Ten-eleven translocation (TET) proteins, the dioxygenase for DNA hydroxymethylation, are important players in nervous system development and diseases. However, their role in myelination and remyelination after injury remains elusive. Here, we identify a genome-wide and locus-specific DNA hydroxymethylation landscape shift during differentiation of oligodendrocyte-progenitor cells (OPC). Ablation of Tet1 results in stage-dependent defects in oligodendrocyte (OL) development and myelination in the mouse brain. The mice lacking Tet1 in the oligodendrocyte lineage develop behavioral deficiency. We also show that TET1 is required for remyelination in adulthood. Transcriptomic, genomic occupancy, and 5-hydroxymethylcytosine (5hmC) profiling reveal a critical TET1-regulated epigenetic program for oligodendrocyte differentiation that includes genes associated with myelination, cell division, and calcium transport. Tet1-deficient OPCs exhibit reduced calcium activity, increasing calcium activity rescues the differentiation defects in vitro. Deletion of a TET1-5hmC target gene, Itpr2, impairs the onset of OPC differentiation. Together, our results suggest that stage-specific TET1-mediated epigenetic programming and intracellular signaling are important for proper myelination and remyelination in mice.
The modified nucleotide 5-hydroxymethylcytosine (5hmC) has been shown to be an intermediate product generated during cytosine demethylation. DNA demethylation, like methylation or demethylation has been established as a common epigenetic modification regulating various processes from development to diseases in a cell-type and context-dependent manner. Dynamic regulation of cytosine demethylation or demethylation has been established as a common epigenetic modification regulating various processes from development to diseases in a cell-type and context-dependent manner. Tet enzymes oxidize 5mC into 5hmC to initiate the DNA demethylation process. Dynamic regulation of cytosine methylation or demethylation has been established as a common epigenetic modification regulating various processes from development to diseases in a cell-type and context-dependent manner. In this study, we demonstrate that there is a genome-wide shift in the 5hmC landscape during OL specification and identify an age-dependent function of TET1 in OL lineage specification and myelination. The mice with Tet1 deletion in OL lineage develop behavioral deficiency. In addition, we show that a TET1-regulated epigenetic program is required for efficient remyelination as depletion of Tet1 in OPCs impairs myelin recovery after demyelinating injury in adult animals. Moreover, Tet1 depletion resulted in genome-wide alterations in 5hmC and transcriptomic profiles that are associated with OPC differentiation and myelination, as well as calcium transport. Ablation of Itpr2, one of the TET1-5hmC targets that responsible for calcium release from endoplasmic reticulum (ER) in the OL lineage significantly impairs OL differentiation. These data suggest that TET1 and DNA hydroxymethylation mediated transcriptional and epigenetic programming regulate OL intracellular signaling and are required for proper myelination and animal behaviors.

Results
Dynamic DNA hydroxymethylation landscape during OL lineage specification. To investigate the 5hmC landscape during OL lineage specification, we carried out antibody-based 5hmC immunoprecipitation (IP) combined with Illumina sequencing (hMeDIP-seq) and analyzed 5hmC distribution across the genome. We compared the 5hmC distribution within OPCs isolated from immunopanning from the cortices at postnatal day 6 (P6) to that in neural progenitor cells (NPCs) and identified 1237 genes that were specifically hydroxymethylated in the promoter or transcription start site (TSS) regions of OPCs but not NPCs (Fig. 1a). Gene ontology (GO) analysis revealed that these genes involved in OPC differentiation are highly associated with terms such as cell projection organization, fatty acid transport, and regulation of cytosolic calcium ion concentration, and with signaling pathways that are essential for OL development such as the G-protein coupled receptor pathway (Fig. 1b). Similarly, gene set enrichment analysis (GSEA) for 5hmC peaks in the gene body regions indicated that genes associated with the bipotent progenitor, OL progenitor, and postmitotic OL were enriched in OPCs (Fig. 1c), while pluripotent stem cell-associated genes were enriched in NPCs (Fig. 1c). Comparison with a neural cell-type transcriptome dataset (Supplementary Fig. 1a–c) showed that the 5hmC signals were higher in OPCs than NPCs, in gene loci of OPC-associated genes, e.g., Csgp4 (chondroitin sulfate proteoglycan 4) (Fig. 1d), immature OL-associated genes, e.g., Kndc1 (kinase non-catalytic C-lobe domain containing 1) (Fig. 1e), and mature OL-associated genes, e.g., Mag (myelin-associated glycoprotein) (Fig. 1f). In contrast, the genes with 5hmC peaks enriched in NPCs were associated with negative regulation of OPC differentiation, such as Ngf and Zfp28 (Fig. 1g). We further verified the presence of loci-specific hydroxymethylation in representative genes with a qPCR assay based on a combination of bisulfite and subsequent cytosine deaminase (APOBEC) treatment (Supplementary Fig. 1d and Supplementary Table 1). These data suggested a unique distribution pattern of genomic 5hmCs in the gene loci associated with OL lineage specification during the transition from NPCs to OPCs.

Deletion of Tet1 in OL lineage causes myelination deficits at early postnatal stages. TET1-3 enzymes are present in OL lineage cells. As TET2 had no detectable effects in OL lineage development, we assessed the functions of Tet1 and Tet3 in OL development. We crossed Tet1lox/lox flox/+ (Tet1cKO) and Tet3lox/lox flox/+ (Tet3cKO) mice with the Olig1-Cre line to knock out the catalytic domains of these TET enzymes early in OL lineage development (Fig. 2a and Supplementary Fig. 2a). The resulting Tet1lox/lox flox/+Olig1Cre+/− (Tet1 cKO) and Tet3lox/lox flox/+Olig1Cre+/− (Tet3 cKO) mice were born at Mendelian ratios and appeared normal at birth. We did not detect significant differences in either the number of CC1+ mature OLs or myelin protein expression between heterozygous Tet1-foxed mice (Tet1lox+/lox flox/+; Olig1Cre+/−), Cre control (Tet1+/+;Olig1Cre+/−), or wild-type mice (Supplementary Fig. 3a, b). Also, we did not detect any Olig2 expression changes within the Olig1-Cre (+/−) heterozygous line (Supplementary Fig. 3c, d). Therefore, heterozygous littersmates were used as controls. To assess Cre-mediated Tet1 deletion in OL lineage, we quantified Tet1 expression in OPCs from Tet1 cKO and control mice at P4. Immunostaining revealed that expression of TET1 in SOX10+ OLs was significantly reduced in Tet1 cKO than in control mice (Fig. 2b, c). Tet1 levels were also decreased in purified OPCs from Tet1 mutant than from control mice assayed by quantitative real-time PCR (Supplementary Fig. 3e). To examine the cell-type or lineage-specificity of Olig1-Cre in the postnatal brain, we have generated Olig1-Cre; R26-tdTomato mice by crossing the Olig1-Cre mice with R26-tdTomato reporter line (Ai14). We found that most Olig1-Cre-TdTomato cells were Olig2-positive OL lineage cells (Supplementary Fig. 3f, g), while TdTatome+ cells rarely co-label with the markers for astrocytes (ALDH1L1+), neurons (NeuN+), or interneurons (PV+, SST+, and VIP+) (Supplementary Fig. 3h, i), indicating that the Olig1-Cre line is predominantly restricted to the OL lineage.

To investigate the effects of TET1 on OL development in the brain, we examined the expression of OL lineage marker SOX10 and mature OL markers CC1 and MBP. The number of CC1+ mature OLs was significantly reduced in juvenile Tet1 cKO mice compared to controls (Fig. 2d, e), but this difference was not observed in adult mice from P52 (Fig. 2e and Supplementary Fig. 4a). Expression of MBP was also substantially decreased in both cortex (gray matter) and corpus callosum (white matter) in Tet1 cKO mice compared to controls at P16 (Fig. 2f), but the levels were similar in P60 adult animals (Supplementary Fig. 4b).
These data are consistent with previous observations with a siRNA-mediated knockdown in rat OPC culture and indicate that Tet1 loss causes a delay in OL maturation. Similar experiments in the Tet3 cKO animals did not show any significant differences between mutants and controls (Supplementary Fig. 2), which is different from previous in vitro data using siRNA-mediated knockdown. We speculate that this might be due to the possible off-target effects of TET3 siRNA on other TET members or other unidentified factors crucial for OPC differentiation. Therefore, we focused on examining the processes underlying the observed myelination defects in Tet1 cKO mice.

In addition, electron microscopy (EM) revealed that the number of myelinated axons was significantly reduced in Tet1 mutants compared to controls at both P14 optic nerves and P27 corpus callosum. However, those myelinated axons in Tet1 cKO mice were characterized by higher G ratios and thinner myelin sheaths than those of control mice. However, the myelin ultrastructure defects were not observed in P60 adult Tet1 cKO animals. Together, these results suggest a stage-dependent function of TET1 in CNS myelination.

Consistent with the observation, we confirmed here that TET1 expression significantly decreased in control adult OLs. However, in Tet1 cKO mice at P60, the number of TET1+ OLs increased to ~29% (Supplementary Fig. 4f, g), which is higher than ~12% in Tet1 cKO animals at P4. This result indicates that OPCs failed to disrupt TET1 expression may contribute to the recovery of myelin formation in older mutant animals.

To evaluate the neurological significance of hypomyelination in Tet1 cKO mice, we analyzed stimulus-evoked compound action potential (CAP) in P14 optic nerves as previously described. Suction electrodes backfilled with artificial cerebrospinal fluid (aCSF) were used for stimulation and recording. In Tet1 mutants, both the peak amplitudes and the CAP areas, which are indexes of excited myelinated axon numbers and nerve function, were significantly lower than controls under all stimulating currents tested. This observation indicates that...
hypomyelination impairs action potential transduction in Tet1 cKO mice.

Loss of TET1 function in OL lineage cells induces behavioral deficiency in the mice. Multiple studies have associate TET-5hmC with psychiatric and cognitive disorders, and multivariable logistic regression showed that ERBB4, BDNF, and TET1 were independent predictors for schizophrenia. To gain insight into the physiological function of TET1 in animal behaviors, Tet1 cKO mice were subjected to behavior tests relevant to schizophrenia. Tet1 cKO mice did not exhibit differences in weight and whisker number in comparison with control littermates.

First, we investigated the performance of juvenile Tet1 mutant in prepulse inhibition (PPI) of the startle test at P42 with myelin deficiency (Supplementary Fig. 5a), which is a common test of sensorimotor gating ability for schizophrenia. Reduced PPI ability due to an exaggerated acoustic startle reflex (ASR) is thought to contribute to schizophrenic conditions. We found that the
Fig. 2 TET1 is required for OL differentiation and myelination. a Schematic diagram of Cre-mediated excision of floxed TET1 exons encoding the critical catalytic domain for dioxygenase activity. b Representative immunostaining for TET1 expression in postnatal day 4 (P4) corpus callosum from control (Ctl) and TET1 cKO (cKO) mice. Upper images show TET1 staining (green), SOX10 (red), and DAPI (blue) staining. Lower images show only TET1. Arrows indicate SOX10+ cells. Scale bar, 20 μm. c Percentage of TET1+ cells among SOX10+ oligodendrocytes in P4 corpus callosum of control and TET1 cKO mice. Data were Means ± SEM (n = 4 animals in each group). ***, Two-tailed unpaired t-test, t = 17.091, df = 6, p = 0.000003. d Representative images of CC1 and SOX10 immunostaining in the corpus callosum of control and TET1 cKO mice at P8. Scale bar, 50 μm. e Quantification of CC1+ cells in P8, P21, P42, P52, and P60 control and TET1 cKO corpus callosum. Data were Means ± SEM (n = 3 animals in P4 and P52 for each group, n = 4 animals in P8, P21, P60, and P42 for each group). Two-tailed unpaired t-test, *: P8: t = 2.724, df = 6, p = 0.034; **: P21: t = 3.794, df = 6, p = 0.009; *: P27: t = 3.702, df = 6, p = 0.010; **: P42: t = 4.071, df = 4, p = 0.015; P52: t = 2.529, df = 4, p = 0.065; P60: t = 1.036, df = 6, p = 0.340 compared to control. f Representative images of MBP immunostaining in TET1 cKO and control mice cortex (upper images) and corpus callosum (lower images) at P16. Scale bar, 100 μm. g Representative electron micrographs of P14 optic nerve and P27 corpus callosum (cc) from control and TET1 cKO mice. Scale bar, 0.5 μm in P14 and 2 μm in P27, respectively. h Quantification of the number of myelinated axons in defined areas from the optic nerve of P14 control and TET1 cKO mice. Data were Means ± SEM (n = 6 slides from three animals per group). ***, Two-tailed unpaired t-test, t = 3.508, df = 10, p = 0.000344 compared to control. i G ratios vs. axonal diameters for P14 optic nerve in control and TET1 cKO mice. Friedman M test, $\chi^2 = 43.251$, df = 1, $p_{\text{between group}} = 0.0001$ (<150 myelinating axon counts/animal from three animals/genotype). j Quantification of the number of myelinated axons in defined areas from corpus callosum of P27 control and TET1 cKO mice. Data were Means ± SEM (n = 6 slides from three animals per group). ***, Two-tailed unpaired t-test, t = 11.050, df = 10, p < 0.0001 compared to control. k G ratios vs. axonal diameters for P27 corpus callosum in control and TET1 cKO mice. Two-way ANOVA, $F_{\text{between group}} (1,588.597) = 10.806$, $F_{\text{within group}} (13.3) = 71.262$, $p_{\text{between group}} = 0.002$ (>120 myelinating axon counts/animal from three animals/genotype). l Representative compound action potential (CAP) series from optic nerves of P14 control and TET1 cKO mice elicited by square voltage pulses of varied amplitudes. m Evoked CAP amplitudes of the second peak (maximal) from control and TET1 cKO mice plotted vs. stimulus intensities. Data were Means ± SD (n = 11 nerves per group). Two-way ANOVA, $F_{\text{between group}} (9,200) = 55.07$, p < 0.0001, ***, $F_{\text{within group}} (1,200) = 1804$, p < 0.0001. n Total CAP area vs. stimulus intensities in controls and TET1 cKO mice at all stimulus intensities compared. Data were Means ± SD (n = 11 nerves per group). Two-way ANOVA, $F_{\text{between group}} (9,200) = 30.31$, p < 0.0001, ***, $F_{\text{within group}} (1,200) = 1337$, p < 0.0001.

input/output function and the ankle response were comparable between control and Tet1-mutant mice (Supplementary Fig. 5b), indicating the normal hearing and motor abilities (i/o function) in Tet1 cKO mice. However, when using a combination of auditory-evoked startle (120 dB) and three levels of prepulse (70, 76, and 82 dB) to compare ARS, we observed that PPI was significantly attenuated in Tet1 cKO mice in comparison with control animals (Supplementary Fig. 5c), suggesting the impaired sensorimotor gating ability in Tet1 mutant.

Since working memory deficits are characteristic features in schizophrenia, adult Tet1 cKO mice and control littersmates at P90 were evaluated for their performance in the Morris water maze (MWM) to assess their working memory. Consistently, the myelin deficiency in Tet1 cKO mice was recovered after P60 (Supplementary Fig. 4), including the hippocampus that is associated with spatial reference memory33 (Supplementary Fig. 5d). Five-day acquisition trials exhibited similar swim paths, swim velocity, and escape latency to the platform between control and Tet1 cKO groups (Supplementary Fig. 5e–g), which indicates that adult Tet1 mutants had normal swimming and learning abilities. However, in the sixth-day probe trial, the escape latency was significantly higher in Tet1 cKO mice than in control mice (Supplementary Fig. 5h), and the number of crossing the position was greatly reduced in mutant mice (Supplementary Fig. 5j, k). These observations indicate that developmental myelogenesisis contribute to working memory deficits in Tet1-mutant animals. Together, our results suggest that the abnormal myelination in Tet1 cKO mice may cause behavioral deficiency with impaired sensorimotor gating ability and working memory.

Ablation of Tet1 results in defects in OPC cell-cycle progression. Concomitant with the myelin deficiency, we observed a marked reduction of OLIG2+ cells from embryonic stage E15.5 and at P1 in Tet1 cKO cortex relative to controls (Fig. 3a, b). Moreover, the number of PDGFRα+ OPCs in the mutant cortex was significantly reduced at E15.5 and P1 (Fig. 3a, c), suggesting a downsized OPC pool in Tet1 cKO brain. Interestingly, the difference of OPC number between Tet1 mutant and control brains was not obvious after P6 (Fig. 3c). We speculate that this may in part be due to the substantial decline in TET1 expression with age particularly after P416, suggesting a less critical role for TET1 in later stages of OPC development. In addition, it is possible that compensation from other TETs (e.g., TET2 and TET3) might also contribute to OPC development in the Tet1 cKO mice.

To determine the underlying defects that led to the observed reduction in the OPC and OL population in juvenile Tet1 mutants, we first tested the possibility that OPCs are more likely to undergo apoptosis in the mutant with the TUNEL assay. Brain sections from E14.5, E17.5, and P1 mice revealed no distinguishable changes in the number of apoptotic cells among OLIG2+ OL lineage cells between Tet1 cKO animals and control littersmates (Supplementary Fig. 6a, b).

Next, we performed a BrdU incorporation assay to examine the proliferation of OPCs. At P1, mice were dosed with BrdU and sacrificed 2 h later. Compared to controls, intriguingly, the percentage of BrdU+ cells in OLIG2+ OL lineage cells showed a significant increase in Tet1 cKO cortex (Fig. 3d, e). However, BrdU incorporation in older mice, e.g., P20 and P60, did not reveal the difference in OPCs between groups (Supplementary Fig. 6c–f). In addition, we examined the proliferation changes in purified OPC cultures. Both BrdU incorporation and Ki67 staining showed increased proliferating OPCs from Tet1 mutants (Supplementary Fig. 6g–i).

The reduction of OPC numbers in Tet1 cKO mice thus promote us to investigate if there is a cell-cycle defect in Tet1-deficient OPCs. Then we performed flow cytometry for purified OPCs in which DNA was stained with propidium iodide. Significant increases in the percentages of cells in the S phase (23.49 ± 0.85%) and G2/M phase (13.39 ± 1.01%) were observed in OPCs from Tet1 mutants compared to the controls (9.02 ± 0.99% for S phase and 8.29 ± 0.62% for G2/M phase) (Fig. 3f, g and Supplementary Fig. 7a, b). Moreover, there was a concomitant reduction in the number of cells in the G1 phase in Tet1 mutants compared to the controls (Fig. 3f, g).

A recent report has suggested the critical role of TETs in regulating the G2 to M phase progression of trophoblast stem cells34, we then tested G2-M progression of Tet1 cKO OPCs in a cell cycle synchronization assay. We arrested cell cycle at G2/M with the CDK1 inhibitor RO3306 for 12 h, followed by a release at specific time points (Fig. 3h). A marker for the M phase,
Fig. 3 Proliferation and cell-cycle progression are defective in Tet1 cKO OPCs. 

- **a** Representative images of OLIG2 staining for embryonic day 15.5 (E15.5) and SOX10/PDGFRα staining for postnatal day 1 (P1) control and Tet1 cKO cortex. Scale bar, 100 μm. 

- **b** Quantification of OLIG2+ cells in cortex from E15.5 and P1 control and Tet1 cKO mice. Data were Means ± SEM (n = 4 animals per group). E15.5: *, Mann–Whitney U-test, z = −2.309, p = 0.021 compared to control; P1: ***, Two-tailed unpaired t-test, t = 17.254, df = 6, p < 0.0001 compared to control. 

- **c** Quantification of PDGFRα+ cells in cortex from E15.5, P1, P6, P14, and P60 control and Tet1 cKO mice. Data were Means ± SEM (n = 4 animals per group). Two-tailed unpaired t-test, E15.5: *, t = 2.519, df = 6, p = 0.045; P1: *, t = 2.682, df = 6, p = 0.036; P60: t = 0.522, df = 6, p = 0.620. Two-tailed unpaired separate variance estimation t-test, P6: t = 0.871, df = 4.823, p = 0.425; P14: t = 2.085, df = 3.770, p = 0.110 compared to control. 

- **d** Representative images of P1 control and Tet1 cKO cortex immunostained for BrdU and OLIG2. Scale bar, 100 μm. 

- **e** Quantification of BrdU+ cells within OLIG2+ OPC population in control and Tet1 cKO brains. Data were Means ± SEM (n = 3 animals per group). *, Two-tailed unpaired t-test, t = −4.380, df = 4, p = 0.012 compared to control. 

- **f** Representative flow cytometry traces of propidium iodide-stained OPCs from control and Tet1 cKO mice. 

- **g** Quantification of the percentage of OPCs from control and Tet1 cKO mice in each phase of the cell cycle. Data were Means ± SEM (n = 4 independent cultures for the control group and n = 3 independent cultures for Tet1 cKO group). Two-tailed unpaired t-test, S: ***, t = −10.556, df = 5, p = 0.00013; G2/M: **, t = −4.506, df = 5, p = 0.006; Mann–Whitney U-test, G1: *, z = −2.141, p = 0.032 compared to control. 

- **h** Diagram showing cell cycle synchronization assay. RO3306, a CDK1 inhibitor, arrests the cell cycle at the G2 phase. Cultures were fixed for immunostaining at A point (Vehicle treatment for 12 h), 0 (RO3306 treatment for 12 h), and 45-, 60-, 80-min after RO3306 withdrawal. Representative H3S10P and OLIG2 double immunostaining for asynchronous and synchronized OPCs from control and Tet1 mutant. Cells were arrested at the G2/M phase with RO3306, 0 h, and released with fresh medium for 45- and 60-min. Enlarged inserts indicate H3S10P+ metaphase nuclei. Scale bar, 100 μm. 

- **i** Quantification of the percentage of H3S10P+ metaphase cells among OLIG2+ cells in asynchronous (A), arrested (0 h), and released control and Tet1 mutant OPCs. Data were Means ± SEM (n = 3 independent experiments for control group at 45 min, n = 4 independent experiments for control at other time points and for Tet1 cKO group). Mann–Whitney U-test, A: *, z = −2.337, p = 0.019; 0 min: ns, z = −1.692, df = 6, p = 0.091; Two-tailed unpaired t-test: 45 min: **, t = 4.469, df = 5, p = 0.0066; 60 min: *, t = 2.897, df = 6, p = 0.0275; 80 min: ns, t = 1.971, df = 6, p = 0.0962.
phosphorylated histone H3 serine 10 (H3S10P), was used for immunostaining. In control OPCs, the percentage of H3S10P+ cells among OLIG2+ cells peaked at 45 min post-release, indicating that most cells had progressed into the early stages of mitosis (Fig. 3i, j). In contrast, less Tet1 cKO OPCs were positive for H3S10P at this time point. At 60 min post-release, control cells had progressed into anaphase as most H3S10P staining disappeared in OLIG2+ cells. In the case of Tet1 cKO OPCs, we find that there is a delay in M-phase entry and withdrawal after cell cycle release (Fig. 3i, j). Thus, these results suggest that Tet1 deletion results in a slower cell-cycle progression in OPCs, specifically during the G2-M transition, which likely leads to the observed reduction of OPC numbers in Tet1 cKO animals.

In addition, we noted that Tet1 deletion in Tet1 cKO mice did not substantially alter the number of other neural cell types in the brain. Western blot and immunostaining with DCX, a marker for newly generated neurons; NeuN, a mature neuron marker; and ALDH1L1, an astrocyte marker, revealed comparable neuron and astrocyte pools between controls and Tet1 mutants (Supplementary Fig. 7c–g). Meanwhile, we validated that TET1 expression in astrocytes and neurons was not significantly decreased in Tet1 cKO mice (Supplementary Fig. 7h, i). Taken together, our data suggest that the abnormal cell-cycle progression of OPCs and delayed OPC differentiation contribute to the reduced OL numbers and hypomyelination in juvenile Tet1-deficient mice.

TET1 regulates the transition from OPCs to OLs. To determine if defects in OL differentiation caused by Tet1 deletion are cell-autonomous, we isolated primary OPCs from the neonatal cortices of control and Tet1 cKO pups and then treated them with T3 to induce differentiation. Immunostaining results showed that the percentage of CNP+ and MBP+ cells among OLIG2+ cells in Tet1-deficient OPCs were significantly decreased when compared with control OPCs at 3 DIV (day in vitro) and 5 DIV (Fig. 4a–c), suggesting that Tet1-depleted OPCs are intrinsically reduced in their differentiation capacity.

Since the impaired myelination in Tet1 cKO mice may result from the downsized OPC pool, to further confirm the role of TET1 in OL differentiation after birth, we bred Tet1fllox/fllox mice with NG2-CreER line58, an OPC-specific tamoxifen-inducible Cre line to generate Tet1fllox/fllox; NG2-CreER (Tet1 OPC-iKO) animals. The Tet1 OPC-iKO mice were treated daily with tamoxifen from P2 through P5 to induce Tet1 deletion (Fig. 4d) and double immunostaining confirmed TET1 loss in SOX10+ cells (Fig. 4e, f). Heterozygous littermates (Tet1fllox/+; NG2-CreER) were served as controls. In the P7 corpus callosum, more PDGFRα+/Ki67+ cells and less MBP+ cells were observed in Tet1 OPC-iKO mice (Fig. 4g–i). Moreover, a reduction in CC1+ OLs and MBP intensity was observed in corpus callosum from P14 Tet1 OPC-iKO mice (Fig. 4k, l). These results indicate that Tet1 is required for the transition from OPCs to OLs.

In Tet1 cKO mice, wherein Tet1 is deleted in the Olig1-Cre expressing early precursors such as pri-OPC from the embryonic stages, we observed a decrease in the number of OPCs, suggesting that TET1 regulates the transition of OPCs from the pri-OPC state. However, in Tet1-iKO mice, wherein NG2-CreER is expressed in the committed OPCs, we observed a defect in OPC differentiation and a corresponding increase in undifferentiated OPCs, suggesting that Tet1 deletion impairs OPC differentiation into mature OLs and maintains them in the OPC state. Thus, Tet1 is involved in the early specification and later differentiation, producing stage-specific effects on OPC numbers.

We further performed the PPI test in Tet1-iKO mice at P42, which were treated daily with tamoxifen from P2 through P7 to induce Tet1 deletion in NG2+ OPCs. Similar to Tet1 cKO mice, the startle response was normal in Tet1-iKO mice, but the percentage of PPI was significantly attenuated (Supplementary Fig. 8a, b). These observations suggest that Tet1 ablation in OL lineage cells leads to behavioral deficiency with impaired sensorimotor gating ability.

To further assess if Tet1 deletion alters OPC fate or differentiation in adult mice, we used R26-EYFP reporter mice to test OL lineage progression in Tet1-iKO mice (Tet1fllox/fllox; NG2-CreER; R26-EYFP). We performed tamoxifen-mediated deletion at P30 in NG2-CreER Tet1-iKO; R26-EYFP mice and analyzed the GFP reporter positive Tet1-iKO cells at P60. We did not detect any significant alteration in the number of OPCs (PDGFRα+) and mature OLs (CC1+) in Tet1-iKO mice (Supplementary Fig. 8c–i). Thus, these data suggest that Tet1 ablation did not change OPC fate or differentiation in adult mice.

Efficient remyelination requires TET1 function. Given the critical role of TET1 in early OL development, we reasoned that Tet1 should also be required in the adult brain for remyelination after an injury that results in demyelination. We induced demyelinated lesions in the corpus callosum via stereotaxic guided lyssolecithin (LPC) injections (Fig. 5a). LPC induces rapid myelin breakdown followed by myelin regeneration through an OPC recruitment phase at 7 days post-lesion (7 dpl) induction and a remyelination phase at 14 dpl. To evaluate the role of Tet1 in remyelination, we used Tet1fllox/fllox; NG2-CreER (Tet1 OPC-iKO) animals mentioned above. To induce recombination in adult mice, 8-week-old Tet1 OPC-iKO mice were injected daily with tamoxifen for 8 days, starting 3 days prior to LPC injection in the corpus callosum (Fig. 5b). Brains were harvested at 7, 14, and 21 dpl from Tet1 OPC-iKO mice and controls. Compared to control mice injected with vehicle, TET1+ cell numbers were increased substantially in the lesion site at 7 dpl (Fig. 5c, d). In particular, the expression levels of TET1 in OLIG2+ cells were higher after LPC treatment (Fig. 5c, d). In Tet1 OPC-iKO mice, immunostaining showed ~97% OLIG2+ cells lack TET1 expression at 7 dpl, indicating the efficient deletion of Tet1 by NG2-Cre (Fig. 5c–e). Notably, TET1+ OLIG2+ OL lineage cells increased in the lesion at 7 dpl, while TET1 expression was detected in a subset of IBA1+ microglia and NESTIN+ cells, but not in GFAP+ astrocytes (Supplementary Fig. 8g).

To determine the extent of remyelination, we examined the expression of OPC markers and myelin-related genes. Loss of Tet1 did not appear to impair the recruitment of PDGFRα+ OPCs, and the numbers of OPCs in the lesions were comparable between control and Tet1 OPC-iKO mice during the recruitment phase at 7 dpl (Fig. 5f, g). In contrast, there were significantly fewer GST-π+ differentiating OLs in the lesion site during the remyelination phase at 14 and 21 dpl in Tet1 OPC-iKO mice relative to controls (Fig. 5f, h). Consistent with a reduction in the number of differentiating OLs, MBP was also reduced in Tet1-iKO lesions compared to those of controls at 21 dpl (Fig. 5i). Notably, far fewer remyelinated axons were detected in the lesions of Tet1-iKO mice than controls at 28 dpl examined by EM (Fig. 5j, k). Furthermore, the thicknesses of newly generated myelin sheaths around axons were significantly reduced in Tet1-iKO mutants (Fig. 5l). These observations indicate that Tet1 is required for the proper remyelination in the context of white matter injury.

Transcriptome alterations and a genome-wide decrease of 5hmC in Tet1-ablated OPCs. To investigate the molecular underpinnings of the observed defects in early OL development, we compared the RNA transcriptomes of OPCs cultured from...
control and Tet1 cKO neonates. There were ~1880 genes downregulated and 881 genes upregulated in Tet1 mutants compared with controls (FDR < 0.05, Log2 > 1 or < −1) (Supplementary Table 2), suggesting that the predominant effect of Tet1 was transcriptional activation. As indicated by GSEA, among the top downregulated TET1 targets were those pertinent to cell division, extracellular matrix, and OL differentiation (Fig. 6a–d), which are in consistent with the abnormal cell cycle and deficient myelin formation in Tet1 mutants. In contrast, GO terms associated with the mitochondria gene module and immune-related functions were upregulated in Tet1 cKO OPCs (Fig. 6a, b, e, f). The observation that TET1-activated genes are related to OL differentiation may reflect a cell-type-specific transcriptional regulation for TET1 in OL differentiation. Quantitative PCR confirmed
representative gene expression changes associated with these gene set enrichments in the RNA-seq data (Fig. 6g–i). Specifically, OL differentiation and myelination-associated genes Mbp, Plp1, Myrf, Enpp2, Cnp, Cldh11, Ugt8a, Kif11, and Bcas1 were markedly downregulated in Tet1-deficient OPCs (Fig. 6g) as were genes involved in cell-cycle regulation, e.g., Ccnb1, Cdc25b, and Cdc25c (Fig. 6h and Supplementary Fig. 6j, k). The transcriptome landscape alterations in Tet1 cKO OPCs were in line with the observations that Tet1 depletion led to cell-cycle progression defects and hypomyelination phenotypes.

Since TET1 mediates DNA hydroxymethylation/demethylation, we next tested the level of 5hmC in OLs from Tet1 mutants. In P27 brain sections, immunostaining of 5hmC simultaneously with the OL marker CC1 revealed a striking reduction in 5hmC intensity in Tet1 cKO OLs (Fig. 7a, b), which strongly suggested that 5hmC is involved in TET1-mediated regulation of OL differentiation. To further compare the genome-wide 5hmC distributions, we performed hMeDIP-seq in OPC cultures from controls and Tet1 mutants. Tet1 cKO OPCs showed a dramatic reduction in 5hmC peak signals compared to controls (Supplementary Fig. 9a). In both groups, most 5hmC peaks resided in intergenic regions; less than 40% of peaks were within gene bodies of annotated RefSeq genes (Supplementary Fig. 9b). This is different from the distribution pattern in mouse embryonic stem
cells and neurons. After plotting the distribution of 5hmC peaks over RefSeq genes, we found that 5hmC was reduced near the TSS and transcriptional start sites (TSSs) in control OPCs and that Tet1 depletion caused reductions of 5hmC, especially in intragenic regions, promoter regions, and TSSs (Figs. 7c and Supplementary Fig. 9b). Heatmap clustering of the 5hmC peak distributions 5-kb upstream and downstream of TSSs revealed five groups; levels of 5hmC signals were lower in all five groups in Tet1 cKO samples than in the control samples (Fig. 7d). Further analysis showed that most differentially hydroxymethylated regions also had a low CpG density of less than 1 CpG per 100 bp (Supplementary Fig. 9c).

We next examined the correlation between 5hmC and gene expression. By integrating RNA-seq data with UmeDIP-seq data, we observed that among the genes that had lower hydroxymethylation in gene body regions in the Tet1 cKO mice than in controls, 12.83% was downregulated (1026 of 7998 genes) and 4.89% was upregulated (391 of 7998 genes) (Supplementary Fig. 9d). The percentages were comparable in promoter regions that were differentially hydroxymethylated (Supplementary Fig. 9d). The percentages were comparable in promoter regions that were differentially hydroxymethylated (Supplementary Fig. 9d). The percentages were comparable in promoter regions that were differentially hydroxymethylated (Supplementary Fig. 9d). The percentages were comparable in promoter regions that were differentially hydroxymethylated (Supplementary Fig. 9d). The percentages were comparable in promoter regions that were differentially hydroxymethylated (Supplementary Fig. 9d).

Among the genes that showed both downregulated mRNA expression and decreased gene body 5hmC levels in Tet1 cKO mice, GO analysis revealed that a majority of them are associated with OL differentiation, cell proliferation, and extracellular matrix (Fig. 7f, i). For example, Mbp, Mbp, and Cnp are myelin-related genes; C12orf2, Myf, and Enpp2/6 are involved in the regulation of OL differentiation in a stage-specific manner within different transcriptional circuits. Snapshots of 5hmC profiles of representative myelination genes and cell division genes showed reduced 5hmC levels in Tet1 cKO group (Fig. 7g and Supplementary Fig. 9f, g). To further test the genome-wide 5hmC landscape changes during OPC-OL transition, we compared hMeDIP-seq data in OLs with that in OPCs. Interestingly, we find increased 5hmC intensity in OL compared to OPC (Supplementary Fig. 10), which indicates that the changes in 5hmC epigenetic marks might contribute to OPC-OL transition. Together, these results are highly in consistent with the hypomyelination phenotypes in Tet1 cKO mice and indicate the significance of Tet1-5hmC mechanisms in OL homeostasis.

To further identify potential TET1 direct targets, we performed CUT & RUN assays for high-resolution mapping of TET1 DNA binding sites. Most TET1 binding sites overlapped with 5hmC modified regions (Fig. 8a), suggesting that TET1 directly binds to these target genes and facilitates hydroxymethylation in the regulatory elements. GO analysis for TET1 and 5hmC overlapped targets in OPCs revealed genes associated with OL differentiation, calcium ion transport, and cell proliferation (Fig. 8b). Furthermore, TET1 binding profiles with TET1 antibody in representative genes were in accordance with 5hmC peaks, and associated with the activating histone mark H3K27ac (Fig. 8c), indicating a transcriptional activation role of TET1 in addition to its DNA methylcytosine dioxygenase activity in the regulation of gene transcription.

**Impaired calcium transport leads to differentiation defects in OPC cultures from Tet1 cKO mice.** When searching for Tet1-5hmC regulated factors that may involve in OL development and homeostasis, we noticed that there was a cluster of calcium transporters genes among the downregulated genes in Tet1 cKO mice and indicate the significance of Tet1-5hmC mechanisms in OL homeostasis. We next examined the correlation between 5hmC expression and gene expression. By integrating RNA-seq data with UmeDIP-seq data, we observed that among the genes that had lower hydroxymethylation in gene body regions in the Tet1 cKO mice than in controls, 12.83% was downregulated (1026 of 7998 genes) and 4.89% was upregulated (391 of 7998 genes) (Supplementary Fig. 9d). The percentages were comparable in promoter regions that were differentially hydroxymethylated (31% of genes downregulated and 6.42% upregulated genes (Supplementary Fig. 9e). These observations indicate that DNA hydroxymethylation is positively correlated with gene expression in OLs.

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was employed. ATP can mobilize Ca^{2+} from ER stores or trigger Ca^{2+} influx across the plasma membrane^{45,46}. To first determine the intracellular calcium release, we used calcium-free aCSF to load Fluo4. In OPC cultures, the application of ATP induced a transient increase of [Ca^{2+}] in both control and Tet1 cKO groups (Fig. 9a, b and Supplementary Movies 1 and 2). The amplitude of ATP-induced calcium rise was significantly higher in control OPCs than in Tet1-mutant OPCs (Fig. 9c). This result indicates a deficit of intracellular calcium mobilization in Tet1-depleted OPCs. Additionally, we compared the relative free Ca^{2+} level of internal stores between control and Tet1 cKO OPCs with mag-Fluo4-AM, a low Ca^{2+} affinity fluorescent indicator for the ER, as previously reported^{47}. Living OPCs from both groups showed similar relative mag-Fluo4-AM fluorescence in the ER, as measured by spectrofluorometer (Supplementary Fig. 11a, b).

To further examine the extracellular calcium influx in Tet1 cKO OPCs, we used Bay K 8644, an L-type calcium channel (L-VOCC) agonist^{48}, to induce synchronous calcium transient in OPCs, which can be blocked by the specific L-VOCC inhibitor Verapamil (Supplementary Fig. 11c). In the mutant OPCs, [Ca^{2+}] increase was of lower amplitude and the kinetics of onset were slower (Supplementary Fig. 11d, e), suggesting the attenuated function of membrane calcium channel in Tet1-ablated OPCs.

To investigate the consequences of impaired calcium rise in Tet1-deficient OPCs, we examined cell differentiation after activating calcium signaling. Consistent with the results of high K^+ application^{43}, three consecutive pulses (5 min/each) daily with 10 μM calcium channel agonist Bay K 8644 significantly promoted differentiation of OPCs and partially rescued the differentiation
defects in Tet1-deficient OPCs as determined by qRT-PCR analysis of myelin genes and MBP+ OL numbers (Supplementary Fig. 11f–h). These data indicate that TET1-modulated [Ca2+]i rise play important roles in the OL differentiation process.

**Ablation ITPR2, a modulator for calcium release from ER, inhibits OL differentiation.** To further distinguish calcium signaling as TET1-5hmC target during OL differentiation, or the results of impaired differentiation in Tet1 cKO mice, we then tested the function of ITPR2, one of TET1-5hmC target calcium transport genes, in myelination. As a modulator for calcium release from ER, ITPR2 showed the highest expression in postmitotic newly formed OLs (OL-1DIV, PDGFRα/CNPase+) (Fig. 9d and Supplementary Fig. 12a) as reported previously. In vitro studies with RNAi methods revealed that transfection of siRNA against Itpr2 in normal OL cultures significantly reduced the expression of myelin genes and impaired OL differentiation (Fig. 9e–g and Supplementary Fig. 12b, c). Additionally, we tested the effect of adenophostin-A (Ada), a high-affinity agonist of ITPR2, on rescuing impaired Tet1 cKO OPC calcium rise and differentiation in cultures. Daily application of Ada for 2 days in Tet1 cKO OPC cultures significantly increased the amplitude of
ATP-induced calcium rise (Fig. 9b, c). Similar to the above rescue assay with Bay K 8644 application, we revealed by immunostaining that the addition of Ada significantly increased the differentiation ability of Tet1 cKO OPCs in culture (Supplementary Fig. 12d–f).

Given that TET1 regulates a set of genes in the calcium transport, activation of a single gene Itpr2 might not be able to fully rescue the calcium defects in Tet1 cKO mice. We then examined the in vivo function of Iftr2 on OL differentiation by generating Iftr2 conditional knockout mice Iftr2 cKO (Iftr2fllox/fllox, Olig1-Cre Δ/Δ). To assess Cre-mediated Iftr2 depletion, we quantified ITPR2 expression in OLs from Iftr2 cKO and control mice at P14. Immunostaining revealed that expression of ITPR2 in the corpus callosum was significantly lower in Iftr2 cKO than in control mice (Fig. 9h, i). Quantitative real-time PCR further confirmed the specific ablation of ITPR2 in purified OPCs, but not in astrocytes (Supplementary Fig. 12g). Examination of the expression of stage-specific OL lineage markers revealed that the percentage of CC1+ mature OLs was significantly reduced in Iftr2 cKO cortices compared to controls at P7 and P14 (Fig. 9j, l), whereas the number of PDGFRα+ OPCs increased (Fig. 9j, k). Myelin protein expression was also substantially decreased in Iftr2 cKO mice at P31 (Fig. 9m). EM of P16 optic nerves revealed that the number of myelinated axons was significantly reduced in Iftr2 mutants (Fig. 9n, o), and the G ratio was higher in mutants (Fig. 9p). Similar to the phenotypes in adult Tet1 cKO mice, the developmental deficiency of Iftr2 mutants was recovered in adult animals, as indicated by the comparable number of CC1+ OLs and NG2+ OPCs between control and Iftr2 mutant animals at P60 (Fig. 9k, l and Supplementary Fig. 12h). Together, these results suggest that as one of the target genes of TET1-5hmC, ITPR2 accumulates after cell cycle withdrawal and is involved in the initiation of OL differentiation, probably by effectively releasing calcium from ER.

Discussion

TET1 functions stage-specifically in OL development and remyelination. Methylation of cytosine on CpG islands in the genome enables stable but reversible transcription repression and is critical for mammalian development.28 Defects in the regulation of DNA methylation are associated with various neurological diseases.30,31 TET enzymes catalyze the first step of DNA demethylation by oxidizing 5mC into 5hmC.10–12 Strikingly, we detected a genome-wide change in the DNA demethylation landscape marked by 5hmC during OPC differentiation from NPCs, suggesting a role of TET-mediated DNA demethylation in the regulation of OL lineage progression.

We found that TET1, but not TET3, is critical for OPC proliferation, differentiation, and myelination during early animal development, suggesting a unique function of TET1 in oligodendrocyte formation and subsequent myelogenesis. Our observation is different from another study that Tet1 deletion has little effect on OPC-to-OL differentiation in the young mouse brain (Tet1loxP/loxP, Olig1Cre+).23 The reasons for different phenotypes between two mouse lines are not immediately clear. We speculate that the use of different Tet1-flxed strains might contribute to the phenotypic discrepancy during early developmental stages. In our study, the Tet1-mutant mice lack exons 11–13, encoding the catalytic domain of TET1 enzyme.24, while Tet1-mutant mice lacking exon 4 (Tet1 Δex4)32 were used in the other study.23 Distinct phenotypes and gene expression changes have been reported among individual Tet1-mutant mice lacking exon 4 or exon 11–12.24 Thus, it is possible that the phenotype discrepancy observed in different Tet1-mutant mice might be due to the nature of the mutations disrupting specific Tet1 isoforms, as suggested by the alternative splicing forms of the Tet1 gene.53 In addition, other possibilities such as differences in Olig1-Cre lines, mosaicism, and mouse backgrounds used in the studies might also contribute to the phenotypic discrepancy.

Despite early developmental defects, we noticed that the developmental myelin deficiencies recovered in adult Tet1 cKO mice. TET1 expression is downregulated in OLs from old mice, and we observed weak TET1 expression in a population of OLs (~29%) in adult Tet1 cKO mice, more than ~12% TET1+ OLs in Tet1 mutants at P4. Thus, myelin deficiencies that recovered in adult Tet1 cKO mice might be in part due to the escape from Cre-mediated Tet1 deletion in a population of OL lineage cells. Similar observations have been suggested in other studies, e.g., for the recovered myelin deficiency in adult Dicer1 cKO mice.54 In addition, it is also possible that the myelination process is not affected in adult OLs lacking TET1 expression, therefore, TET1 may not be essential for myelogenesis in mature OLs. Furthermore, the remyelination capacity after injury was compromised in adult Tet1 OPC-iKO brains, suggesting that TET1 is also critical for the myelin regeneration process.

Although OL differentiation defects were not due to increased apoptosis in the Tet1-mutant brain, we found that OPC cell cycle progression, especially G2 to M phase transition, was impaired in developing Tet1 cKO animals. In line with this result, we showed that factors involved in cell cycle regulation, Ccng2, Cdc42, Ccn2, Ccnb1, and Cdc25b, were 5hmC enriched genes in OPCs and were downregulated in Tet1 cKO OPCs. In particular, CCNB1 (Cyclin B1) is the regulatory subunit of CDK1 serine/threonine kinase, and accumulation of CCNB1 is a prerequisite for mitotic initiation in the late G2
phase. Consistently, TET1 has been also implicated in stabilizing CCNB1 in trophoblast stem cells and can act as a facilitator of mitotic cell-cycle progression. Therefore, it is possible that TET1 may regulate the expression of cell cycle regulators, CCNB1 stability, or both, in OPCs. Although the precise mechanism for cell cycle transition block in Tet1 cKO OPCs remains unknown, our observations suggest an important role of TET1 in OPC cell-cycle progression.

TET1-5hmC functions in psychological disorders. In recent years, TET-5hmC association with psychiatric and cognitive disorders has gained increasing recognition. A cohort of new autosomal recessive genes for intellectual disability, including the missense mutations in TET1, has been identified. A previous report using the same Tet1loxP line indicated that Tet1 KO mice exhibited delayed spatial learning and deficient short-term memory retention in MWM. In contrast, in another study using a different Tet1 KO line (Δ Exon 4), no significant differences were observed between groups during the training and the probe trials; but short-term memory extinction was impaired in this Tet1 KO mice.

TET1 together with other factors such as ERBB4, BDNF was identified as independent predictors of schizophrenia and serves as a high-risk gene for schizophrenia. Loss of normal PPI of startle, in particular, is widely accepted as an endophenotype of schizophrenia and considered indicative of disrupted sensorimotor gating, a precognitive process to prevent sensory overload and cognitive fragmentation. Besides, the prodromal cognitive symptoms of schizophrenia often precede the occurrence of psychosis, and the range of cognitive deficits in schizophrenia suggests an overarching alteration in cognitive control, the ability...
to adjust thoughts or behaviors in order to achieve goals\(^{66-68}\). We
find that mice with Tet1 loss in the OL lineage exhibit impaired
PPI and working memory deficits, two of the core symptoms
related to schizophrenia. Thus, our studies demonstrate a crucial
function of TET1-mediated epigenetic modifications in OLs for
psychological disorders.

**Locus-specific alterations of DNA hydroxymethylation in
OPCs of Tet1 mutants.** Levels of 5hmC are variable in the pro-
moter and gene body regions\(^ {69}\) and impact gene expression in a cell
type-dependent manner\(^ {18,70,71}\). Our comparison of differentially
hydroxymethylated genes with transcriptome profiles indicated that
5hmC signals in gene bodies are more significantly associated with
gene expression changes than those in promoter regions in
OPCs. This suggests that 5hmC modification by TET1 regulates
locus-specific gene expression programs necessary for OPC differ-
entiation. Although the exact mechanism of gene body 5hmC in
regulating gene expression is unclear, there are many observations
indicating the association between enriched 5hmC in the gene
body and the active transcription in different tissues\(^ {36,72-75}\). One
possibility is that 5mC oxidation relieves a repressive effect on
transcription, perhaps by counteracting spurious intragenic anti-
sense transcription\(^ {76}\). Other explanations may include the fact
that 5hmC has a destabilizing effect on DNA structure that
potentially favors the opening of the double helix by the tran-
scription apparatus\(^ {77,78}\). Moreover, 5hmC enriched in the gene
body cannot be bound by transcriptionally repressive methyl-CpG
binding domain (MBD) proteins in vitro\(^ {79,80}\). These studies sug-
gested that gene body 5hmC levels may regulate the transcription
rate by modifying the accessibility of genic chromatin to tran-
scriptional machinery or by inhibiting the binding of repressive
methyl-CpG binding proteins (MBDs). How 5hmCs cooperate with
other epigenetic regulators for OPC differentiation remains to be
determined.
Fig. 9 Impaired calcium transport in Tet1 cKO OPCs and depletion of ITPR2, a TET1-5hmC target gene, inhibits developmental myelination. a Representative serum image was obtained after the addition of 100 μM ATP to OPCs from control or Tet1 cKO mice. b Representative traces of Fluo4 intensity in OPCs from control and Tet1 cKO mice following application of ATP. Agonist for ITPR2, Ada, is added to rescue impaired calcium rise in Tet1 cKO mice. c Amplitude changes (ΔF/F) after ATP treatment of control and Tet1 cKO OPCs. ITPR2 agonist Ada is used to rescue calcium rise deficiency in Tet1 cKO mice. Data were Means ± SD (n = 97 cells in Ctrl group from three independent cultures, n = 99 cells in cKO group from three independent cultures, n = 100 cells in cKO - Ada group from three independent cultures). One-way ANOVA, f(2,99) = 102.001, p < 0.000001. Tukey’s multiple comparisons test: Ctrl vs. cKO: **, q = 2.9233, p < 0.000001, Ctrl vs. cKO + Ada: ***, q = 1.7517, p < 0.000001, cKO vs. cKO + Ada: ***, q = −1.17160, p < 0.000001. d Representative images of ITPR2 immunostaining during OPC differentiation in vitro. Arrows indicate the highest level of ITPR2 in newly formed CNPase+ oligodendrocytes from DIV differentiation cultures, which does not appear in other stages. Scale bar, 10 μm. e Quantitative real-time PCR identified the efficiency of a siRNA-Itpr2 duplex in reducing the mRNA level of Itpr2 in normal OPCs and in repressing the expression of two myelin genes, Mbp and Plp1, in differentiating OLs. Successfully. Transfection with nontargeting duplex was used as negative control (NC). Data were Means ± SEM (n = 3 transfections). Two-tailed unpaired t-test. Itpr2: ***, t = 9.174, df = 4, p = 0.000784; Mbp: ***, t = 8.083, df = 4, p = 0.001273; Plp1: ***, t = 7.528, df = 4, p = 0.001667. f Immunostaining for MBP and OLIG2 in OL cultures after transfected with siRNA-Itpr2 duplex for 4 days. Scale bar, 100 μm. g Quantification of the percentage of MBP+ cells among OLIG2+ cells after siRNA transfection. Note the significant decrease of differentiated cells in the siRNA-Itpr2 transfected group. Data were Means ± SEM (n = 3 transfections). Two-tailed unpaired t-test, *, t = 3.372, df = 4, p = 0.028 compared to control. h Representative double immunostaining for ITPR2 and OLIG2 in P14 corpus callosum from control and Itpr2 cKO mice. Scale bar, 50 μm. i Percentage of ITPR2+ cells among OLIG2+ OLs in P14 corpus callosum of control and Itpr2 cKO mice. Data were Means ± SEM (n = 4 animals in each group). Two-tailed unpaired t-test, **, t = −25.304, df = 6, p < 0.00001 compared to control. j Immunostaining for OPC marker PDGFRα and double immunostaining for OL lineage marker OLIG2 and mature OL marker CC1 in corpus callosum from P14 Itpr2 cKO mice. Scale bar, 50 μm. k Quantification of the density of PDGFRα+ cells in P7 and P14 mice revealed a significant reduction in Itpr2 cKO mice. No difference in density of NG2+ cells was observed in P60 adult mice. Data were Means ± SEM (n = 4 animals per group). Two-tailed unpaired t-test, P7: ***, t = −5.431, df = 6, p = 0.002; P14: ***, t = −4.668, df = 6, p = 0.003; P60: t = −0.336, df = 6, p = 0.748 compared to control. l Quantification of the percentage of CC1+ cells among OLIG2+ cells from P7 to P31 mice revealed significant reduction in Itpr2 cKO mice. No difference was observed at P60 adult mice. Data were Means ± SEM (n = 4 animals in control group and three animals in mutant). P7: **, Mann–Whitney U-test, z = −2.141, p = 0.032. Two-tailed unpaired t-test, P14: ***, t = 7.414, df = 4, p = 0.002; P31: ***, t = 7.931, df = 5, p = 0.0005; P60: t = 0.178, df = 6, p = 0.865 compared to control. m Representative images for MBP staining in corpus callosum from P31 Itpr2 cKO mice. Scale bar, 50 μm. n Representative electron micrographs of P16 optic nerves from control and Itpr2 cKO mice. Scale bar, 1 μm. o Quantification of the number of myelinated axons in defined areas of optic nerves of control and Itpr2 cKO mice. Data were Means ± SEM (n = 8 slides from three animals per group). Two-tailed unpaired t-test, ***, t = 5.035, df = 14, p = 0.00018 compared to control. p G ratios vs. axonal perimeters for control and Itpr2 cKO mice reveal a significant difference. Friedman M test, χ² = 39.035, df = 1, p = 0.00001 (110 myelinating axon counts from three animals each genotype).

Intriguingly, ablation of Tet1 led to the upregulation of a set of genes in OPCs, indicating that TET1 may also function as a transcriptional repressor. Consistent with our data, inhibition of Tet1 expression increased the level of a set of genes in ESCs81,82. Tet1-mediated repression might involve recruitment of the MBDB3/Numr repressor complex, which was shown to co-localize with Tet1 in ESCs83. Tet1 may also coordinate with the SIN3A co-repressor complex, which shows a similar binding profile to Tet1 and is required for a subset of Tet1-repressed target genes81,84. A recent study indicates that Tet1-mediated transcriptional repression could channel through the JMJD8 demethylase transcriptional repressor and is independent of Tet1 catalytic activity during epiblast differentiation.85 The mechanisms of locus-specific transcriptional regulation by Tet1 during OL development remain to be further defined.

TET1-5hmC regulates calcium transport to control proper OL differentiation. Calcium signaling is important for OPC migration, differentiation, and initiation of myelin formation.86–89. In fact, blocking of voltage-gated Ca2+ entry in OPCs inhibits their maturation and myelin formation ability.42. Similarly, an increase of the resting [Ca2+]i, through membrane depolarization facilitates MBP synthesis in OPCs.87. We found that Tet1 deletion led to the downregulation of multiple calcium transporter genes in OPCs, which mediated the impaired intracellular store release and extracellular influx in response to a stimulus. Moreover, treatment with a calcium channel agonist reversed the differentiation defects in Tet1-deficient OPCs, thus indicating the significance of TET1-5hmC regulated calcium transport in OLs.

We find that ITPR2, an intracellular ER calcium channel that is exclusively expressed in postmitotic OLs43,44, is one of the TET1-5hmC targets. Expression of ITPR2 is upregulated during a motor learning task44, indicating the participation of ITPR2+ OLs in myelin plasticity. We find that deletion of ITPR2 in the OL lineage greatly reduces OPC differentiation, suggesting that ITPR2 is critical for the initiation of myelination. In addition to ITPR2, we have also detected solute carrier (Slc) family gene members as TET1-5hmC targets. Among these genes, Slc12a2 is reported responsible for defective myelin regeneration detected in old Tet1 cKO mice. Therefore, TET1-mediated genome-wide DNA hydroxymethylation may regulate multiple distinct targets including Itpr2 and Slc12a2 for optimal myelination or at different stages of OL lineage cells.

Together, TET1-mediated 5hmC modification, or DNA hydroxymethylation, can modulate the process of oligogenesis and myelogenesis through at least two critical processes, by fine-tuning cell cycle progression for OPC proliferation and by regulating OL homeostasis e.g., ITPR2-mediated calcium transport, for OL myelination.

Methods

Animals, immunohistochemistry, and EM. All animal experiment protocols were approved by the Animal Care and Use Committee of the Fourth Military Medical University and were conducted in accordance with the guidelines for the care and use of laboratory animals. All mice used in this study were kept under stable 12-h cycles of darkness and light in the respective facilities. Room temperature was kept between 20–24 °C and air humidity between 45–60% as documented in daily controls. NG2CreER2lox/ox line91 offspring, that were then bred with Tet1fllox/lox or Tet1fllox/lox mice to produce Tet1 cKO (Tet1fllox/lox;NG2CreER2lox/lox) or Tet1 cKO (Tet1fllox/lox;Olig2CreER2lox/lox) offspring, respectively. NG2CreER mice48, R26-EYFP mice (Stock No: 006148), and R26tdTomato mice (Ai14) (Stock No: 007914) were from Jackson lab. Itp2flox/flox39 was provided by the RIKEN BRC (RBRC10293) through the National Bio-Resource Project of the MEXT/AMED, Japan. It was crossed with heterozygous Olig2-Cre mice56 to generate Tet1fllox/lox;Olig2CreER2lox/lox mice and Tet1fllox/lox;Olig2CreER2lox/lox mice, which were bred with Tet1fllox/lox or Tet1fllox/lox mice to produce Tet1 cKO (Tet1fllox/lox;Olig2CreER2lox/lox) or Tet1 cKO (Tet1fllox/lox;Slc12a2CreER2lox/lox) offspring. For optimal myelinogenesis, or at different stages of OL lineage cells.

Together, TET1-mediated 5hmC modification, or DNA hydroxymethylation, can modulate the process of oligogenesis and myelogenesis through at least two critical processes, by fine-tuning cell cycle progression for OPC proliferation and by regulating OL homeostasis e.g., ITPR2-mediated calcium transport, for OL myelination.

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For immunohistochemistry, cryosections (14 µm) of brains were fixed in 4% paraformaldehyde and processed for antigen retrieval. Sections were treated with a citrate buffer (pH 6.0) at 90°C for 10 min in a microwave and cooled down in room temperature. Then sections were washed three times in PBS, blocked in 3% BSA with 0.3% Triton X-100 (blocking buffer) for 1 h at RT, and incubated with primary antibodies in blocking buffer overnight at RT. The next day, sections were washed three times in PBS, incubated with secondary antibodies at RT for 1 h, incubated then counterstained with DAPI for 15 min. Sections were washed three times in PBS and mounted. Images were taken on an Olympus FV1200 Confocal microscope. We used antibodies against OLG2 (Millipore AB9619, 1:500), SOX10 (Santa Cruz 17342, 1:50), TET1 (Genetex GTX630201, 1:50), PDGFαR (Abcam ab61219, 1:500), CC1 (Oncogene Research OP80, 1:200), MBP (Abcam ab7349, 1:500), Shh (Active motif 39769, 1:1000), NeuN (Millipore ABN78, 1:800), PV (Abcam ab11427, 1:200), SST (Millipore AB5494, 1:50), VIP (TST 63269, 1:200), ALDH1L1 (ProteinTech 17390-1-AP, 1:100), CNPase (Sigma C9522, 1:500), Ki67 (Abcam ab16667, 1:500), GFAF (Millipore MA360, 1:1000), GST-pi (Abcam AB53943, 1:500), NESTIN (Genetex GTX3201, 1:500), DCX (Millipore ab2253, 1:500), ITPR2 (Millipore ab9000, 1:500), and H3S10P (Cell Signaling Technology 9706, 1:500).

For BrDU pulse labeling in P1 pups, animals were injected intraperitoneally with 100 mg BrDU/kg body weight 2 h prior to sacrifice. For the staining of BrdU, as well as Shh, before permeabilization, sections were subjected to DNA denaturation with 2 M hydrochloric acid at 37°C for 20 min and then neutralized with 2.5 M sodium borate at pH 8.5 for 2 x 10 min. The G44 monoclonal antibody (anti-BrdUrd, 1:40) was obtained from the Developmental Studies Hybridization Bank developed under the auspices of the NICHD and maintained by The University of Iowa.

For TUNEL/OLIG2 double staining, OLG2 immunostaining were applied after the DeadEnd™ Fluorometric TUNEL System kit (Promega, G2350) to reveal TUNEL positive cells in OL lineage.

For EM, tissues were dissected and fixed in 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4°C for 24 h. The brain tissue was postfixed with 1% osmium tetroxide overnight before being subjected to a standard protocol for epoxy resin embedding. Tissues were sectioned at 1 µm and stained with toluidine blue. Ultrathin sections were cut onto copper grids and stained with uranyl acetate before being examined with a 120-120 electron microscope (JEOL LTD, Tokyo, Japan), which is equipped with a CCD camera and its application software (832 SC1000, Gatan, Warrendale, PA).

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Western blot assay. For Western blot assay, whole cell lysates were prepared from tissue or cultures using RIPA buffer (150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 1% NP40, 1% PMSF, 1x Roche complete mini protease inhibitor cocktail, and 1x Pierce phosphatase-inhibitor cocktail). Protein concentrations in centrifugation-treated samples were measured by the BCA Protein Assay Kit (Pierce) and equal amounts of protein (10 µg) were separated on SDS-gel and transferred to Hybond PVDF (Amersham Biosciences). For protein blotting, we used antibodies against OLG2 (Millipore MABN50, 1:1000), MBP (Abcam ab7349, 1:1500), Neun (Millipore ABN78, 1:2000), ALDH1L1 (ProteinTech 17390-1-AP, 1:1000), DCX (ProteinTech 13925-1-AP, 1:1000), CCNB1 (Booster BA076-2, 1:500), β-actin (ProteinTech PA0309-1, 1:5000), GAPDH (ProteinTech 60004-1, 1:50000), and Tubulin (Abkmune A01030, 1:1000). Signals were developed with horseradish peroxidase-conjugated secondary antibodies (Abkine), followed by an ECL kit (Zeta LIFE). The band intensity was calculated with Tanon5200 imager and quantified with ImageJ 1.52p software. The intensity was normalized to β-actin or Tublin level expressed as relative fold change against control.

RNA extraction and qRT-PCR. Total RNAs were purified from tissues or cell cultures using TRIzol reagent according to the manufacturer’s instruction (Invitrogen). For qRT-PCR, RNA was transcribed to cDNA with the PrimeScript Premix Ex Taq (Takara) in CFX96 Touch Real-Time PCR Detection System (Bio-Rad). Relative gene expression was calculated by Bio-Rad CFX Manager and normalized to control β-actin. Primer sequences for SybrGreen probes of target genes are listed in Supplementary Table 3.

Behavior test. Startle response/PII tests. A startle reflex measurement system (Labmaze ZS-ZJT, Beijing) was used to test the startle response and PII for P42 mice. XeyeStartle software was used to record and analyze the data. Throughout the session, the startle system delivered a constant background white noise of 68 dB. The startle response was recorded for 300 ms (measuring the response every 1 ms) with the onset of the stimulus and a startle response was defined as the peak response during the 300 ms period.

The acoustic startle begins by placing a mouse in the undisturbed chamber for 5 min. The test consists of ten 20 ms bursts of white noise varied in level from 65–125 dB sound stimuli in steps of 5 dB, plus no-stimulus trials. The order of the stimuli was randomized, and the interval between trials was 15 s. The PPI test session began with a 5-min acclimation period followed by three consecutive blocks of test trials. Block 1 and 3 consisted of six startle stimulus-alone trials. Block 2 contained ten startle stimulus-alone trials, ten prepulse + startle trials per prepulse intensity, and ten no-stimulus trials. Three combinations of prepulse and startle stimuli (72 dB, 82 dB, and 92 dB) were employed. Trials were presented in a pseudorandom order, ensuring that each trial was presented ten times and that no two consecutive trials were identical. Intertrial intervals ranged from 30 to 45 s. Basal startle amplitude was determined as the mean amplitude of the ten startle stimulus-alone trials. PPI was calculated according to the formula: 100 /[1 – (PPx/P120)] in which PPx means the mean of the ten PPI trials (PP70, PP76, or PP82 and P120 was the basal startle amplitude).
The swim training consisted of 5 days of trials, during each day mice were released from four random locations around the edge of the tank with an intertrial interval of about 30 s. Mice were allowed to swim for 15 min and then returned to the platform. Afterward, mice were allowed to stay on the platform for 15 s. A probe trial was performed 24 h after the last day of training. During the probe trial, mice were allowed to swim in the pool without the escape platform for 60 s. The performance was expressed as the percentage of time spent in each quadrant of the MWM and swim distance in the target quadrant, which were automatically recorded. Moreover, the latency to reach the platform position (using 10 cm diameter) and the number of crossings through the position were manually recorded.

RNA-seq and data analysis. RNA-seq was performed by RibBio Co., Ltd. (Guangzhou, China). Briefly, libraries were prepared using Illumina RNA-seq Preparation Kit (TruSeq RNA Sample Prep Kit) and sequenced by HiSeq 3000 sequencer. RNA-seq reads were mapped to mm10 using TopHat 2.1.1 with settings of “read mismatches=2” and “read gap length=2” (http://ccb.jhu.edu/software/tophat/index.shtml). TopHat output data were then analyzed by DESeq (1.39.0) package to compare the changes of gene expression between Tet1 EKO and control, based on the calculated RPKM values for known transcripts in mouse genome reference. Heatmap of gene differential expression was generated using R Package (http://www.r-project.org). GO analysis was performed using ToppGene Suite (http://toppgene.cchmc.org) and GSEA 4.0.1 (http://www.broadinstitute.org/gsea/index.jsp).

BOWTIE software (V2.1.0). Aligned reads were used for peak calling, both mRNA Solexa CHASTITY quality MinElute columns, after which a 5-methylenesulfonate (CMS). Further treatment with specific treatment, unmodified cytosines such as 5C and 5C5C. The primer’s extension follows the same criteria for Bisulfite conversion-based methylation-specific PCR (MSP) and selected with MethPrimer (http://www.urogene.org/methprimer/index.html). Primers were listed in Supplementary Table 1. Two pairs of primers are needed for modified and hydroxymethylated DNA, and then using the newest UCSC RefSeq database. Differentially hydroxymethylated regions associated hMeDIP enriched regions (peaks) were annotated by the nearest gene using the standard LncRNA sample prep kit) and sequenced by HiSeq 1800. The library was denatured with 0.1 M NaOH to generate single-stranded DNA and used for qPCR of loci-specific hydroxymethylated DNA. We calculate 5mC abundance using the following equation:

\[
\text{Relative 5mC abundance} = \frac{2^{(Ct\text{OPC unmethylated} - Ct\text{NPC unmethylated})}}{Ct\text{OPC unmethylated}}
\]

Cut & Run-seq and data processing. Cut & Run-seq was performed following the published protocol97. Two million OPCs were centrifuged (800g, 3 min). 10 µl of a solution was loaded onto the gel and stained with ethidium bromide. DNA bands were visualized under UV light and images were captured with a gel documentation system. The intensity of the DNA bands was quantified using ImageJ software. The fold change in DNA methylation was calculated using the following equation:

\[
\text{DMS} = \left( \frac{F_{\text{OPC}}}{F_{\text{NPC}}} \right)_{\text{CtOPC unmethylated}}\text{/Ct}\text{NPC unmethylated}
\]

For sequencing, the library was denatured with 0.1 M NaOH to generate single-stranded DNA, and loaded onto a gel to assess the quality of the flow cell and plate concentrations, amplified in situ using TruSeq Rapid SR Cluster Kit (GD-402-4001, Illumina). Sequencing was carried out by running 150 cycles on Illumina HiSeq 2500 using TruSeq Rapid SBS Kit (FC-402-4001, Illumina) according to the manufacturer’s instructions.

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nontargeting siRNA (GenePharma, Shanghai) using Lipofectamine RNAiMAX (Invitrogen). Six hours after transfection, the cultures were changed to a differentiation medium. Two or four days later, cultures were harvested for qRT-PCR assay or immunocytochemistry as indicated.

**Statistics and reproducibility.** All statistical analyses were performed in Prism v8.0 (GraphPad Software, Inc.) and SPSS v.21.0 (IBM Inc.). The normality test was performed by the Shapiro–Wilk test and the homogeneity of variance test was performed by Levene’s test. All data met normality and homogeneity of variance were compared using a two-tailed unpaired t-test and RMANOVA. Data sets that were normal distribution but not homogeneity of variance were compared using a two-tailed unpaired separate variance estimation t-test. Data that didn’t meet normal distribution were analyzed with Mann–Whitney U-test or Friedman M test. Numerical values were described using mean ± SEM and presented as bar graphs. Significance is denoted as *p<0.05, **p<0.01, or ***p<0.001 in the figures.

For all quantifications in immunostaining and EM, n=3–5 animals, independent cultures, or transfections were examined as indicated in the figure legends (3–6 images were analyzed and averaged per mouse or culture, for each staining). All statistical details for each graph can be found in the figure legends. qRT-PCR on cell cultures have been replicated three times in independent experiments.

**Repporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The transcriptome, DNA hydroxymethylation profiling, and Cut & Run-seq data generated in this study have been deposited in the Gene Expression Omnibus database under accession code GSE122838. Previously published and deposited data for hMe-Seal and TET1-mediated DNA hydroxymethylation regulate adult remyelination in mice. Nat. Commun. 12, 3359 (2021).

14. Sun, W., Zang, L., Shu, Q. & Li, X. From development to diseases: the role of 5-hydroxymethylcytosine in mammalian myelination. J. Neurosci. 34, 11929–11947 (2014).

15. Mogha, A., D’Rozario, M. & Monk, K. R. G. Protein-coupled receptors in myelinating glia. Trends Pharm. Sci. 37, 977–987 (2016).

16. Zhao, Y. et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947 (2014).

17. Ficz, G. et al. Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. Nature 473, 398–402 (2011).

18. Gu, T. P. et al. The role of TET1 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature 477, 606–610 (2011).

19. Li, X. et al. Ten-eleven translocation 2 interacts with forkhead box O3 and regulates adult neurogenesis. Nat. Commun. 8, 15903 (2017).

20. Chen, Y. et al. The oligodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination. Nat. Neurosci. 12, 1398–1406 (2009).

21. Mogha, A., D’Rozario, M. & Monk, K. R. G. Protein-coupled receptors in myelinating glia. Trends Pharm. Sci. 37, 977–987 (2016).

22. Chang, Y. et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947 (2014).

23. Moyon, S. et al. TET1-mediated DNA hydroxymethylation regulates adult remyelination in mice. Nat. Commun. 12, 3359 (2021).

24. Zhang, R. R. et al. TET1 regulates adult hippocampal neurogenesis and cognition. Cell Stem Cell 13, 237–245 (2014).

25. Xin, M. et al. Myelogenesis and axonal recognition by oligodendrocytes in brain are uncoupled in Olig1-null mice. J. Neurosci. 25, 1354–1365 (2005).

26. Devaux, J., Gola, M., Jacquet, G. & Crest, M. Effects of K+ channel blockers on developing rat myelinated CNS axons: identification of four types of K+ channels. J. Neurophysiol. 87, 1376–1385 (2002).

27. Devaux, J. & Gow, A. Tight junctions potentiate the insulative properties of small CNS myelinated axons. J. Neurosci. 183, 909–921 (2008).

28. Rustad, S. R., Papade, L. A. & Alisch, R. S. DNA methylation and hydroxymethylation and behavior. Curr. Top. Behav. Neurosci. 42, 51–82 (2019).

29. Madrid, A., Papade, L. A. & Alisch, R. S. New hope: the emerging role of 5-hydroxymethylcytosine in mental health and disease. Epigenomics 8, 981–991 (2016).

30. Antunes, C., Sousa, N., Pinto, L. & Marques, C. J. TET enzymes in neurobiology and brain function. Neurosci. Biobehav. Rev. 102, 337–344 (2019).

31. Li, C. et al. Assessment of a combination of serum proteins as potential biomarkers to clinically predict Schizophrenia. Int. J. Med. Sci. 15, 900–906 (2018).

32. Mená et al. Reduced prepulse inhibition as a biomarker of schizophrenia. Front. Behav. Neurosci. 10, 202 (2016).

33. Vorhees, C. V. & Williams, M. T. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. 4, 848–858 (2009).

34. Chrysanthou, S. et al. A critical role of TET1/2 proteins in cell-cycle progression of trophoblast stem cells. Stem Cell Reports 10, 1355–1368 (2018).

35. Zha, X. et al. Age-dependent fate and lineage restriction of single NG2 cells. Development 138, 745–753 (2011).

36. Hahn, M. A. et al. Dynamics of 5-hydroxymethylcytosine and chromatin marks in Mammalian neurogenesis. Cell Rep. 3, 291–300 (2013).

37. Hornig, J. et al. The transcription factors Sox10 and Myrf define an essential regulatory network module in differentiating oligodendrocytes. PLoS Genet. 9, e1003907 (2013).

38. Morita, J. et al. Structure and biological function of ENPP6, a choline-specific glycosylphosphatidylinositol-phosphodiesterase. Sci. Rep. 6, 20995 (2016).

39. Skene, P. J. & Henikoff, S. An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. Elife 6, e21856 (2017).

40. Larson, V. A., Zhang, Y. & Bergles, D. E. Electrophysiological properties of NG2(+)-cells: matching physiological studies with gene expression profiles. Brain Res. 1638, 138–160 (2016).

41. Cheli, V. T. et al. Conditional deletion of the L-type calcium channel Cav1.2 in oligodendrocyte progenitor cells affects postnatal myelination in mice. J. Neurosci. 36, 10853–10869 (2016).

42. Cheli, V. T., Santiago Gonzalez, D. A., Speuer, V. & Paez, P. M. Voltage-gated Ca2+ entry promotes oligodendrocyte progenitor cell maturation and myelination in vitro. Exp. Neurol. 265, 69–83 (2015).

43. Zeisel, A. et al. Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science 347, 1138–1142 (2015).

44. Marques, S. et al. Oligodendroglial heterogeneity in the mouse juvenile and adult central nervous system. Science 352, 1326–1329 (2016).

45. Kirischruch, S., Scherer, J., Kettenmann, H. & Verkhantsky, A. Activation of P2-purinoceptors triggered Ca2+ release from InsP3-sensitive internal stores in astrocytes. J. Physiol. 483, 41–57 (1995).

46. Stevens, B., Porta, S., Haak, L. L., Gallo, V. & Fields, R. D. Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36, 855–868 (2002).
Ding, Z. et al. Binding of inositol 1,4,5-trisphosphate (IP3) and adenophostin A to the N-terminal region of the IP3 receptor: thermodynamic analysis using fluorescence polarization with a novel IP3 receptor ligand. *Mol. Pharmacol.* 77, 995–1004 (2010).

Gopalakrishnan, S., Van Embrugh, B. O. & Robertson, K. D. DNA methylation in development and human disease. *Mutat. Res.* 647, 30–38 (2008).

Liu, H., Liu, X., Deng, Y. & Qing, H. DNA methylation, a hand behind neurodegenerative diseases. *Front. Aging Neurosci.* 5, 85 (2013).

Towers, A. J. et al. Epigenetic dysregulation of Oxt in Tett-deficient mice has implications for neuropsychiatric disorders. *JCI Insight* 3, e120592 (2018).

Zhang, W. et al. Isoform switch of TET1 regulates DNA demethylation and mouse development. *Cell* 66, 1062–1073 (2016).

Dugas, J. C. et al. Dicer1 and miR-219 are required for normal oligodendrocyte differentiation and myelination. *Neuron* 65, 597–611 (2010).

Bennin, D. A. et al. Cyclin G2 associates with protein phosphate 2 A catalytic and regulatory B subunits in active complexes and induces nuclear aberrations and a G1/S phase cell cycle arrest. *J. Biol. Chem.* 277, 27449–27467 (2002).

Boutros, R. et al. CDC25B overexpression stabilises centrin 2 and promotes the formation of excess centriolar foci. *PLoS ONE* 8, e67822 (2013).

Kalaszczyńska, I. et al. Cyclin A is redundant in fibroblasts but essential in hematopoietic and embryonic stem cells. *Cell* 138, 352–365 (2009).

Bennin, D. A. et al. Tett is crucial for olfactory nerve-regulated gene expression and memory extinction. *Cell Mol. Neurobiol.* 30, 987–990 (2010).

Huang, S. et al. Tet1 is required for Rb phosphorylation during G1/S phase transition. *Biochem. Biophys. Res. Commun.* 434, 241–244 (2013).

Harripaul, R. et al. Mapping autosomal recessive intellectual disability: combined microarray and exome sequencing identifies 26 novel candidate genes in 192 consanguineous families. *Mol. Psychiatry* 23, 973–984 (2018).

Rudenko, A. et al. Tom-1, a trigger of mitotic entry, is degraded during G1 via the APC. *Cell* 138, 1069–1081 (2009).

Moore, J. D., Kirk, J. A. & Hunt, T. Unmasking the S-phase-promoting potential of cyclin B1. *Science* 300, 987–990 (2003).

Huang, S. et al. Tett is required for Rb phosphorylation during G1/S phase transition. *Biochem. Biophys. Res. Commun.* 434, 241–244 (2013).

Kaliszewski, J. & Kopieciewicz, A. CUI/LASS confirms CATIE. *Arch. Gen. Psychiatry* 64, 978 (2007). (author reply 979–980).

Lesh, T. A., Niendam, T. A., Minzenberg, M. J. & Carter, C. S. Cognitive control deficits in schizophrenia: mechanisms and meaning. *Neuropsychopharmacol.* 36, 316–338 (2011).

Shi, D., Qin, A., Li, L., Tang, J. & Yang, W. C. New insights into 5hmC DNA methylation: generation, distribution and function. *Front. Genet.* 8, 106 (2017).

Stroud, H., Feng, S. H., Kinney, S. M., Pradhan, S. & Jacobsen, S. E. 5-Hydroxymethylcytosine is associated with enhancers and gene bodies in human embryonic stem cells. *Genome Biol.* 12, R34 (2011).

Tan, L. et al. Genome-wide comparison of DNA hydroxymethylation in mouse embryonic stem cells and neural progenitor cells by a new comparative hMeDIP-seq method. *Nucleic Acids Res.* 41, e84 (2013).

Song, C. X. et al. Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. *Nat. Biotechnol.* 29, 68–72 (2011).

Nestor, C. E. et al. Tissue type is a major modifier of the 5-hydroxymethylcytosine content of human brain. *Genome Res.* 22, 467–477 (2012).

Jin, S. G., Wu, X., Li, A. X. & Pfeifer, G. P. Genomic mapping of 5-hydroxymethylcytosine in the human brain. *Nucleic Acids Res.* 39, 5015–5024 (2011).

Xu, Y. et al. Genome-wide regulation of 5hmC, 5mC, and gene expression by Tet1 hydroxylase in mouse embryonic stem cells. *Mol. Cell* 42, 451–464 (2011).

Kang, K. E., Kim, S. J. & Kim, J. S. 5-hydroxymethylcytosine and its potential roles in development and cancer. *Epigenetics Chromatin* 6, 10 (2013).

Liu, H., Liu, X., Deng, Y. & Qing, H. DNA methylation, a hand behind neurodegenerative diseases. *Front. Aging Neurosci.* 5, 85 (2013).

Loeper, C. M., Lloyd, A. J., Leonard, K. & Wilkinson, M. J. Differential effect of three base modifications on DNA thermotolerance revealed by high resolution melting. *Anal. Chem.* 84, 7336–7342 (2012).

Valinluck, V. et al. Oxidative damage to methyl-CpG sequences inhibits the binding of the methyl-CpG binding domain (MBD) of methyl-CpG binding protein 2 (MeCP2). *Nucleic Acids Res.* 32, 4100–4108 (2004).

Jin, S. G., Kadam, S. & Pfeifer, G. P. Examination of the specificity of DNA methylation profiling techniques towards 5-methylcytosine and 5-hydroxymethylcytosine. *Nucleic Acids Res.* 38, e125 (2010).

Williams, K. et al. TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity. *Nature* 473, 343–348 (2011).

Wu, H. et al. Dual functions of Tet1 in transcriptional regulation in mouse embryonic stem cells. *Nature* 473, 389–393 (2011).

Yildirim, O. et al. Mbd3/NURD complex regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells. *Cell* 147, 1498–1510 (2011).

Neri, F. et al. Genome-wide analysis identifies a functional association of Tett1 and Polycomb repressive complex 2 in mouse embryonic stem cells. *Genome Biol.* 14, R91 (2013).

Khoeuire, R. et al. Lineage-specific functions of TET1 in the postimplantation mouse embryo. *Nat. Genet.* 49, 1061–1072 (2017).

Butt, A. M. Neuregulin-mediated calcium signalling in oligodendrocyte physiology and pathology. *Glia* 54, 666–675 (2006).

Fries, M. et al. Intracellular ion signaling influences myelin basic protein synthesis in oligodendrocyte precursor cells. *Cell Calcium* 60, 322–330 (2016).

Li, T. et al. Dynamic calcium release from endoplasmic reticulum mediated by ryanodine receptor 3 is crucial for oligodendrogial differentiation. *Front. Mol. Neurosci.* 16, 182 (2018).

Zhang, M., Liu, Y. & Wu, S. & Zhao, X. Ca(2+)-signaling in oligodendrocyte development. *Cell Mol. Neurobiol.* 39, 1071–1080 (2019).

Yu, Y. et al. Olig2 targets chromatin remodelers to enhancers to initiate oligodendrogial differentiation. *Cell* 152, 248–261 (2013).

Futagami, A. et al. Tett receptor 3 and 3 mediate excitone secretion underlying energy metabolism. *Science* 309, 2232–2234 (2005).

Emery, B. & Dugas, J. C. Purification of oligodendrocyte lineage cells from mouse cortices by immunopanning. *Cold Spring Harb. Protoc.* 2013, 854–868 (2013).

Ranscht, B., Clapshaw, P. A., Price, J., Noble, M. & Seifert, W. Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. *Proc. Natl. Acad. Sci. USA* 79, 2709–2713 (1982).

Sommer, I. & Schachner, M. Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. *Dev. Biol.* 83, 311–327 (1981).

Evans, R. D., Weston, D. A., McLaughlin, M. & Brown, A. M. A non-linear regression analysis method for quantitative resolution of the stimulus-evoked compound action potential from rodent optic nerve. *J. Neurosci. Methods* 188, 174–178 (2010).

Down, T. A. et al. A Bayesian deconvolution strategy for immunoprecipitation-based DNA methylation analysis. *Nat. Biotechnol.* 26, 779–785 (2008).

Li, L. C. & Dahiya, R. MethPrimer: designing primers for methylation PCR. *Bioinformatics* 18, 1427–1431 (2002).

Brahma, S. & Henikoff, S. RSC-associated subnucleosomes define MNase-sensitive promoters in yeast. *Mol. Cell* 73, 238–249 e233 (2019).

Rossi, A. M. & Taylor, C. W. Reliable measurement of free Ca(2+) concentrations in the ER lumen using Mag-Fluo-4. *Cell Calcium* 87, 102188 (2020).
