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Kif1b is essential for mRNA localization in oligodendrocytes and development of myelinated axons

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Abstract

The kinesin motor protein Kif1b has previously been implicated in the axonal transport of mitochondria and synaptic vesicles1,2. More recently ***kif1b*** has been linked with susceptibility to Multiple Sclerosis (MS) 3. Here we show that Kif1b is required for the localization of ***myelin basic protein*** mRNA to processes of myelinating oligodendrocytes in zebrafish. We observe the ectopic appearance of myelin-like membrane in ***kif1b*** mutants, coincident with the ectopic localization of myelin proteins in ***kif1b*** mutant oligodendrocyte cell bodies. These observations suggest the hypothesis that oligodendrocytes localize certain mRNA molecules, namely those encoding small basic proteins such as ***mbp***, to prevent aberrant effects of these proteins elsewhere in the cell. We also find that Kif1b is required for outgrowth of some of the longest axons in the peripheral and central nervous systems. Our data demonstrate new functions of ***kif1b in vivo*** and provide insights into its possible roles in Multiple Sclerosis.

The myelin sheath is essential for the normal function of the vertebrate nervous system4. Disruption of myelin underlies the symptoms of many human diseases, including multiple sclerosis (MS). In fish and mammals, a few specific messenger RNA molecules, including *myelin basic protein* (*mbp*) mRNA, are localized to processes of myelinating oligodendrocytes (Supplementary Fig. 1)5,6. Previous studies carried out in cell culture have implicated microtubules and associated motors in the localization of *mbp* mRNAs to myelinating oligodendrocyte processes7,8,9. Nonetheless, the identity of specific factors required for the localization of endogenous *mbp* mRNAs in vivo has remained unclear. In addition, the function of this evolutionarily conserved mRNA localization mechanism is not known.

In a zebrafish genetic screen, we previously identified a mutation (named *st43*) that disrupts localization of *mbp* mRNA in the central nervous system (CNS) and outgrowth of the posterior lateral line nerve (PLLn) in the peripheral nervous system (PNS)10. Using positional cloning, we identified the gene disrupted by the *st43* mutation. High-resolution mapping showed that *st43* is tightly linked to *kif1b*, which encodes a kinesin motor. Sequence analysis showed that the *st43* mutation disrupts zebrafish *kif1b* (Fig. 1a), converting a conserved threonine to a proline, which is predicted to break a highly conserved alpha helix in the putative microtubule interaction site of the kinesin motor11,12. *kif1b* has two prominent isoforms, *kif1bα* and *kif1bβ*, which are expressed widely in the

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developing nervous system (Fig. 1b,c, and data not shown). Zebrafish Kif1bα is 78% identical to human Kif1bα and zebrafish Kif1bβ is 87% identical to human Kif1bβ. These Kif1b isoforms share a common region, which includes the motor domain disrupted by *st43*, and are distinguished by distinct tail domains that are thought to confer cargo specificity2,13. To confirm the specific requirement for *kif1b* during *mbp* mRNA localization and peripheral nerve outgrowth, we designed an antisense morpholino oligonucleotide (MO) to block translation of all *kif1b* isoforms, and MOs that targeted splice junctions specific to *kif1bα* and *kif1bβ*. Injection of either the common-region MO or the *kif1bβ* specific MO disrupted *mbp* mRNA localization in the CNS and peripheral nerve outgrowth in the PNS (Fig. 1f,g, Fig. 2a–d and data not shown), whereas MOs targeting *kif1bα* did not (Fig. 2c). *kif1b*^{st43} mutant larvae do not have overt morphological defects at 4 days post fertilization (dpf), but fail to inflate their swim bladders (Fig. 1d,e) and die between 12–14 dpf.

In addition to the axonal outgrowth defect we also noted a reduction in the number of neurons in the *kif1b*^{st43} mutant PNS. The posterior lateral line ganglion (PLLg) contained an average of 27 neurons (s.d.+/− 2) in wildtype siblings at 28 hpf (n=15) compared to 20 (s.d. +/− 5) in *kif1b*^{st43} mutants (n=12), and 38 (s.d.+/− 1) in wildtype (n=13) compared to 25 (s.d.+/− 6) in *kif1b*^{st43} mutants (n=6) at 36 hpf. To determine whether *kif1b* functioned in neurons or Schwann cells to mediate normal peripheral nerve development we performed chimeric analyses, whereby wildtype cells were transplanted into either *kif1b* morphant or *kif1b*^{st43} mutant hosts. Analyses of chimeras showed that wildtype axons could grow significantly further than those with disrupted *kif1b* function in individual nerves. Whereas *kif1b* morphant axons never grew past somite ten, wildtype axons could grow to the tip of the tail in the same nerves (Fig. 2e–g, n=4 chimeras). Wildtype neurons were also sufficient to restore the migration and differentiation of *kif1b*^{st43} mutant Schwann cells (Fig. 2g,h, n=7 chimeras). These data demonstrate that *kif1b* is required autonomously in neurons to mediate normal PLLn development.

Analyses of the pan-neuronal marker Tg(HuC:kaede)14 revealed disruption in the outgrowth of some long axons in the *kif1b*^{st43} mutant spinal cord (Fig. 3a,b). Transplantation studies showed that *kif1b* functions autonomously in neurons to ensure normal outgrowth of these axons. Whereas *kif1b* morphant axons did not grow past somite sixteen, wildtype axons could grow to the posterior tip of the spinal cord in the same animals (Fig. 3c,d, n=7 chimeras). Previous studies have implicated Kif1b in the transport of mitochondria and synaptic vesicles1,2. Our marker and ultrastructural analyses, however, failed to reveal an obvious defect in the distribution of these cargoes in *kif1b*^{st43} mutant axons prior to or during disruption of (Fig. 2j–m, Fig. 3d,e, Fig. 5a,b and Fig. 6a–d). Furthermore, *kif1b*^{st43} mutants did not have overt disruption in axonal microtubules (data not shown), in contrast to mutants for *kbp*15, which encodes a putative binding partner of Kif1b. It is also possible, therefore, that the axonal and neuronal defects observed in *kif1b*^{st43} mutants might relate to unknown Kif1b cargo, or functions independent of its motor activity, precedent for which has recently been demonstrated16,17,18.

In light of its role in neurons, we investigated whether *kif1b* functions autonomously in oligodendrocytes or non-autonomously in neurons to mediate normal *mbp* mRNA localization. Microarray analyses show that *kif1b* is upregulated in myelinating oligodendrocytes19, but there is no previously defined role for *kif1b* in these glia. We generated chimeras in which wildtype oligodendrocytes and/or neurons were present in *kif1b*^{st43} mutants. In 5 such chimeras we observed *mbp* mRNA localized in distal oligodendrocyte processes (Fig. 4a,b). In each of these cases, the oligodendrocyte(s) with normally localized *mbp* mRNA derived from a wildtype animal. We never saw a case where wildtype neurons restored normal *mbp* mRNA localization to a *kif1b*^{st43} mutant

oligodendrocyte (n>100 chimeras). These data indicate that *kif1b* is required autonomously in oligodendrocytes to localize *mbp* mRNA. To test the possibility that *kif1b* is required for oligodendrocytes to extend myelinating processes, we analyzed the expression of Tg(olig2:gfp)20, which labels oligodendrocytes, and an antibody directed against MBP protein21. The number and distribution of oligodendrocytes was similar in *kif1b*^{st43} mutants and wildtype animals from 3 through 9 dpf, stages when mRNA was always mislocalized in *kif1b*^{st43} (Fig. 4c,d, and data not shown). Furthermore, MBP protein was localized in myelinating processes of *kif1b*^{st43} mutant oligodendrocytes (Fig. 4c,d,i and j) showing that its translation is not dependent on localization of its mRNA. To investigate whether the localization of other myelin mRNAs requires *kif1b* function, we analyzed the distribution of *36k*, the other mRNA known to be localized to myelinating processes in zebrafish22 (Fig. 4e). As for *mbp*, *36k* mRNA was also restricted to oligodendrocyte cell bodies in *kif1b*^{st43} mutants (Fig. 4f). At later stages of development we noticed a striking accumulation of both MBP and 36K protein in cell bodies and proximal processes of *kif1b*^{st43} mutant oligodendrocytes, in contrast to wildtype, where these proteins are largely restricted to myelinating processes (Fig. 4g–j). These data indicate that *kif1b* is specifically required in oligodendrocytes to localize mRNAs encoding a subset of myelin proteins, including MBP and 36K.

To determine if *kif1b* is required for the formation of myelin in CNS, we analyzed ultrastructure of myelinated axons by TEM. The number of myelinated axons was reduced at 9 dpf in *kif1b*^{st43} mutants in both the anterior hindbrain (wildtype average of 57 (s.d.+/−4), n=4, compared to *kif1b*^{st43} mutant average of 42 (s.d.+/− 7), n=4) and ventral spinal cord (wildtype average of 59 (s.d.+/− 3), n=4, compared to *kif1b*^{st43} mutant average of 50 (s.d.+/−6), n=4). We also noted a small but significant reduction in the amount of myelin surrounding those axons in both the hindbrain and ventral spinal cord (Fig. 5 a–d). Axonal cross sectional area was almost identical between wildtype and *kif1b*^{st43} mutants in the hindbrain and spinal cord (Fig. 5 c,d, and data not shown), suggesting that the reduction in myelin did not result from a simple delay in axonal development.

Our ultrastructural analyses also revealed striking ectopic myelin-like membranes in *kif1b*^{st43} mutants (Fig. 6a–f) at the same time that we observed robust ectopic expression of myelin proteins in *kif1b*^{st43} mutant oligodendrocyte cell bodies. Myelin-like membrane was present in processes that did not ensheath axons, but extended over distances of several microns (Fig. 6b,d,e,f). In almost all of these cases the elongate myelin-like membranes were continuous with “normal” myelin surrounding axons (Fig.6d,f). In addition, we observed neuronal cell bodies surrounded by myelin-like membrane (Fig. 6e). These aberrant membranes were observed in every *kif1b*^{st43} mutant examined at 9 dpf (n=8) and were never observed in the wildtype. These observations suggest an unexpected function for *kif1b* in preventing the ectopic production of myelin-like membrane along primary oligodendrocyte processes (Fig. 6g–h). Thus, in addition to localizing specific mRNAs in order to maintain myelin production, mRNA may be localized to myelinating processes to exclude certain proteins from other regions of the oligodendrocyte, where they may exert deleterious effects. It is interesting in this regard that both MBP and 36K are small, highly basic proteins, as is MOBP, another protein whose mRNA is localized to myelinating processes in mammalian oligodendrocytes23. MBP is sufficient to compact model cell membranes in vitro24, emphasizing the potential need to restrict its localization to the proper region of the cell. Future analyses will identify additional Kif1b cargo(es) and define the minimal set of factors required to generate myelin-like membrane.

In conclusion, our study shows that *kif1b* is required to localize myelin mRNA to oligodendrocyte processes, to elaborate the correct amount of myelin around axons, and to prevent the ectopic production of myelin-like membrane. We also show that *kif1b* is

required for the normal development of certain axons in the PNS and CNS. How these functions might relate to increasing susceptibility to MS remains unclear. Given increasing evidence that some of the most debilitating symptoms of MS may derive from damage to axons25 it is possible, for example, that *kif1b* related disruption of axons or neurons could contribute to the disease. In addition, our analysis supports the possibility that a function for *kif1b* in oligodendrocytes might underlie a role in MS. Disruption of *kif1b* might also reduce the capacity of oligodendrocytes to remyelinate demyelinated axons characteristic of MS, and thus exacerbate symptoms of the disease. It is also possible that Kif1b may play a causal role in onset of the disease. MBP, for example, is a prominent autoantigen for human T-cells26, and certain MBP epitopes can activate specific autoimmune defects in MS patients27,28. Therefore mislocalization of MBP or other factors following disruption of *kif1b* may play a role in MS by increasing autoimmune attacks characteristic of the disease directly, or indirectly, by damaging the oligodendrocyte. Future work on the role of *kif1b* in MS susceptibility should consider its functions in both oligodendrocytes and neurons.

Methods

Positional Cloning of *kif1b*^{st43}

We previously mapped *st43* to linkage group 2310. To pursue the positional cloning of the mutated gene, we scored polymorphic markers in the region of *st43* in large mutant mapping crosses. This analysis identified a marker approximately 0.1 cM (1 recombinant among 1092 meioses) from *st43*. Partial sequencing of candidate genes in the vicinity of this marker indicated that the *st43* mutation converts a conserved threonine to a proline in zebrafish *kif1b*. The *st43* mutation was scored by PCR as described (Supplementary Methods and Supplementary Table 1). Full-length *kif1bβ* was cloned from larval cDNA using Phusion (Finnzyme), (Supplementary Methods and Supplementary Table 1).

Immunohistochemistry

The following antibodies were used: mouse anti-acetylated-tubulin (1:1000, Sigma), rabbit anti-MBP21, mouse anti-SV2 (1:200, DSHB), rabbit anti-36K22, mouse and rabbit anti-GFP (Molecular probes), anti-Oregon green (Molecular probes), AlexaFluor-conjugated secondary antibodies (1:2000, Molecular Probes).

Synaptic vesicle protein labeling

25 pg of plasmid DNA encoding synaptophysin:GFP was injected into embryos at the one cell stage.

Kif1b morpholinos

Antisense MOs (Gene Tools) were designed to target a region surrounding the start codon of *kif1b*, the *kif1bα* specific splice junction and a *kif1bβ* specific splice junction (Supplementary Methods). The efficacy of specific splice junction MOs was assayed by RT-PCR (Supplementary Methods and Supplementray Table 1).

In situ hybridization

PCR products specific to the common domain of *kif1b*, to *kif1bα*, and to *kif1bβ* were cloned into pCRII-TOPO (Invitrogen) and sequenced. Riboprobes were synthesized using SP6 RNA polymerase for antisense and T7 for sense.

Transplants

Transplants were carried out as described 15 with modifications outlined in Supplementary Methods.

Transmission electron microscopy was carried out as described15. We used the g-ratio to assess the extent of myelination of axons (see Supplementary Methods).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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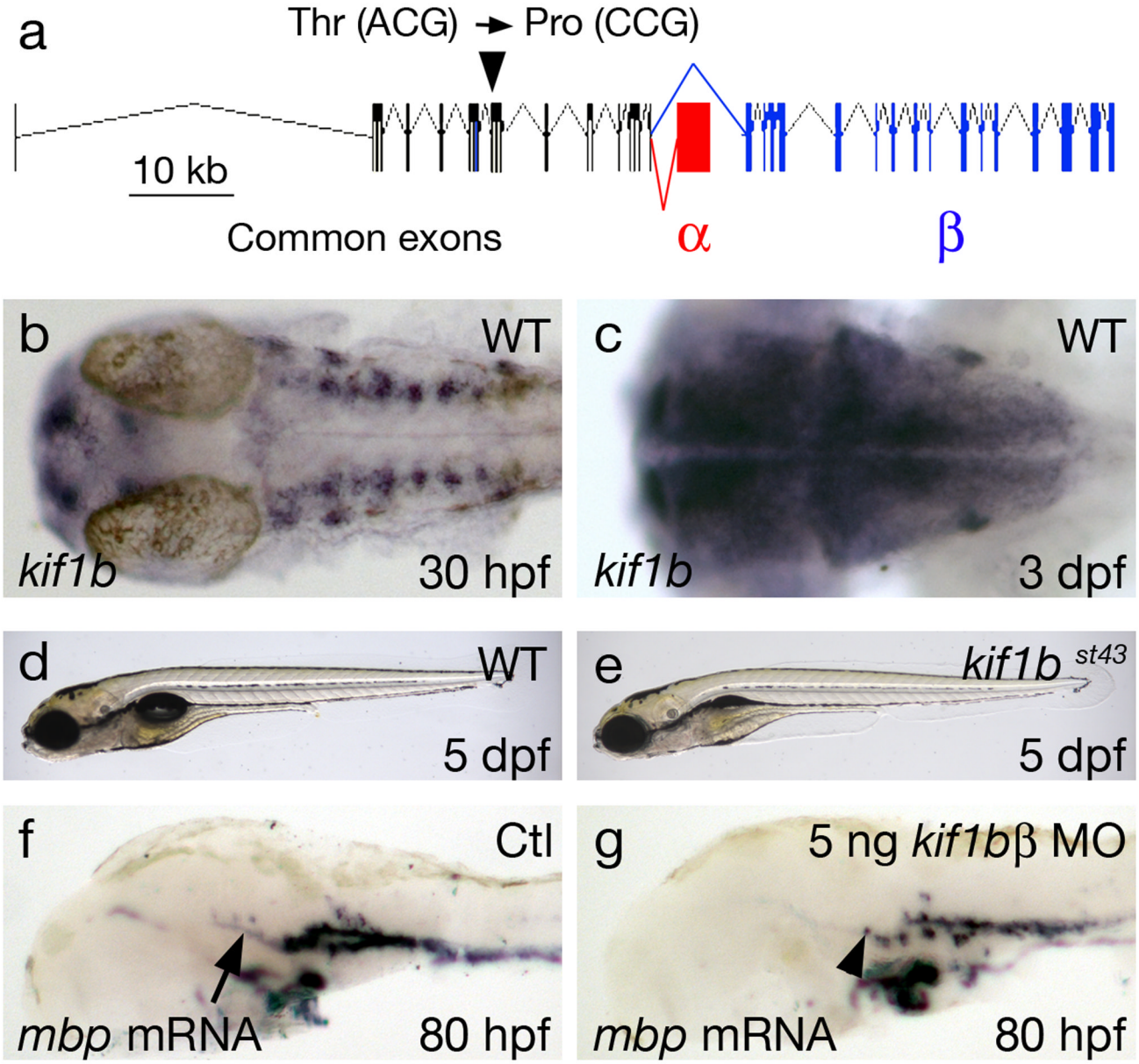

Figure 1. *kif1b* is essential for *myelin basic protein* mRNA localization in the CNS

a. Genomic structure of zebrafish *kif1b*. Exons common to *kif1bα* and *kif1bβ* are black, those specific to *kif1bα* are red and to *kif1bβ* blue. The *st43* mutation changes a threonine codon (ACG) to a proline codon (CCG) in an exon common to both isoforms (arrowhead).

b and c. Dorsal views of *kif1b* mRNA expression at 30 hours post fertilization (hpf) (b) and 3 dpf (c). Expression is strongest in differentiated neurons at 30 hpf and is widely expressed in the nervous system at 3 dpf.

d and e. Lateral views of live zebrafish larvae at 5 dpf shows that $kif1b^{st43}$ mutants (e) are morphologically similar to wildtype (d) despite not inflating their swim bladder.

f and g. Lateral views of dissected zebrafish heads at 80 hpf showing that *mbp* mRNA expression is localized primarily to cell bodies (arrowhead, g) in animals injected with a

kif1bβ specific morpholino but localized to cell bodies and myelinating processes (arrow, f) in controls.

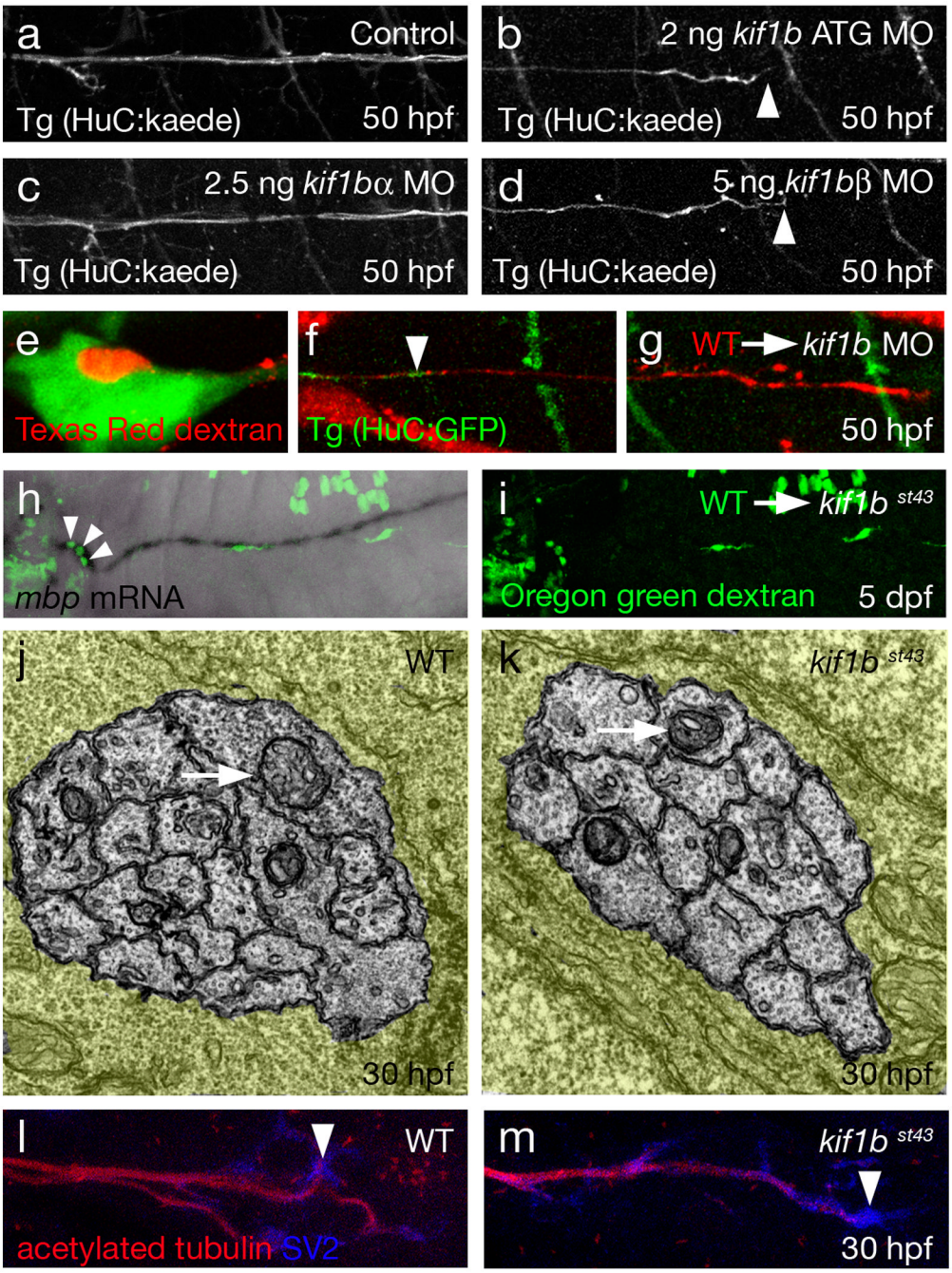

Figure 2. Kif1b is required for normal axonal outgrowth in the PNS

a–d Lateral views of larvae at 50 hpf expressing Tg(HuC:kaede) show that antisense morpholino oligonucleotide (MO) knockdown of the common start region of *kif1b* (b) and blocking a splice junction specific to *kif1bβ* (d) cause truncation of the PLLn (arrowheads). Disruption of *kif1bα* does not affect PLLn outgrowth (c).

e–g Three images of a PLLn chimera. Wildtype cells were transplanted into a Tg(HuC:GFP)-expressing *kif1b* ATG morphant. Wildtype axons (red) grow over a longer distance than *kif1b* morphant axons (green). Arrowhead in f (somite level 9) points to the tip of the longest *kif1b* morphant axon in the chimeric PLLn. g shows a wildtype axon at somite 24. Morphant axons never grew beyond the level of somite 10 (n=52 chimeras).

h and i. Wildtype neurons (arrowheads) transplanted into a *kif1b*^{st43} mutant are sufficient to restore *mbp* expressing Schwann cells along the length of the PLLn.
j and k. TEM images of transverse sections cut through the PLLn show mitochondria (arrows) present in *kif1b*^{st43} mutant axons (k) and wildtype (j) at 30 hpf.
l and m. Lateral views of embryos at 30 hpf stained with acetylated tubulin (red) and SV2 show that SV2 is present in growth cones at the leading tips of growing axons of the PLLn in both wildtype (1) and *kif1b*^{st43} mutant (m) embryos.

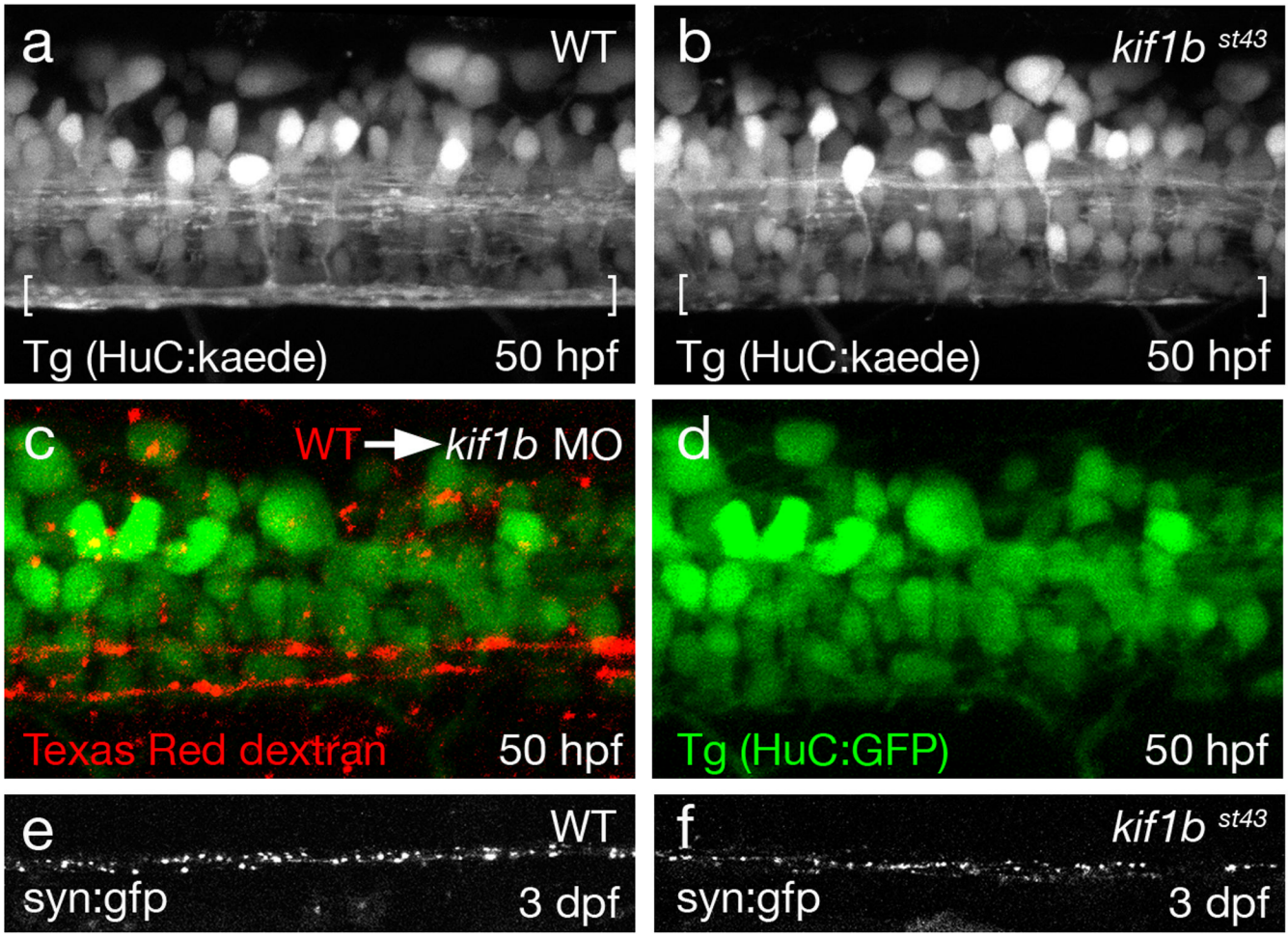

Figure 3. Kif1b is required for normal axonal outgrowth in the CNS

a–b. Lateral views of Tg(HuC:kaede) expressing larvae at 50 hpf showing a reduction in the number of axons in the ventral spinal cord (brackets) in *kif1b*^{st43} mutants (b) compared to wildtype (a).

c–d. Images of the posterior spinal cord of a chimera where wildtype cells were transplanted into a Tg(HuC:GFP) expressing *kif1b* ATG morphant. Wildtype axons (red) grow further than *kif1b* morphant axons (green), which cannot be seen in the ventral spinal cord at this posterior level (somite 27–28).

e–f. Lateral views of embryos injected with the transgene synaptophysin:gfp show similar distribution of this synaptic vesicle protein in *kif1b*^{st43} mutant (f) and wildtype (e) axons.

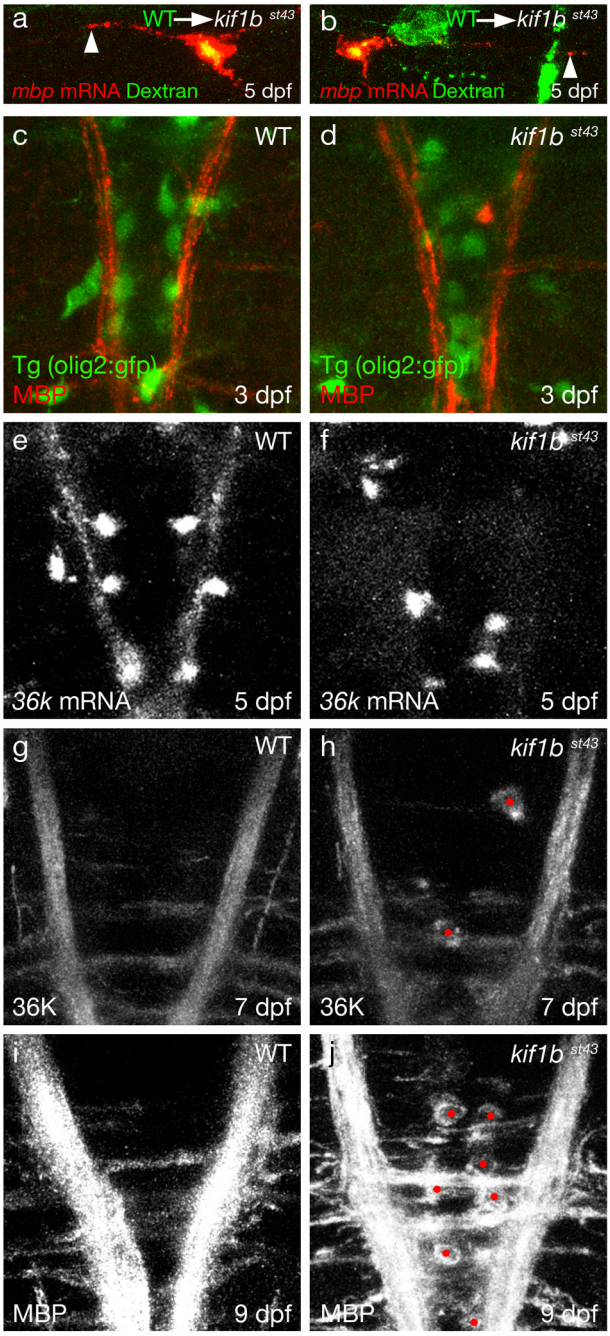

Figure 4. Kif1b functions in oligodendrocytes to localize myelin mRNA and protein

a and b. Confocal images of wildtype oligodendrocytes (yellow) transplanted into a *kif1b*^{st43} mutant. Arrowheads indicate *mbp* mRNA localized in distal processes of the wildtype oligodendrocyte. Such distribution was never seen in mutant oligodendrocytes.

c and d. Dorsal views of the hindbrain at 3 dpf shows that expression of Tg(olig2:gfp) and Myelin Basic Protein (MBP) is similar in wildtype (c) and *kif1b*^{st43} mutants (d).

e and f. Dorsal views of the hindbrain of larvae at 5 dpf shows *36k* mRNA localized to oligodendrocyte cell bodies and myelinating processes in wildtype (e), but only to oligodendrocyte cell bodies in *kif1b*^{st43} mutants (f).

g and h. Dorsal views of the hindbrain at 7 dpf show robust expression of 36K protein in myelinating oligodendrocyte processes in both wildtype (g) and *kif1b*^{st43} mutant (h) larvae, and ectopic expression of 36K protein in *kif1b*^{st43} mutant cell bodies (red dots).
i and j. Dorsal views of the hindbrain at 9 dpf show robust expression of MBP protein in myelinating oligodendrocyte processes in both wildtype (g) and *kif1b*^{st43} mutant (h) larvae, and ectopic expression of MBP protein in *kif1b*^{st43} mutant cell bodies (red dots).

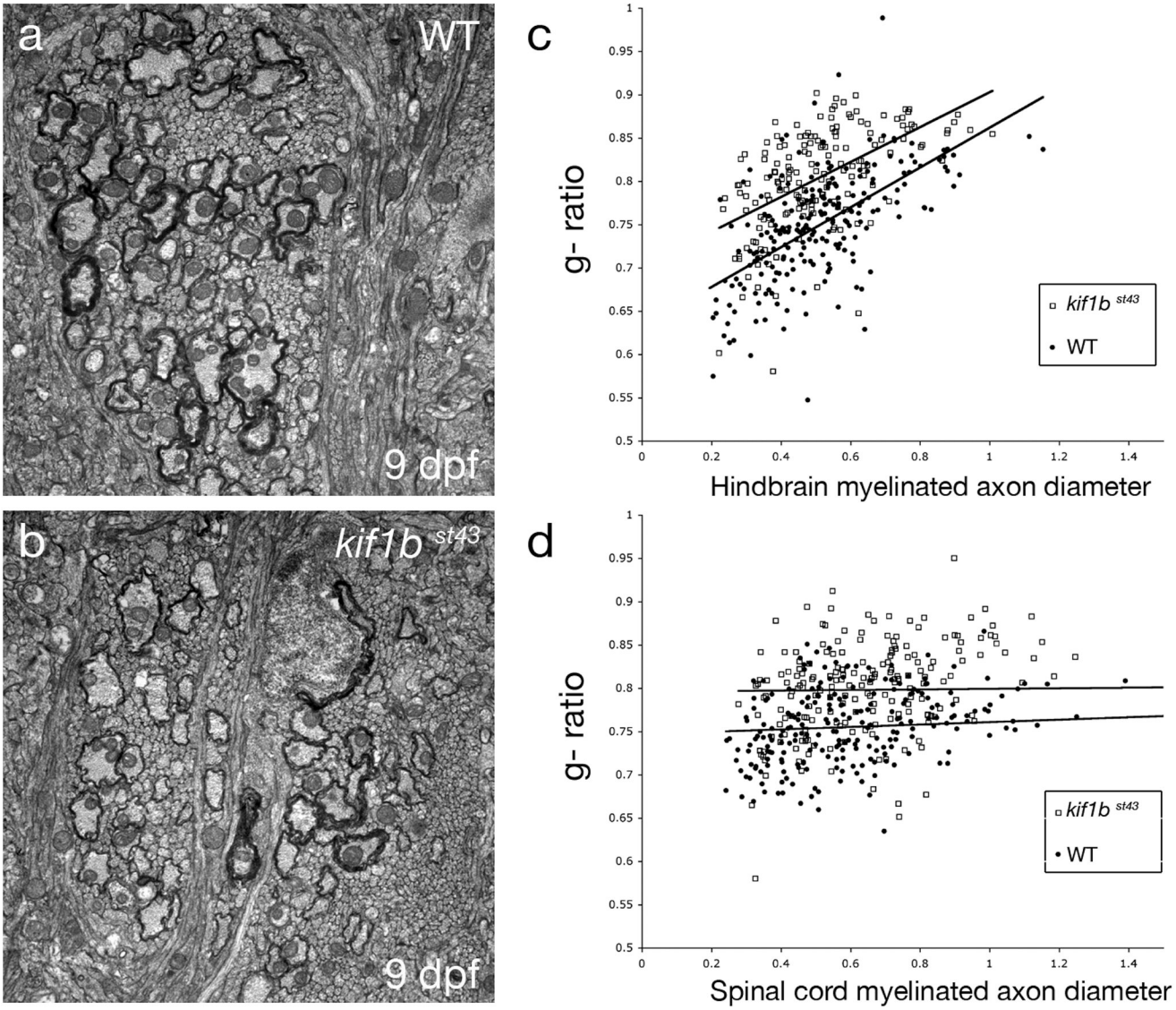

Figure 5. Abnormalities in myelinated axons in *kif1b*^{st43} mutants

a and b. TEM images of transverse sections through myelinated axon tracts in the anterior hindbrain in (a) wildtype and (b) *kif1b*^{st43} mutant animals. There are fewer myelinated axons in the in the *kif1b*^{st43} mutant and these axons also have a slight reduction in myelin relative to wildtype.

c and d. Scatter plots of myelinated axon g-ratios at different axon diameters in the anterior hindbrain (c) and spinal cord (d). There is an overall increase in g-ratio (reduction in myelin thickness) in *kif1b*^{st43} mutant axons (higher trend lines in c and d) compared to wildtype. No myelinated axons have a diameter greater than 1.5μm in either the wildtype or mutant anterior hindbrain at the stage examined. The small number of reticulospinal axons with a diameter greater than 1.5μm in the spinal cord at this stage have been omitted from (d).

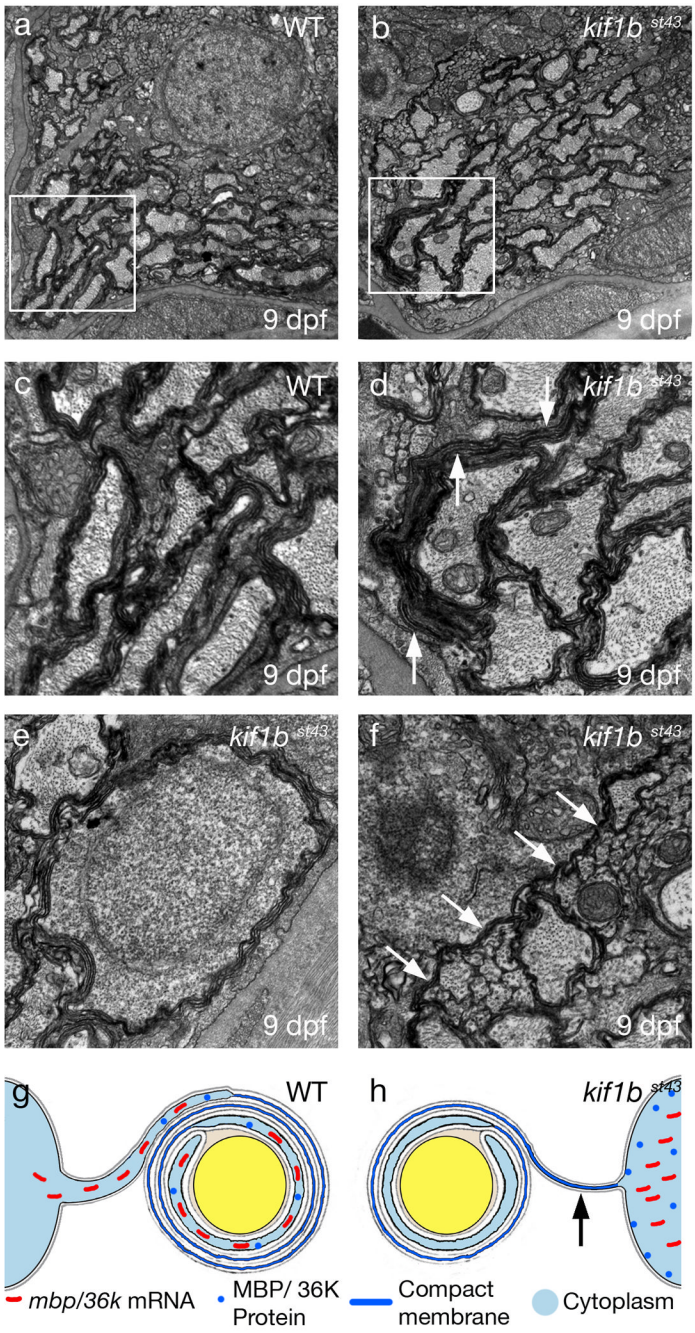

Figure 6. Ectopic myelin–like membrane in *kif1b*^{st43} mutants

a and b. TEM images of transverse sections through myelinated axon tracts in the anterior spinal cord. There is a similar distribution of large-diameter axons in wildtype (a) and *kif1b*^{st43} mutants (b). Boxes indicate regions of higher magnification in c and d.

c and d. Higher magnification views of areas outlined by white boxes in a and b respectively. Ectopic myelin-like membranes are present in the *kif1b*^{st43} mutant (arrows).

e. TEM image of a transverse section through the spinal cord shows a neuronal cell body surrounded by myelin-like membrane in a *kif1b*^{st43} mutant.

f. TEM image of a transverse section through the anterior spinal cord showing another ectopic process of myelin-like membrane (arrows).

g–h. Cartoon depicting defects of *kif1b*^{st43} mutant oligodendrocytes. Wildtype oligodendrocytes (g) localize *mbp* and *36k* mRNAs to their myelinating processes. *mbp* and *36k* mRNA is localized almost exclusively in the cell body of *kif1b*^{st43} mutants (h). *kif1b*^{st43} mutants have less myelin surrounding axons than wildtype, and also have ectopic myelin-like membrane along oligodendrocyte processes (arrow). MBP and 36K proteins are expressed ectopically in *kif1b*^{st43} mutant oligodendrocyte cell bodies.