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

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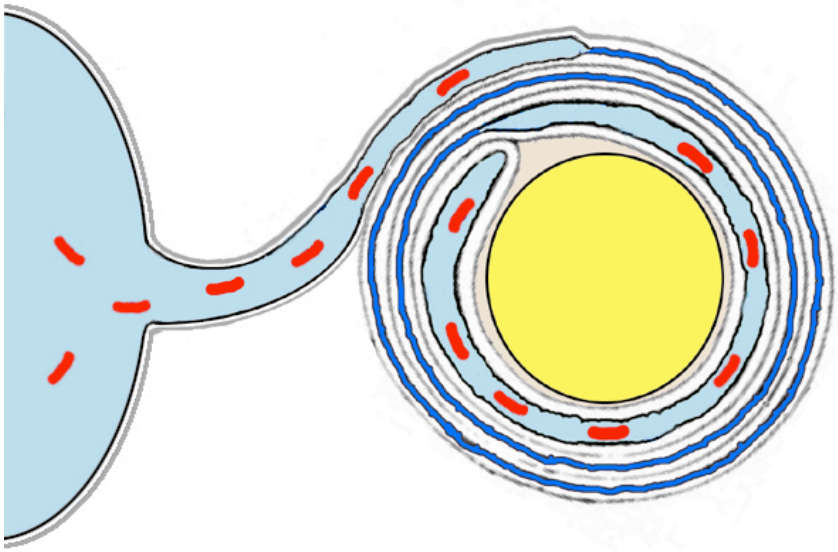
Supplementary Figure 1. Cartoon of mRNA localization in a wildtype oligodendrocyte.



Certain mRNAs (red) including those of *myelin basic protein (mbp)* are localized to the cytoplasm (light blue) of myelinating processes in addition to that of the oligodendrocyte cell body. Compact myelin is indicated in dark blue surrounds an axon (yellow).

Supplementary Methods

kif1b ^{st43} genotyping

The *st43* mutation was scored throughout using the "*st43* mutation" primers listed in Supplementary Table 1. The 180 bp mutant PCR product is digested with BsrB1 into two fragments, 97 bp and 83 bp long. The wildtype product is not cut by BsrB1.

Kif1b Morpholinos

Morpholino antisense oligonucleotides (Gene Tools) targeted the region surrounding the common *kif1b* start codon, the *kif1bα* specific splice junction, and a *kif1bβ* specific splice junction (see MO sequences in Supplementary Table 1).

2 ng of the common *kif1b* ATG MO was injected into each embryo at the 1 cell stage.
2.5 ng of the *kif1bα* specific MO was injected into each embryo at the 1 cell stage.
5 ng of the *kif1bβ* specific MO was injected into each embryo at the 1 cell stage.
No morphological defects were detected at these concentrations.

The penetrance of abnormal axon outgrowth in the PLLn and ventral spinal cord was 100% for the *kif1b* ATG MO and the *kif1bβ* specific MO at all stages examined (n=154 and n=83 respectively). The *kif1bα* specific MO never generated an axon outgrowth defect (n>50). The efficacy of specific splice junction Morpholinos was assayed by RT-PCR (see Supplementary Table 1 for primer sequences). RT-PCR confirmed the aberrant splicing of both *kif1bα* and *kif1bβ*. Some correctly spliced *kif1bβ* was observed at 80 hpf. This is likely to account for the incomplete penetrance of the *mbp* mRNA mislocalization phenotype observed at this stage (60%, n=20).

Transplantation

Three distinct sets of transplant experiments were carried out in this study. To assay axon outgrowth directly in chimeras wildtype embryos were injected with 0.5% Texas Red Dextran (Invitrogen). Cells from these embryos were transplanted into Tg(HuC:GFP) expressing embryos that had been injected with 2ng of the *kif1b* ATG MO. Chimeras were imaged live at 50 hpf.

To examine *mbp* mRNA expression along the PLLn, 1% w/v Oregon green dextran (Invitrogen) was used as a lineage tracer to label host wildtype embryos embryos. Larvae were fixed at 5 dpf, and *mbp* mRNA was labeled by in situ hybridization, and Oregon green with an anti-Oregon green antibody.

To examine *mbp* mRNA localization in oligodendrocytes host wildtype embryos were also labeled with 1% Oregon green dextran and fixed at 5 dpf for *mbp* in situ hybridization. In this case embryos were dissected to expose the brain prior to fluorescent in situ hybridization. Oregon green was detected by an anti-Oregon green antibody.

Determination of g-ratio

The g-ratio is the numerical value given to the diameter of an axon divided by the diameter of that axon plus its myelin sheath. In order to determine the G-ratio we measured the cross sectional area of individual axons and also of those axons plus their myelin sheathes. We determined the value of respective diameters from these data. Measurements were performed using ImageJ.

Supplementary Table 1. Morpholino and primer sequences.

Morpholinos	**Sequence**	
kif1b ATG MO	CCAGACATGGTTGAAGCTGTTGTGA	
kif1bα MO	CCATCCTTGAGAGAACAAAACAGGA	
kif1bβ MO	GCAATAAATGACTAGCGTACCTCCC	
Primers	**Forward (5'-3')**	**Reverse (5'-3')**
st43 mutation	AATGTGCTTGTTTACCAGGAGG	CATGGAAAAGCACAAGTTACTG
kif1bα MO RT-PCR	CAGAAATGGAGATCCTGTACAAA	AGATGGTCACCCATTTGGAG
kif1bβ MO RT-PCR	GGACCAGATGCGAGAGATGT	TGAACCATGGAGAACGATCA
Full-length *kif1bβ*	CGTGCAGGATTTCTATGGT	CACACTTGGTACGCTGTAAAGG
kif1b common domain probe	ACTTGGGCCGTATGTTGAAG	CTGATTCCTTGAGCCTCTCG
kif1bα probe	AACCAACGGTAGAGGTGTGG	TCCGGTTTCCAGAATTGAAG
kif1bβ probe	GGAGGATGAAGTGCCCTGTA	GTGAGCTCCCAACCAGCTTA