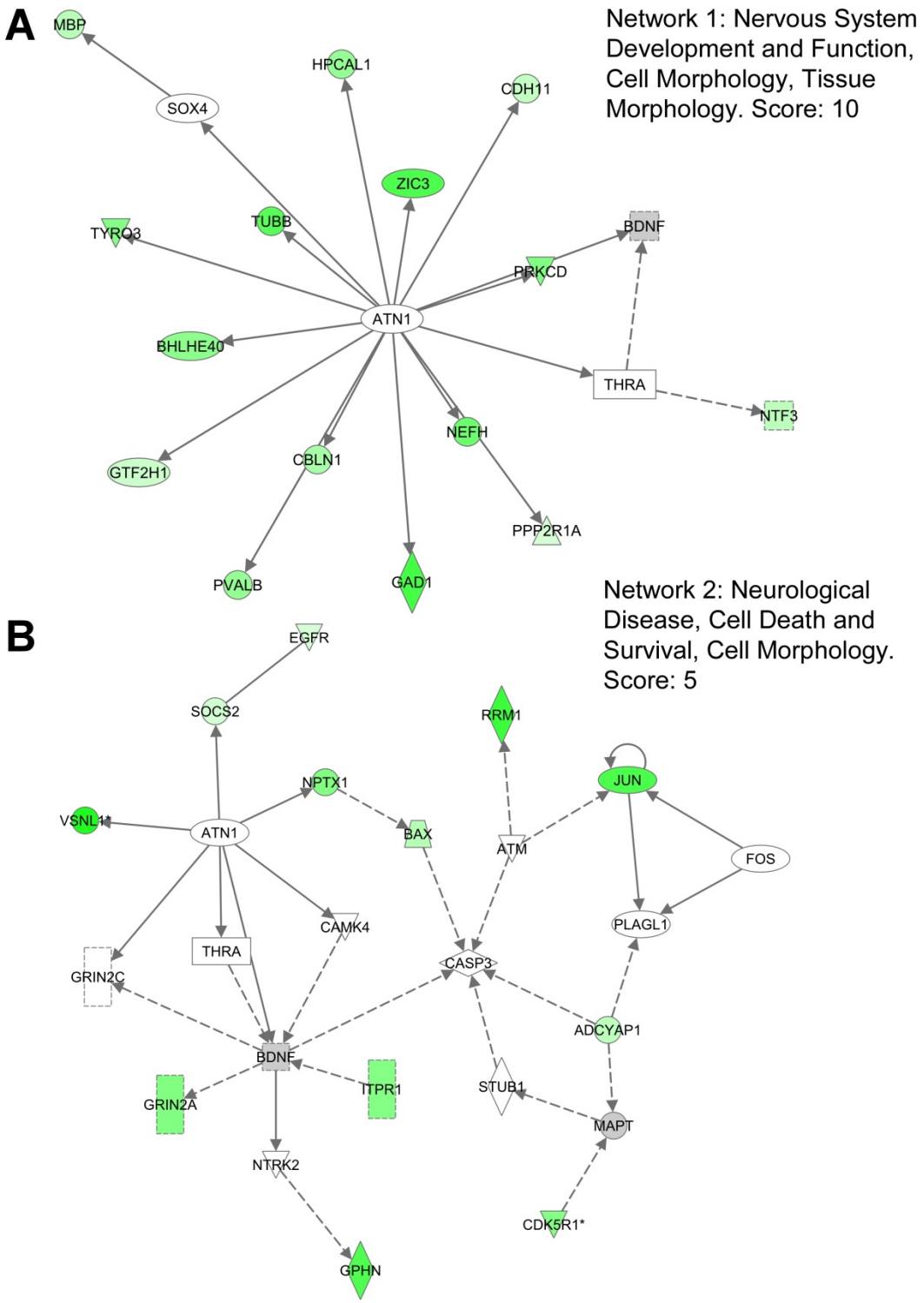

Figure S8. Top two networks identified by the overlapping methyl marks as described in Figure 6. Genes selected from **Figure 6G** were loaded, along with $\log_2$ fold change values and false discovery rate, into Ingenuity Pathway Analysis software (Qiagen). Results of a core analysis are summarized in the Supplemental material.

The top two predicted networks are shown here. Shades of green depict the $\log_2$ fold change values from selected genes.

Figure S9

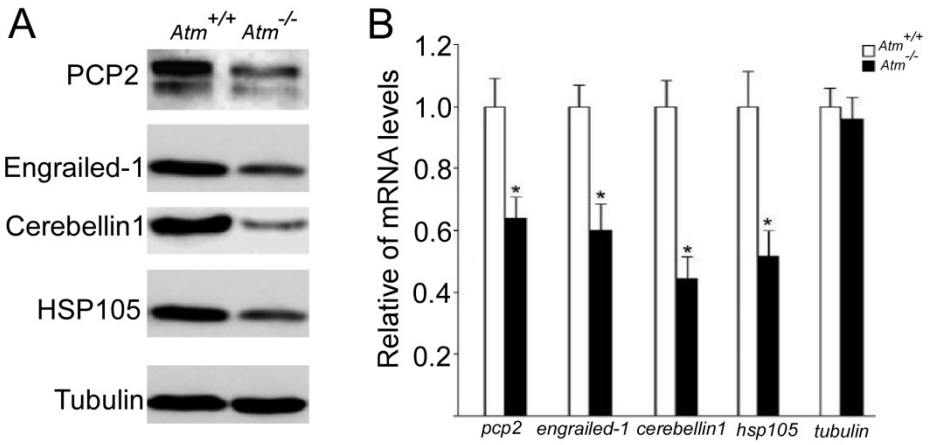

Figure S9. Decreased enrichment of 5hmC genomic pattern is related to downregulated Purkinje cell-specific genes in *Atm*$^{-/-}$ mouse.（**A**）Protein extracts from wild type and *Atm*$^{-/-}$ cerebellar cortex were assayed by Western blot for the presence of PC-specific expressed genes. (**B**) Quantitative real-time PCR was performed with specific primers for target genes. Total RNA was extracted from mouse cerebellar cortex. Data represent mean ± s.d. of three independent experiments. **P*= 0.003, unpaired *t*-test.

Figure S10

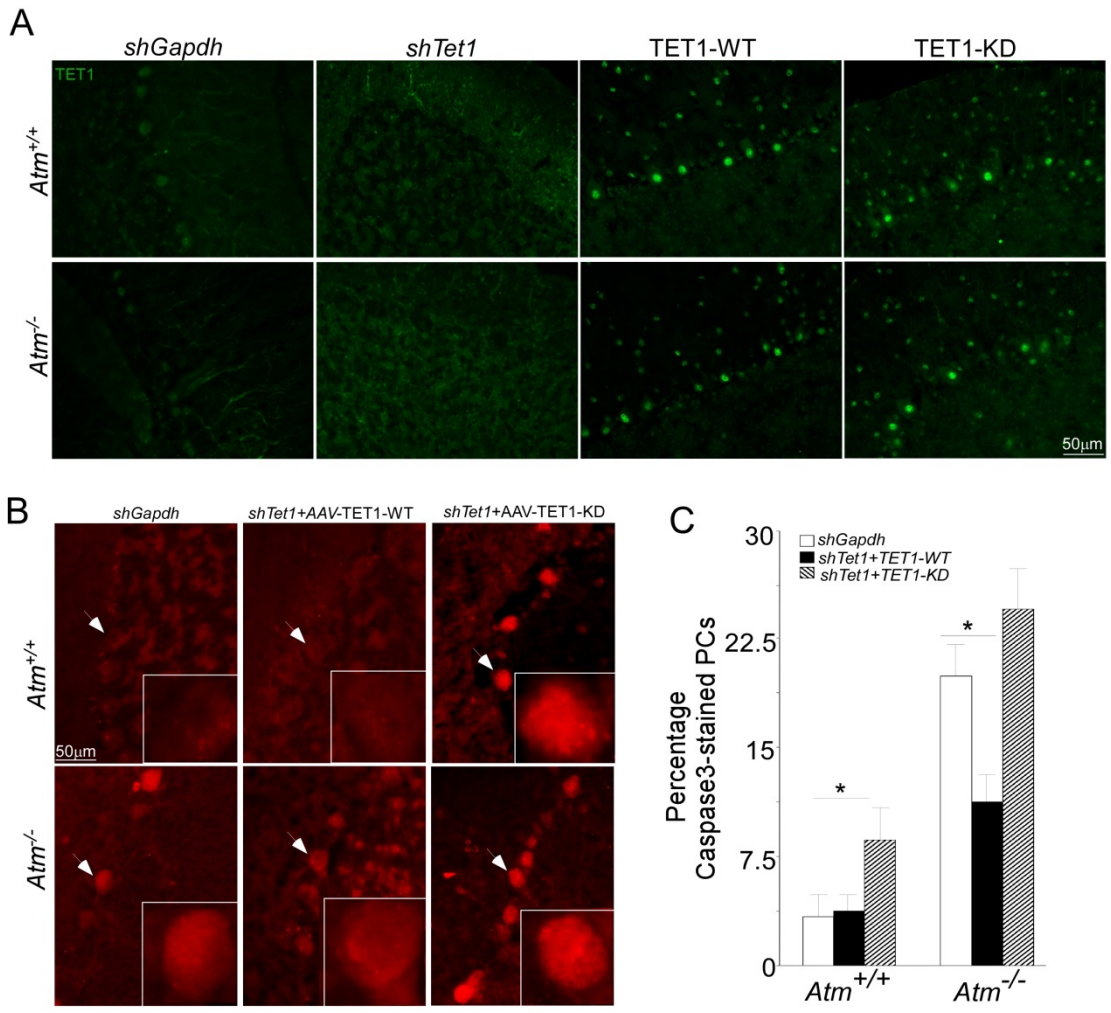

Figure S10. TET1 regulates the degenerative process of PCs in ATM-deficiency. (**A**) Images of TET1 expression in mice cerebellar slice cultures show the efficiency of gene transfer. Lentiviral *shgapdh*, *shtet1* as well as adenoviral TET1-WT and KD were co-infected into mouse cerebellar slice cultures at DIV 0, the slices were fixed for analysis after 2 weeks. (**B**) Representative images of cleaved caspase3-tained PCs show the effects of the overexpression of AAV-TET1-WT and AAV-TET-KD on cell death in PCs. (**C**) Quantification of the number of cleaved caspase3-stained PCs shown in panel **B**). Error bars represents s.d. between slides. 3-5 images per slide, 3 slides per mouse. Data are presented as mean ± s.e.m. (n=3-5 mice). *$P < 0.05$. Scale bar represents 50 μm.

Extended Experimental Procedures

Antibodies and chemical regents

Antibodies against Aldolase C，NMDA-NR1, TET1, TET2，TET3, 5mC, 5hmC, Acet-H3, H3K27me3, BrdU and cleaved caspase3 were obtained from Abcam. Antibodies against TET1 and 5hmC were also obtained from Active Motif. HA tag and PCNA antibody was ordered from Santa Cruz. Secondary antibodies used for immunocytochemistry were as follows: Alexa 488-labeled chicken anti-mouse or anti-rabbit; Alexa 594-labeleddonkey anti-mouse or anti-rabbit (Invitrogen, Eugene, OR); all were used at a dilution of 1:500. DAPI (4', 6'-diamidino-2-phenylindol) was used as a nuclear counterstain at 1 μg/ml. FITC Conjugation Kit f was from Abcam.

Constructs and Plasmids

GFP-TET1 plasmid was provided by Dr. Heinrich Leonhardt (Ludwig Maximilians University Munich). The Flag-ATM wild type and kinase dead (KD) plasmids were kindly provided by Dr. Michael B. Kastan ((Duke University School of Medicine)). TET1 wild type (pAAV-EF1a-HA-hTet1CD-WPRE-PolyA) and kinase dead mutant (pAAV-EF1a-HA-hTet1CDmu-WPRE-PolyA) constructs were ordered from Addgene. MA). TET1 CRISPR/Cas9 KO (knock out) Plasmids (mouse and human) (GFP-Cas9-Tet1) were ordered from Santa Cruz. GST-TET1was cloned from human brain mRNA by SuperScript III one-step RT-PCR system (Invitrogen). Site-direct mutation of GST-TET1 on S116 and S116A/S262A/S546A (3SA) were performed by Quik Change Mutagenesis Kit (Stratagene, La Jolla, CA).

Human subjects

Human autopsy tissue from four individuals diagnosed with A-T and four age-matched controls was obtained from UCLA Medical Center and Children's Hospital Los Angeles. Brain tissue was routinely fixed, paraffin embedded, and

sectioned at 10 μm. All A-T cases were confirmed both clinically and by post-mortem examination. The average age of the subjects was 21 years for the A-T patients and 25years for non-A-T controls. The non-A-T controls were free of any known CNS neurological diseases at the time of death. Human frozen cerebellar and frontal cortex tissue were secured from 4 additional individuals diagnosed with A-T and four age-matched controls; samples were obtained from NICHD Brain and Tissue Bank of Developmental Disorders at the University of Maryland, Baltimore, MD.

Animals

Atm$^{tm1Bal/+}$ mice were a gift from Dr. Yang Xu. Timed pregnancies were established from these matings; the date of appearance of a vaginal plug was considered embryonic day 0.5. Embryos were taken at embryonic day 16.5 (E16.5) for either cortical cultures or histology. Animals were allowed to survive for 7 days before sacrifice. All animal procedures were carried out in accordance with Rutgers University IACUC standards. The animal facilities at Rutgers University are fully AAALAC accredited. And animal procedures were also approved by the Animal Care and Use Committee of Kunming Institute of Zoology, Chinese Academy of Sciences.

Mouse embryonic fibroblasts preparation

MEFs were isolated and prepared from E13.5 wild-type or *Atm*$^{-/-}$ mice embryos. The tissues taken from only forelimbs and forelegs were manually dissociated and incubated in 0.25% trypsin (Gibco) at 37℃ for 10–15 min. Cells from each embryo were plated onto a 10 cm tissue culture dish in DMEM medium (Invitrogen) containing 10% fetal bovine serum (Hyclone), β-mercaptoethanol (Sigma), nonessential amino acids (Invitrogen). In all experiments, cells were not passaged more than four times.

Immunohistochemistry

For DAB/bright field staining, all paraffin-embedded human sections were deparaffinized in xylene and then rehydrated through graded ethanol to water.The sections were pretreated in 0.3% hydrogen peroxide in methanol for 30 min to remove endogenous peroxidase activity, rinsed in Tris-buffered saline (TBS), and then treated with 0.1 M citrate buffer in a microwave at sufficient power to keep the solution at 100°C for 20 min. Sections were cooled in the same buffer at room temperature (RT) for 30 min and rinsed in TBS. Slides were incubated in 10% goat serum in PBS blocking solution for1 h at RT, after which primary antibody was applied to the sections that were then incubated at 4°C overnight. The sections were washed three times in TBS before applying the secondary antibody (Vector Laboratories). Secondary antibody was applied for 1h at RT. Afterwards, sections were rinsed three times in TBS. Rinsed sections were then incubated in Vectastain ABC Elite reagent for 1 h and developed using diaminobenzidine, according to the protocol of the manufacturer (Vector Laboratories). The sections were counterstained with hematoxylin, and after dehydration all sections were mounted in Permount under a glass cover slip. Control sections were subjected to the identical staining procedure, except for the omission of the primary antibody.

Immunofluorescence

All paraffin-embedded human sections were deparaffinized in xylene and then rehydrated through graded ethanol to water and PBS. For the mouse, cryostat sections were first rinsed in PBS, Subsequent steps were identical for paraffin and cryostat material. After rehydration, sections were treated in antigen unmasking solution (low pH) for 30 min at 100°C.After the slides had cooled in buffer for 30 min at room temperature, slides were rinsed in PBS. Then sections were incubated in 10% goat serum in PBS to block nonspecific binding for 1 h at room temperature. All primary antibodies were diluted in PBS containing 0.5% TritonX-100 and 5% goat serum and incubated with sections overnight at 4°C. After rinsing in PBS, they were incubated for 1 h with secondary antibody, which was conjugated with various fluorescent

labeled secondary antibodies. All sections were mounted in ProLong® Gold anti-fade reagent with DAPI (Invitrogen) under a glass cover slip. All experiments were conducted in triplicates.

In vitro kinase assay

N2A cells overexpressing wild type Flag-ATM were lysed in TNN buffer and immunoprecipitated with protein G Dynabeads beads (20 µl) and anti-Flag antibody (Sigma). Kinase reactions were performed using precipitated ATM on beads (~ 15 µl) with GST-tagged TET1, GST-tagged TET1^{S116A} and GST-tagged 3SA purified from bacteria (~ 1 µg protein) in a kinase buffer (50 mMHepes at pH 7.4, 150 mMNaCl, 6 mM $MgCl_2$, 4 mM $MnCl_2$, 10% glycerol, 1 mMdithiothreitol, 0.1 mMNaOV and 15 µCi of [γ-^{32}P] ATP] and incubated at 30 °C for 30 min. Proteins were separated by SDS–PAGE, transferred to nitrocellulose membranes and visualized by autoradiography.

BrdU incorporation

For BrdU labeling, 10 µM BrdU was added to the Neurobasal media at DIV6. After labeling for 24 hours, the primary neurons were then fixed, and DNA was hydrolyzed by exposing the cells to 2N HCl for 10 minutes. Specimens were then neutralizing in 0.1M sodium borate (pH 8.6) for 10 minutes and then rinsed extensively in PBS (3X) for 45 min before treatment with blocking reagent. Non-specific antibody binding was blocked by exposing the fixed cells to 5% normal goat serum in 0.1% Triton-X for 1 hour before application of the BrdU primary antibody. Total cells number was based on DAPI-positive nuclei. Counts were made of BrdU-positive, DAPI-positive and double-positive cells.

Human fibroblast, N2a and HT22 cell culture

All human control and A-T primary fibroblast cell lines described in this paper were purchased from the Coriell Cell Repository. Fibroblasts were cultured in fibroblast medium (Dulbecco's modified Eagle's medium [DMEM] supplemented with 15% fetal bovine serum [FBS; Hyclone], 1 mM glutamine [Invitrogen], 1% nonessential amino acids [Invitrogen], and penicillin/streptomycin [Invitrogen]).N2a and HT22 cells were cultured in DMEM medium supplemented with 10% FBS. To differentiate N2a and HT22 cells, 24 hours after seeding, culture medium was replaced by DMEM with 1mM dbcAMP and 0.25% FBS. Cells were kept in this differentiation medium for 3-7 days before use.

Primary Neuronal Cultures

Embryonic cortical neurons were isolated by standard procedures. For ATM-deficient cultures, all embryos from an $Atm^{tmBal/+}$ x $Atm^{tm1Bal/+}$ mating were harvested and treated separately then retrospectively genotyped by PCR. Isolated E16.5 embryonic cerebral cortices were treated with 0.25% Trypsin-EDTA and dissociated into single cells by gentle trituration. Cells were suspended in Neurobasal medium supplemented with B27 and 2 mM glutamine, then plated on cover slips or dishes coated with poly-L-Lysine (5 mg/mL). All cultures were grown for a minimum of 5 days *in vitro* (DIV) before any treatment. All experiments were performed on a minimum of three cultures from three separate embryos; each condition was examined in triplicate. All transfections were carried out using Lipofectamine 2000 after 4-5 DIV. Live imaging of GFP was performed on a heated stage in a controlled 5% CO_2 atmosphere at 7-10 DIV.

shRNA knockdown in primary neurons

The hairpin sequences for MISSION® shRNA Lentiviral Transduction Particles including *shTet1-1 and -2, shTet2-1 and -2, shTet3-1 and -2,* and *shgapdh* (Table S1) were from Sigma. After 5-7 days in culture, primary neurons or cerebellar slices were infected using a multiplicity of infection (moi) between 5 and 10 to provide an

efficiency of infection above 70%. Samples were collected 7-10 days later and different assays were performed.

RNA extraction and reverse transcription PCR

RNA was prepared using PureLink micro-to-midi total RNA purification system (Invitrogen). Semi-quantitative RT-PCR was performed with Superscript III one-step RT-PCR system with platinum Taq High Fidelity (Invitrogen).For quantitative Real-time PCR, cDNA was prepared by using oligonucleotide (dT), random primers, and a Thermo Reverse Transcription kit (Signal way Biotechnology). The mRNA level of tubulin was used as an internal control.