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## m<sup>6</sup>A mRNA methylation is essential for oligodendrocyte maturation and CNS myelination

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### SUMMARY

The molecular mechanisms that govern the maturation of oligodendrocyte lineage cells remain unclear. Emerging studies have shown that N<sup>6</sup>-methyladenosine (m<sup>6</sup>A), the most common internal RNA modification of mammalian mRNA, plays a critical role in various developmental processes. Here, we demonstrate that oligodendrocyte lineage progression is accompanied by dynamic changes in m<sup>6</sup>A modification on numerous transcripts. *In vivo* conditional inactivation of an

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Author contributions

H.X. led the project, wrote the manuscript and was involved in all aspects of the study. Y.D. contributed to RNA scope data and neurofascin immunohistochemistry. A.S. performed mRNA alternative splicing analysis. Y.-L.W. performed m<sup>6</sup>A-seq and contributed to bioinformatics analysis. R.B.K. and Q.L.F. contributed to data collection and analysis. B.E. contributed to project design and interpretation of the experimental data. J.S.J. contributed to the bioinformatics analysis of m<sup>6</sup>A-seq and RNA-seq data. Y.I.L. contributed to the critical discussion about alternative splicing analysis. X.X.Z. and C.H. contributed *Mettl14<sup>fl/fl</sup>* mice and critical discussion. G.-I.M. contributed to the m<sup>6</sup>A-seq experiments and project design. B.P. conceived and designed the project. All authors contributed to the preparation of the manuscript.

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Declaration of Interests

The authors declare no competing interests.

essential m<sup>6</sup>A writer component, METTL14, results in decreased oligodendrocyte numbers and CNS hypomyelination, although oligodendrocyte precursor cell (OPC) numbers are normal. *In vitro* *Mettl14* ablation disrupts post-mitotic oligodendrocyte maturation and has distinct effects on OPC and oligodendrocyte transcriptomes. Moreover, the loss of *Mettl14* in oligodendrocyte lineage cells causes aberrant splicing of myriad RNA transcripts, including that which encodes the essential paranodal component neurofascin 155 (NF155). Together, our findings indicate that dynamic RNA methylation plays an important regulatory role in oligodendrocyte development and CNS myelination.

### eTOC Blurp:

Xu et. al. show that oligodendrocyte development is associated with dynamic changes in posttranscriptional mRNA methylation. Moreover, they demonstrate that the m<sup>6</sup>A epigenetic RNA mark has considerable impact on the myelinating cell's transcriptome and is essential for normal CNS myelination.

### Keywords

*Mettl14*; m(6)A; mRNA methylation; RNA epigenetic regulation; oligodendrocyte precursor cells; oligodendrocytes; oligodendrocyte development; alternative splicing; NF155

## INTRODUCTION

Oligodendrocytes are glial cells in the CNS that are responsible for myelination of axons, which allows for rapid saltatory conduction (Nave and Werner, 2014; Simons and Nave, 2015). Oligodendrocytes develop from oligodendrocyte precursor cells (OPCs), which originate from discrete regions of the embryonic neural tube (Rowitch, 2004). To become mature myelinating oligodendrocytes, OPCs first exit the proliferation state and differentiate into pre-myelinating oligodendrocytes, resulting in the expression of major myelinating proteins such as myelin basic protein (MBP) and proteolipid protein (PLP). A series of morphological changes then allows these oligodendrocytes to extend a number of processes that wrap axons with the multilayered myelin sheath (Zuchero and Barres, 2015). Elucidating the key events involved in oligodendrocyte lineage progression is critical to understand the cellular and developmental biology of myelin production and regeneration.

Following a defined series of steps, oligodendrocyte lineage progression is tightly controlled in time and space (Liu et al., 2016). The exact mechanism by which oligodendrocyte lineage progression is regulated, however, has yet to be fully elucidated. A number of intrinsic and extrinsic factors have been found to be critical for regulating oligodendrocyte development. Growth factors, such as Sonic Hedgehog (SHH), bone morphogenetic proteins (BMPs), and platelet-derived growth factor (PDGF), have been shown to influence the maturation of oligodendrocyte lineage cells from early progenitors to mature, myelinating cells (Nishiyama et al., 2009). Transcription factors, such as Nkx-2.2, Olig1, Olig2, Sox10, Myrf and ZFP24, are required for maturation of oligodendrocytes (Elbaz and Popko, 2019; Mitew et al., 2014). In addition, epigenetic mechanisms including chromatin remodeling by DNA methylation, histone deacetylases, and gene silencing by non-coding RNAs have been shown

to play critical roles in oligodendrocyte differentiation and function during development and remyelination (Li and Richardson, 2009; Marin-Husstege et al., 2002; Moyon and Casaccia, 2017; Ye et al., 2009; Zhao et al., 2010b).

Although reversible chemical modification of DNA and histone proteins is known to influence gene expression and a multitude of biological processes, a similar role for the chemical modification of RNA has only recently been identified (Fu et al., 2014). The discovery of reversible *N*<sup>6</sup>-methyladenosine (m<sup>6</sup>A) mRNA methylation has revealed a new dimension of post-transcriptional regulation of gene expression (Yue et al., 2015). Emerging studies have demonstrated this m<sup>6</sup>A “mark” influences various aspects of mRNA metabolism, including stability, translation, localization, and splicing (Roundtree et al., 2017; Wang et al., 2014; Xiao et al., 2016; Zhao et al., 2014; Zhou et al., 2018). By controlling the turnover and/or translation of transcripts during cell-state transitions, m<sup>6</sup>A modification of mRNA plays key regulatory roles during embryonic and adult stem cell differentiation (Frye et al., 2018). Recent studies have highlighted the function of m<sup>6</sup>A in lineage fate decisions during cell development, such as embryonic stem cell pluripotency exit, T cell differentiation, hematopoietic fate transition, and gametogenesis (Batista et al., 2014; Geula et al., 2015; Ivanova et al., 2017; Li et al., 2017; Weng et al., 2018b; Xu et al., 2017; Zhang et al., 2017). Importantly, a recent study in neural stem cells revealed that conditional inactivation of the gene that encodes METTL14, a core component of the m<sup>6</sup>A methyltransferase complex (Liu et al., 2014), disrupts cortical neurogenesis (Yoon et al., 2017), thus revealing a critical role of the m<sup>6</sup>A mark in CNS neuronal development.

In this study, we sought to elucidate the role that m<sup>6</sup>A mRNA methylation plays in oligodendrocyte lineage progression by conditionally inactivating the *Mettl14* gene specifically in these cells using a *Mettl14* conditional (floxed) mouse line in combination with oligodendrocyte Cre driver lines. *In vivo*, we found myelin abnormalities and altered oligodendrocyte numbers in the *Mettl14* mutants. Despite these findings, OPC numbers were not affected. *In vitro*, OPCs lacking *Mettl14* did not properly differentiate into mature oligodendrocytes, suggesting that m<sup>6</sup>A plays a critical role in oligodendrocyte differentiation. RNA-seq and m<sup>6</sup>A -seq revealed that OPC and oligodendrocyte transcripts encoding transcription factors, DNA epigenetic regulators and signaling pathways that are critical for oligodendrocyte lineage progression were m<sup>6</sup>A marked, and differentially affected by the *Mettl14* deletion. We also found pervasive, aberrant mRNA splicing in the *Mettl14*-deleted OPCs and oligodendrocytes. Importantly, we discovered that the critical paranode component, NF155, is differentially spliced and significantly disrupted during myelination in the *Mettl14* ablated mutants.

## RESULTS

### Oligodendrocyte lineage progression is accompanied by changes in m<sup>6</sup>A modification on numerous transcripts

To characterize changes of the m<sup>6</sup>A mark and its role in gene expression during oligodendrocyte lineage progression, we performed m<sup>6</sup>A-seq and RNA-seq on both purified OPCs and mature, cultured oligodendrocytes. Using an immunopanning approach (Emery and Dugas, 2013), we purified OPCs from neonatal mouse pups. These cells were

maintained under proliferating conditions with the addition of the OPC mitogen PDGF-AA. We obtained mature oligodendrocytes by promoting OPC differentiation via removal of PDGF-AA and addition of the T3 hormone to the culture media (Fig.1 A). SMART2 single cell RNA-seq was used for m<sup>6</sup>A mRNA profiling (Picelli et al., 2014; Weng et al., 2018b), which detected 3,554 m<sup>6</sup>A marked transcripts in OPCs and 2606 m<sup>6</sup>A marked transcripts in oligodendrocytes. Gene ontology analyses indicated that these m<sup>6</sup>A marked transcripts have important functions for cell development in both OPCs (Fig.1 B) and oligodendrocytes (Fig.1 C). The m<sup>6</sup>A-seq data also revealed transcripts present in both OPCs and oligodendrocytes that were differentially marked by m<sup>6</sup>A, demonstrating the dynamic nature of this mRNA modification. We found 2,806 transcripts with the m<sup>6</sup>A mark in OPCs that were present but not marked in oligodendrocytes (Fig.1 D), and 1,626 transcripts that possessed the m<sup>6</sup>A mark in oligodendrocytes but not in OPCs (Fig.1 E). Only 23 of the shared transcripts (Fig.1 F), showed the m<sup>6</sup>A mark in both OPCs and oligodendrocytes. The dynamic nature of the m<sup>6</sup>A mark in oligodendrocyte lineage cells suggests that it may play an important role in regulating oligodendrocyte differentiation and CNS myelination.

In order to investigate the role of the m<sup>6</sup>A mark in CNS myelinating cells, we generated mouse lines in which the gene encoding an essential m<sup>6</sup>A writer component, METTL14, was conditionally inactivated at distinct oligodendrocyte developmental stages. We crossed mice carrying a conditional allele of *Mettl14* (*Mettl14<sup>fl/fl</sup>*) (Koranda et al., 2018) with mice expressing the Cre recombinase under the transcriptional control of oligodendrocyte transcription factor 2 (*Olig2*), which is expressed throughout the oligodendrocyte lineage (Schüller et al., 2008) (Fig.1 G). The *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mouse line allows us to study the role of m<sup>6</sup>A in developing oligodendrocyte lineage cells (Bergles and Richardson, 2015). We also generated *Mettl14<sup>fl/fl</sup>;CNP-Cre* mice (Fig.1 G), in which *Mettl14* is conditionally eliminated by Cre under the transcriptional control of the myelin protein CNP primarily in post-mitotic oligodendrocytes (Lappe-Siefke et al., 2003), allowing us to study the role of m<sup>6</sup>A in maturing oligodendrocytes.

### ***Mettl14* ablation leads to reduction of mature oligodendrocytes but not OPCs**

We first examined whether the *Mettl14* gene was efficiently inactivated via the *Cre-loxP* genetic strategy by examining METTL14 expression in the CNS using immunohistochemistry. We observed a reduction of METTL14 expression in both *Mettl14<sup>fl/fl</sup>;Olig2-Cre* and *Mettl14<sup>fl/fl</sup>;CNP-Cre* mutants, compared to their *Mettl14<sup>fl/fl</sup>* littermate controls at postnatal day P12 (Fig.S5 A–F), and across different CNS white matter regions at postnatal day 18 (P18) (data not shown), a time point at which myelin is still undergoing development.

To gain insight into how *Mettl14* inactivation affects oligodendrocyte lineage cell development, we used immunohistochemistry to detect the oligodendrocyte lineage cell marker *Olig2* in P18 mice (Fig.2 A, B). We found a decreased percentage of *Olig2*/METTL14 double positive cells (Fig.2 C) accompanied by a reduction of *Olig2*+ cell numbers in the corpus callosum in *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.2 D) mutants. Similarly, P18 *Mettl14<sup>fl/fl</sup>;CNP-Cre* (Fig.S1 A,B) mutants also showed decreased percentage of *Olig2*/METTL14 double positive cells (Fig.S1 C) and decreased *Olig2*+ cell numbers (Fig.S1 D)

and in the corpus callosum. The reduction of oligodendrocyte lineage cells in both *Mettl14<sup>fl/fl</sup>;Olig2-Cre* and *Mettl14<sup>fl/fl</sup>;CNP-Cre* mutant corpus callosum indicates that *Mettl14* is important in oligodendrocyte lineage development.

During early development, Olig2+ pMN progenitors produce both motor neurons and oligodendrocytes (Ravanelli and Appel, 2015). Therefore, we explored whether the *Mettl14* deletion affects motor neuron development. We used choline acetyltransferase (ChAT) immunohistochemistry to identify motor neurons in the lumbar spinal cord at P12, and we found no difference in motor neuron numbers in both *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.S7 A,B,E) and *Mettl14<sup>fl/fl</sup>;CNP-Cre* (Fig.S7 C,D,F) mutant mice compared to controls. In addition, motor neurons express METTL14 in the mutants of both strains (Fig.S7 B,D), suggesting that Cre recombination has limited effect in motor neurons.

To identify the oligodendrocyte lineage stage(s) that was affected by *Mettl14* inactivation, we examined the number of OPCs and post-mitotic oligodendrocytes in P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* and *Mettl14<sup>fl/fl</sup>;CNP-Cre* corpus callosum. CC1 antibody immunostaining specific for mature oligodendrocytes, showed that the mutants had significantly fewer mature oligodendrocytes as compared to controls in the corpus callosum of both *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.2 E,G) and *Mettl14<sup>fl/fl</sup>;CNP-Cre* mice (Fig.S1 E,G). Interestingly, the number of cells positive for PDGF-receptor-alpha (PDGFR- $\alpha$ ), a marker for OPCs, showed no difference in the mutants in both *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.2 F,H) and *Mettl14<sup>fl/fl</sup>;CNP-Cre* mice (Fig.S1 F,H), indicating that the loss of *Mettl14* does not disrupt OPC formation.

To investigate *Mettl14*'s role in OPC proliferation, we co-stained CNS tissue sections with Ki-67, a marker of cellular proliferation, and PDGFR- $\alpha$  to detect OPCs. We found no significant difference in the numbers of proliferating OPCs between mutants and controls in both P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.2 F,I) and P18 *Mettl14<sup>fl/fl</sup>;CNP-Cre* mice (Fig.S1 F,I). We further examined the effects of *Mettl14* ablation on OPCs and proliferating OPCs at an earlier time point P12, when a larger percentage of OPCs are normally proliferative compared to P18. The quantitative analysis of P12 sections revealed results similar to those seen at P18, with no significant difference of OPC and proliferating OPC numbers between mutants and controls in both *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig. S6 C, D, K, M) and *Mettl14<sup>fl/fl</sup>;CNP-Cre* mice (Fig.S6 G, H, L, N). In addition, similarly to P18 mutants, both P12 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.S6 A, B, I) and *Mettl14<sup>fl/fl</sup>;CNP-Cre* (Fig.S6 E, F, J) mice showed reduced numbers of CC1<sup>+</sup> mature oligodendrocytes in the corpus callosum.

Together, our findings demonstrate that *Mettl14* ablation leads to the reduction of oligodendrocyte lineage cells, in which mature oligodendrocyte numbers, as opposed to OPC numbers, are predominantly affected.

### ***Mettl14* ablation in oligodendrocyte lineage cells leads to hypomyelination**

To characterize the pathological consequences caused by *Mettl14* ablation during myelin development, we examined two different CNS regions, the corpus callosum and optic nerve, in both P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* and *Mettl14<sup>fl/fl</sup>;CNP-Cre* mice using electron microscopy (EM). We found hypomyelination in both corpus callosum and optic nerve in

*Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.3 A) and *Mettl14<sup>fl/fl</sup>;CNP-Cre* P18 mutants (Fig.S2 A), indicating that pathological changes start when myelin is developing. Quantitative analyses showed significantly increased g-ratios, the ratio between the inner axonal and outer total diameter of the myelin sheath, of myelinated axons (Fig.3 B, Fig.S2 B) and a significantly increased percentage of unmyelinated axons in both P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (corpus callosum: Fig.3 D, optic nerve: Fig.3 E) and *Mettl14<sup>fl/fl</sup>;CNP-Cre* (corpus callosum: Fig.S2 D, optic nerve: Fig.S2 E) mutants. These results revealed decreased thickness of the myelin sheath and fewer myelinated axons upon *Mettl14* ablation. CNS hypomyelination was further supported by Western blot analysis, which revealed significant reductions of the myelin proteins MBP and myelin-associated glycoprotein (MAG) expression levels in the *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.3 H, I), and *Mettl14<sup>fl/fl</sup>;CNP-Cre* (Fig.S2 H, I) P18 mutant brains.

To explore whether the pathological change caused by *Mettl14* ablation persists in adulthood, we analyzed adult CNS regions using EM. The *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mutant animals are clinically normal until about 6 months (P180) of age, when they begin to display occasional hind limb flexion, slight ataxia, and mild tremor. The *Mettl14<sup>fl/fl</sup>;CNP-Cre* mutant animals start to display tremor and hind limb clenching at around 4 months of age, with symptoms becoming progressively worse (e.g. ataxic phenotype). The earlier appearance of clinical symptoms in the *Mettl14<sup>fl/fl</sup>;CNP-Cre* mutant animals is likely the result of Cre expression in Schwann cells in the peripheral nervous system of the CNP-Cre mice (Brockschneider et al., 2004). Similar to P18 animals, both *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (P180) and *Mettl14<sup>fl/fl</sup>;CNP-Cre* (P150) adult mutants displayed myelin abnormalities (Fig.3 A, Fig.S2 A), with increased g-ratios (Fig.3 C) and non-myelinated axon percentages in both corpus callosum (Fig.3 F, Fig.S2 F) and optic nerve (Fig.3 G, Fig.S2 G). Western blot analysis also revealed decreased myelin protein levels in both P180 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (Fig.3 H, J) and P150 *Mettl14<sup>fl/fl</sup>;CNP-Cre* (Fig.S2 H, J) mutants. Together, our results demonstrate that *Mettl14* is important in CNS myelination.

### ***Mettl14* ablation prevents oligodendrocyte differentiation**

To further determine the role of *Mettl14* in different oligodendrocyte lineage stages, we turned to *in vitro* cultures. We found that the *Mettl14* mutant OPCs were isolated as efficiently as control OPCs, and that the mutant cells displayed similar mitotic activity and bipolar morphology in the presence of PDGF-AA (Fig.4 A, B). Interestingly, the *Mettl14* deleted cells did not develop into MBP<sup>+</sup> mature oligodendrocytes after 5 days of differentiation following removal of PDGF-AA from the culture media (Fig.4 D). The mutant cells did not send out the extensive membrane structure seen with control oligodendrocytes (Fig.4 C). Indeed, only rare cells (less than 7%, data not shown) that escaped Cre recombination and were METTL14<sup>+</sup> in the mutant cell cultures developed into MBP<sup>+</sup> cells (Fig.4 E). Our Western blot data confirmed the almost complete elimination of METTL14 in the mutant oligodendrocytes, as well as the dramatic reduction of major myelin protein (MAG and MBP) expression (Fig.4 F, G). These results correlate with our *in vivo* findings, strongly suggesting that *Mettl14* is critical for oligodendrocyte maturation.

We next examined cell morphology and maturation from early to late post-mitotic differentiation stages of oligodendrocytes *in vitro* to further explore the effects of *Mettl14* on oligodendrocyte lineage cell maturation. We used O1, an antibody specific for the myelin galactolipid galactocerebroside, to detect oligodendrocyte morphology (Sommer and Schachner, 1981) from day 1 to day 5 after cells were plated in differentiation media. Interestingly, *Mettl14* deleted cells (Fig.5 C) showed O1 immunoreactivity but did not display the morphological changes of control cells, which progressively extended their membrane structures to form complex membrane sheets (Fig.5 A). The *Mettl14* ablated cells did not express appreciable levels of MBP (Fig.5 D), whereas control cells matured gradually from day 1 to day 5 with increasing MBP expression (Fig.5 B). These results together with our *in vivo* data strongly suggest that *Mettl14* plays an important role in post mitotic oligodendrocyte maturation.

### ***Mettl14* ablation differentially alters the OPC and oligodendrocyte transcriptomes**

To elucidate the effects of *Mettl14* on oligodendrocyte lineage cell gene expression during development at the transcriptome level, we performed RNA-seq with both purified OPCs and cultured mature oligodendrocytes from *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant mice. The quantification of differential expression in the *Mettl14* mutant transcriptome revealed distinct differences in the OPCs and myelinating oligodendrocytes. For quantification analysis we defined significance of differentially expressed transcripts using the following three criteria: 1. a 99.9% confidence interval, adjusted for false discovery, as a q-value using methods previously described (Benjamini and Yekutieli, 2005); 2. fold changes (FC) that exceeded 2.0 fold ( $\log_2 |\text{FC}| > 1$ ) in expression and 3. an expression level that exceeded two counted transcripts per million ( $\log_2 |\text{CPM}| > 1$ ). Of the 11,809 transcripts present in the OPC transcriptome, 586 were expressed at significantly higher levels and 177 were expressed at significantly lower levels in the mutant cells (Fig.6 A). Among the 12,542 transcripts present in mature oligodendrocytes, 1,388 transcripts were significantly upregulated and 1,247 were downregulated in the mutant cells (Fig.6 B). Interestingly, among the significantly downregulated oligodendrocyte transcripts, many are normally highly expressed in myelinating oligodendrocytes, such as *Mbp*, *Mog*, *Mag*, *Plp1*, and *Cnp* (Fig.6 B). The downregulation of these myelin transcripts correlates with the downregulation of myelin protein expression observed in the *Mettl14* ablated mutant animals.

The m<sup>6</sup>A mark has been shown to play a role in reducing the stability of m<sup>6</sup>A-containing transcripts (Wang et al., 2014, 2018; Weng et al., 2018a; Yoon et al., 2017; Zhang et al., 2017). Accordingly, many transcripts in the *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mutants had higher relative expression levels. We compared the m<sup>6</sup>A-seq data and RNA-seq data, and found that among the 3,554 m<sup>6</sup>A marked OPC transcripts, 46 transcripts had significantly downregulated expression levels, and 108 transcripts had significantly upregulated expression levels (Fig.6 C). Among the 2,606 m<sup>6</sup>A marked oligodendrocyte transcripts, 221 had significantly downregulated expression levels, and 217 transcripts had significantly upregulated expression levels (Fig.6 D). Gene ontology analysis of significantly altered m<sup>6</sup>A marked transcripts revealed many important functions such as glia cell development in OPCs (Fig.6 E) and myelination in oligodendrocytes (Fig.6 F). These results indicate that the m<sup>6</sup>A mark differentially regulates the OPC and oligodendrocyte transcriptomes.

## ***Mett14* regulates OPC and oligodendrocyte transcripts that are critical for oligodendrocyte lineage progression**

In order to find clues of how the m<sup>6</sup>A mark regulates oligodendrocyte lineage development, we examined the expression of factors that play a critical cell-autonomous role in oligodendrocyte lineage progression by cross-comparing our m<sup>6</sup>A-seq and RNA-seq datasets. We identified a number of transcripts that encode transcriptional factors implicated in oligodendrocyte lineage progression as being dynamically marked by m<sup>6</sup>A at different oligodendrocyte lineage stages. For example, *Hey1*, *Klf19*, *Sox2*, *Sox5*, *Srebf1*, *Tcf19*, *Zeb2* are marked by m<sup>6</sup>A in OPCs, but not in oligodendrocytes; *Hes1*, *Nkx6.2*, *Olig2* and *Yy1* are marked by m<sup>6</sup>A only in oligodendrocytes, but not in OPCs (Table S1). The dynamic m<sup>6</sup>A marked status of these transcription factor transcripts suggests a time-specific, post-transcriptional regulatory role of m<sup>6</sup>A during oligodendrocyte lineage progression and may contribute to the differentially altered transcriptome in OPCs and oligodendrocytes following *Mett14* deletion.

Studies have shown that DNA epigenetic regulation mechanisms, such as chromatin remodeling and histone modifications, are important for oligodendrocyte lineage progression (Koreman et al., 2018). Our RNA-seq and m<sup>6</sup>A-seq analyses revealed that many transcripts encoding histone modification regulators bear an m<sup>6</sup>A mark, and were significantly differentially expressed in the mutant transcriptome (Table S2). We detected transcripts of histone “writers” such as histone acetyltransferases (HATs) *Hat1*, histone methyltransferases (HMTs) *Smyd2*, *Prdm2*, *Setdb1*, *Suv39h1*, *Ash11*, *Dot11*; histone “erasers” such as histone deacetylases (HDACs) *Hdac3*, *Hdac7*, *Hdac8*, *Hdac9*, and lysine demethylases (KDMs) *Kdm2b*, *Kdm5c*, *Kdm3b*, *Kdm4a*, *Kdm4c*, *Kdm6a* that had the m<sup>6</sup>A mark and had significantly altered mRNA levels in the *Mett14* ablated mutants: these transcripts encode proteins with important regulator functions in oligodendrocyte development (Hernandez and Casaccia, 2015). Thus, our findings suggest a possible link between m<sup>6</sup>A RNA modification and histone modifications in the regulation of oligodendrocyte lineage development.

We also examined key signaling pathways that are critically involved in oligodendrocyte lineage progression. We found transcripts that were significantly altered by *Mett14* ablation in the bone morphogenetic proteins (BMPs), ERK/MAPK, fibroblast growth factor families (FGFs), Notch/Delta, Sonic hedgehog (Shh) and Wnt signaling pathways in OPCs (Table S3); and P13K/AKT/mTOR, BMPs, ERK/MAPK, insulin-like growth factor-1 (IGF-1), Notch/Delta, Shh and Wnt signaling pathways in oligodendrocytes (Table S4). The alternation of critical gene expression levels in these signaling pathways provided us with important clues regarding disruption of oligodendrocyte maturation displayed by the *Mett14<sup>fl/fl</sup>;Olig2-Cre* and *Mett14<sup>fl/fl</sup>;CNP-Cre* animals. Indeed, many transcripts that encode critical components of these signaling pathways have the m<sup>6</sup>A mark (Fig.6 G) (Table S3, S4), suggesting that m<sup>6</sup>A may regulate these signaling pathways to promote oligodendrocyte lineage progression.

### ***Mett14*'s possible mechanism of actions in oligodendrocyte lineage cells**

We also wished to explore potential mechanism(s) of action of the m<sup>6</sup>A mark in regulating oligodendrocyte lineage cell development and function in addition to the disruption of the



cells' transcriptomes discussed above. Previous studies have shown m<sup>6</sup>A's role in increasing the translational efficiency of the marked transcripts in various systems (Coots et al., 2017; Shi et al., 2017; Wang et al., 2015; Zhou et al., 2018). In order to investigate this potential mechanism, we compared transcriptional and translational levels of a subset of the m<sup>6</sup>A marked transcripts that encode proteins critical for oligodendrocyte development (Bujalka et al., 2013; Zhou and Anderson, 2002). We found significantly decreased levels of these proteins both *in vivo* (Fig.3 H,I,J) and *in vitro* (Fig.4 F,G; Fig.S8 A,B), however, the observed reductions correlated with the levels of mRNA reduction found in the oligodendrocyte transcriptome (Table S5), suggesting that translational regulation may not be a key feature of m<sup>6</sup>A gene regulation in oligodendrocyte lineage cells. Nevertheless, a more comprehensive proteomics assessment will be required to determine the global impact of the m<sup>6</sup>A mark on translational efficiency in oligodendrocytes.

MBP, a predominant and critical protein of myelin, is translated locally in the myelin compartment (Colman et al., 1982), which requires the active transport of its mRNA into oligodendrocyte processes (Carson et al., 1997). This transport requires the RNA binding protein heterogeneous nuclear ribonucleoprotein (hnRNP) A2 (Hoek et al., 1998; Müller et al., 2013). Importantly, it was recently discovered that hnRNPA2B1, an isoform of hnRNP A2 that is expressed in oligodendrocyte lineage cells (Han et al., 2010), is an m<sup>6</sup>A reader (Alarcón et al., 2015), suggesting that the m<sup>6</sup>A mark might have a role in regulating *Mbp* mRNA transport in oligodendrocytes. In order to investigate this possibility, we used the RNAscope approach to determine the distribution of *Mbp* and *Myrf* mRNA in oligodendrocytes of the corpus callosum. In controls, *Myrf* mRNA is localized in the oligodendrocyte cell bodies; whereas, *Mbp* mRNA distributes to the complex, web-like oligodendrocyte processes (Fig.S3 A). In P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mutant oligodendrocytes, *Myrf* and *Mbp* mRNA levels are clearly reduced (Fig.S3 B), as expected, but the distribution of these transcripts does not appear altered, suggesting that the absence of the m<sup>6</sup>A mark has not disrupted the transport of the *Mbp* mRNA into the myelin compartment. In addition to MBP mRNA, a number of key oligodendrocyte transcripts has been shown to be localized in the myelin sheath (Thakurela et al., 2016). A more extensive analysis of the myelin mRNA content of the *Mettl14* mutants will help determine the role of the m<sup>6</sup>A mark in the establishment of myelin transcriptome.

Functional variant isoforms of myelin proteins are generated by alternative splicing to ensure precise oligodendrocyte lineage progression (Montague et al., 2006; Zhang et al., 2014; Zhao et al., 2010a). Previous studies have shown that m<sup>6</sup>A plays a critical role in regulating mRNA splicing in various cellular systems (Hausmann et al., 2016; Xiao et al., 2016; Zhao et al., 2014; Zhou et al., 2019). In order to investigate the potential role of the m<sup>6</sup>A mark in regulating differential splicing during oligodendrocyte development, we used LeafCutter (Li et al., 2018) to identify altered splicing events in OPC and oligodendrocyte transcriptomes. LeafCutter identifies alternatively excised intron clusters and compares differentially excised intron levels between controls and mutants. Differential splicing is measured by changes in the percent spliced in (change, or delta, dPSI) (Li et al., 2018). Global comparison of alternative splicing events revealed numerous statistically significant changes in *Mettl14* ablated mutants versus controls (Stouffer's Z-score = 40.86 in OPCs and 105.76 in oligodendrocytes). In addition, we found that 1,372 splicing events in 364 genes in

OPCs, and 1,930 splicing events in 485 genes in oligodendrocytes were differentially spliced upon *Mett14* deletion ( $q < 0.01$ ). A number of significant differentially alternative spliced transcripts were previously shown to encode proteins with important functions in the myelinating process, such as protein tyrosine phosphate receptor type Z1 (*Ptprz*) in OPCs (Harroch et al., 2002) and neurofascin (*Nfasc*) in oligodendrocytes (Table.S6,S7). Interestingly, the neurofascin protein (NF), which is essential in the establishment and maintenance of node of Ranvier domains (Howell et al., 2006; Pillai et al., 2009; Sherman et al., 2005; Thaxton et al., 2010; Zonta et al., 2008), has the most significantly altered isoforms and bears the highest differential dPSI level in the oligodendrocyte transcriptome (Fig.7 A, Table.S7).

To further investigate the role of m<sup>6</sup>A mRNA methylation in regulating the distribution of NF isoforms during development, we used RT-PCR to confirm the differential distribution of distinct splicing products (Fig.7 B) in purified oligodendrocyte mRNA from mutants and controls. This analysis confirmed the presence of the predicted altered spliced *Nfasc* mRNA product in the mutant oligodendrocytes. Since NF155 is the glial NF isoform that is required for the assembly of paranodal domains (Sherman et al., 2005), we examined NF155 expression levels in different developmental stages *in vitro* and *in vivo*. *Mett14<sup>fl/fl</sup>;Olig2-Cre* mutant oligodendrocytes that had been cultured in differentiation media for 5 days had a significant reduction of NF155 expression compared to controls (Fig.7 C,F). *In vivo*, 1-month old animals (P30) showed significantly decreased NF155 levels in the *Mett14<sup>fl/fl</sup>;Olig2-Cre* mutants (Fig.7 D,G). To further analyze nodal and paranodal domains with immunohistochemistry we used antibodies to the voltage gated sodium channel (NaCh) and Caspr to identify the nodal and paranodal domains, respectively (Fig.8 A,B). We found a significant reduction in the number of nodes at P30 in the mutants compared to controls (Fig.8 E). We also used pan-NF antibody to characterize NF localization and morphology in both nodal and paranodal domains (Fig.8 A,B). We found no difference in node and paranodal size in the mutants as compared to controls (Fig.8 F).

We further investigated whether NF155 expression abnormalities persist to adulthood (P180) in *Mett14<sup>fl/fl</sup>;Olig2-Cre* mutants. Western blot results revealed significantly reduced expression levels of NF155 in P180 mutants (Fig.7 E,H). Next, we measured nodal and paranodal domains via immunohistochemistry in these animals (Fig.8 C,D). We found significant reduction of node numbers in the mutants (Fig.8 G). In addition, both nodal and paranodal domains showed significantly increased sizes in the mutants (Fig.8 H), suggesting widespread pathological changes at the node of Ranvier in adult *Mett14<sup>fl/fl</sup>;Olig2-Cre* mutants.

## DISCUSSION

RNA modifications have recently emerged as critical post-transcriptional regulatory mechanism to modulate gene expression (Frye et al., 2018). Among all post-transcriptional mRNA modifications, m<sup>6</sup>A is the most abundant internal alteration found in eukaryotic mRNA (Yue et al., 2015). In contrast to DNA and protein methylation, m<sup>6</sup>A methylation has the potential to have a very rapid influence on transcriptome changes during cell state transitions (Frye et al., 2018; Zhao and He, 2017).

In this report we show that oligodendrocyte lineage progression is accompanied by changes in m<sup>6</sup>A modification on numerous transcripts. We also show that *Mettl14*, which encodes an essential m<sup>6</sup>A writer component, is critical in regulating oligodendrocyte development and CNS myelination. We demonstrate altered oligodendrocyte numbers and hypomyelination in both oligodendrocyte lineage cell specific *Mettl14* ablated mice. Nevertheless, OPC numbers were not altered by *Mettl14* ablation. We also show that *Mettl14* ablated OPCs lacked the ability to differentiate into mature MBP-positive myelin-forming oligodendrocytes *in vitro*. These results indicate that the m<sup>6</sup>A RNA modification is essential for post-mitotic oligodendrocyte differentiation.

Interestingly, our data revealed a more severe developmental phenotype *in vitro* than *in vivo*, suggesting communication with other CNS cell types may mitigate the effects of the *Mettl14* deletion *in vivo*. In addition, it is curious that when we cultured *Mettl14<sup>fl/fl</sup>;CNP-Cre* OPCs (data not shown), in which the *Mettl14* gene is inactivated later in the oligodendrocyte lineage, the mutant cells had the capacity to differentiate into MBP-positive, mature oligodendrocytes as efficiently as control cells, suggesting that the severe block in maturation that occurs in the *Mettl14<sup>fl/fl</sup>;Olig2-Cre* OPCs *in vitro* is the result of an m<sup>6</sup>A deficiency early in the oligodendrocyte lineage. This is supported by the failure of enforced METTL14 expression in the *Mettl14<sup>fl/fl</sup>;Olig2-Cre* OPCs to fully rescue the severe *in vitro* differentiation phenotype (Fig. S4). Our future efforts will be devoted to elucidating the effects of the m<sup>6</sup>A mark at distinct stages of the oligodendrocyte lineage.

Oligodendrocyte differentiation involves many steps that must be regulated in time and space (Zuchero and Barres, 2013). Our RNA-seq and m<sup>6</sup>A-seq data revealed changes of the m<sup>6</sup>A marked status on numerous transcripts that encode critical transcription factors in OPCs and oligodendrocytes, suggesting that m<sup>6</sup>A mRNA modification contributes to transcriptional changes during oligodendrocyte development. Indeed, emerging studies have shown that the turnover and/or translation of transcripts during cell-state transitions regulated by the m<sup>6</sup>A mark represent an important developmental mechanism (Frye et al., 2018).

The functional role of various histone modifiers in oligodendrocyte differentiation is stage-dependent, yet the underlying regulatory role of these factors is unknown (Coprav et al., 2009; Hernandez and Casaccia, 2015). Our study revealed that transcripts that encode a number of histone modifiers are dynamically marked by m<sup>6</sup>A in OPCs and oligodendrocytes (Table S2), suggesting that m<sup>6</sup>A RNA modifications may play a role in regulating the expression of epigenetic modifiers at distinct oligodendrocyte lineage stages. Consistent with this possibility, a recent study revealed cross-talk between m<sup>6</sup>A RNA modification and histone modification (Wang et al., 2018).

In addition, we also found numerous oligodendrocyte lineage signaling pathway transcripts that are dynamically marked by m<sup>6</sup>A and expressed at significantly altered levels in the absence of METTL14. The alternation of m<sup>6</sup>A marked transcripts is accompanied by significant alterations of other important pathway transcripts that do not bear an m<sup>6</sup>A mark, suggesting that m<sup>6</sup>A RNA modification may have a primary or secondary effect on gene expression. Together, our RNA-seq and m<sup>6</sup>A-seq results indicate that m<sup>6</sup>A RNA

modifications modulate the expression of multiple transcriptional regulators, DNA epigenetic modifiers and signaling pathways to facilitate oligodendrocyte lineage progression.

In addition to the effect on mRNA levels discussed above, we examined several additional potential functions of the m<sup>6</sup>A mark on gene expression in oligodendrocyte lineage cells. We explored whether the m<sup>6</sup>A mark affects translational efficiency of important myelin genes and their regulators, since it had previously been shown that translational efficiency of marked transcripts is increased (Shi et al., 2017; Wang et al., 2015). Surprisingly, we did not detect dramatic alternations of protein levels when compared with mRNA levels of m<sup>6</sup>A marked transcripts. The m<sup>6</sup>A mark has also been shown to play a role in intracellular mRNA transport (Roundtree et al., 2017). Therefore, we examined the transport of *Mbp* mRNA into oligodendrocyte processes, which has been shown to be critical for CNS myelination. Nevertheless, using RNAscope we were unable to detect a significant decrease in the efficiency with which the *Mbp* mRNA is transported into the myelin domain in the *Mettl14* mutant animals. Although these initial efforts did not reveal a role for the m<sup>6</sup>A mark in mRNA translation or sub-cellular transport, more thorough analyses may uncover alterations in these processes in the *Mettl14* mutant oligodendrocytes.

We did, however, detect widespread changes in the *Mettl14* mutant oligodendrocyte lineage cells that were related to aberrant RNA splicing. In fact, 283 out of 364 (~78%) aberrantly spliced OPC transcripts, and 311 out of 485 (~64%) aberrantly spliced oligodendrocyte transcripts are marked by m<sup>6</sup>A. Many of these altered transcripts have been described as crucial for oligodendrocyte lineage development and function, such as *Ptprz1*, *Gsn* (Brown and Verden, 2017) and *Map2* (Müller et al., 1997). Importantly, we discovered the transcript encoding NF, a critical cell adhesion protein involved in node of Ranvier establishment and maintenance, was differentially spliced under the regulation of m<sup>6</sup>A. The mouse *Nfasc* gene contains 39 exons, and the inclusion or exclusion of different *Nfasc* exons results in transcripts that encode functionally distinct isoforms (Suzuki et al., 2017). NF186 is expressed by neurons and is critical for node assembly, and NF155, which is expressed by the myelinating cells, is critical to the stability of the paranodal domain (Howell et al., 2006; Kawamura et al., 2013; Kira et al., 2018; Pomicter et al., 2010). The disruption of NF isoform distribution results in pathological changes in myelinated axons (Howell et al., 2006; Pillai et al., 2009; Thaxton et al., 2010). We identified aberrantly spliced *Nfasc* RNA isoforms in the *Mettl14* deleted oligodendrocytes, and provided *in vivo* evidence of altered NF155 protein expression that correlated with morphological abnormalities of the paranodal domain. In particular, the CNS nodes of Ranvier of adult *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mutants displayed widespread abnormalities, strikingly reminiscent of NF155-deficient mice (Pillai et al., 2009). These results indicate m<sup>6</sup>A RNA methylation regulates *Nfasc155* splicing, and plays a role in establishing and maintaining normal function of critical axonal-oligodendrocyte interactions. Interestingly, changes in NF155 expression have recently been suggested to be central to adult myelin remodeling associated with altered impulse transmission (Fields and Dutta, 2019). This raises an intriguing possibility that the m<sup>6</sup>A epigenetic mark plays a critical role in activity-dependent myelin remodeling.

A recent study reported that the proline rich coiled-coil 2 A (Prrc2a) protein is an m<sup>6</sup>A reader that participates in oligodendrocyte specification and myelination by regulating the stability of its critical downstream target *Olig2* (Wu et al., 2019). Nevertheless, the clinical and pathological phenotypes of the oligodendrocyte-specific mouse mutants of *Prrc2a* are considerably more severe than that observed for the *Mett14* writer mutants described here, and the alteration in *Olig2* expression is also much more significant in the *Prrc2a* mutants. This raises the possibility that Prrc2a participates in other functions in addition to its putative role as an m<sup>6</sup>A reader in oligodendrocyte lineage cells.

In conclusion, our study demonstrates that m<sup>6</sup>A RNA modification is essential for normal oligodendrocyte maturation and CNS myelination. We show that the m<sup>6</sup>A mark plays an important role in regulating various aspects of gene expression in oligodendrocyte lineage cells, with the most profound effects on mRNA levels and splicing. Rapid alterations to the m<sup>6</sup>A landscape have the potential to quickly modify a cell's phenotypic properties (Geula et al., 2015; Licht and Jantsch, 2016; Yoon et al., 2017). Therefore, in addition to its critical role during development, the m<sup>6</sup>A mark may participate in oligodendrocyte plasticity in adults. Future characterization of m<sup>6</sup>A RNA epigenetic regulation should provide important insight to our growing understanding of the myelination process and demyelinating diseases.

## STAR Methods

### LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Brian Popko (brian.popko@northwestern.edu)

**Materials Availability Statement**—This study did not generate new unique reagents.

### ANIMALS

All animals were housed under pathogen-free conditions, and all animal procedures and animal care were conducted in accordance with guidelines approved by the University of Chicago's Institutional Animal Care and Use Community (IACUC). All mice were on the C57BL/6 background, and both female and male mice were used.

*Mett14*<sup>fl/fl</sup> mice (Koranda et al., 2018; Weng et al., 2018b; Yoon et al., 2017) were crossed with *Olig2*-Cre mice (Schüller et al., 2008), and *CNP*-Cre mice (Lappe-Siefke et al., 2003). *Mett14*<sup>fl/fl</sup>;*Olig2*-Cre and littermate control *Mett14*<sup>fl/fl</sup> mice as well as *Mett14*<sup>fl/fl</sup>;*CNP*-Cre and littermate control *Mett14*<sup>fl/fl</sup> mice were used for experiments.

### METHOD DETAILS

**OPC isolation and culture**—OPCs were isolated and purified (95% purity) from postnatal day 6 (P6) mouse brains using an immunopanning protocol (Emery and Dugas, 2013). In brief, mice pups (both female and male) were genotyped and marked at P4–P5. At P6, pups were deeply anaesthetized on ice and cortices were collected, diced and digested with papain at 37° C. Cells were then triturated into a single cell suspension, then sequentially immunopanned in Ran-2, GalC, and O4 antibodies from hybridoma

supernatant. The remaining O4<sup>+</sup>GalC<sup>-</sup> cells (OPCs) were then trypsinized and plated in poly-d-Lysine (PDL)-coated plates with proliferation media. Once OPCs numbers reach sufficient amount, they were split and plated in differentiation media.

**OPC electroporation**—Amaxa cell nucleofector II device and an electroporation kit for primary mammalian glial cells (Lonza, Cat# VPI-1006) were used as per the manufacturer's instructions for OPC electroporation. For each biological replicate, 5 million OPCs were collected and transfected with both pmaxGFP Vector (Lonza, Cat# VPI-1006) and *Mettl14* plasmid (Origene, Cat# MR207291) or pmaxGFP Vector alone. Transfected OPCs were then plated in PDL coated plates with differentiation media. Transfection efficiency was about 40%, measured by GFP positive cell number versus total cell number after 96 hours of electroporation.

**Immunohistochemistry and cell counts**—Mice were deeply anaesthetized with 2.5% avertin (Cat#T48402, Sigma Aldrich) in dH<sub>2</sub>O. Upon the loss of nociceptive reflexes, mice were transcardially perfused with 0.9% saline followed by ice-cold 4% paraformaldehyde (PFA). Brains were collected and post-fixed overnight in 4% PFA at 4°C, followed by incubation in 30% sucrose until saturation. Tissues were then embedded in optimal cutting temperature compound (OCT) and sectioned at 10µm. Prior to Rabbit-anti-METTL14 (1:300, Sigma, Cat# HPA038002), Goat-anti-PDGFR-α (1:100, R and D systems, Cat# AF1062), Rabbit-anti-Ki67 (1:100, Abcam, Cat# ab15580), Chicken-anti-Neurofascin (1:50, R and D systems, Cat# AF3235), Mouse-anti-sodium channel (1:100, Sigma, Cat# S8809) and Rabbit-anti-Caspr (1:300, Abcam, Cat# ab34151) immunostaining, tissue sections were processed with an antibody retrieval protocol in which sections were treated with 10 mM trisodium citrate buffer (pH 6.0) at 90 °C for 30 minutes. After cooling at room temperature (RT) for 30 minutes, sections were then incubated in 10 mM glycine (in TBS with 0.25% Triton X-100) for 1hour at RT. Slices were then blocked with TBS containing 5% normal donkey serum, 1% BSA and 0.25% Triton X-100 (blocking buffer) for 2 hours at RT, followed by incubation in primary antibody(s) diluted in blocking buffer for 48 hours at 4°C. Immunohistochemistry with Rabbit-anti-MBP (1:500, Abcam, Cat# ab40390), Mouse-anti-CC1 (1:50, Milipore, Cat# OP80), Mouse-anti-Olig2 (1:100, Milipore, Cat# MABN50) and Rabbit-anti-ChAT (1:300, Milipore, Cat# AB144P) antibodies, were followed by immunostaining protocol without antigen retrieval.

Stained tissue sections were imaged with a Mariana Yokogawa-type spinning disk confocal microscope or Leica TCS SP5 two-photon confocal microscope. All experimental and littermate control tissues were imaged with the same parameters, followed with the same adjustments in Image J (NIH). Cells counts data was converted to cells/100µm<sup>2</sup>.

**Immunocytochemistry**—Cells cultured on cover slips were rinsed with PBS and fixed with ice cold 4% PFA for 10 minutes at RT, washed with PBS, then air dried and stored at -80°C until immunostaining. The primary antibodies used were Rabbit-anti-MBP (1:500, Abcam, Cat# ab40390), Goat-anti-PDGFR-α (1:100, R and D systems, Cat# AF1062) and Mouse-anti-O1 (1:100, R and D systems, Cat# MAB1327). Immunocytochemistry was conducted using the normal immunostaining protocol as described above, without the antigen retrieval process.

**RNA scope**—Mouse *Myrf* mRNA probe (ACDbio, Cat# 524061), mouse *Mbp* mRNA probe (ACDbio, Cat# 451491) and RNAscope Multiplex Fluorescent Reagent Kit V2 assay (ACDbio, Cat# 323110) were purchased from ACDbio company. Fluorophores were purchased from Akoya biosciences (Opal 520: Cat# FP1487001KT, Opal 620: Cat# FP1495001KT). Mice were processed for RNAscope as follows. Deeply anesthetized animals were transcardially perfused with 0.9% saline followed by ice-cold 4% PFA as described above. Brains were immediately dissected out and transferred to 10% NB formalin solution (Sigma, Cat# HT5011) at RT for exactly 24 hours. Brains were then transferred to freshly made 70% ethanol for 24 hours at RT and processed for paraffin embedding. Sections were cut at 5  $\mu$ m. RNAscope assay was performed as per manufacturer's specifications.

**Electron Microscopy (EM) and analysis**—Mice were deeply anesthetized with 2.5% avertin, followed by perfusion with 0.9% saline and 0.1M sodium cacodylate buffer containing 4% PFA and 2.5% glutaraldehyde (EM buffer). Corpus callosum and optic nerve were then post-fixed overnight at 4°C. Tissues were dissected and washed with 0.1M sodium cacodylate buffer for 3 times, followed by post fixation with 1% osmium tetroxide (diluted with 0.1M sodium cacodylate) for 2 hours and another 3 times of wash with 0.1M sodium cacodylate buffer. These tissue samples then went through dehydration steps with 30%, 50%, 70%, 90%, 95%, 100% ethanol and propylene oxide (PO), followed by permeation with 1:1 PO/Epon 812 and 1:2 PO/Epon 812 for 2 hours each, and in Epon 812 overnight at RT. The next morning, samples were permeated with Epon 812 for another 4 hours at RT, and then embedded with labeled paper strips in fresh Epon 812. These samples were then cured for 48 hours in a 60°C oven. After EM processing steps, samples were sectioned (1  $\mu$ m) and stained with toluidine blue, before sectioned into ultrathin slices (60–90nm) and stained with uranyl acetate-lead citrate. FEI Tecnai F30 scanning transmission electron microscope (FEI company) was used to take EM images. Image J was used to analyze the EM images for g-ratio and axon counting.

**Total protein isolation**—Cells in culture were rinsed with sterile PBS 2 times, then lysed with ice-cold RIPA buffer containing protease inhibitors (Thermo Fisher Scientific, Cat# 78430) and phosphatase inhibitors (Sigma, Cat# P2850 and P5726)(lysis buffer), and then scraped and collected in microcentrifuge tubes for 10 min incubation on ice. Cell lysates were then centrifuged at 13,000 g for 15 min at 4°C, and supernatant was collected and stored in –80°C until measurement. To collect brain tissues, mice were deeply anesthetized with 2.5% avertin and perfused with ice-cold sterile PBS, followed by brain isolation into microcentrifuge tubes and immediately frozen in liquid nitrogen. Brain samples were then stored in –80°C until homogenization. Brain tissue protein lysates were prepared as follows: homogenized in lysis buffer, incubated on ice for 15 min and centrifuged at 13,000g for 15 min at 4°C, then collect supernatants. Protein concentration was determined by using a BCA Protein Assay Kit (Thermo Fisher Scientific, Cat# 23255) as per the manufacturer's instructions.

**Western blot**—Protein lysates were boiled for 5 min in Laemmli sample buffer (Bio-Rad, Cat# 161–0737) with  $\beta$ -mercaptoethanol (Sigma, Cat# M6250), separated by SDS-PAGE,

transferred to nitrocellulose membrane and immunoblotted. The primary antibodies used were Rabbit-anti-METTL14 (1:1000, Sigma, Cat# HPA038002); Rabbit-anti-MBP (1:1000, Abcam, Cat# ab40390), Rabbit-anti-MAG (1:1000, Thermo Fisher Scientific, Cat# AB\_2533179), Mouse-anti-Olig2 (1:1000, Millipore, Cat# MABN50), Mouse-anti-MYRF (1:5000, gift from Dr. Ben Emery), GAPDH(1:2000, Cell signaling, Cat# 2118S). Western blot bands were analyzed in Image Lab software (Bio-Rad laboratories).

**RNA isolation**—Cell and tissue RNA samples were prepared and isolated following the manufacture's protocol (Bio-Rad, Cat# 732–6820). RNA quality was confirmed by 2100 Bioanalyzer using a model 6000 Nano kit (Agilent technologies, Cat# 5067–1511). Samples with RNA integrity number >8 were used.

**RNA-seq and analysis**—Bulk RNA-seq was performed on RNA isolated from cultured OPCs and oligodendrocytes as previously described (Aaker et al., 2016). Libraries were prepared and sequenced using the Illumina HiSeq 4000 at the University of Chicago Genomics Core facility. Reads were mapped using both STAR v2.6.1a and Kallisto v.0.44.0 using bowtie 2 aligner (Bray et al., 2016; Dobin et al., 2013). Mapped reads were further analyzed with the Bioconductor suite v3.7 by the University of Illinois at Chicago Bioinformatics Core facility (Huber et al., 2015). Q-values were determined as false discovery rate adjusted p-values using the method previously described (Benjamini and Yekutieli, 2005). Results were compared with the m<sup>6</sup>A-SMART-Seq analysis and visualized in R v.3.5.1 using the plot.ly, ggplot2, and venn.diagram packages. Values for expression, fold change and statistical significance were adapted for visualization using a log<sub>2</sub> transformation.

**m<sup>6</sup>A-SMART-seq and analysis**—mRNA from total RNA of OPCs and oligodendrocytes was purified with Dynabeads Oligo (dT)<sub>25</sub> (Thermo Fisher Scientific, Cat# 61006). The purified mRNA was then processed for m<sup>6</sup>A-SMART-seq and analyzed as previously described (Weng et al., 2018b). Z scores were calculated for each m<sup>6</sup>A mark and filtered with a threshold value of 0.

**Differential alternative splicing analysis**—Differential splicing analysis was performed between OPCs versus OPCs lacking *Mettl14* and oligodendrocytes versus oligodendrocytes lacking *Mettl14*. In brief, exon-exon junctions from mapped RNA-seq reads, which are representative of introns that are removed from pre-mRNA, were extracted. Next, alternatively excised introns, which are comprised of two more overlapping introns (e.g. introns that share a splice site), were clustered together. Finally, differential intron excision events across conditions were tested using LeafCutter (Li et al., 2018).

**Statistical analysis**—All immunohistochemistry and electron microscopy data obtained from experimental and control mice were compared with a two-tailed unpaired Student's t-test. Data were presented as mean + SEM. A p value of less-than 0.05 was considered significant. Analysis was done using GraphPad Prism version 6.00 for Windows (GraphPad Software) and Microsoft Office Excel 2010.



**Data Availability**—The sequencing data have been deposited to the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database under accession number: GSE124244.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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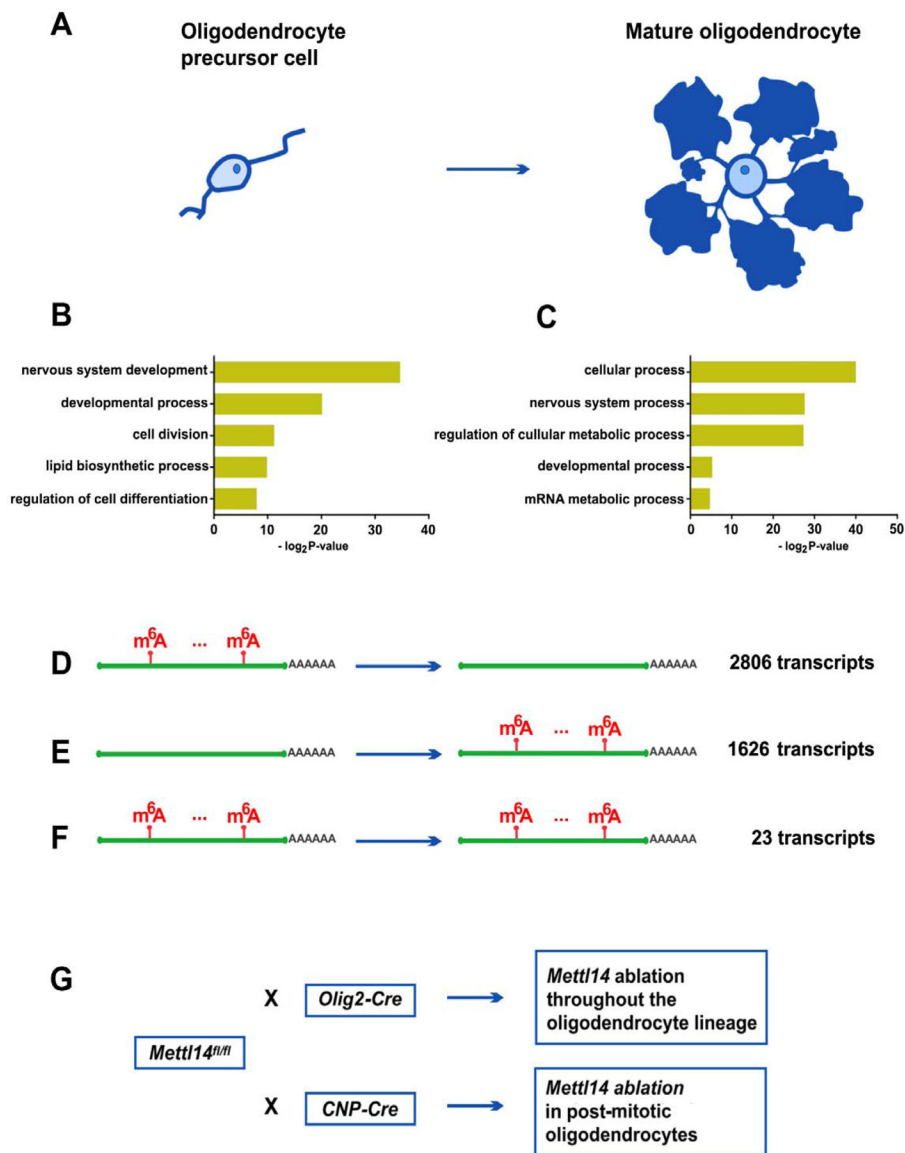
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**Highlights:**

- Oligodendrocyte maturation is accompanied by modifications in m<sup>6</sup>A mRNA methylation
- m<sup>6</sup>A mRNA methylation is required for oligodendrocyte maturation and CNS myelination
- m<sup>6</sup>A mRNA methylation regulates the transcriptomes of oligodendrocyte lineage cells
- Proper *neurofascin* mRNA splicing in oligodendrocytes requires m<sup>6</sup>A methylation



**Figure 1. Oligodendrocyte lineage progression is accompanied by changes in m<sup>6</sup>A modification on numerous transcripts.**

(A) Schematic drawing of an OPC and mature oligodendrocyte.

(B–C) The gene ontology categories of the m<sup>6</sup>A marked transcripts that belong to OPCs (B) and oligodendrocytes (C) ( $\log_2 |\text{CPM}| > 1$ , Z-score > 0).

(D) Of the 11,502 transcripts that are expressed both in OPCs and oligodendrocytes, 2806 transcripts bear the m<sup>6</sup>A mark in OPCs, but not in oligodendrocytes. ( $\log_2 |\text{CPM}| > 1$ , Z score > 0).

(E) Of the 11,502 transcripts that are expressed both in OPCs and oligodendrocytes, 1626 transcripts bear the m<sup>6</sup>A mark in oligodendrocytes, but not in OPCs.

(F) Of the 11,502 transcripts that are expressed both in OPCs and oligodendrocytes, 23 transcripts bear the m<sup>6</sup>A mark in both OPCs and oligodendrocytes.

(G) Mouse lines generated for this study. *Mett14<sup>fl/fl</sup>* mouse line was crossed with *Olig2-Cre* and *CNP-Cre* mouse lines, to conditional eliminate *Mett14* in oligodendrocyte lineage cells and post-mitotic cells, respectively.

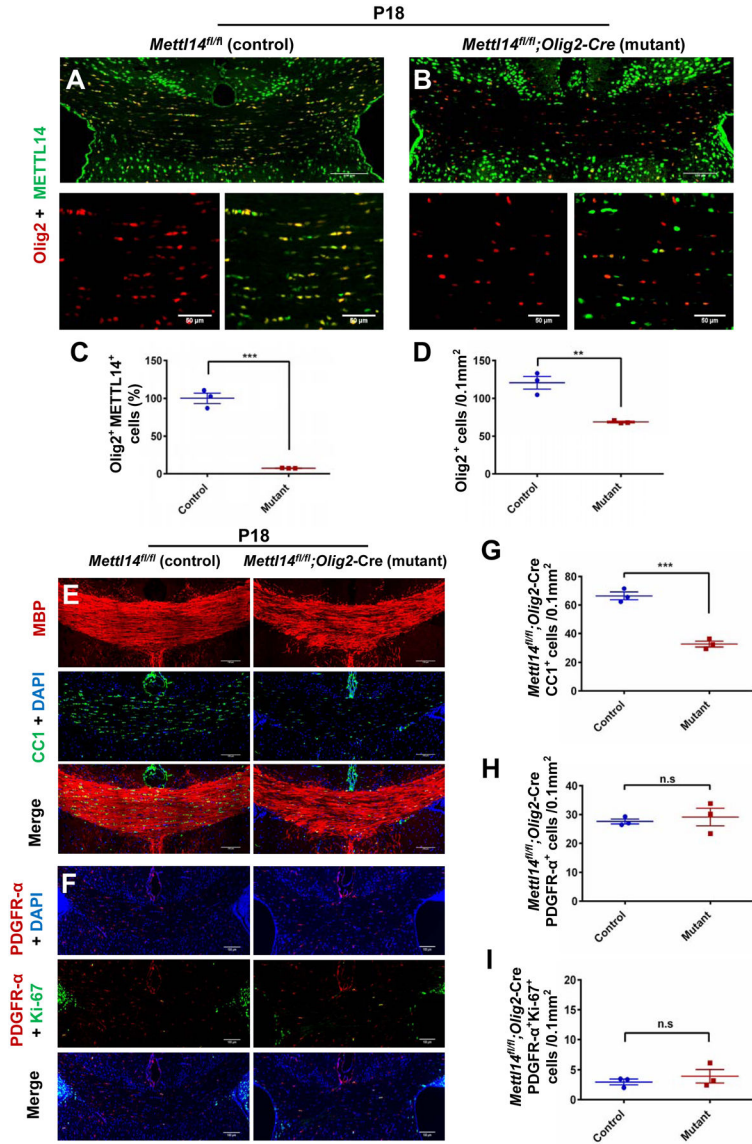
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**Figure 2. Oligodendrocyte lineage cell-specific ablation of *Mettl14* results in loss of oligodendrocytes.**

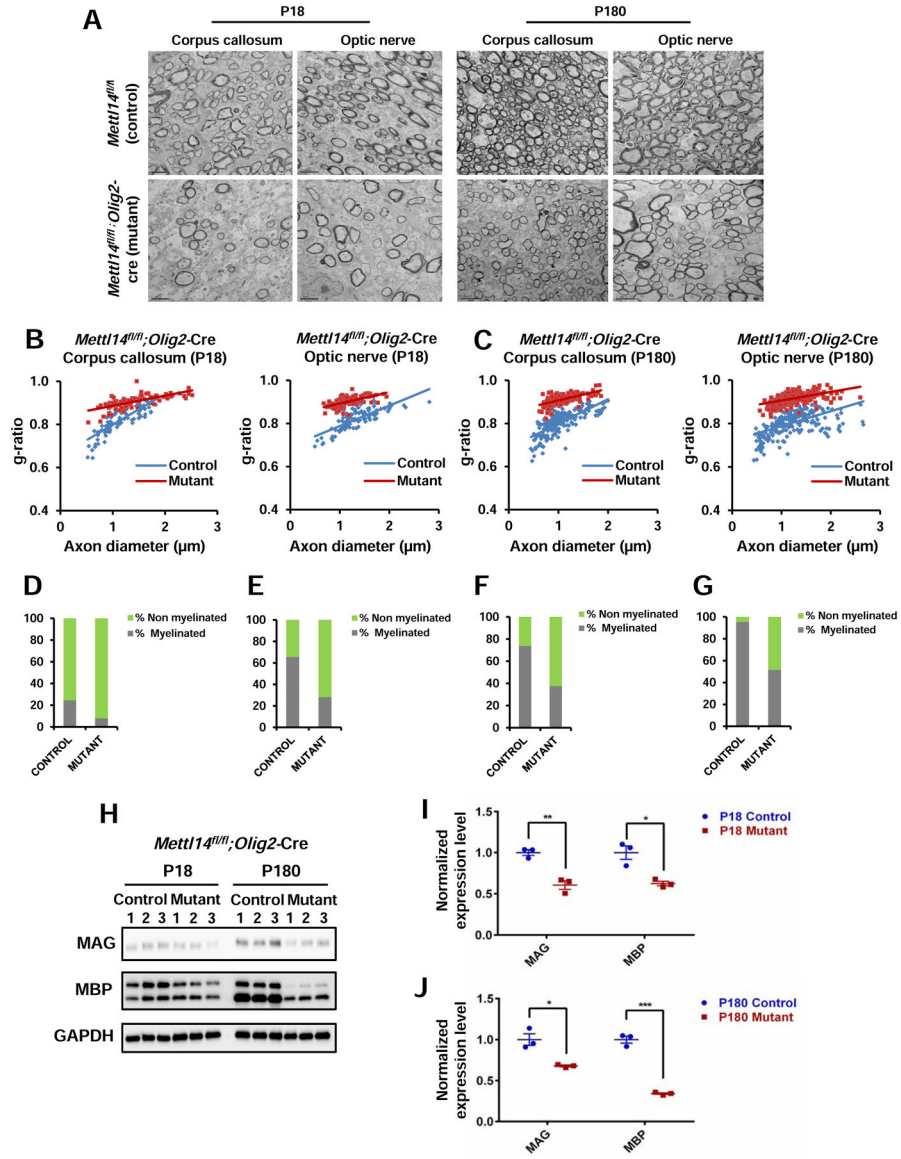
(A–B) Representative METTL14 (green) and Olig2 (red) immunostaining in the corpus callosum of P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control (A) and mutant (B) mice (Scale bar=100μm, 50μm).

(C) Quantification analysis showing a significantly reduced percentage of Olig2<sup>+</sup>/METTL14<sup>+</sup> double positive cells in the mutants. Values represent mean ± SEM (n=3; \*\*\*p<0.001; unpaired Student’s t-test).

(D) Quantification analysis showing a statistically significant reduction of total oligodendrocyte lineage cells (Olig2<sup>+</sup> cells). Values represent mean ± SEM (n=3; \*\*p<0.01; unpaired Student’s t-test).

(E) Representative CC1 (green) and MBP (red) immunostaining in the corpus callosum of P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant mice. Mutant corpus callosum showed visible reduction of oligodendrocytes (CC1<sup>+</sup> cells) and patchy myelin (MBP) (Scale bar=100μm).

- (F)** Representative PDGFR- $\alpha$  (red) and Ki-67 (green) immunostaining in the corpus callosum of P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant mice (Scale bar=100 $\mu$ m).
- (G)** Quantification showing a significant reduction of CC1<sup>+</sup> cells (OLs) in P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mutant corpus callosum. Values represent mean  $\pm$  SEM (n=3; \*\*\*p<0.001; unpaired Student's t-test).
- (H)** Quantification showing no significant difference between control and mutant numbers of PDGFR- $\alpha$ <sup>+</sup> cells (OPCs) in P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mice. Values represent mean  $\pm$  SEM (n=3; p>0.05; unpaired Student's t-test).
- (I)** Quantification showing no significant difference between control and mutant numbers of PDGFR- $\alpha$ <sup>+</sup> and Ki67<sup>+</sup> double positive cells in P18 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mice. Values represent mean  $\pm$  SEM (n=3; p>0.05; unpaired Student's t-test).
- See also Figure S1, Figure S5–S7.



**Figure 3. *Mettl14* ablation leads to hypomyelination.**

(A) Representative EM images of corpus callosum and optic nerve in P18 and P180 *Mettl14<sup>fl/fl</sup>; Olig2-Cre* control and mutant animals. Mutant corpus callosum and optic nerve had thinner myelin and fewer myelinated axons in both ages (Scale bar=2 $\mu\text{m}$ ).

(B) g-ratio analyses showing significantly higher g-ratios in both P18 *Mettl14<sup>fl/fl</sup>; Olig2-Cre* mutant corpus callosum (Mutant g ratio=0.91, control g ratio=0.80) and optic nerve (mutant g ratio= 0.90, control g ratio= 0.82), (n=3; \*\*\*p<0.001; unpaired Student's t test).

(C) g-ratio analyses showing significantly higher g-ratios in both P180 *Mettl14<sup>fl/fl</sup>; Olig2-Cre* mutant corpus callosum (Mutant g ratio=0.91, control g ratio=0.80) and optic nerve (mutant g ratio= 0.91, control g ratio= 0.83), (n=3; \*\*\*p<0.001; unpaired Student's t test).

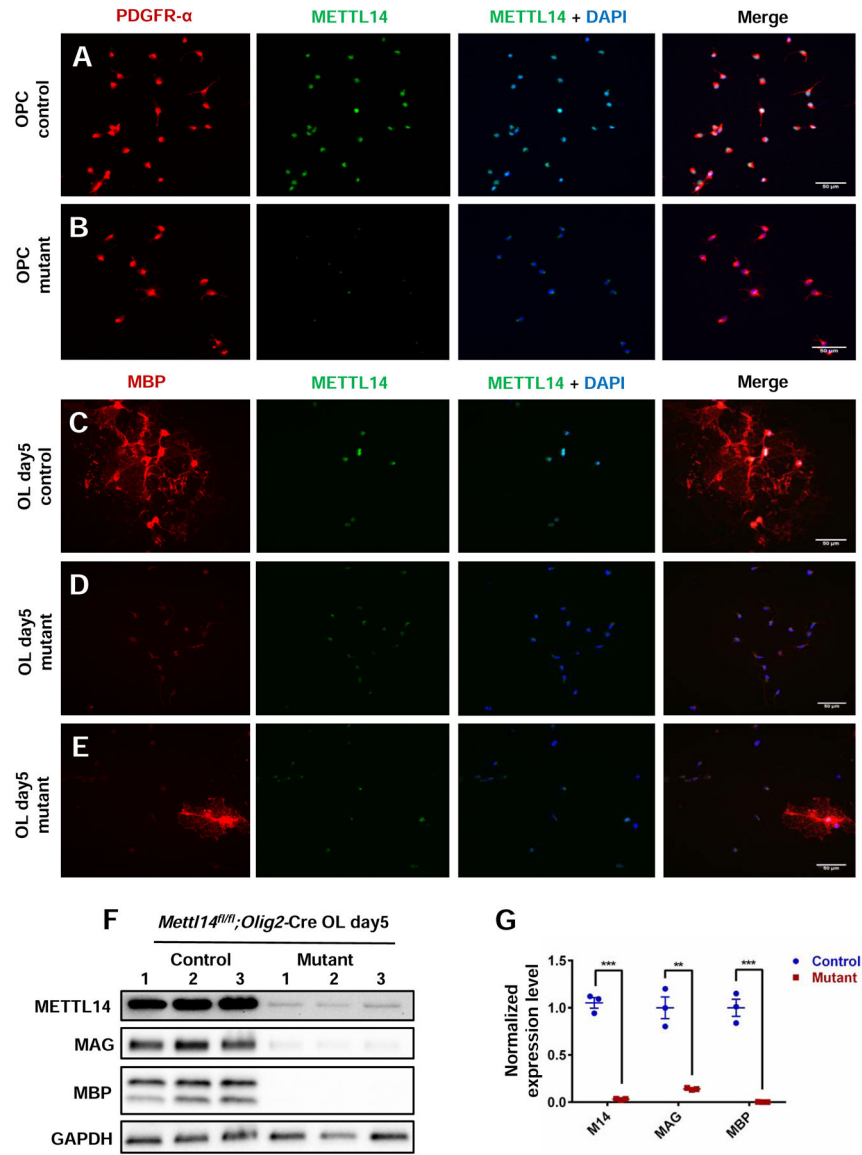
(D–G) Percentage of myelinated axons in corpus callosum and optic nerve (D. P18 *Mettl14<sup>fl/fl</sup>; Olig2-Cre* corpus callosum, E. P18 *Mettl14<sup>fl/fl</sup>; Olig2-Cre* optic nerve, F. P180

*Mett114<sup>fl/fl</sup>;Olig2-Cre* corpus callosum, G. P180 *Mett114<sup>fl/fl</sup>;Olig2-Cre* optic nerve). (n=3, \*\*\*p<0.001; unpaired Student's t test).

**(H)** Western blot showing myelin protein expression (MAG, MBP) levels in both P18 and P180 *Mett114<sup>fl/fl</sup>;Olig2-Cre* control and mutant animals (n=3).

**(I–J)** Quantification of immunoblots. MAG and MBP expression levels were normalized to GAPDH expression levels. Both MAG and MBP were significantly reduced in P18 (I) and P180 (J) *Mett114<sup>fl/fl</sup>;Olig2-Cre* mutants. Values represent mean ± SEM (n=3; \*p<0.05; \*\*p<0.01; \*\*\*p<0.001; unpaired Student's t test).

See also Figure S2.



**Figure 4. *Mettl14* ablated OPCs fail to develop into mature oligodendrocytes *in vitro*.** (A–B) PDGFR- $\alpha$  and METTL14 immunostaining of *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant OPCs in culture. METTL14 was eliminated from the mutant OPCs, which showed no morphological changes compared to control OPCs (Scale bar=50 $\mu$ m). (C–D) MBP and METTL14 immunostaining of *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant oligodendrocytes that had been cultured in differentiation media for 5 days (oligodendrocyte day5). Mutant cells fail to develop into MBP-positive cells (Scale bar=50 $\mu$ m). (E) Only rare cells (white arrow pointed) that had escaped Cre-mediated recombination and thus expressed METTL14 in the mutant day5 OL group successfully differentiated into MBP expressing oligodendrocytes (Scale bar=50 $\mu$ m). (F) Western blot showing METTL14, MAG, MBP and GAPDH expression levels in control and mutant OL day5 groups.

**(G)** Quantification of immunoblots showing significant reduction of METTL14, MAG and MBP expression in mutant OL day5 group. METTL14, MAG and MBP expression levels were normalized to GAPDH expression level. Values represent mean  $\pm$  SEM (n=3; \*\*p<0.01; \*\*\*p<0.001, unpaired Student's t test).

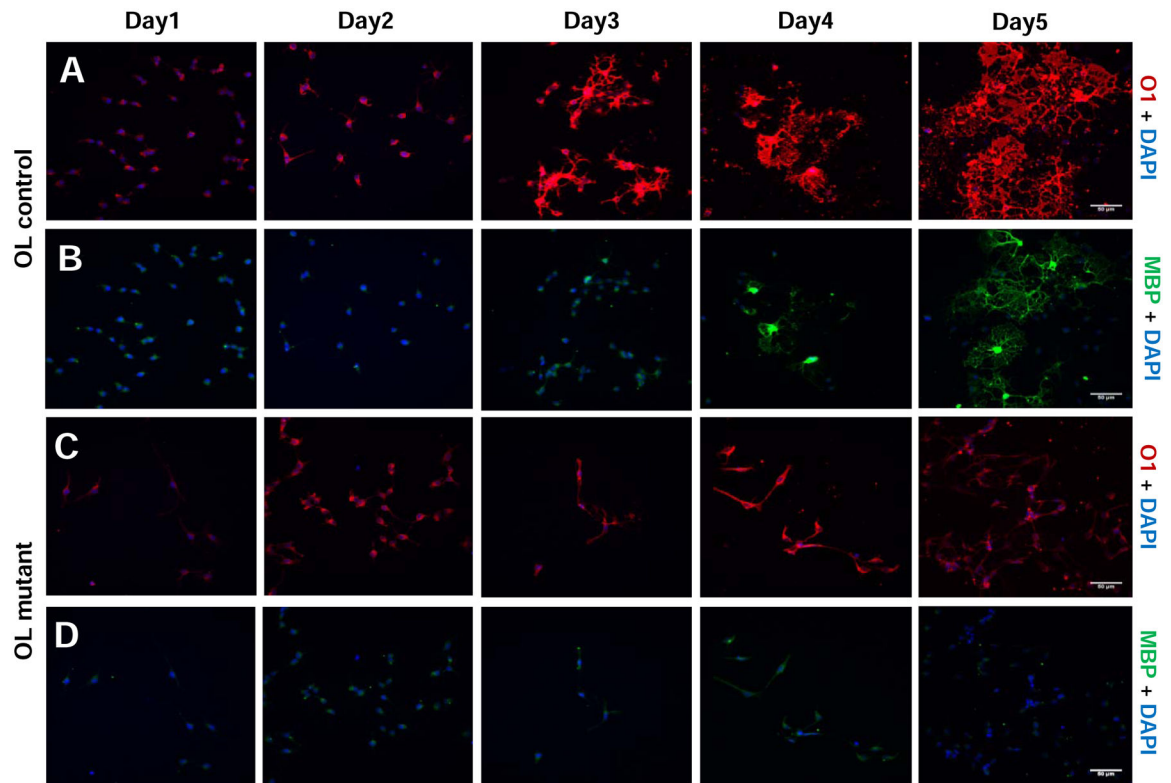
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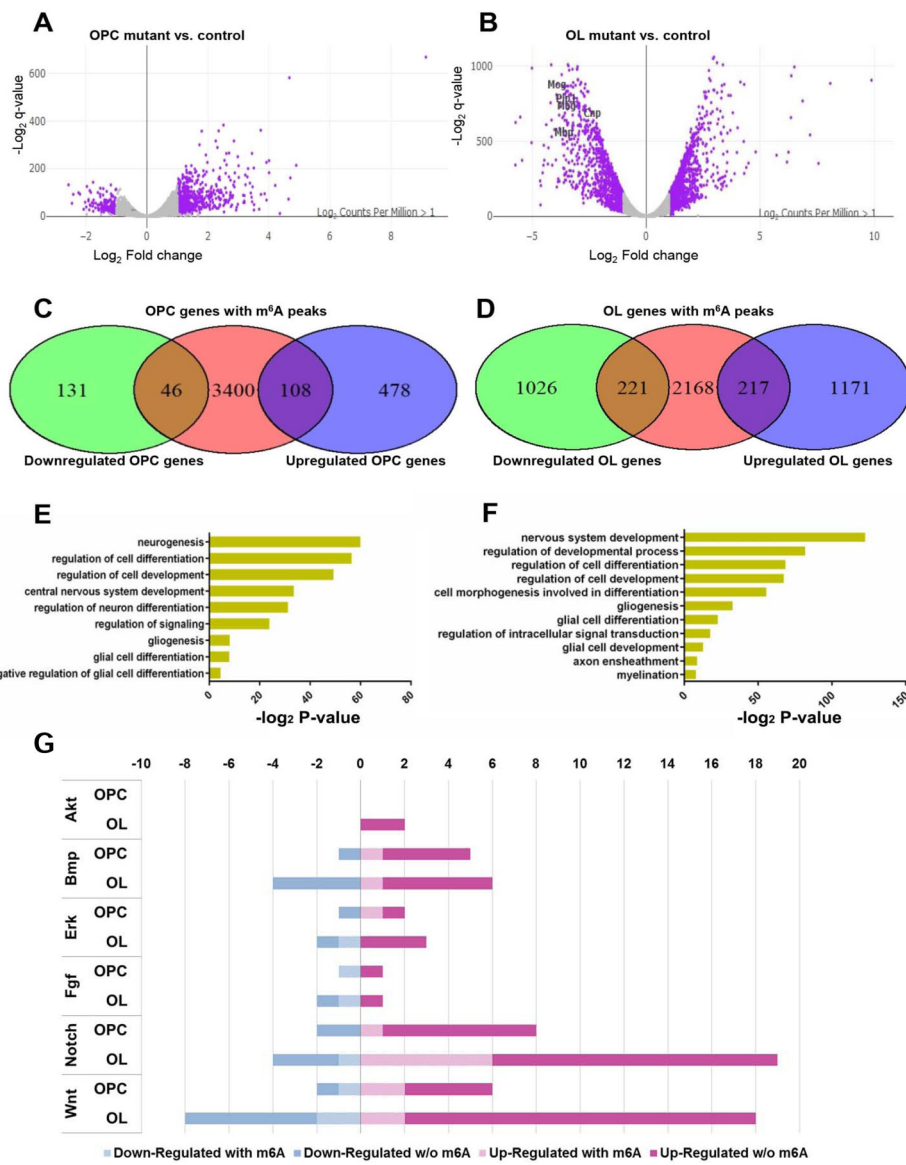
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**Figure 5. *Mettl14* ablation prevents oligodendrocyte differentiation.**

(A–B) O1 and MBP immunostaining of *Mettl14<sup>fl/fl</sup>* (control) oligodendrocytes that had been seeded in differentiation media for 1–5 days (day1–5). Control cells progressively differentiated into mature oligodendrocytes (Scale bar=50 $\mu$ m).

(C–D) O1 and MBP immunostaining of *Mettl14<sup>fl/fl</sup>;Olig2-Cre* (mutant) oligodendrocytes that had been seeded in differentiation media for 1–5 days (day1–5). Mutant cells did not differentiate into MBP positive oligodendrocytes, and never formed membrane sheath structures like control cells (Scale bar=50 $\mu$ m).



**Figure 6. *Mettl14* deletion differentially alters OL and OPC transcriptome.**

(A–B) Volcano plots display the differentially expressed genes in the *Mettl14*<sup>fl/fl</sup>; *Olig2*-Cre OPCs (A) and oligodendrocytes (B) mutants versus controls (n=3). The highlighted genes (purple) are significantly (q-value<0.001, log<sub>2</sub> |CPM|>1) regulated and have a notable fold change (log<sub>2</sub> |FC|>1) in their expression in the mutants. Selected myelin genes are labeled.

(C–D) Venn diagram shows the numbers of significantly downregulated or upregulated OPC (C) and oligodendrocyte (OL) (D) transcripts that also have the m<sup>6</sup>A mark.

(E–F) The ontology categories of the m<sup>6</sup>A marked transcripts that are significantly altered in the OPCs (E) and oligodendrocytes (F). (log<sub>2</sub> |FC|>1, log<sub>2</sub> |CPM|>1, q-value <0.001, Z-score>0).

(G) Bar graph shows the number of m<sup>6</sup>A marked transcripts in the selected altered signaling pathways in OPCs and oligodendrocytes. (log<sub>2</sub> |FC|>1, log<sub>2</sub> |CPM|>1, q-value<0.001, Z-score>0).



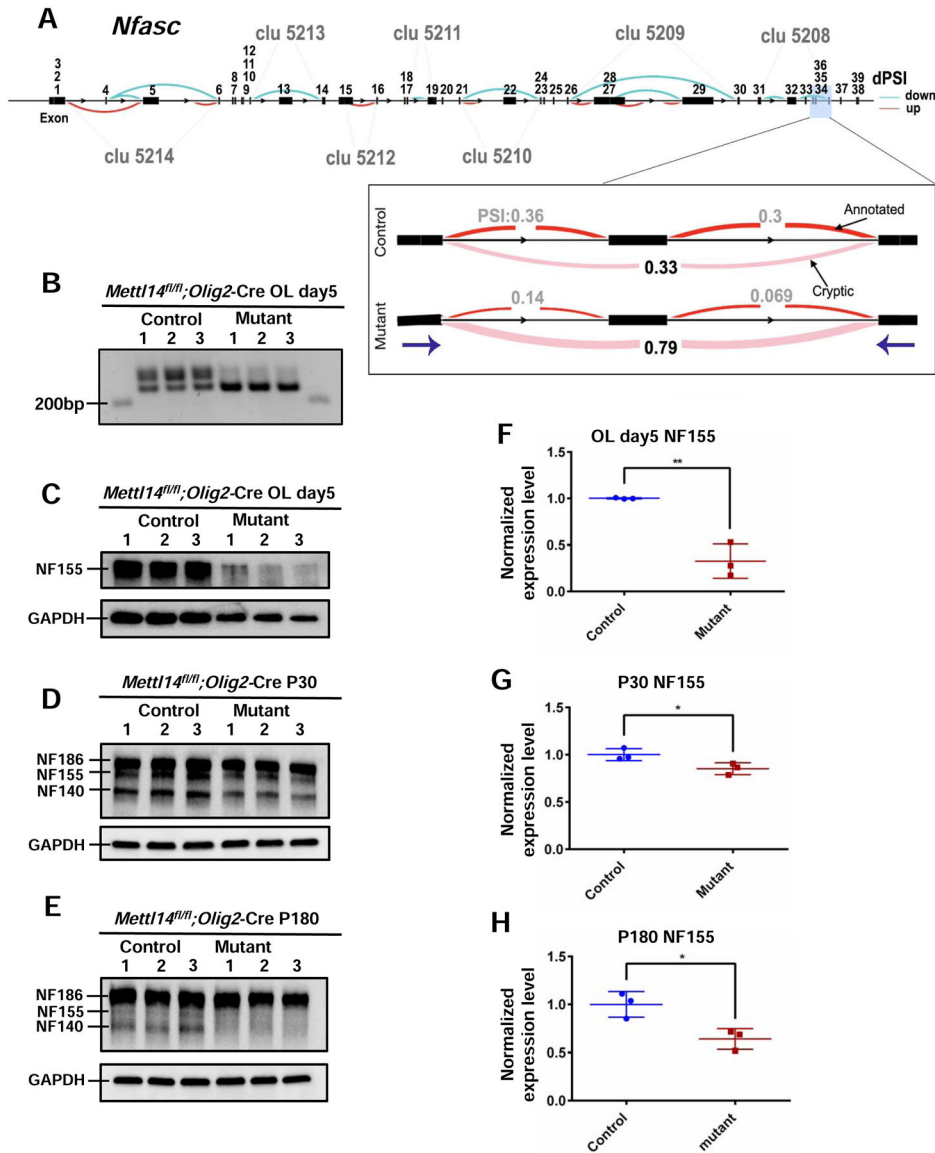
See also Figure S8, Table S1–S5

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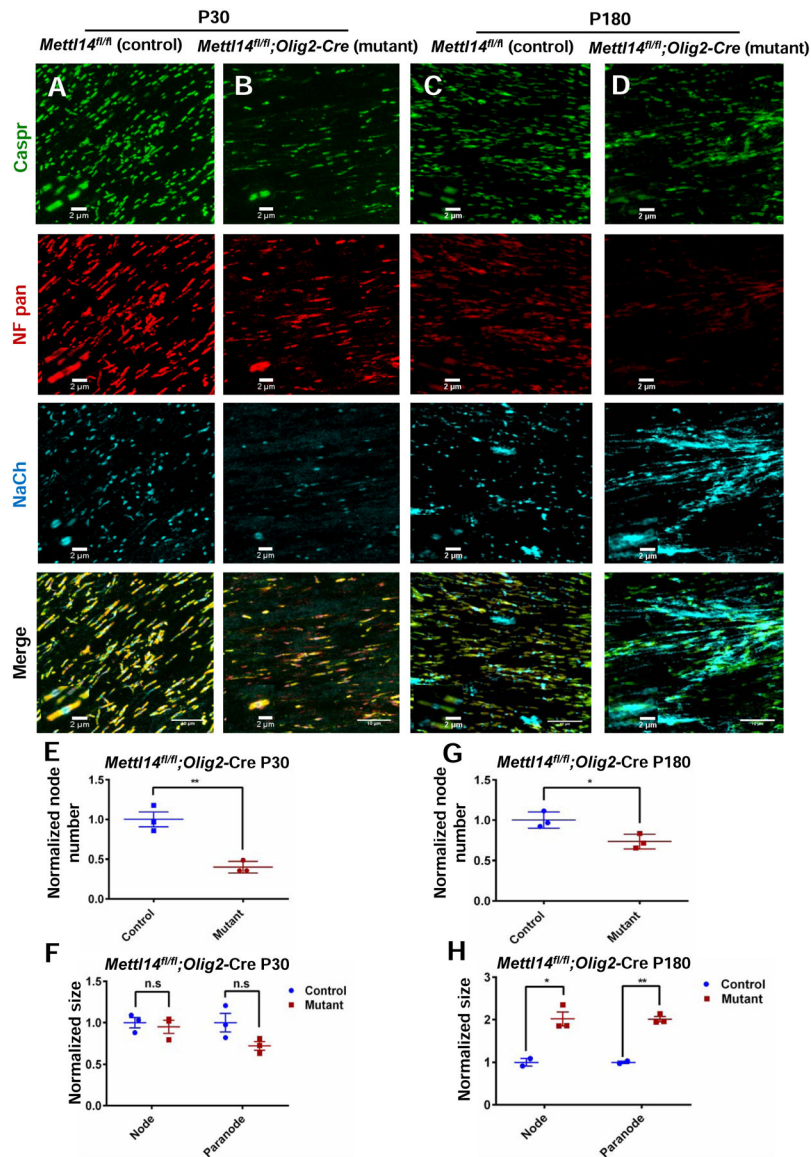
**Figure 7. *Mettl14* deletion differentially alters *Nfasc155* alternative splicing and expression.** (A) Schematic view of differentially spliced sites in the *Nfasc* gene in control versus *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mutant day5 oligodendrocytes. The 39 *Nfasc* exons are labeled above the exons. Each cluster (i.e. abbreviated as “clu X”) represents a group of introns that display alternative excision events. Specifically, these are introns that share a donor site (canonical 5’ splice site, AT) or acceptor site (canonical 3’ splice site, GA). Blue curves represent cases that have fewer splicing events in the mutants, while the red represent cases with more splicing events in the mutants ( $p < 0.05$ ). The magnified window shows the sample cluster (clu 5208) that we examined for the presence of aberrant spliced isoforms in the mutants in panel B. Purple arrows represent the start points for reverse and forward primers that we used for RT-PCR in (B). (B) Differentially spliced *Nfasc* isoform were detected by RT-PCR and agarose gel electrophoresis in the *Mettl14<sup>fl/fl</sup>;Olig2-Cre* day5 oligodendrocyte mutants (218kb).

(Primers used: Forward: ACTGGGAAAGCAGATGGTGG Reverse: ACATGAGCCCCGATGAACCAG).

**(C–E)** Western blot results of NFASC *in vitro* (C) and *in vivo* (D:P30, E:P180)

**(F–H)** Quantification of NF155 expression *in vitro* (F) and *in vivo* (G:P30, H:P180). NF155 expression level was normalized to GAPDH expression level. NF155 had significant reduction in both P30 and P180 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* mutants. Values represent mean  $\pm$  SEM (n=3; \*p<0.05; \*\*p<0.01; unpaired Student's t test).

See also Figure S3, Table S6–S7.



**Figure 8. *Mettl14* deletion results in aberrant node and paranode morphology.** (A–B) Representative immunostaining with Caspr, Nfasc and NaCh in P30 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant corpus callosum. Representative node(s) of Ranvier are shown in magnified windows. (Scale bar=10µm, 2µm) (C–D) Representative immunostaining with Caspr, Nfasc and NaCh in P180 *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant corpus callosum. Representative node(s) of Ranvier are shown in magnified windows. (Scale bar=10µm, 2µm) (E, G) Quantification of node number (NaCh positive) in P30(E) and P180(G) *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant corpus callosum. Normalized number = mutant count / control count (=1) (n=3; \*p<0.05; \*\*p<0.01, unpaired Student’s t-test). (F, H) Quantification of node (Caspr, NaCh positive) and paranode (Nfasc, Caspr double positive) size in P30 (F) and P180 (H) *Mettl14<sup>fl/fl</sup>;Olig2-Cre* control and mutant corpus

callosum. Normalized size = mutant size / control size (=1) (control n=2 mutant n=3;  
\*p<0.05; \*\*p<0.01, unpaired Student's t-test).

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## KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-METTL14	Sigma-Aldrich	Cat# HPA038002, RRID:AB_10672401
Goat anti-PDGFR- $\alpha$	R and D systems	Cat# AF1062, RRID:AB_2236897
Rabbit anti-MBP	Abcam	Cat# ab40390, RRID:AB_1141521
Mouse anti-CC1	Milipore	Cat# OP80, RRID:AB_2057371
Mouse anti-Olig2	Milipore	Cat# MABN50, RRID:AB_10807410
Mouse anti-O1	R and D systems	Cat# MAB1327, RRID:AB_357618
Rabbit anti-Ki67	Abcam	Cat# AB15580, RRID:AB_805388
Rabbit anti-MAG	Thermo Fisher Scientific	Cat# 34-6200, RRID:AB_2533179
Mouse anti-MBP	Biologend	Cat# SMI 99, RRID:AB_2314771
Mouse anti-MYRF	Generous gift from Dr. Ben Emery	Cat# 4G4, RRID:AB_2814997
Mouse anti-GAPDH	Cell signaling	Cat# 2118, RRID:AB_561053
Chicken anti-Neurofascin, pan	R and D systems	Cat# AF3235, RRID:AB_10890736
Rabbit anti-Caspr	Abcam	Cat# ab34151, RRID:AB_869934
Mouse anti-Sodium channel, pan	Sigma-Aldrich	Cat# S8809, RRID:AB_477552
Rabbit anti-Choline acetyltransferase(ChAT)	Milipore	Cat# AB144P, RRID:AB_2079751
Goat anti-mouse IgG+IgM	Jackson ImmunoResearch	Cat# 115-055-044, RRID:AB_2338532
Goat anti-mouse IgM	Jackson ImmunoResearch	Cat# 115-005-020, RRID:AB_2338450
Mouse IgG HRP Linked Whole Antibody	GE Healthcare	Cat# NA931, RRID:AB_772210
Rabbit IgG HRP-Linked Whole Antibody	GE Healthcare	Cat# NA934, RRID:AB_772206
Rabbit anti-Chicken IgY (H+L) Secondary Antibody, HRP	Thermo Fisher Scientific	Cat# 31401, RRID:AB_228385
Donkey Anti-mouse IgG (H+L), Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-21202, RRID:AB_141607
Donkey Anti-Rabbit IgG (H+L) Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-21206, RRID:AB_2535792
Donkey Anti-Mouse IgG (H+L), Alexa Fluor 594	Thermo Fisher Scientific	Cat# A-21203, RRID:AB_2535789
Donkey Anti-Goat IgG (H+L), Alexa Fluor 594	Thermo Fisher Scientific	Cat# A-11058, RRID:AB_2534105
Donkey Anti-Rabbit IgG (H+L), Alexa Fluor 594	Thermo Fisher Scientific	Cat# A-21207, RRID:AB_141637
Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor 594	Thermo Fisher Scientific	Cat# A-11042, RRID:AB_2534099
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
RNAscope® Probe -Mm-Myrf	ACD bio	Cat# 524061
RNAscope® Probe -Mm-Mbp	ACD bio	Cat# 451491
Dynabeads Oligo(dT) <sub>25</sub>	Thermo Fisher Scientific	Cat# 61006
Dynabeads Protein A	Thermo Fisher Scientific	Cat# 1001D
N <sup>6</sup> -Methyladenosine 5'-monophosphate sodium salt	Sigma-Aldrich	Cat# M2780
Poly-D-lysine	Sigma-Aldrich	Cat# P6407
Platelet derived growth factor	PeoroTech	Cat# 100-13A

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Neurotrophin-3	PeproTech	Cat# 450-03
Ciliary neurotrophic factor	PeproTech	Cat# 450-13
Forskolin	Sigma-Aldrich	Cat# F6886
B27	Life technologies	Cat# 17504044
Fetal bovine serum	Altanta biologicals	Cat# S11050
Normal donkey serum	Jackson Immunoresearch	Cat# 017-000-121
Protease inhibitor cocktail	Thermo Fisher Scientific	Cat# 78430
Phosphatase inhibitors	Sigma-Aldrich	Cat# P2850 and P5726
Laemmli sample buffer	Bio-Rad Laboratories	Cat# 161-0737
$\beta$ -mercaptoethanol	Sigma-Aldrich	Cat# M6250
Trizol reagent	Thermo Fisher Scientific	Cat# 15596018
Triiodothyronine	Sigma-Aldrich	Cat# T6397
Trypsin 0.05%	Thermo Fisher Scientific	Cat# 25300-054
Trypsin 2.5%	Thermo Fisher Scientific	Cat# 15090046
Trypsin inhibitor	Worthington	Cat# LS003086
Deoxyribonuclease I	Worthington	Cat# LS002007
Papain	Worthington	Cat# LS003126
Apo transferrin	Sigma-Aldrich	Cat# T1147
ProLong gold abtifaide reagent with DAPI	Life technologies	Cat# P36931
Paraformaldehyde	Thermo Fisher Scientific	Cat# T353-500
Osmium Tetroxide	Electron microscopy Science	Cat# 19152
Propylene Oxide	Electron microscopy Science	Cat# 20401
Sodium Cacodylate Buffer	Electron microscopy Science	Cat# 11652
Epon 812	Electron microscopy Science	Cat# 14900
<b>Critical Commercial Assays</b>		
BCA Protein Assay Kit	Thermo Fisher Scientific	Cat# 23255
Aurum Total RNA mini Kit	Bio-Rad Laboratories	Cat# 732-6820
Agilent RNA 6000 Nano kit with chips	Agilent	Cat# 5067-1511
RNA Clean & Concentrator	Zymo	Cat# R1015
NEBNext Ultra RNA library Prep kit for Illumina	New England Biolabs	Cat# 61011
RNA Fragmentation Reagents	Thermo Fisher Scientific	Cat# AM8740
Dynabeads Oligo(dT) <sub>25</sub>	Thermo Fisher Scientific	Cat# 61006
SuperSignal West Dura Extended Duration Substrate	Thermo Fisher Scientific	Cat# 34076
Pierce™ ECL Western Blotting Substrate	Thermo Fisher Scientific	Cat# 32209
SMARTScribe Reverse Transcriptase	Clontech	Cat# 639537
Protein A Dynabeads	Thermo Fisher Scientific	Cat# 0002D
Advantage 2 Polymerase Mix	Thermo Fisher Scientific	Cat# 639201
Agencourt AMPure XP	Beckman Coulter	Cat# A63880
RNAScope® Multiplex Fluorescent V2 Assay kit	ACD bio	Cat# 323110

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Basic Nucleofector™ Kit for Primary Mammalian Glial Cells	Lonza	Cat# VPI-1006
Opal™ 520	Akoya Biosciences	Cat# FP1487001KT
Opal™ 620	Akoya Biosciences	Cat# FP1495001KT
<b>Deposited Data</b>		
Raw and analyzed data	This paper	GEO: GSE124244
<b>Experimental Models: Organisms/Strains</b>		
Mouse: <i>Mett14<sup>fl/fl</sup></i>	Generous gift from Dr. Xiaoxi Zhuang	( Koranda et al., 2018; Weng et al., 2018b; Yoon et al., 2017)
Mouse: <i>Olig2-Cre</i>	Generous gift from Dr. David Rowitch	The Jackson Laboratory: 011103
Mouse: <i>CNP-Cre</i>	Generous gift from Dr. Klaus Amin Nave	MGI: 3051635
<b>Oligonucleotides</b>		
RT-PCR primers for <i>Nfasc</i> aberrant spliced locus, see Figure 7. Forward: ACTGGGAAAGCAGATGGTGG Reverse: ACATGAGCCCGATGAACCAG	This paper	N/A
<b>Recombinant DNA</b>		
Mett14 (NM_201638) Mouse Tagged ORF Clone	OriGene	Cat# MR207291
pmaxGFP™ vector	Lonza	Cat# VPI-1006
<b>Software and Algorithms</b>		
Image J	National Institutes of Health	RRID:SCR_003070
Image Lab	Bio-Rad Laboratories	RRID:SCR_014210
R v3.5.1	R core team	RRID:SCR_001905
Bioconductor v.3.7	(Huber et al., 2015)	RRID:SCR_006442
STAR v2.6.1a	(Dobin et al., 2013)	RRID:SCR_015899
Trimmomatic	(Bolger et al., 2014)	RRID:SCR_011848
Kallisto v0.44.0	(Bray et al., 2016)	RRID:SCR_016582
GraphPad Prism 6	GraphPad Software	RRID:SCR_002798
LeafCutter	(Li et al., 2018)	RRID:SCR_017639