Dual regulatory switch through interactions of Tcf7l2/Tcf4 with stage-specific partners propels oligodendroglial maturation

Chuntao Zhao1,2,*, Yaqi Deng1,2,*, Lei Liu1,3,*, Kun Yu1, Liguo Zhang2, Haibo Wang2, Xuelian He2, Jincheng Wang2,4, Changqing Lu1, Laiman N. Wu2, Qinjie Weng4, Meng Mao3, Jianrong Li5, Johan H. van Es6, Mei Xin2, Lee Parry7, Steven A. Goldman8, Hans Clevers6 & Q. Richard Lu2,3,9

Constitutive activation of Wnt/β-catenin inhibits oligodendrocyte myelination. Tcf7l2/Tcf4, a β-catenin transcriptional partner, is required for oligodendrocyte differentiation. How Tcf7l2 modifies β-catenin signalling and controls myelination remains elusive. Here we define a stage-specific Tcf7l2-regulated transcriptional circuitry in initiating and sustaining oligodendrocyte differentiation. Multistage genome occupancy analyses reveal that Tcf7l2 serially cooperates with distinct co-regulators to control oligodendrocyte lineage progression. At the differentiation onset, Tcf7l2 interacts with a transcriptional co-repressor Kaiso/Zbtb33 to block β-catenin signalling. During oligodendrocyte maturation, Tcf7l2 recruits and cooperates with Sox10 to promote myelination. In that context, Tcf7l2 directly activates cholesterol biosynthesis genes and cholesterol supplementation partially rescues oligodendrocyte differentiation defects in Tcf7l2 mutants. Together, we identify stage-specific co-regulators Kaiso and Sox10 that sequentially interact with Tcf7l2 to coordinate the switch at the transitions of differentiation initiation and maturation during oligodendrocyte development, and point to a previously unrecognized role of Tcf7l2 in control of cholesterol biosynthesis for CNS myelinogenesis.
Oligodendrocyte (OL) myelination permits salutary propagation of nerve signals and is critical for cognitive and motor functions in the vertebrate central nervous system (CNS)1–3. During myelination, OLs pass through multiple developmental stages, including OL precursor cell (OPC), immature premyelinating OL and mature myelinating OL stages. A series of signalling pathways including Wnt/β-catenin, BMP/Id and Notch/Hes signalling have been shown to negatively regulate OL differentiation4–11. Hyperactivation of canonical Wnt signalling leads to the inhibition of OL differentiation and myelination through constitutively activated β-catenin6–10. Wnt3a ligand treatment11–13 or the loss of signalling inhibitors, as observed in ApcMin (ref. 14) or Apc knockout mice15. Intriguingly, the loss of the β-catenin (Apc) gene results in the stabilization and subsequent nuclear translocation of β-catenin16,17. Nuclear β-catenin, raising the paradox of how Tcf7l2 might exert β-catenin activity17 and is highly expressed by OL lineage cells8,9. Although hyperactive Wnt signalling inhibits OL differentiation, intriguingly, the loss of the β-catenin effector Tcf7l2 leads to a block of OL differentiation in Tcf7l2-null animals9,18. At present, the mechanisms underlying Tcf7l2 regulation of CNS myelination and remyelination remain elusive. Importantly, direct transcriptional targets of Tcf7l2 have not been identified in OLs, a particular concern, as Tcf7l2 is a known partner of β-catenin, raising the paradox of how Tcf7l2 might exert functions opposing inhibitory functions of β-catenin.

By generating mice lacking the Tcf7l2 DNA-binding transcription-activating domain, we showed that Tcf7l2 transcriptional activity is crucial for OL myelination and remyelination. We further conducted genome-wide chromatin immunoprecipitation sequencing (ChIP-seq) profiling to comprehensively map the Tcf7l2 direct targets at different stages of spinal cord development and find that Tcf7l2 engages the OL genome through its sequential interactions with stage-specific co-regulators, including the non-canonical Wnt signalling regulator Kaiso/Zbtb33 at the early stage of OPC differentiation and a differentiation-promoting factor, Sox10, later in OL differentiation. Our data further suggest that Tcf7l2 interacts with Kaiso to antagonize Wnt signalling activity at the differentiation onset, while coordinating with Sox10 to promote myelin gene expression during OL maturation. Furthermore, we find that Tcf7l2 and Sox10 interaction controls the cholesterol biosynthesis pathway for myelinogenesis. Thus, our studies define stage-dependent functions of Tcf7l2 during OL lineage development mediated through switching binding partners and provide a molecular framework for understanding the context-specific control of CNS myelination.

Results

Tcf7l2 transcriptional activity is vital for OL myelination. Tcf7l2 consists of several functional domains including the β-catenin-binding domain, Groucho/TLE-binding domain and HMGI (high mobility group) DNA-binding domain (Fig. 1a)19. To assess the role of Tcf7l2 transcriptional activity during OL development, we generated mutant mice carrying a transcriptionally inactive Tcf7l2, in which the floxed Tcf7l2 exon 11 encoding the DNA-binding HMGI box20,21 was excised by an OL lineage-expressing Olig1-Cre22,23, to generate a Tcf7l2 in-frame mutant without the HMGI domain. This yielded control (Tcf7l2+/+; Olig1-Cre−/−) and mutant (Tcf7l2+/−; Olig1-Cre−/+′′) mice (designated as Tcf7l2AHMG, Fig. 1a). We confirmed the excision of the exon 11 in complementary DNAs of OPcs isolated from Tcf7l2AHMG neonates by quantitative reverse transcriptase–PCR (qRT–PCR), using primers spanning the deleted exon 11 (Fig. 1b). Expression of Tcf7l2AHMG, but not full-length Tcf7l2 protein, was detected in Tcf7l2-mutant spinal cords, although its level is lower when compared with that of control animals by western blot analysis (Fig. 1b).

To investigate OL differentiation in Tcf7l2 mutants, we first examined myelin gene expression in the brain. In the Tcf7l2AHMG cortex, we found that in contrast to their robust expression in the control, Mbp and Plp1 (proteolipid protein 1) expression was remarkably reduced at postnatal stages (Fig. 1c). The dysmyelinating phenotype persisted to adulthood (Fig. 1c). Myelin basic protein (MBP) expression or green fluorescent protein (GFP) signals from the CNP-mGFP transgenic line23 was also reduced at P14 (Fig. 1d,e), consistent with the sustained decrease in the number of Plp1+ OLs at P60 (Fig. 1f).

Impaired terminal OL differentiation in Tcf7l2 mutants might be due to a shortage in OPCs. We then assessed the formation of cortical OPCs using platelet-derived growth factor receptor-α (PDGFRα) immunolabelling and a PDGFRα-GFP reporter24. In the Tcf7l2AHMG cortex at P7, the number of PDGFRα+ OPCs was comparable to that in the control (Fig. 1g-j). The rate of OPC proliferation was also unaltered, as shown by 5-bromodeoxyuridine (BrDU) incorporation (Fig. 1j). Furthermore, inactivation of Tcf7l2 in the OL lineage did not affect the formation of neurons, astrocytes or microglia, as immunolabelled by NeuN, glial fibrillary acidic protein and Iba1, respectively (Supplementary Fig. 1). These results indicated that Tcf7l2 is not essential for OPC formation.

In light of OL differentiation deficits noted in the Tcf7l2 mutants, we used electron microscopy to analyse myelin sheath morphologies in the corpus callosum and in optic nerves. Consistent with the decrease in myelin gene expression in the brain, the optic nerves of mutant mice exhibited a significantly reduced proportion of myelinated axons at early postnatal stages such as P14, this relative hypomyelination persisted into adulthood (Fig. 1k). Similarly, hypomyelination was also observed in the corpus callosum at P60 (Fig. 1l), in which the percentage of myelinated axons remained significantly lower than controls (Fig. 1m). These data suggest that the loss of Tcf7l2 transcriptional activity impairs myelination in both developing and adult brains.

To further verify the stage-specific function of Tcf7l2 in OL differentiation, we inactivated Tcf7l2 using other OL-lineage expressing Cre drivers, including Olig2-Cre, the expression of which begins in early OL progenitors8. Similar to Olig1-Cre-mediated mutagenesis, ablation of Tcf7l2 by Olig2-Cre resulted in a substantial reduction in the expression of the myelin genes Plp1 and Mbp in the mutant cortex at P14 (Supplementary Fig. 2a). In addition, in mice with Tcf7l2 ablated by a CNP-Cre line25, wherein Cre expression commences at early postmitotic OPC stages, there was a significant decrease in the number of CC1+ or MAG+ myelinating OLs (Supplementary Fig. 2b,c). Together, these observations suggest that Tcf7l2 regulates myelination-associated gene expression and is critical for OPC differentiation.
Quantification of Plp1 and Tcf7l2 enhanced GFP (EGFP) driven by a CNP promoter in the cortices from control and immunostaining in the cortices from control and terminal epitope) and glyceraldehydes 3-phosphate dehydrogenase (GAPDH; loading control). (Supplementary Fig. 3). In adulthood at P60, however, Tcf7l2 activity is required for OL remyelination observed spinal cord of Tcf7l2ΔHMG mice by in situ hybridization. Arrows indicate the cerebral white matter in coronal sections, except P60 ΔHMG mice at P14 assayed by immunohistochemistry. (e) Expression of Mbp and Plp1 in cortices from control and Tcf7l2ΔHMG mice by in situ hybridization. Arrows indicate the cerebral white matter in coronal sections, except P60 Mbp in sagittal sections. (d) MBP immunostaining in the cortices from control and Tcf7l2ΔHMG mice at P14 assayed by immunohistochemistry. (e) Expression of membrane-anchored enhanced GFP (EGFP) driven by a CNP promoter in the cortices from control and Tcf7l2ΔHMG mice carrying the CNP-mGFP transgene at P14.

Tcf7l2 activity is required for OL remyelination. In the developing spinal cord of Tcf7l2ΔHMG mice, expression of Mbp was substantially reduced at P0 and P7 (Fig. 2a), whereas the number of PDGFRα+ OPCs was comparable to controls at P7 and P14 (Supplementary Fig. 3). In adulthood at P60, however, Mbp expression was similar to control (Fig. 2b). Consistently, myelin ultrastructure, the percentage of myelinated axons and their g-ratios were all comparable between control and adult Tcf7l2ΔHMG spinal cord at P60 (Fig. 2c–e), indicating a delayed myelination process in the Tcf7l2-mutant spinal cord.
As myelination in the spinal cord fully caught up when the Tcf7l2 mutants reached adulthood, we then capitalized on this phenotype to assess the function of Tcf7l2 in remyelination by employing the lysolecithin (LPC)-induced demyelination. Local injection of LPC in the white matter induces rapid myelin breakdown and removal of myelin from adult CNS; myelin regenerates through an OPC recruitment phase at 7 days post lesion (dpl) and a remyelinating phase at 14 dpl. In adult

Figure 2 | Tcf7l2 ablation impairs remyelination in LPC-induced demyelinating animal model. (a,b) Expression of Mbp in the spinal cord from control (Ctrl) and Tcf7l2ΔHMG mice at indicated neonatal and adult ages by in situ hybridization. (c) Electron microscopy of the spinal white matter of control and Tcf7l2ΔHMG mice at P60. The percentages of myelinated axons (d) and g-ratio (e) in the spinal white matter of control and Tcf7l2ΔHMG mice at P60. The data represent the means ± s.e.m.; n = 3 animals per genotype. (f) Left: the location of LPC-induced lesion (DAPI counterstaining, dashed lines) in the spinal cord. Right: in situ hybridization analysis showed re-expression of Tcf7l2 in the LPC-induced demyelinating lesions (demarcated with dashed lines) and uninjured regions at 7 and 14 dpl in spinal cords of P60 mice. (g) In situ hybridization analysis of PDGFRα, Mbp and Plp1 in the lesion regions (demarcated with dashed lines) at 14 dpl in spinal cords of P60 Ctrl and Tcf7l2ΔHMG mutant mice; n = 5 animals for each genotype. (h) Representative electron micrographs of spinal cords of 8-week-old control and Tcf7l2ΔHMG mice at 14 dpl. Arrow indicates the newly formed thin myelin sheath. (i) Quantification of the percentage newly myelinated axons in spinal lesions of 8-week-old control and Tcf7l2ΔHMG mice at 14 dpl; n = 3 animals for each genotype. (j) Quantification of g-ratio of newly myelinated axons in spinal lesions of 8-week-old control and Tcf7l2ΔHMG mice at 14 dpl; n = 3 animals for each genotype. P < 0.001, Student’s t-test. Data are presented as mean ± s.e.m. **P < 0.01, ***P < 0.001; Student’s t-test. Scale bars, 100 μm (a,b), 2 μm (c,j) and 50 μm (f,g).
control mice, Tcf7l2 was drastically upregulated at 7 and 14 dpi within the LPC lesions (Fig. 2f), consistent with the previous findings. To determine whether Tcf7l2/HMG is required for myelin repair, we analysed myelin gene expression in the lesions at 14 dpi, a phase of OL regeneration and remyelination. Compared with controls, we detected substantially lower levels of Mbp and Plp1 during remyelination in Tcf7l2/HMG mice (Fig. 2g,i), although PDGFRx+ OPCs were generated normally (Fig. 2g,h). Importantly, many fewer myelinated axons were detected in the lesions of Tcf7l2/HMG mice than in controls (Fig. 2j). The percentage and thickness of newly generated myelin sheaths around axons were significantly reduced in the Tcf7l2 mutants (Fig. 2k,l). These observations indicate that Tcf7l2 activity is critical for remyelination after demyelinating injury.

**Downregulation of myelin genes in Tcf7l2/HMG mutants.** In light of our data demonstrating impaired re/myelination capacity in the absence of Tcf7l2, we sought to identify the Tcf7l2-regulated genes. We carried out RNA-sequencing (RNA-seq) analysis using the OL-enriched optic nerves from control and Tcf7l2/HMG mice at P12, to identify differentially expressed genes. In accordance with dysmyelinating phenotypes, expression of myelin genes such as Cnp, Mbp, Ugt8a2, and Mag, and myelin-regulatory genes such as Sox10, Olig1 and Myrf, was significantly reduced in Tcf7l2 mutants (Fig. 3a,b). In contrast, we observed an upregulation of OL differentiation inhibitors, including Id2, Notch2, Tgfbr1/Tgfr3 and Bmp6, as well as Wnt signalling pathway components including Wnt4/6, Axin2, Ctnnb1, Ccnd1 and Sp5 (Fig. 3a,b). Gene ontology analysis of downregulated genes identified cholesterol biosynthesis, axon ensheathment, OL differentiation and myelination (Fig. 3c), congruent with impaired OL differentiation in Tcf7l2 mutants. We further confirmed the downregulation of these myelination-associated genes and differentiation regulators by qRT–PCR analysis (Fig. 3d). Moreover, overexpression of Tcf7l2 in OPCs enhanced expression of myelin genes such as Mbp, Cnp, Plp1 and Mag, while repressing Id2 expression (Fig. 3e). These observations suggest that Tcf7l2 transcriptional activity is both necessary and sufficient for OPC maturation.

**Stage-specific Tcf7l2 targeting for OL lineage progression.** To determine the expression pattern of Tcf7l2 during OL lineage progression, we treated rat OPCs with triiodothyronine (T3) for different durations. Tcf7l2 messenger RNA upregulated in differentiating OPCs after 1 and 3 days of T3-induced differentiation (Fig. 3f), but fell to a lower level in terminally differentiated OLs 5 days after T3 treatment. Similarly, Tcf7l2 immunoreactivity was weakly detected in PDGFRx+ OPCs but increased substantially in Cnp+ differentiating OLs and then decreased in terminally differentiated Mbp+ OLs (Fig. 3g).

To gain insights into the direct targets regulated by Tcf7l2, we carried out ChIP-seq analysis for Tcf7l2-chromatin occupancy in OPCs, immature OLs (iOL, OPC exposure to T3 for 1 day) and maturing OLs (mOLs, OPC exposure to T3 for 3 days) (Fig. 3h). The closest annotated gene to each Tcf7l2-binding site was identified as a presumed target. The number of Tcf7l2-targeted sites was ~1,125 in iOLs and 14,541 in mOLs, respectively (Fig. 3i). The majority of Tcf7l2-binding peaks in iOLs overlapped with those identified in mOLs, but were of lower intensity (Fig. 3h). The increase in targeted sites and signal intensity from OPC to mOL correlated with the progression of OPC differentiation. In contrast, Tcf7l2-targeted sites in iOL and mOLs, respectively, overlapped by only 10 and 3% with those in other cell types such as H4IIIE liver cells (Fig. 3i), suggesting that Tcf7l2 targets unique sets of genes in the OL lineage and possesses a distinct role in the control of OL differentiation.

The Tcf7l2-binding sites were co-localized with evolutionarily conserved enhancer elements marked by an activating histone mark H3K27ac in iOL and mOL cells (Fig. 3j,k). To evaluate the global distribution of Tcf7l2-binding loci, we plotted the number of Tcf7l2 sites against their distance to the nearest transcription start site (TSS). We detected a strong enrichment for Tcf7l2 binding around TSSs within 5-kb promoter regions of genes marked by the histone mark H3K4me3 (ref. 29), in particular in mOLs (Fig. 3l). These observations indicate that Tcf7l2 targets primarily to enhancer/promoter regions to regulate target gene expression.

**Wnt inhibitor Kaiso is a Tcf7l2 co-factor in iOLs.** To investigate whether certain DNA motifs were enriched in Tcf7l2-binding sites, we applied a motif-discovery algorithm, HOMER. The sequence motif ACTG/TACCAAG identified in iOLs matches the consensus-binding motif for Tcf7l2 in previous ChIP-seq data sets (Fig. 4a) (ref. 30). Enhancer regions typically have binding sites for co-factors, which bound within ~100 bp of the Tcf7l2 peak summit. We found that a substantial proportion (~28%) of Tcf7l2-binding sites were significantly overrepresented with the binding-motif of Zbtb33/Kaiso (Fig. 4a). Kaiso is a transcriptional repressor of Wnt signalling, it interacts with TCF factors to inhibit β-catenin-dependent activation of transcription. Consistently, we observed that Tcf7l2 targets primarily to enhancer/promoter regions to regulate target gene expression.
To determine the effect of Kaiso on the genome occupancy of Tcf7l2 on different target genes, we performed Tcf7l2 ChIP-qPCR in primary rat OPCs transfected with a Kaiso-expressing vector. We found that Tcf7l2 occupancy on the promoter of Wnt target genes (that is, Sp5, Ctnnb1, Ccnl1, Wnt11 and Wnt10a) was reduced in Kaiso-overexpressing cells (Fig. 4l). In contrast,
Figure 4 | Tcf7l2 coordinates with Kaiso to inhibit Wnt/β-catenin signalling during OL differentiation. (a) Kaiso and Tcf7l2/Tcf4 consensus sites are most overrepresented in Tcf7l2-binding regions of iOLs. (b) qRT-PCR analysis of Kaiso in OPCs under T3-containing differentiation conditions for indicated days; n = 3 independent experiments. (c) Kaiso and CNP expression at indicated days after T3 treatment of rat OPCs. (d) Co-expression of Kaiso with Tcf7l2, PDGFRα, CNP and MBP at indicated days after T3 treatment of OPCs. Arrows indicate co-labelled cells. (e) Biological processes overrepresented in Tcf7l2-occupied regions in iOLs. (f) Tcf7l2-binding profiles in OPCs, iOLs and mOLs on representative Wnt-signalling gene loci. (g) Kaiso occupancy on Tcf7l2-binding sites in iOLs by ChIP-qPCR. Control: genomic segment lacking Tcf7l2-binding sites. (h) Topflash luciferase activity in Hek293T cells transfected with expression vectors for Kaiso, β-catenin or both together. (i) Kaiso co-immunoprecipitated with Tcf7l2 in iOLs. (j) Co-immunoprecipitation with anti-Tcf7l2 from Hek293T cells transfected with Tcf7l2 with β-catenin and Kaiso for 48 h. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a loading control. (k) qRT-PCR analysis of myelination-related genes in OPCs transfected with control or Kaiso-expressing vectors for 48 h; n = 3 independent experiments. (l) Tcf7l2 occupancy by ChIP-PCR in rat OPCs transfected with control or Kaiso-expressing vectors on the promoters of targeted genes; n = 3 independent experiments. (m) qRT-PCR analysis of myelination-related and Wnt signalling genes in OPCs transfected with scrambled control and Kaiso siRNAs; n = 3 independent experiments. Expression of Kaiso and Tcf7l2 (n), as well as myelination-related genes (o) in Oli-neu cells transfected with control and Kaiso-overexpressing vectors, and/or scrambled control and Tcf7l2-siRNAs; n = 3 independent experiments. (p,q) The corpus callosum (arrows) of control and Kaiso−/− mutants at P7 were immunostained with CC1 and MBP (p). Panel q depicts the percentage of PDGFRα+ and CC1+ cells in the corpus callosum at P7, n = 3 animals per genotype. Data are presented as mean ± s.e.m. *P<0.05, **P<0.01 and ***P<0.001; Student’s t-test, except in o with analysis of variance (ANOVA) and Newman-Keuls multiple comparison test. Scale bars, 50 μm (d) and 100 μm (p,q).
Tcf7l2 occupancy on the promoter of a myelination-promoting factor Myrf was enhanced (Fig. 4I), suggesting that Kaiso expression levels modulate the genome occupancy of Tcf7l2 in OPCs. To further examine the effect of Kaiso knockdown on the OL differentiation programme, small interfering RNA (siRNA) targeting Kaiso was transfected into rat OPCs and cultured under differentiation conditions for 72 h. Kaiso knockdown resulted in the downregulation of myelination-associated genes that included Mbp, Pip1, Cnp, Mag and Myrf, while upregulating the Wnt signalling effectors Sp5, Wnt10a, Wnt4 and Wnt11 (Fig. 4m), all as compared with scrambled siRNA-transfected OPCs. Furthermore, we found that Kaiso overexpression enhanced the expression levels of myelin-associated genes, while Tcf7l2 knockdown blocked the ability of Kaiso to promote their expression (Fig. 4n,o), suggesting that the effect of Kaiso on enhancing OL differentiation gene expression depends on Tcf7l2.

In addition, to determine the in vivo role of Kaiso in OL development, we analysed the phenotype of Kaiso-null mice39, and found that the number of CC1⁺ differentiating OLs and MBP signal intensity were substantially reduced in the corpus callosum of Kaiso mutants at P7 (Fig. 4n,o) compared with controls, despite normal PDGFRα⁺ OPC formation (Fig. 4g). Collectively, these data suggest that Kaiso promotes OPC differentiation programmes, while repressing Wnt signalling activity and is required for normal OL differentiation.

Sox10 is a Tcf7l2 co-regulator for OL maturation. As in iOLs, we found that Tcf7l2-binding peaks in mOLs match its consensus DNA-binding motif (Fig. 5a); however, ~38% of Tcf7l2-binding sites present in mOLs were predominantly enriched with the Sox-consensus binding motif C(T/A)TTG(T/A) (T/A), which matches most significantly with an OL differentiation-promoting factor Sox10 motif40,41 in the HOMER-motif discovery programme (Fig. 5a), but to a lesser extent with the Kaiso-binding motif. To determine whether Sox10 and Tcf7l2 co-target to the same regulatory elements in mOLs, we then performed genome-wide occupancy of Sox10 in mOL using ChIP-seq. We found that ~44% of Tcf7l2 peak sets overlapped with those of Sox10 occupancy (Fig. 5b and Supplementary Fig. 5). Sox10 motifs were enriched in Tcf7l2-binding sites in mOLs (Fig. 5b,c), suggesting that Tcf7l2 and Sox10 co-target the same elements in mOLs. To further determine whether Tcf7l2 may co-associate with Sox10, we performed co-immunoprecipitation assays and showed that Tcf7l2 and Sox10 were present in the same complex in mOLs (Fig. 5d), suggesting that Sox10 is a co-factor of Tcf7l2 during OL maturation.

A large proportion of Tcf7l2/Sox10 co-occupancy was within the regulatory elements of myelination-associated genes including Mbp, Myrf, Olig1, Utg8 and Zfp191 in mOLs, but not in OPCs or iOLs (Fig. 5e). In addition, although Sox10 or Tcf7l2 expression in rat OPCs elevated the expression level of myelin-associated genes such as Mbp and Cnp, co-expression of Sox10 and Tcf7l2 further increased their levels (Fig. 5f), suggesting that Sox10 and Tcf7l2 cooperate to promote myelin gene expression.

How does Tcf7l2 coordinate distinct co-factors to control OL lineage progression? Kaiso expression increases in iOLs, but downregulates in mOL, while the Sox10 expression level is maintained throughout the OL lineage. To determine whether the temporal sequence of Tcf7l2 recruitment of Kaiso and Sox10 depends on their expression levels, we co-transfected Tcf7l2 with a varied amount of Kaiso, while keeping the Sox10 level constant in 293T cells. We found that Tcf7l2 was associated preferentially with Kaiso when Kaiso expression levels were high (Fig. 5g). As the Kaiso level fell, Tcf7l2 was found to interact with Sox10 (Fig. 5g). Furthermore, we found that overexpression of Sox10 substantially reduced association of Tcf7l2 with Kaiso in Oli-neu cells (Supplementary Fig. 4b), suggesting dosage-dependent competitive binding of Tcf7l2 with Kaiso and Sox10. Thus, there appears to be a two-step recruitment process in which Tcf7l2 association with ‘early’ and ‘late-binding’ transcription factors, Kaiso and Sox10, respectively, is responsible for OL lineage progression. Together, these observations suggest that Tcf7l2 coordinates the stepwise OL differentiation process through interacting with Kaiso to suppress inhibitory Wnt signalling in OPCs, while associating with Sox10 to promote myelination-associated programmes during OL maturation (Fig. 5h).

Tcf7l2 activates cholesterol biosynthesis for OL maturation. Superimposing Tcf7l2 ChIP-seq from mOLs and RNA-seq data revealed that ~174 targeted genes with substantial changes of expression in Tcf7l2AHMG mutants also exhibited strong Tcf7l2 binding to their proximal promoter regions (Fig. 6a). These genes were overrepresented in the functional categories of steroid biosynthesis and cholesterol metabolism (Fig. 6b). Among these Tcf7l2 targets in mOLs, we identified a cohort of genes that encode enzymes involved in de novo cholesterol biosynthesis, including hmgcs1, Fdps, Fdfr1, Lss, Cyp51, Hsd17b7 and Dhc24 (Fig. 6c,d). Although Tcf7l2 did not appear to directly target the gene locus of Hmgcr (Supplementary Fig. 6a), it was highly enriched on the promoter region of Sreb2 (Supplementary Fig. 6b), a key upstream regulator of Hmgcr expression42,43.

When comparing Tcf7l2 occupancy on the promoters of cholesterol pathway genes with that of Olig2, an OL specification factor, we found that Tcf7l2 targeting was substantially enriched in parallel with OL maturation, and that these promoters were marked with the activating histone marks H3K4me3 and H3K27Ac28,29 (Fig. 6d). In contrast, Olig2 binding on these promoter regions was barely detectable in mOLs (Fig. 6d), suggesting a distinct role between Olig2 and Tcf7l2 in the differentiation of mOLs.

We next asked whether Tcf7l2 acted with partners to support oligodendrocytic cholesterol synthesis and found that in mOLs, Tcf7l2 co-occupied the promoters of cholesterol pathway genes with Sox10 (Fig. 6d); ChIP–qPCR further confirmed the enrichment of Tcf7l2 and Sox10 binding on the promoter elements of these cholesterol synthetic genes (Fig. 6e). The co-occupancy of the gene loci by Tcf7l2 and Sox10 is consistent with observations that Tcf7l2 and Sox10 form a complex in mOL.

The presence of H3K4me3 and H3K27Ac in the targeted promoters, both of which are indicators of active transcription state28,29, suggests that Tcf7l2 positively regulates expression of cholesterol biosynthesis genes (Fig. 6d). Accordingly, the mRNA expression levels of these Tcf7l2-targeted cholesterol biosynthesis genes was significantly reduced in Tcf7l2AHMG mutant optic nerves (Fig. 6f).

To compare the activity of Tcf7l2 with a key regulator for the cholesterol biosynthesis Sreb2/Sreb2 on target gene expression, we cloned the promoter regions carrying Tcf7l2-binding sites of cholesterol biosynthesis genes including Sreb2, Hmgcs1, Hmgcr, Fdps, Lss, Cyp51, Hsd17b7 and Dhc24 into a luciferase reporter system. We found that similar to Sreb2, Tcf7l2 stimulated the luciferase reporter activity driven by these regulatory elements/enhancers (Fig. 6g). In contrast, these effects could not be observed with Tcf7l2AHMG (Fig. 6g).

To further explore the relative contribution of Tcf7l2 to expression of cholesterol biosynthesis genes in OLs, we next transfected purified OPCs with control and expression vectors carrying Tcf7l2 and found that overexpression of Tcf7l2 induced...
the expression of a number of genes encoding essential enzymes for cholesterol synthesis, including Lss, Cyp51, Hsd17b7 and Dhc24 (Fig. 6h), as well as Axin2, a generic Wnt/Tcf7l2 target gene, and the myelination-promoting gene Myrf, suggesting that activation of Tcf7l2 promotes expression of cholesterol biosynthesis genes for OL differentiation.

**Tcf7l2-induced cholesterol synthesis for OL differentiation.** Cholesterol biosynthesis is required for myelin sheath outgrowth as shown in Hmgrs mutant zebrafish44 and Fdft1 mutant mice45. To determine the role of other Tcf7l2-regulated cholesterol biosynthesis genes in OL differentiation, we carried out siRNA knockdown during OPC differentiation. Knockdown of Cyp51,
**Figure 6** | **Tcf7l2 regulates the expression of cholesterol biosynthetic genes in OLs.** (a) Venn diagram shows the overlap between Tcf7l2-targeting genes and substantially altered genes detected by RNA-seq in Tcf7l2ΔHMG optic nerves (fold change >1.5 between Ctrl and mutant). (b) GO analysis of pathways overrepresented by direct Tcf7l2 target genes in mOLs. (c) Schematic view of de novo cholesterol biosynthetic pathway; the putative direct Tcf7l2 target genes are highlighted in red. (d) Genome browser view of the distribution of Tcf7l2, Sox10, Olig2, H3K27ac and H3K4me3 binding to promoter regions of cholesterol biosynthesis genes over IgG controls; n = 3 independent experiments. (e) ChIP–PCR assay for the enrichment of Tcf7l2 and Sox10 binding on the promoters of cholesterol biosynthesis genes over IgG controls; n = 3 independent assays. (f) qRT–PCR analysis of the expression of cholesterol biosynthetic genes in Tcf7l2ΔHMG optic nerves versus controls; n = three animals per genotype. (g) Luciferase reporter activity driven by Tcf7l2-binding promoter/enhancer regions was assessed in 293T cells co-transfected with control and pcDNA3 expression vectors for Tcf7l2 or ΔHMG mutant Tcf7l2, or Srebfl; n = 3 independent experiments. (h) qRT–PCR assay for the expression of cholesterol biosynthetic genes in OPCs transected with the pcDNA3 expression vectors for control and Tcf7l2; n = 3 independent experiments. Data are presented as mean ± s.e.m. *P < 0.05, **P < 0.01 and ***P < 0.001; Student’s t-test, except in g with analysis of variance (ANOVA) and Newman–Keuls multiple comparison test.

**Dhcr24, Hsd17b7 and Lss** (Fig. 7a) reduced expression of myelin genes including *Mbp, Plp1, Cnp* and *Mag*, although *Id2* and *Id4* expression was not significantly altered (Fig. 7b).

As Tcf7l2 activated the expression of cholesterol biosynthesis genes, we hypothesized that dysregulation of cholesterol synthesis might account for the impairment of OL maturation in Tcf7l2ΔHMG mice. Previous studies reported that cholesterol, a rate-limiting lipid component for myelin sheath growth, is not imported into the brain from the circulation but rather synthesized locally by myelin-forming OLs. On that basis, we next asked whether exogenous cholesterol would restore the differentiation capacity of OPCs isolated from Tcf7l2 mutants.
Under differentiation conditions, control OPCs readily differentiated into CNP\(^+\) and MBP\(^+\) mature OLs, whereas the majority of Tcf7l2-mutant OPCs failed to mature into OLs with elaborate membrane processes (Fig. 7c). In addition, the proportion of CNP\(^+\) or MBP\(^+\) OLs was significantly reduced in Tcf7l2 mutants compared with controls (Fig. 7d,e). Strikingly, exogenous cholesterol partially restored the percentage of CNP\(^+\) and MBP\(^+\) OLs in mutant cultures (Fig. 7c–e). Consistently, expression of myelin-associated genes increased in Tcf7l2-mutant OPCs treated with cholesterol (Fig. 7f). To further determine whether cholesterol supplementation could rescue the OL differentiation defect in Tcf7l2-mutant animals, we fed the pregnant mice carrying control and Tcf7l2ΔHMG mutants with cholesterol-enriched diet during gestation, to allow cholesterol intake into the CNS. The pups were harvested at P14 for analysis. Although cholesterol supplementation did not alter the number of OLs in the spinal cords of control animals, it significantly increased the number of CC1\(^+\) OLs and expression of MBP in Tcf7l2 mutants (Fig. 7g,h). These observations suggest that cholesterol is responsible, at least in part, for Tcf7l2-dependent...
control of OL differentiation, and that Tcf7l2 directly activates the genes encoding cholesterol biosynthesis enzymes to promote OL differentiation and maturation.

Discussion

Wnt/β-catenin/Tcf7l2 signalling has been suggested to positively or negatively regulate OL development, which may depend on the context9,44–51. Identification of Tcf7l2 direct targets and its co-regulators is of vital importance to understand the Tcf7l2-mediated process for OL development. Using integrative unbiased genomics analyses of multiple stages in the OL lineage, we present the first genome occupancy mapping of stage-specific targets of Tcf7l2 during OL lineage progression and further identify Tcf7l2-engaging partners that control transcriptional switches at the transitions of differentiation initiation and OL maturation. Strikingly, we uncover a non-canonical Wnt signalling co-regulator Kaiso as a Tcf7l2 partner at the OL differentiation state. At the onset of OPC differentiation, Kaiso is upregulated the transition of OPCs from an undifferentiated to differentiating stepwise OL differentiation. Importantly, our studies further provide evidence that Tcf7l2 directly targets and activates cholesterol metabolism-associated genes and thereby regulates de novo cholesterol biosynthesis necessary for myellogenesis, pointing to a previously unappreciated role of Tcf7l2 for cholesterol homeostatic control of CNS myelination.

Constitutive activation of Wnt/β-catenin signalling inhibits OL differentiation, suggesting that Wnt signalling may need to be attenuated for developmental OL differentiation. We found that the association of Kaiso with Tcf7l2 reverses the role of the β-catenin/Tcf7l2-mediated transcriptional complex from a differentiation repressor to an activator, which then promotes the transition of OPCs from an undifferentiated to differentiating state. At the onset of OPC differentiation, Kaiso is upregulated and appears to act as a competitive inhibitor of β-catenin, to oppose Wnt signalling activation by disrupting the β-catenin/Tcf7l2 complex. Kaiso has been shown to interact with the NCoR/Hdac1 complex to repress target gene expression. It is possible that Tcf7l2 forms a potent repressive complex with Kaiso and Hdac1, and perhaps Hdac-associated Groucho/TLE repressors as well, so as to inhibit Wnt/β-catenin signalling.

Our data indicate that Tcf7l2 targets a number of prototypical Wnt-responsive genes, including Axin2, Ccnd1, Ctnnb1, Lef1 and Sp5 on OPC differentiation into iOLs and persists with maturation (Fig. 4f). This suggests that Tcf7l2 may continue to modulate β-catenin activation and downstream target expression during OL differentiation. Persistent control of Wnt target gene expression and signalling by Tcf7l2 and its co-factors probably generates a high degree of regulatory complexity in response to different Wnt signalling inputs over the course of OPC differentiation.

We found that the transcriptional activity of Tcf7l2 is critical for OL differentiation and myelin repair. Although we could not rule out a potential dominant-negative effect of the Tcf7l2 mutant, the Tcf7l2AHMG mutant mice yielded a dysmyelinating phenotype similar to that of full-length Tcf7l2-deletion mutants. Of note, we did not observe any discernable phenotype in Tcf7l2AHMG heterozygous mice. It thus seems unlikely that any dominant-negative effect would significantly contribute to the dysmyelinating phenotype. Although Tcf7l2AHMG was able to interact with Kaiso and Sox10 when overexpressed in 293T cells (Supplementary Fig. 7a,b), it could not activate myelin gene expression as wild-type Tcf7l2 (Supplementary Fig. 7c), but rather blocks Kaiso and Sox10 activity for myelin gene activation (Supplementary Fig. 7d,e). This suggests that Tcf7l2 activity is required for OL differentiation-promoting machinery. Tcf7l2AHMG mutant protein appears at a lower level in the Tcf7l2-mutant spinal cord compared with its wild-type counterpart (Fig. 1b). This is probably due to the severe reduction of differentiating OLs, where Tcf7l2 is highly expressed, in the mutants.

During OL maturation, we detect stronger signal intensity and an increase in Tcf7l2-targeted binding sites, which largely overlap with those identified in rat spinal cord (Supplementary Fig. 8). We find that Sox10 acts a co-regulator with Tcf7l2 in mOLs, and that Tcf7l2 and Sox10 cooperate to promote myelogenic programmes, consistent with the notion that Tcf7l2 has a β-catenin independent function during OL differentiation. These findings suggest that Tcf7l2 functions through the sequential operation of two interlaced gene regulatory networks: one blocking inhibitory Wnt signalling activity at iOL stages through recruiting transcriptional repressors such as Kaiso, and the other promoting a ‘terminal’ differentiation process in committed OLs in cooperation with Sox10. The switch of Tcf7l2 co-regulators from Kaiso to Sox10 while transitioning from iOL to mOLs may lead to the further induction of myelin-specific genes, thus facilitating OL maturation (Fig. 5b). Cooperation with stage-specific, transiently expressed transcriptional partners may also explain the distinct stage-specific role of Tcf7l2 in the initiation and maintenance of OL differentiation.

Besides the temporally dynamic nature of Tcf7l2 interactions with other transcriptional modulators, we have also observed a distinct role for Tcf7l2 in regulating the extent of myelination. In contrast to persistent myelination defects in the brain of Tcf7l2 mutants, Tcf7l2 appears dispensable for myelination in the adult spinal cord, where Tcf7l2 is hardly detectable. At present, the underlying mechanisms for this regional specificity remain undetermined. The efficient Tcf7l2 allele recombination in the spinal cord (Fig. 1b) indicates that a region-specific function of Tcf7l2 rather than incomplete recombination accounts for the phenotypic difference.

A novel finding of our present study is that Tcf7l2 directly regulates de novo cholesterol biosynthesis and metabolism. We find that Tcf7l2 target genes are selectively enriched on the promoter regions of genes involved in cholesterol biosynthesis in mOLs. In contrast, in liver cells, Tcf7l2 targets are enriched on the promoter/enhancers of classical lipid synthesis-related genes such as Fabp1, Apol, Sod1, Mtp, Hnf4a and Fas, which are associated primarily with lipid metabolic pathways, such as the synthesis of ketone bodies, transport of fatty acids and gluconeogenesis (Supplementary Fig. 9). This suggests a conserved yet distinct role for Tcf7l2 in the control of lipid biosynthesis and metabolism, by regulating different sets of genes in a cell-type-specific manner.

Cholesterol is a rate-limiting lipid component for OL myelination and myelin sheath growth. Defects in myelination has been observed in mutants in the genes responsible for cholesterol biosynthesis such as in Fdft1 mutant mice and in Hmgcs1 mutant zebrafish. We find that cholesterol supplementation could, at least in part, rescue OL differentiation defects in Tcf7l2AHMG mutants. Interestingly, a similar observation was recently reported that cholesterol injection could rescue the axon wrapping defect in hmgcs1 mutant zebrafish, suggesting that cholesterol synthesis is necessary for OL maturation and axon wrapping. A cell sensing insufficient cholesterol could block OL differentiation and expression of myelination-associated genes. It is possible that a negative feedback or ‘check point’ mechanism exists, whereby sufficient cholesterol biosynthesis for myelin biosynthesis must be
verified before transcription of myelination-promoting factors. Given that Tcf7l2 mutations lead to downregulation in the expression of cholesterol biosynthesis enzymes, the requirement of Tcf7l2 for OL myelination can be mediated, at least partially, through its role as a regulator of cholesterol biosynthesis during OL maturation. Collectively, Tcf7l2 may have a parallel role in regulating the expression of cholesterol biosynthesis genes in addition to directly activating the myelinogenic transcriptional programme.

Previous studies show that Tcf7l2 expression rose during active remyelination in both human patients and rodent models3–56, and yet is completely absent in the lesions of chronic multiple sclerosis67. This observation suggested the potential requirement of Tcf7l2 for active myelin repair. Here we provide evidence that Tcf7l2 transcriptional activity is crucial for remyelination after demyelination, as well as to normal myelin formation during brain development. Indeed, as the impact of the Tcf7l2 knockout on myelination is more severe in the brain than in the spinal cord, it would seem likely to be that the remyelination defects observed in the Tcf7l2ΔHMG spinal cord should manifest in the brain as well.

Recent studies indicate that TCF7L2 is a key regulator of the metabolic gene programme that controls transcriptional responses to metabolic challenge in the liver, muscle or white adipose tissues32. For instance, TCF7L2 single-nucleotide polymorphism variants and mutations contribute to type 2 diabetes mellitus88–60. Yet, type 2 diabetes impairs OL regeneration in the white matter lesions after ischaemic injury61. Our findings suggest then that Tcf7l2 mutations or single-nucleotide polymorphism variants might modify both the degree of ischaemic myelin damage in patients with type 2 diabetes and their ability to remyelinate. Together, integrative analyses of Tcf7l2 occupancy, gene expression profiling and binding motifs at multiple OL stages reveal that Tcf7l2 appears to exercise its functions by cooperating with stage-specific co-regulators to control OL differentiation programmes. More broadly, the present genome-wide Tcf7l2 target identification should provide us a framework to identify novel therapeutic avenues for myelin repair in the CNS.

Methods

Generation of Tcf7l2 conditional knockout mice. Tcf7l2 floxed mice20 were crossed with Olig1-Cre23, Olig2-Cre62 (Olig2 < t-myc (TVA, creRti) > / Jackson Laboratory) and CNP-Cre23 (gift of Dr Klaus-Armin Nave) mice to generate Tcf7l2ΔHMG (Tcf7l2fl/CNP-Cre1/1) and heterozygous control (Tcf7l2flfl/CNP-Cre1/+) mice. The mouse strain carrying Cre-expressing Kaiso and Myc-tag Kaiso-transfected 293T cells by western blotting and immunostaining (Supplementary Fig. 10a,b). In addition, Kaiso expression was abolished in the brain of Kaiso-null mice by western blot analysis (Supplementary Fig. 10c), confirming the antibody specificity.

RNA in situ hybridization was performed using digoxigenin-labelled riboprobes as described previously63. The probes were used as follows: murine PGDFRζ, Pip1-Dm-20 and Mhp.

Oligodendroglial cell culture and transfection. Isolation of primary rat OPCs from cortices of P2 pups was performed as previously described44. OPCs were differentiated in DiIoliferation medium (Sato medium supplemented with 15 nM T3 and 10 mg/ml ciliary neurotrophic factor) for 24 and 72 h to become ifc OLi cultures. OLs as the initial phase of OLs as described previously27. Mouse primary OPCs was harvested and transduced with retrovirus vectors expressing Kaiso and Myc-tag Kaiso-transfected 293T cells by western blotting and immunostaining (Supplementary Fig. 10a,b). In addition, Kaiso expression was abolished in the brain of Kaiso-null mice by western blot analysis (Supplementary Fig. 10c), confirming the antibody specificity.

RNA in situ hybridization was performed using digoxigenin-labelled riboprobes as described previously63. The probes were used as follows: murine PGDFRζ, Pip1-Dm-20 and Mhp.

Oligodendroglial cell culture and transfection. Isolation of primary rat OPCs from cortices of P2 pups was performed as previously described44. OPCs were differentiated in DiIoliferation medium (Sato medium supplemented with 15 nM T3 and 10 mg/ml ciliary neurotrophic factor) for 24 and 72 h to become ifc OLi cultures. OLs as the initial phase of OLs as described previously27. Mouse primary OPCs was harvested and transduced with retrovirus vectors expressing Kaiso and Myc-tag Kaiso-transfected 293T cells by western blotting and immunostaining (Supplementary Fig. 10a,b). In addition, Kaiso expression was abolished in the brain of Kaiso-null mice by western blot analysis (Supplementary Fig. 10c), confirming the antibody specificity.

RNA in situ hybridization was performed using digoxigenin-labelled riboprobes as described previously63. The probes were used as follows: murine PGDFRζ, Pip1-Dm-20 and Mhp.

Co-immunoprecipitation and luciferase assays. Co-immunoprecipitation and western blotting were performed as described previously67. Briefly, for co-immunoprecipitation in iOLs and MSLA, 60 μg of cell lysate proteins were incubated with 3 μg anti-Tcf7l2, anti-Kaiso or anti-Sox10 for immunoprecipitation of control. Each analysis was performed in triplicates and the results were normalized as an internal control. Gapdh

Co-immunoprecipitation and luciferase assays. Co-immunoprecipitation and western blotting were performed as described previously67. Briefly, for co-immunoprecipitation in iOLs and MSLA, 60 μg of cell lysate proteins were incubated with 3 μg anti-Tcf7l2, anti-Kaiso or anti-Sox10 for immunoprecipitation of control. Each analysis was performed in triplicates and the results were normalized as an internal control. Gapdh

Quantitative real-time PCR analysis. RNAs were isolated with Trizol (Invitrogen Inc.) from cells or snap-frozen tissues. Reverse transcription was performed with the cdNA Reverse Transcription Kit (Bio-Rad) with iQ SYBR Green (17Q 8980). qRT-PCR was carried out using the ABI Prism 7900 Sequence Detector System (Perkin-Elmer Applied Biosystems) using Gapdh as an internal control. Each analysis was performed in triplicates and the results were normalized to Gapdh for each sample. The qRT-PCR primer sequences are listed in Supplementary Table 1.

RNA-seq and data analysis. We isolated RNAs from the optic nerves of control mice and Tcf7l2ΔHMG mutants at P12 and subjected samples to RNA deep sequencing and data analysis as previously described67. RNA-seq libraries were prepared using Illumina RNA-seq Preparation Kit (Illumina) and sequenced on the HiSeq 2000 sequencer. RNA-seq reads were mapped using TopHat with default settings (TopHat-1.0-0.1.0.1) and stringently aligned by Cufflinks to (1) calculate fragments per kilobase of transcript per million mapped reads (FPKM) for known transcripts in mouse genome reference and (2) test the
changes of gene expression between Tcf7l2A/2A and control. Heatmap of gene differential expression was generated using R language (http://www.r-project.org).

ChIP-seq and ChiP-qPCR. ChiP-seq libraries were performed as previously described, with minor modifications27. Briefly, OLGs, iOLs and mOLs (~20 million cells) were fixed for 10 min at room temperature with 1% formaldehyde-containing medium. Nuclei were isolated and sonicated in sonication buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.5 mM EGTA and protease inhibitor cocktail). Sonicated chromatin (~300 μg) was used for immunoprecipitation by incubation with appropriate antibodies (4 μg) overnight at 4°C. Ten per cent of chromatin used for each ChiP reaction was kept as input DNA. Precipitated magnetic protein A/G beads (50 μl) were added to each ChiP reaction and reactions were incubated for 1 h at 4°C. The beads were then incubated in 200 μl elution buffer at 65°C for 20 min to elute immunoprecipitated material. We performed replicative ChiP-seq assays using chromatin from at least two different cell cultures. The ChiP-seq libraries were prepared using NEBNext ChiP-seq Library Prep Master Mix Set for Illumina (NEB catalogue number E1124) and then run on the Illumina sequencer HS2000. The antibodies used were as follows: Kaiso (Abcam, ab12723), TCF7L2 (Santa Cruz, sc-6631) and Sox10 (Abcam, ab515279). The primers used for ChiP–PCR are listed in Supplementary Table 1.

ChIP-seq peak-calling and data analysis. All sequencing data were mapped to rat genome assembly rn5 and peak calling was performed as previously described27 using MACS (Model-based Analysis of ChiP-seq) version 1.4.2 (http://liulab.dfci.harvard.edu/MACS) with default parameters, to get primary binding regions. Spinal cord tissues carrying the lesions were collected at time points as described, with minor modifications27. Briefly, OPCs, iOLs and mOLs (L-a-lysophosphatidylcholine; 0.5 μl) via a Hamilton syringe attached to a glass micropipette was injected into the ventrolateral white matter via a stereotactic apparatus. Spinal cord tissues carrying the lesions were collected at time points as follows: 7 dpl, representing peak OPC recruitment, and 14 dpl, representing OL differentiation and new myelin sheath formation (at least 5 mice per control and mutant groups were used for each time point analysis).

Electron microscopy. Electron microscopy was performed essentially as previously described27. Anaesthetized mice were perfused briefly with 0.1 M cacodylate and followed by 2.5% glutaraldehyde/2.5% PFA in 0.1 M cacodylate (pH 7.2). The optic nerves and spinal cord were removed and fixed in fresh fixative overnight at 4°C. Tissues were rinsed in PBS, postfixed in 1% OsO4 in PBS for 1 h, dehydrated in a graded ethanol series, infiltrated with propylene oxide and embedded in Epon. Semi-thin sections were stained with toluidine blue and thin sections were stained with lead citrate.

Statistical analysis. All analyses were done using Microsoft Excel or GraphPad Prism 6.00 (San Diego, CA, www.graphpad.com). Quantifications were carried from at least three independent experimental groups and data were presented as means ± s.e.m. Student’s two-tailed t-tests and one-way analysis of variance analysis with a Newman–Keuls multiple comparison test were used to compare two data sets and more than two data sets, respectively. P-value <0.05 was considered to be statistically significant.

References
1. Zalc, B. & Colman, D. R. Origins of vertebrate success. Science 288, 271–272 (2000).
2. Molófsky, A. V. et al. Astrocyte-encoded positional cues maintain sensorimotor circuit integrity. Nature 509, 189–194 (2014).
3. McKenzie, I. A. et al. Motor skill learning requires active central myelination. Science 346, 318–322 (2014).
4. Bercury, K. K. & Macklin, W. B. Dynamics and mechanisms of CNS myelination. Dev. Cell 32, 447–458 (2015).
5. Han, X. et al. Forebrain engrafment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. Cell Stem Cell 12, 342–353 (2013).
6. Zuccher, J. B. & Barnes, R. A. Intrinsic and extrinsic control of oligodendrocyte development. Curr. Opin. Neurobiol. 23, 914–920 (2013).
7. He, L. & Lu, R. Q. Coordinated control of oligodendrocyte development by extrinsic and intrinsic signaling cues. Neurosci. Bull. 29, 129–143 (2013).
8. Fancy, S. P. et al. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. Genes Dev. 23, 1571–1585 (2009).
9. Ye, F. et al. HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin-TCF interaction. Nat. Neurosci. 12, 829–838 (2009).
10. McClain, C. R., Sim, F. J. & Goldman, S. A. Pleiotrophin suppression of receptor protein tyrosine phosphatase-beta/zeta maintains the self-renewal competence of fetal human oligodendroglial progenitor cells. J. Neurosci. 32, 15066–15075 (2012).
11. Shimizu, T. et al. Wnt signaling controls the timing of oligodendrocyte development in the spinal cord. Dev. Biol. 282, 397–410 (2005).
12. Feigenson, K., Reid, M., See, J., Crenshaw, 3rd E. B. & Grinspan, J. B. Wnt signaling is sufficient to perturb oligodendrocyte maturation. Mol. Cell. Neurosci. 42, 255–265 (2010).
13. Azim, K. & Butt, A. M. GSK3beta negatively regulates oligodendrocyte differentiation and myelination in vivo. Glia 59, 540–553 (2011).
14. Fancy, S. P. et al. Axin2 as regulatory and therapeutic target in newborn brain injury and remyelination. Nat. Neurosci. 14, 1009–1016 (2011).
15. Lang, J. et al. Adenomatous polyposis coli regulates oligodendroglial development. J. Neurosci. 33, 3113–3130 (2013).
16. Clevers, H. & Nusse, R. Wnt/beta-catenin signaling and disease. Cell 149, 1192–1205 (2012).
17. Schuijers, J., Mokry, M., Hatzis, P., Cuppen, E. & Clevers, H. Wnt-induced transcriptional activation is exclusively mediated by TCF/LEF. EMBO J. 33, 146–156 (2014).
18. Hammond, E. et al. The Wnt effector transcription factor 7-like 2 positively regulates oligodendrocyte differentiation and myelination in vivo. Glia 59, 540–553 (2011).
19. Arce, L., Yokoyama, N. N. & Waterman, M. L. Diversity of LEF/TCF action in development and disease. Oncogene 25, 7492–7504 (2006).
20. Vizcaino, E. A. et al. Critical role of the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal. Mol. Cell. Biol. 32, 1918–1927 (2012).
21. Korinek, V. et al. Deletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat. Genet. 19, 379–383 (1998).
22. Xin, M. et al. Myelino genesis and axonal recognition by oligodendrocytes in brain tissue uncoupled in Olig2 null mice. J. Neurosci. 25, 1354–1365 (2005).
23. Deng, Y. et al. Direct visualization of membrane architecture of myelinating cells in transgenic mice expressing membrane-anchored EGFP. Genesis 52, 341–349 (2014).
24. Hamilton, T. G., Klinghoffer, R. A., Corrin, P. D. & Soriano, P. Evolutionary divergence of platelet-derived growth factor alpha receptor signaling.

25. Lappe-Siefke, C. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

26. Franklin, R. J. Why does remyelination fail in multiple sclerosis? 

27. Yu, Y. et al. Molecular basis of the selective vulnerability of oligodendrocytes in multiple sclerosis.

28. Hatzis, P. et al. Genome-wide pattern of TCF7L2/TCF4 chromatin occupancy in colorectal cancer cells. Mol. Cell Biol. 26, 2737–2744 (2006).

29. Boj, S. F. et al. Huges gene and Wnt effector Tcft2/TCF4 controls hepatic response to perinatal and adult metabolic demand. Cell 151, 1595–1607 (2012).

30. Ruzov, A. et al. The interaction of xKaiso with xTcf: a revised model for integration of epigenetic and Wnt signalling pathways. Development 136, 723–739 (2009).

31. Korinek, V. et al. Canonical Wnt/beta-catenin signaling.

32. Yu, J. et al. Disruption of Cnp1 uncouples oligodendroglial functions in ischaemic white matter lesions due to type 2 diabetes. 

33. Santos-Rosa, H. et al. Active genes are tri-methylated at K4 of histone H3. 

Nature 419, 407–411 (2002).

34. Molex, S. et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell 38, 576–589 (2010).

35. Hatzis, P. et al. Groucho binds two conserved regions of the histone H3K27ac regulatory element binding protein 2 in cholesterol gallstone-susceptible mice. Annu. Rev. Genet. 45, 645–660 (2012).

36. Jurevics, H. & Morell, P. Cholesterol for synthesis of myelin is made locally, not exported. J. Neurosci. 34, 2015–2025 (2014).

37. Stein, M. E. & Hasinoff, C. M. Inhibitors of cholesterol synthesis and myelin development.

38. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

39. Lappe-Siefke, C.et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

40. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

41. Hatzis, P. et al. Genome-wide pattern of TCF7L2/TCF4 chromatin occupancy in colorectal cancer cells. Mol. Cell Biol. 26, 2737–2744 (2006).

42. Boj, S. F. et al. Huges gene and Wnt effector Tcft2/TCF4 controls hepatic response to perinatal and adult metabolic demand. Cell 151, 1595–1607 (2012).

43. Ruzov, A. et al. The interaction of xKaiso with xTcf: a revised model for integration of epigenetic and Wnt signalling pathways. Development 136, 723–739 (2009).

44. Park, J. I. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

45. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

46. Hatzis, P. et al. Genome-wide pattern of TCF7L2/TCF4 chromatin occupancy in colorectal cancer cells. Mol. Cell Biol. 26, 2737–2744 (2006).

47. Stein, M. E. & Hasinoff, C. M. Inhibitors of cholesterol synthesis and myelin development.

48. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

49. Lappe-Siefke, C. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

50. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

51. Hatzis, P. et al. Genome-wide pattern of TCF7L2/TCF4 chromatin occupancy in colorectal cancer cells. Mol. Cell Biol. 26, 2737–2744 (2006).

52. Boj, S. F. et al. Huges gene and Wnt effector Tcft2/TCF4 controls hepatic response to perinatal and adult metabolic demand. Cell 151, 1595–1607 (2012).

53. Ruzov, A. et al. The interaction of xKaiso with xTcf: a revised model for integration of epigenetic and Wnt signalling pathways. Development 136, 723–739 (2009).

54. Park, J. I. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

55. Lappe-Siefke, C. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

56. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

57. Hatzis, P. et al. Genome-wide pattern of TCF7L2/TCF4 chromatin occupancy in colorectal cancer cells. Mol. Cell Biol. 26, 2737–2744 (2006).

58. Boj, S. F. et al. Huges gene and Wnt effector Tcft2/TCF4 controls hepatic response to perinatal and adult metabolic demand. Cell 151, 1595–1607 (2012).

59. Ruzov, A. et al. The interaction of xKaiso with xTcf: a revised model for integration of epigenetic and Wnt signalling pathways. Development 136, 723–739 (2009).

60. Park, J. I. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

61. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

62. Lappe-Siefke, C. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

63. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

64. Hatzis, P. et al. Genome-wide pattern of TCF7L2/TCF4 chromatin occupancy in colorectal cancer cells. Mol. Cell Biol. 26, 2737–2744 (2006).

65. Boj, S. F. et al. Huges gene and Wnt effector Tcft2/TCF4 controls hepatic response to perinatal and adult metabolic demand. Cell 151, 1595–1607 (2012).

66. Ruzov, A. et al. The interaction of xKaiso with xTcf: a revised model for integration of epigenetic and Wnt signalling pathways. Development 136, 723–739 (2009).

67. Park, J. I. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

68. Franklin, R. J. Why does remyelination fail in multiple sclerosis?

69. Lappe-Siefke, C. et al. Disruption of Cap1 uncouples oligodendrogial functions in axonal support and myelination. 

70. Franklin, R. J. Why does remyelination fail in multiple sclerosis?