

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	The western blot results were collected by Image Studio Ver 5.2 on a LI-COR Odyssey CLx instrument. The CellTiter Glo viability results were collected by i-control 1.10 on a TECAN infinite M200 PRO plate reader. The qRT-PCR results were collected by Bio-Rad CFX Manager 3.1 on a Bio-Rad C1000 Touch Thermal Cycler. The sequencing data was demultiplexed using bcl2fastq v2.20 software from Illumina. No software was used for collection of genomic sequencing data.
Data analysis	Flow cytometry data were analyzed using FlowJo v10. Statistical analysis was performed with Prism 10.4.2 and R v.4.4.2. Foci analysis was performed with ImageJ/Fiji v2.16.0. Sequencing data analysis was conducted using publicly available software packages: Trimmomatic v0.39, Trim Galore! v0.6.7, Cytoscape v3.9.1, MAGeCK v0.5.7, Bowtie v1.3.1, Bowtie2 v2.5.3, STAR v2.7.11b, BSMAP v2.90, Samtools v1.18, Bedtools v2.25.0, UCSC tools v407, MACS2 v2.2.9.1, DeepTools v3.5.5, pysamstats v1.1.2, CIBERSORTx, fastq-join tool v1.3.1, BWA-MEM v0.7.17, SigProfiler, and MethPipe v5.0.0. Genome alignment files were visualized using the UCSC Genome Browser and Integrative Genomics Viewer (IGV) v2.16.2. Further analysis of sequencing data was performed with Rsubread v2.18.0, DESeq2 v1.44.0, clusterProfiler v4.12.6, DOSE v3.28.0, Seurat v5.1.0, and MethySeekR v1.44.0 R packages in R v4.4.2.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The CRISPRi screen, END-seq/S1-END-seq, RNA-seq, LAM-HTGTS, ATAC-seq, SAR-seq, targeted deep sequencing (amplicon-seq), PS-seq, and CUT&RUN data have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE293256. Data obtained from publicly available sources include: single-nucleus RNA sequencing in spinocerebellar ataxia type 1 (SCA1) cerebellar tissues (GSE246183); BS-seq and oxBS-seq in Purkinje cells (GSE166423); ATAC-seq in Purkinje and granule cells (GSE174842); ChIP-seq of H3K4me1 in primary cortical neurons (GSE164265); ChIP-seq of H3K4me1 (GSE167259), and ddC-S1-END-seq and ddC-END-seq in iNeurons (GSE210317). The hg19 (human) and mm10 (mouse) genome assemblies were used as references for alignment.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For the CRISPRi screen, 5–7 million cells were used in both the non-treated control and treated groups, providing > 1000x coverage for the AX227 sgRNA library, which contains 1573 sgRNAs.
 For immunofluorescence (IF) assays, more than 200 cells were counted in both control and treated groups, ensuring sufficient samples for statistical analysis.
 For the CellTiter Glo viability assay, 300,000 cells were used per well in both control and treated groups, resulting in adequate fluorescent intensity for detection.
 For END-seq, S1-END-seq, LAM-HTGTS, and targeted deep sequencing assays, 10 million cells were used in both control and treated groups, yielding sufficient DNA for library preparation.
 For RNA-seq and ATAC-seq assays, 20,000–30,000 nuclei isolated from a single mouse brain were used per sample in both control and treated groups, generating sufficient RNA or DNA for library preparation.
 For CUT&RUN assays, 300,000 nuclei isolated from 10 mice were used per sample in both control and treated groups, providing ample DNA for library preparation.
 For END-seq assays in Purkinje cells, 400,000–450,000 nuclei isolated from 20 mice were used per sample in both control and treated groups, yielding sufficient DNA for library preparation.
 For RNA-seq in primary cortical neurons and iPSC-derived neurons, 1 million cells were used per sample in both control and treated groups, generating sufficient RNA for library preparation.
 For PS-seq, 10 million iPSC-derived neurons and 300,000–450,000 sorted Purkinje nuclei were used per sample, generating sufficient genomic DNA for library preparation.
 For SAR-seq, 15 million iPSC-derived neurons were used per sample, generating sufficient genomic DNA for library preparation.

Sample sizes were determined based on previously published studies (Wang, D. et al. Active DNA demethylation promotes cell fate specification and the DNA damage response. *Science* 378, 983–989 (2022). <https://doi.org/10.1126/science.add9838>; Wu, W. et al. Neuronal enhancers are hotspots for DNA single-strand break repair. *Nature* 593, 440–444 (2021). <https://doi.org/10.1038/s41586-021-03468-5>;

Hussmann, J. A. et al. Mapping the genetic landscape of DNA double-strand break repair. *Cell* 184, 5653-5669 e5625 (2021). <https://doi.org/10.1016/j.cell.2021.10.002>; Vega-Sendino, M. et al. The homeobox transcription factor DUXBL controls exit from totipotency. *Nat Genet* 56, 697-709 (2024). <https://doi.org/10.1038/s41588-024-01692-z>), which demonstrated that comparable group sizes are sufficient to detect biologically relevant effects in similar experimental settings.

Data exclusions	In the differential gene expression analysis of Purkinje nuclei following PBS or AraC treatment, one AraC-treated mouse (1 out of 6) was excluded due to low γ H2AX detection.
Replication	For RNA-seq in primary cortical neurons and iPSC-derived neurons, 2-3 replicates were used in both control and treated groups. For PS-seq, 4 regions covering 25 CpG sites were tested for iPSC-derived neurons, and 7 regions covering 55 CpG sites were tested for sorted Purkinje nuclei. For the CRISPRi screen, 3 replicates from each collection point were used in both the non-treated control and treated groups. For immunofluorescence (IF) assays, 2 replicates from each genotype or treatment were used in both the control and treated groups. For the CellTiter Glo viability assay, 3 wells per genotype or treatment were used in both the control and treated groups. For the LAM-HTGTS assays, 3 replicates were used in both control and treated groups. For targeted deep sequencing assays, 6 independent loci were examined in both control and treated groups. For RNA-seq assays, 6 mice were used for Purkinje and Golgi cells in both control and treated groups, and 3 mice were used for whole brain neurons. For ATAC-seq assays, 3 mice were used in both control and treated groups. For CUT&RUN assays, 1 pooled replicate (300,000 nuclei) from 10 mice was used to map H3K27ac. For END-seq assays in Purkinje cells, 2 pooled replicates (400,000-450,000 nuclei) from 20 mice each were used in both control and treated groups. For in vitro biochemical reconstitution assays, 3 replicates were performed independently. For FACS analysis assays, 2-3 mice were used in both the control and treated groups. All replication attempts were successful and yielded consistent results.
Randomization	Randomization was not necessary. Cells and mice were allocated based on treatments or genotypes determined by PCR screening, qRT-PCR, or western blot.
Blinding	Blinding was not necessary. Because the outcome measurements were objective and did not involve subjective interpretation. The treatments or genotypes were clearly defined and measurable, there was no risk of bias influencing the results.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies:

Anti-phospho-histone H2AX (gamma H2AX, 1:5000 for IF, 1:1000 for FACS, Millipore, clone JBW301, cat. No. 05-636, RRID:AB_309864);
 Anti-NeuN antibody (1:1000 for IF, 1:250 for FACS, Abcam, cat. No. ab279297);
 Anti-DNA Ligase IV/LIG4 antibody [EPR16531] (1:1000, Abcam, cat. No. ab193353);
 Anti-Actin (1:5000, Sigma, cat. No. A5441, RRID:AB_476744);
 Anti-PAR (1:500, Sigma-Aldrich MABE1031, RRID:AB_2665467);
 Anti-Dnmt1 [60B1220.1] (1:1,000, Abcam, cat. No. ab13537);
 Anti-TDG [EPR8774] (1:1,000, Abcam, cat. No. ab154192);
 Anti-XRCC1 (1:1,000, NOVUS, cat. No. NB120-1838);
 Anti-Tubulin (1:10,000, Sigma-Aldrich, cat. No. T5168);
 Anti-Histone H3 (Acetyl-K27) antibody (1:200, Abcam, cat. No. ab4729).

Secondary antibodies:

Alexa Fluor 568 goat anti-mouse (1:1000, Invitrogen cat. No. A11031, RRID: AB_144696);
 Alexa Fluor 488 chicken anti-rat (1:1000, Invitrogen cat. No. A21470, RRID: AB_2535873);
 Goat anti-Rabbit IgG Secondary Antibody (1:10,000, IRDye cat. No. 926-32211);
 Alexa Fluor 488 goat anti-rabbit (1:1,000, Invitrogen no. A11034, RRID:AB_2576217);

Goat anti-Mouse IgG Secondary Antibody (1:10,000, IRDye cat. No. 926-32210).

Conjugated antibody:

Coralite Plus 647-conjugated ITPR1-specific Polyclonal antibody (1:200, Proteintech, cat. No. CL647-19962).

Validation

Primary antibodies:

Anti-phospho-histone H2AX (gamma H2AX, Millipore, clone JBW301, cat. No. 05-636, RRID:AB_309864) – validated by the manufacturer in ChIP, ICC, IF, and WB; reacts with vertebrate samples.

Anti-NeuN antibody (Abcam, cat. No. ab279297) – validated by the manufacturer in IHC-P, ICC/IF, WB, and Flow Cytometry; reacts with human, mouse, and rat samples.

Anti-Actin (Sigma, cat. No. A5441, RRID:AB_476744) – validated by the manufacturer in ELISA, IF, IHC, and WB; reacts with sheep, carp, feline, chicken, rat, mouse, *Hirudo medicinalis*, rabbit, canine, pig, human, bovine, and guinea pig samples.

Anti-Histone H3 (Acetyl-K27) (Abcam, cat. No. ab4729) – validated by the manufacturer in western blotting, IHC, IF, and ChIP; suitable for human, mouse, and rat samples.

Anti-PAR (Sigma-Aldrich MABE1031, RRID:AB_2665467) - validated by the manufacturer in Western Blotting, Immunocytochemistry and Dot Blot; reacts with human and mouse.

Anti-Dnmt1 [60B1220.1] (Abcam, cat. No. ab13537) - validated by the manufacturer in flow cytometry, WB and IHC-P; reacts with human, mouse samples.

Anti-TDG [EPR8774] (Abcam, cat. No. ab154192) - validated by the manufacturer in WB and flow cytometry; reacts with mouse, rat and human samples.

Anti-XRCC1 (NOVUS, cat. No. NB120-1838) - validated by the manufacturer in immunohistochemistry, immunoprecipitation and WB; reacts with human samples.

Anti-Tubulin (Sigma-Aldrich, cat. No. T5168) - validated by the manufacturer in immunofluorescence, western blotting, immunoblotting and immunolabelling cells in electron microscopy; reacts with mouse, chicken, *Chlamydomonas*, African green monkey, human, rat, bovine, sea urchin and kangaroo rat.

Conjugated Antibody:

Coralite Plus 647-conjugated ITPR1-specific Polyclonal antibody (Proteintech, cat. No. CL647-19962) – validated by the manufacturer in Flow Cytometry; reacts with human, mouse, and rat samples.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

The induced pluripotent stem cell (iPSC) experiments were conducted using the WTC11 line, which was derived from a healthy male donor and obtained from the Coriell Cell Repository. All procedures followed the NIH Intramural Program's guidelines for the registration and use of this iPSC line. Additionally, the Lig4 knockout iPSC line was generated in this study. Lenti-X-HEK293T cell was a generous gift from Michael E. Ward.

Authentication

The WTC11 iPSC line was not authenticated through genome sequencing, but it was validated to possess a normal male karyotype.

Mycoplasma contamination

The iPSC lines and Lenti-X-HEK293T cells used in this study were confirmed to be mycoplasma-free, as determined using the Lonza 'MycoAlert' mycoplasma testing kit.

Commonly misidentified lines (See [ICLAC](#) register)

No commonly misidentified cell lines were used in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Species: *Mus musculus*.

Strain: C57BL/6J wild type; Pcp2-Cre, Tet1^{fl/fl}, Tet2^{fl/fl}, and Tet3^{fl/fl} (Tet triple floxed); Ubc-CreERT2, Tdg^{fl/fl}. Ubc-CreERT2, Tet1^{fl/fl}, Tet2^{fl/fl}, and Tet3^{fl/fl} (Tet triple floxed); Pcp2-Cre, TDG^{fl/fl}.

Age: One-month or six-month-old, as indicated.

Source: C57BL/6J wild type mice were obtained from the Jackson Laboratory; Pcp2-Cre, Tet1^{fl/fl}, Tet2^{fl/fl}, and Tet3^{fl/fl} (Tet triple floxed) mice were generated in a previous study (Stoyanova, E., Riad, M., Rao, A., & Heintz, N. "5-Hydroxymethylcytosine-mediated active demethylation is required for mammalian neuronal differentiation and function." *eLife* 10 (2021). <https://doi.org/10.7554/eLife.66973>); Ubc-CreERT2, Tdg^{fl/fl} mice were generated in a previous study (Onodera, A. et al. "Roles of TET and TDG in DNA demethylation in proliferating and non-proliferating immune cells." *Genome Biology* 22, 186 (2021). <https://doi.org/10.1186/s13059-021-02384-1>); Ubc-CreERT2, Tet1^{fl/fl}, Tet2^{fl/fl}, and Tet3^{fl/fl} (Tet triple floxed) mice were generated in a previous study (Georges, R. O. et al. Acute deletion of TET enzymes results in aneuploidy in mouse embryonic stem cells through decreased expression of Khdc3. *Nat Commun* 13, 6230 (2022). <https://doi.org/10.1038/s41467-022-33742-7>). Pcp2-Cre, TDG^{fl/fl} mice were generated in this study.

Housing condition: Mice were housed under a 12-hour light/dark cycle at room temperatures maintained between 68–79 °F (20–26 °C) and relative humidity of 30–70%.

Wild animals

The study did not involve wild animals.

Reporting on sex

In all animal experiments, an equal distribution of male and female mice was used to ensure balanced representation of both sexes.

Field-collected samples	The study did not involve field collected samples.
Ethics oversight	All animal procedures were conducted according to the NIH Guide for the Care and Use of Laboratory Animals, under Animal Study Proposal no. 23-042 approved by the NIH Animal Care & Use Committee (ACUC).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Isolated cerebellum or other brain tissues from mice were placed in 4 ml of homogenization buffer [0.25 M sucrose, 25 mM KCl, 5 mM MgCl ₂ , 20 mM Tris-HCl (pH 7.5), 0.2% BSA and protease inhibitor cocktail tablet (complete Mini, Roche Diagnostics)] and homogenized for 10 strokes with a glass dounce homogenizer, then added 32 µl of 5% NP-40 and homogenized for another 5 strokes. Tissue homogenates were strained through a 40-µm nylon filter (Falcon, cat. No. 352340), centrifuged at 500g for 5min and incubated in 1ml blocking buffer (1%BSA/PBS) for 15min at 4°C. Nuclei were incubated with anti-NeuN antibody (1:250, Abcam, cat. No. ab279297) and γ-H2AX antibody (Millipore, clone JBW301, no. 05-636, RRID:AB_309864, 1:1000) at 4°C for 1h. Subsequently, nuclei were pelleted at 500g for 5min and incubated with secondary antibodies Alexa Fluor 488 chicken anti-rat (1:1,000, Invitrogen no. A21470, RRID: AB_2535873), Alexa Fluor 568 goat anti-mouse (1:1,000, Invitrogen no. A11031, RRID: AB_144696) for 20 min at room temperature and CoraLite Plus 647-conjugated ITPR1-specific Polyclonal antibody (1:200, Proteintech, cat. No. CL647-19962) for Purkinje nuclei isolation. Nuclei were then stained by DAPI (1 µg/ml, Thermo Fisher Scientific no. 62248) for 2min and collected by centrifuge. Cell type-specific nuclei were independently analyzed using BD LSRFortessa SORP or Cytex Amnis Imaging Flow Cytometers, with excitation at 488, 568, and 647 nm. Nuclei sorting were performed on BD FACSria II with an UV or a Violet laser.
Instrument	FACS analysis was performed using the BD LSRFortessa SORP, cell sorting was carried out on the BD FACSria II with either a UV or violet laser, and flow imaging was conducted using the Cytex Amnis Imaging Flow Cytometer.
Software	FlowJo v10.
Cell population abundance	Between 20,000 and 30,000 Purkinje or Golgi cell nuclei were collected by FACS from each mouse.
Gating strategy	Cell debris and dead cells were excluded based on forward scatter area (FSC-A) and side scatter area (SSC-A), while cell doublets were removed using forward scatter area (FSC-A) and forward scatter height (FSC-H). The DAPI-positive population was selected, and specific cell types exhibiting DNA damage were identified using the neuronal marker NeuN-488, the Purkinje-specific marker ITPR1-647, and the DNA damage marker γ-H2AX-568.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.