DYNLRB1 is essential for dynein mediated transport and neuronal survival

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ABSTRACT

The cytoplasmic dynein motor complex transports essential signals and organelles from the cell periphery to the perinuclear region, hence is critical for the survival and function of highly polarized cells such as neurons. Dynein Light Chain Roadblock-Type 1 (DYNLRB1) is thought to be an accessory subunit required for specific cargos, but here we show that it is essential for general dynein-mediated transport and sensory neuron survival. Homozygous Dynh1 null mice are not viable and die during early embryonic development. Furthermore, heterozygous or adult knockout animals display reduced neuronal growth, and selective depletion of Dynh1 in proprioceptive neurons compromises their survival. Conditional depletion of Dynh1 in sensory neurons causes deficits in several signaling pathways, including β-catenin subcellular localization, and a severe impairment in the axonal transport of both lysosomes and retrograde signaling endosomes. Hence, DYNLRB1 is an essential component of the dynein complex, and given dynein's critical functions in neuronal physiology, DYNLRB1 could have a prominent role in the etiology of human neurodegenerative diseases.

1. Introduction

Molecular motors perform a critical function in eukaryotic cells, ensuring the anterograde (kinesins) and retrograde (dyneins) transport of cargoes, organelles and cellular components. Dynein motors are divided into two major classes: axonal dyneins, which are involved in ciliary and flagellar movement, and cytoplasmic dyneins, minus-end microtubule molecular motors involved in vesicular transport, cell migration, cell division and maintenance of Golgi integrity (Hirokawa et al., 2010; Kardon and Vale, 2009; Reck-Peterson et al., 2018). Cytoplasmic dynein is the main retrograde motor in all eukaryotic cells and is particularly important in highly polarized cells such as neurons, where it carries essential signals and organelles from the periphery to the cell body (Terenzio et al., 2017). Cytoplasmic dynein consists of two heavy chains (HC) associated with intermediate chains (IC), which provide direct binding to dynactin (Karki and Holzbaur, 1995; King et al., 2003; Siglin et al., 2013), four light intermediate chains (LIC) and several light chains (LC) (Reck-Peterson et al., 2018). There are three families of dynein light chains, LC7/road-block, LC8 and Tctx1/rp3, the latter playing a role in the direct binding of cargoes to the dynein motor complex (Olenick and Holzbaur, 2019; Reck-Peterson et al., 2018; Tai et al., 1999).

Dynein Light Chain Roadblock-Type 1 (DYNLRB1), also known as Km23, and DNLC2A (Jiang et al., 2001; Tang, 2002), was first identified in Drosophila during a genetic screen for axonal transport mutants (Bowman et al., 1999). Roadblock mutants exhibited defects in intracellular transport, including intra-axonal accumulation of synaptic cargoes, severe axonal degeneration and aberrant chromosome segregation (Bowman et al., 1999). DYNLRB protein sequence is highly conserved among different species; with two isoforms, DYNLRB1 and DYNLRB2, sharing 98% sequence similarity in mammals (Susalka et al., 2002). Mammalian DYNLRB1 has been studied mainly as an adaptor linking specific modules to the dynein complex, including SMAD2 complexes activated by TGFβ receptors (Jin et al., 2013, 2012, 2009, 2007, 2005; Tang, 2002) and Rab6 or N-acetyl-D-glucosamine kinase (NAGK) for interactions with the Golgi compartment (Islam et al., 2020).
In previous work, we identified a role for the dynein complex in regulating axon growth rates (Perry et al., 2016; Rishal et al., 2012). A screen of all dynein accessory subunits now shows that knockdown of Dynlrb1 reduces neurite outgrowth in cultured sensory neurons. Surprisingly, a complete knockout of Dynlrb1 was found to be embryonic lethal, suggesting the existence of non-redundant functions between DYNLRB1 and DYNLRB2. Conditional knockout or acute knockdown of Dynlrb1 compromised survival of proprioceptive neurons, together with changes in subcellular localization of transported cargos, and major impairments in the axonal transport of lysosomes and retrograde signaling endosomes. Thus, DYNLRB1 is an essential component of the dynein complex, and the conditional allele described here enables selective targeting of dynein functions.

2. Results

2.1. Depletion of Dynlrb1 impairs neurite outgrowth in cultured DRG neurons

We performed an siRNA screen in cultured dorsal root ganglion (DRG) neurons to investigate the effects of downregulating individual dynein subunits on axonal growth. Knockdowns of Dync1i2, Dynclii2, Dynlrb1 and Dctn6 reduced the extent of axon outgrowth in cultured DRG neurons (Fig. 1A), with Dynlrb1 knockdown providing the most robust effect (Fig. 1A, B). We then sought to validate this finding in a knockout mouse model for Dynlrb1 generated by the European Conditional Mouse Mutagenesis Program (EUCOMM) as part of the International Mouse Knockout Consortium. The gene targeted Dynlrb1tm1a(EUCOMM)Wtsi allele, hereafter referred to as Dynlrb1tm1a, results in a complete knockout of Dynlrb1 with concomitant expression of β-galactosidase from the same locus; this allele also allows the subsequent generation of a floxed conditional allele (Dynlrb1tm1a) upon application of Flp.
recombinase (Supplementary Fig. 1A)(Skarnes et al., 2011). Homozygous complete knockout mice (Dynlrb<sup>tm1a/tm1a</sup>) were not viable, and analyses of early pregnancies showed loss of homozygous null embryos before E9.5, while heterozygous animals were viable. The β-galactosidase expression pattern in heterozygous mice showed that DYNLRB1 was highly expressed in DRGs of E12.5 mouse embryos (Supplementary Fig. 1B). We then crossed (Dynlrb<sup>tm1a/tm1a</sup>) heterozygous mice with Thy1- yellow fluorescent protein (YFP) mice (Feng et al., 2000) to allow live imaging of DRG neuron growth in culture. Similarly to Dynlrb<sup>1</sup> knockout neurons (Fig. 1A, B), Thy1-YFP- Dynlrb<sup>tm1a/tm1a</sup> DRG neurons had significantly reduced axon outgrowth compared to littermate controls (Fig. 1C, D).

2.2. Dynlrb<sup>1</sup> deletion affects survival of proprioceptive neurons

We proceeded to cross Dynlrb<sup>tm1a/tm1a</sup> heterozygotes with mice expressing the Flp recombinase (Farley et al., 2000) to recombine the FRT sites, thereby removing the LacZ sequence (Sadowski, 1995). This generated a conditional allele, Dynlrb<sup>tm1c</sup> in which LoxP sites flank exon 3 of Dynlrb<sup>1</sup> (Supplementary Fig. 1A). Dynlrb<sup>tm1c</sup> mice were then crossed with a RNX3-Cre driver line (Levanon et al., 2011), to examine the effect of Dynlrb<sup>1</sup> deletion in proprioceptive sensory neurons. In contrast to the full knockout, RNX3-Dynlrb<sup>tm1a/tm1a</sup>, hereafter referred to as RNX3-Dynlrb<sup>1</sup>−/− mice were viable to adulthood. However, these mice displayed abnormal hind limb posture (Fig. 2A), and an uncoordinated walking pattern with abdomen posture close to the ground (Supplementary movie S1). A battery of tests of motor activity and proprioception showed that RNX3-Dynlrb<sup>1</sup>−/− mice were unable to balance themselves in the rotarod (Fig. 2B) and exhibited an abnormal walking pattern in the catwalk (Fig. 2C, D). There was no difference between genotypes in basal motor activity (Supplementary Fig. 2A), but RNX3-Dynlrb<sup>1</sup>−/− mice displayed lower speed and covered less distance than their wild type counterparts in the open field (Supplementary Fig. 2B-D). Overall, these tests reveal a strong impairment of proprioception in adult RNX3-Dynlrb<sup>1</sup>−/− mice.

To understand the mechanistic basis of this impairment, we performed histological analyses on RNX3-Dynlrb<sup>1</sup>−/− mice. There was no difference between genotypes in neuromuscular junction size or numbers (Supplementary Fig. 2E, F), and in total (TuJ1 positive) or nociceptive (CGRP positive) skin innervation (Supplementary Fig. 2G, H). However, the number of proprioceptive NFH-positive neurons in DRG ganglia was greatly reduced in RNX3-Dynlrb<sup>1</sup>−/− mice (Fig. 2E, F), while CGRP-positive nociceptive neuron numbers did not differ between genotypes (Fig. 2E, F). These data reaffirm the specificity of the RNX3-Cre driver and indicate that the proprioceptive impairment observed in RNX3-Dynlrb<sup>1</sup>−/− mice is due to a loss of this class of sensory neurons.

2.3. Acute viral-mediated knockdown of Dynlrb<sup>1</sup> causes motor-balance defects in vivo

The RNX3-Cre driver is activated early in development. In order to determine whether Dynlrb<sup>1</sup> deletion has similar effects upon acute depletion in adult proprioceptive neurons, wild type mice were injected intrathecally with adeno-associated virus (AAV) harboring an shRNA against Dynlrb<sup>1</sup> or an shControl sequence. We used AAV serotype 9 for neuronal-specific knockdown (Naso et al., 2017; Snyder et al., 2011), and observed transduction rates of 25–35% in DRG ganglia (Fig. 3A, B). Efficiency of the knockdown was found to be in the range of 40% decrease in Dynlrb<sup>1</sup> mRNA levels in cultured DRG (Fig. 3C). 32 days after the injection, mice transduced with AAV-shDynlrb<sup>1</sup> developed motor problems and abnormal hind limb posture (Fig. 3D). AAV-shDynlrb<sup>1</sup> transduced mice showed significant impairment in rotarod and catwalk analyses when compared with AAV-shControl transduced animals (Fig. 3E-G). Overall these data show that Dynlrb<sup>1</sup> is critical for adult sensory neurons and its depletion by knockdown recapitulates the phenotype observed in the RNX3-Dynlrb<sup>1</sup>−/− mice.

2.4. Gene expression changes in Dynlrb<sup>tm1a/tm1a</sup> DRG neurons

We then sought to obtain insights on the physiological role(s) of DYNLRB1. Since complete depletion of DYNLRB1 is lethal, we focused on Dynlrb<sup>tm1a/tm1a</sup> DRG neurons, which are viable and display reduced neurite outgrowth. RNA-seq of Dynlrb<sup>tm1a/tm1a</sup> versus wild type DRG neurons at 12, 24 and 48 h in culture showed almost no differences between the two genotypes at 12 and 24 h, while significant differences were observed at 48 h (Fig. 4A, B, Supplementary Table S1). Bioinformatic analyses of the differentially expressed genes dataset revealed changes in a number of signaling and transcriptional pathways, including the canonical Wnt – β-catenin pathway (Fig. 4B, C, Supplementary Table S2). Interestingly, direct interaction between a Dynein intermediate chain and β-catenin has been shown in epithelial cells (Ligon et al., 2001), where the complex tethers microtubules at adherens junctions. Thus, DYNLRB1 might be part of a dynein complex carrying β-catenin from the periphery to the nucleus. Indeed, β-catenin nuclear accumulation at 48 h in culture is reduced in Dynlrb<sup>tm1a/tm1a</sup> DRG neurons compared to their wild type littermates (Fig. 4D, E).

2.5. Depletion of Dynlrb<sup>1</sup> has significant effects on axonal retrograde transport

The broad spectrum of gene expression changes in Dynlrb<sup>tm1a/tm1a</sup> DRG neurons argues for a general role of this subunit in the dynein complex. We therefore investigated the effect of DYNLRB1 depletion on dynein-mediated axonal retrograde transport, using acute depletion of Dynlrb<sup>1</sup> in culture to circumvent the loss of neurons observed in RNX3-Dynlrb<sup>1</sup>−/− mice. To this end, we transduced Dynlrb<sup>tm1c/tm1c</sup> DRG neurons with Adenovirus 5 (Ad5) expressing a Cre-GFP, to induce recombination of the Dynlrb<sup>tm1</sup> allele to Dynlrb<sup>mid/mid</sup> in infected cells. We first optimized the transduction protocol using neurons from the A9 mouse (Madsen et al., 2010), which expresses td-Tomato under the control of a LoxP-flanked STOP cassette and showed that 72 h of incubation with Ad5 Cre-GFP are sufficient to express the Cre and trigger the recombination of LoxP sites in cultured DRG neurons (Supplementary Fig. 3A, B). We then tracked axonal retrograde transport of acidic organelles, including lysosomes, late endosomes and autophagosomes in Ad5-Cre: Dynlrb<sup>mid/mid</sup> DRG neurons using Lysotracker Red. Indeed, Ad5-Cre: Dynlrb<sup>mid/mid</sup> transduced neurons 96 h in culture showed a significant impairment of axonal retrograde transport of these organelles, both in terms of velocity of individual carriers and the overall fraction of moving carriers (Fig. 5A-C).

We further examined transport properties using a marker for neurotrophin signaling endosomes, the binding fragment of tetanus neurotoxin (H<sub>T</sub>) fragment (Deinhardt et al., 2006; Lalli and Schiavo, 2002). Whilst we could not detect any significant differences in the rates of transport of H<sub>T</sub>-positive retrograde axonal carriers in Ad5-Cre: Dynlrb<sup>mid/mid</sup> neurons (Fig. 5D, E), the percentage of stationary carriers was significantly increased (Fig. 5F), as was also found for Lyso tracker Red-positive organelles (Fig. 5C). Thus, depletion of Dynlrb<sup>1</sup> has significant effects on retrograde transport of a broad spectrum of dynein cargos.

3. Discussion

The dynein complex has essential roles in all multicellular organisms, most prominently in long distance axonal transport in neurons. Studies on specific roles of dynein carriers have suffered from a lack of appropriate mouse models, since homozygous deletion or mutation of Dyn1h1 results in early embryonic death (Hafezparast et al., 2003; Harada et al., 1998). We have characterized a new conditional knockout model for the DYNLRB1 subunit of dynein and use this model to show that DYNLRB1 is required for retrograde axonal transport of a
Fig. 2. Conditional Dynlrb1 depletion affects survival of proprioceptive neurons in vivo.

A: Abnormal clenched hind-limb phenotype in RNX-Dynlrb1−−/− mice suspended from the tail, compared to the typical limb extension of wild type animals.

B: Rotarod test (acceleration set at 20 rpm in 240 s) showing that RNX-Dynlrb1−−/− mice are unable to balance themselves on the rod. Mean ± SEM, *** p < .001, n > 6 mice per genotype, two-way ANOVA.

C: Representative catwalk gait traces for wild type and RNX-Dynlrb1−−/− mice. The position of the paws are shown in different colors as indicated, RF - right front, RH - right hind, LF - left front, LH - left hind.

D: Step sequence regularity index and Paw stands from the catwalk gait analyses of C shows impairment in RNX-Dynlrb1−−/− mice compared to wild type. Mean ± SEM, * p < .05 *** p < .001, n > 6 mice per genotype, one-way ANOVA followed by Tukey’s HSD post hoc correction for multiple comparisons.

E: Representative DRG ganglia sections from wild type and RNX-Dynlrb1−−/− mice, stained with either NFH (green) for proprioceptive neurons or CGRP (red) for nociceptors. Scale bar, 50 μm.

F: Quantification of NFH and CGRP positive neurons from the experiment described in G. Mean ± SEM, * p < .05, n = 3 per genotype, unpaired t-test.
broad spectrum of cargos in sensory axons, as well as for neuronal survival. These findings were surprising given the notion that DYNRLB1 should function mainly as a binding adaptor for TGFβ signaling complexes and other specific cargos (Jin et al., 2013, 2012, 2009, 2007, 2005; Tang, 2002), whereas our data suggests that DYNLRB1 is essential for dynein-mediated axonal transport of lysosomes, late endosomes and signaling endosomes.

The hindlimb proprioception phenotype observed in both RNX3-Dynlrb1<sup>−/−</sup> mice and Dynlb1 viral-mediated knockdown is highly reminiscent of dynein heavy chain mutant models, such as Dynch1<sup>Loa</sup> and Dynch1<sup>Cra1</sup> (Dupuis et al., 2009; Hafezparast et al., 2003). Both models show deficits in endosomal axonal retrograde transport (Hafezparast et al., 2003) and proprioception (Dupuis et al., 2009; Ilieva et al., 2008), as we have shown above for knockout or

(caption on next page)
knockdown of Dynlrb1. In addition, depletion of Roadblock in Drosophila was described to cause defects in intra-axonal accumulation of cargoes and severe axonal degeneration (Bowman et al., 1999). The distal enrichment of axonal accumulations observed in Roadblock mutants was suggested to arise from axonal transport deficits originating at the synapse (Bowman et al., 1999). Moreover, incorporation of DYNLRB1 and DYNLRB2 into the Dynactin-2 complex, which drives retrograde protein trafficking in primary cilia, was recently shown to be crucial for dynein-2 transport function (Tsurumi et al., 2019). While the precise mechanism by which dynein activity is impaired upon loss of Dynlrb1 is yet to be determined, it is interesting to note that this subunit can bind directly to core components of the dynein complex such as the intermediate chains DYNC1I1 and DYNC1I2 (IC74 – 68097 NM_026556) (Susalka et al., 2002). The latter directly associate with the dynactin complex, which facilitates interactions of numerous cargos with the main motor complex, and expedites processive transport along microtubules (Karki and Holzbaur, 1995; Peck-Peterson et al., 2018).

Our findings show that Dynlrb1 is critical not only for embryonic development, but also for maintenance of sensory neurons in adulthood. Deficits in dynein functions have been implicated in the pathogenesis of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) (Bilsland et al., 2010; Gershoni-Emek et al., 2016; Hoang et al., 2017; Schiavo et al., 2013; Teuling et al., 2008; Xie et al., 2018). The new mouse model described herein will expand the options for studying such roles of dynein, using conditional targeting of Dynlrb1 for both temporal and tissue/cell type specificity in studies in vivo.

4. Materials and methods

Key resource table

| Reagent or resource | Source | Identifier |
|---------------------|--------|------------|
| Antibodies          |        |            |
| NFH (Chicken)       | Millipore | Cat Number: AB114P |
| Tuji (Rabbit)       | Covance  | RRID: AB:2313773 |
| GCRP (Goat)         | Abcam   | RRID: AB:725807 |
| β-catenin (Mouse)   | A. Ben-Ze’ev lab (Johnson et al., 1992; Simcha et al., 1998) | Clone 5H10 |
| GFP (Rabbit)        | MBL International | RRID: AB:10597267 |
| Alexa Fluor 488 secondary antibody (Donkey anti-rabbit) | Thermo Fisher Scientific | RRID: AB:2556546 |

siRNA screen Dharmacon smartpools

| Catalog number | Gene symbol | Locus ID | Accession |
|----------------|-------------|----------|-----------|
| D-001210-02    | Non-targeting siRNA | N/A | N/A |
| M-043143-01    | Dyncl1      | 13426    | NM_010663 |
| M-043171-01    | Dyncl2      | 13277    | NM_010644 |
| M-051314-01    | Dyncl3      | 235661   | NM_042797 |
| M-056180-01    | Dyncl4      | 234663   | NM_010138 |
| M-062961-01    | Dyncl5      | 213575   | NM_172256 |
| M-055779-01    | Dyncl6      | 216487   | NM_093442 |
| M-059901-01    | Dyncl7      | 67017    | NM_029975 |
| M-044820-01    | Dyncl8      | 67068    | NM_029974 |
| M-050822-01    | Dyncl9      | 75465    | NM_029979 |
| M-063157-01    | Dyncl10     | 56455    | NM_019682 |
| M-059592-01    | Dyncl11     | 68097    | NM_026556 |
| M-024820-01    | Dyncl12     | 13191    | NM_007835 |
| M-056851-01    | Dyncl13     | 69654    | NM_027151 |
| M-063022-00    | Dyncl14     | 53958    | NM_016890 |
| M-063074-00    | Dyncl15     | 67665    | NM_026302 |
| M-063270-00    | Dyncl16     | 59288    | NM_021608 |
| M-047876-00    | Dyncl17     | 22428    | NM_011722 |
| M-049777-00    | Dyncl18     | 56444    | NM_019785 |

Contact for reagent and resources sharing

Further information and requests for resources and reagents should be directed to Marco Terenzio (marco.terenzio@oist.jp).

Experimental model and subject details

Animal subjects

All animal procedures were approved by the IACUC of the Weizmann Institute of Science. The knockout mouse model for Dynnlrb1 was generated by the European Conditional Mouse Mutagenesis
RNA-seq of cultured Dynlrb<sup>tm1a</sup> DRG neurons highlights signaling deficits.

A: Bar charts representing the numbers of differentially expressed genes (contrast analysis in EdgeR, FDR < 0.1) in wild type and Dynlrb<sup>tm1a</sup> DRG neurons at the indicated time points in culture.

B: Heat map representing the differential expressed genes in wild type and Dynlrb<sup>tm1a</sup> mice at 48 h in culture (Log10(FPKM)).

C: Ingenuity pathway analyses on the 48 h differentially expressed genes dataset, ranked by −log(p values). The Wnt – β-catenin pathway (highlighted in red) was selected for further validation.

D: Representative cell bodies of wild type and Dynlrb<sup>tm1a</sup> DRG neurons cultured for 48 h before immunostaining as shown. Scale bar, 20 μm.

E: Quantification of nuclear versus cytoplasmic β-catenin in the experiment described in C. Mean ± SEM, * p < .05 ** p < .01, n > 3 mice per genotype, one-way ANOVA followed by Tukey’s HSD post hoc correction for multiple comparisons.
Fig. 5. Axonal retrograde transport deficits after Dynlrb depletion.
A: Representative kymographs of Lysotracker Red tracking on wild type and Dynlrb\textsuperscript{tm1c/tm1c} DRG neurons transduced for 96 h in culture as indicated (Ad5-Cre, Adenovirus5 expressing Cre-GFP) for 96 h. Diagonal lines indicate retrogradely moving carriers.
B: Velocity distributions from the experiment shown in A. Mean ± SEM, n > 32 axons per group over 3 independent biological repeats.
C: Fraction of moving versus stationary carriers in the experiment described in A. Mean ± SEM, *** p < .001, n > 32 axons per group over 3 independent biological repeats, two-way ANOVA.
D: Representative kymographs of TeNT H\textsubscript{T} tracking on wild type and Dynlrb\textsuperscript{tm1c/tm1c} DRG neurons transduced for 96 h in culture as indicated.
E: Velocity distributions from the experiment shown in D. Mean ± SEM, n > 36 axons per group over 3 independent biological repeats.
F: Fraction of moving versus stationary carriers in the experiment described in D. Mean ± SEM, ** p < .01, n > 36 axons per group over 3 independent biological repeats, two-way ANOVA.
Program (EUCOMM) as part of the International Mouse Knockout Consortium (IKMC, allele: Dynlrb1tm1a(EUCOMM)Wtsi) using a KO first targeting vector (MPGS8001_A.B08, IKMC Project 25156) (Skarnes et al., 2011). All mouse strains used were bred and maintained at the Veterinary Resource Department of the Weizmann Institute of Science. For live imaging of DRG outgrowth (Fig. 1A-D) C57BL/6J YFP16 mouse were used (Feng et al., 2000). All control mice were age-, sex-matched littermates. For testing Adenovirus5 Cre-GFP infectivity Ai9 mice were used (Madsen et al., 2010). Mice utilized for behavioral experiments were kept at 24.0 ± 0.5°C in a humidity-controlled room under a 12-h light–dark cycle with free access to food and water. Experiments were carried out on 2-6 months old male mice, unless specified otherwise.

**Generation of the Dynlrb1m1c and RNX3- Dynlrb1m1d/m1d lines**

The Dynlrb1 mice were acquired from the International Mouse Knockout Consortium (IKMC, allele: Dynlrb1tm1a(EUCOMM)Wtsi). The gene targeted Dynlrb1tm1a(EUCOMM)Wtsi allele, hereafter referred to as Dynlrb1m1a results in a complete knockout of Dynlrb1 with concomitant expression of β-galactosidase from the same locus; this allele also allows the subsequent generation of a floxed conditional allele (Dynlrb1m1s) upon application of the Flp recombinase (Skarnes et al., 2011). Dynlrb1m1s heterozygous mice were crossed with a mouse harboring the Flp recombinase in all tissues (FLP1ER - 129/129Sv/J (OSA2Z6SOR-P2P1/J)) (Farley et al., 2000), which was provided by the veterinary services of the Weizmann Institute of Science to recombine the FRT sites and so remove the LacZ sequence, generating a conditional allele, Dynlrb1m1c in which loxP sites flank a likely critical exon, exon 3, of Dynlrb1 (Skarnes et al., 2011, Suppl. Fig. 1A). We further crossed the Dynlrb1m1c mice with the propioceptive Cre-driver R26lacZ/Rnx3flcm1a (Levanon et al., 2011) to generate the RNX3-Dynlrb1m1d/m1d line, which we refer to in the text as RNX3-Dynlrb1 (Suppl. Fig. 1A) (Skarnes et al., 2011), in which Dynlrb1 is depleted in proprioceptive neurons.

**Methods**

**DRG culture**

Adult mice DRGs were dissociated with 100 U of papain followed by 1 mg/ml collagenase-II and 1.2 mg/ml dispase. The ganglia were then triturated in HBSS, 10 mM Glucose, and 5 mM HEPES (pH 7.35). Neurons were recovered through Percoll, plated on laminin, and grown in F12 medium (ThermoFisher Scientific) supplemented with 5 μM cytosine β-D-arabinofuranoside hydrochloride (AraC, Sigma-Aldrich, C6645) to inhibit glial proliferation. Adenovirus5 Cre-GFP was added to the neurons 4 h after plating. 96 h after infection with the virus, cells were washed in warm HBSS medium (ThermoFisher Scientific). LysoTracker™ Red DND-99 (ThermoFisher Scientific) (200 nM concentration) or AlexaFluor555-conjugated HCT Red DND-99 (ThermoFisher Scientific) was added to the neurons 4 h after infection. The ganglia were dissociated as previously described (Ben-Yaakov et al., 2012; Hanz et al., 2003; Perslon et al., 2005).

**siRNA screen**

Adult C57BL/6J YFP16 mouse DRG were dissociated and an siRNA screen was conducted as previously described (Rishal et al., 2012). Briefly, mouse siGenome smart pools (Dharmacon) were used for transfection of neuronal cultures two hours after plating, using DharmaFect 4 as the transfection reagent. 24 hours after siRNA transfection the neurons were plated in new poly-L-lysine and Laminin treated glass bottom 96 well plates at the same density (80,000-100,000 cells per well) and imaged 72 h after transfection. Images were captured at X10 magnification on ImageXpress Micro (Molecular Devices) and analyzed using the Metamorph software (Molecular Devices). The total outgrowth parameter was considered, defined as the sum of lengths of all processes and branches per cell. Statistical analyses were carried out with Student’s t-test and One Way ANOVA using the statistical software GraphPad Prism.

**Growth rate analysis**

Adult C57BL/6J YFP16 mouse DRG were dissociated as previously described (Rishal et al., 2012), and neurons were plated on MatTek glass bottom dishes (MatTek corporation). YFP6 - Wild type and YFP16-Dynlrb1+/− neurons were imaged at 1h intervals in a Fluoview (FV10i, Olympus) automated confocal laser-scanning microscope with built-in incubator chamber. Neuronal morphology was subsequently quantified using the Metamorph software (Molecular Devices). The total outgrowth parameter was considered, defined as the sum of lengths of all processes and branches per cell. Statistical analyses were carried out with a Two-way ANOVA test using the statistical software GraphPad Prism.

**Axonal transport experiments**

Wild type and Dynlrb1m1c DRG ganglia were dissociated as previously described and neurons were plated on MatTek glass bottom dishes (MatTek corporation) in F12 medium (ThermoFisher Scientific) supplemented with 10 μM cytosine β-D-arabinofuranoside hydrochloride (AraC, Sigma-Aldrich, C6645) to inhibit glial proliferation. Adenovirus5 Cre-GFP was added to the neurons 4 h after plating. 96 h after infection with the virus, cells were washed in warm HBSS medium (ThermoFisher Scientific). Adenovirus infection of DRG neurons from Ai9 mice harboring a LoxP-flanked STOP cassette preventing transcription of a CAG promoter-driven red fluorescent protein variant (td-Tomato). The virus was added directly into the F12 culture medium immediately after plating. Infected cultures were then incubated at 37°C for 48 and 72 h. After each time point, neurons were washed in PBS, fixed in 4% PFA in PBS for 30 min and immunostained with an α-GFP antibody (1:1000) as previously described. Coverslips were then mounted with Fluoromount-G™ and imaged using the ImageXpress (Molecular Devices) automated microscope at 10X magnification or using a confocal laser-scanning microscope (Olympus FV1000, 60x oil-immersion objective Olympus UPLSAO - NA 1.35). Image quantification was performed using the proprietary software Metamorph (Molecular Devices). Statistical analysis was carried out with a one-way ANOVA followed by Tukey post-hoc multiple comparison test using the statistical software GraphPad Prism.

**β-Catenin nuclear accumulation in Dynlrb1+/− DRG neurons**

Wild type and Dynlrb1+/− DRG ganglia were dissociated as previously described and cells were plated on coverslips in F12 medium (ThermoFisher Scientific). 48 h after plating cells were washed in PBS, fixed in 4% PFA in PBS for 30 min and immunostained for β−catenin (1:100 dilution), NFH (1:2,000 dilution) and DAPI as previously described. Coverslips were then mounted with Fluoromount-G™ and imaged using a confocal laser-scanning microscope (Olympus FV1000, 60x oil-immersion objective Olympus UPLSAO - NA 1.35). Image quantification was performed using ImageJ software. Nuclei were identified using a mask drawn on the DAPI staining and cytoplasm was masked using the NFH staining. Nuclear and cytoplasmic intensity of β−catenin was recorded. Statistical analysis was carried out with a one-way ANOVA followed by Tukey post-hoc multiple comparison test using the statistical software GraphPad Prism.
Design and production of AAV shDynlrb1

Design of AAV shRNA constructs was based on AAV-shRNA-ctrl (addgene, plasmid #85741, (Yu et al., 2015)). sh-Ctrl sequence was replaced by a sequence targeting Dynlrb1 using the BamHI and XbaI restriction sites. The primers used for cloning were:

Forward: GATCCGATCCAGAATCTGAAGCTTGTATTC AGTTGATCTGATCTCCTTTTTT.
Reverse: CTAGAAAAAGATCCAGATATCCAACTGATACACGGCT CTAATCGATCGATCTGATCTCCTTTTTT.

Purified adeno associated virus (AAV) was produced in HEK 293t cells (ATCC®), with the AAVpro® Purification Kit (All Serotypes) from TaKaRa (#6666). For each construct 10 plates (15 cm) were transfected with 20 μg of DNA (AAV-plasmid containing our construct of interest + 2 helper plasmids for AAV9) using jetPEI® (Polyplus) in DMEM medium without serum or antibiotics. Medium (DMEM, 20% FBS, 1 mM sodium pyruvate, penicillin-streptomycin) was added on the following day to a final concentration of 10% FBS and extraction was done 3 days post transfection. Purification was performed according to the manufacturer’s instructions (TaKaRa, #6666).

Immunohistochemistry of tissue samples

Tissue samples from muscle, skin, DRGs and sciatic nerve were harvested and fixed in 4% PFA in PBS (30 min for skin at RT, overnight at 4°C for muscles, DRG ganglia and sciatic nerve). The tissues were then washed in PBS and equilibrated in 30% w/v sucrose in PBS, embedded in Optimal Cutting Temperature Compound (O.C.T.™, Tissue-Tek®) and finally cryo-sectioned at a thickness ranging from 10-50 μm. Sections were then rehydrated in PBS, blocked and permeabilized with 5% goat serum, 1% BSA, 0.1% Triton X-100 in PBS, or donkey serum, 0.1% Triton X-100 in PBS for 1 h and incubated with primary antibody overnight at 4°C. Sections were then washed 3 times in PBS and incubated for 2 h with secondary antibodies from Jackson Immunoresearch (Alexa Fluor 647 or Alexa Fluor 488 conjugated, 1:500 dilution in PBS). Slides were then washed in PBS, mounted with Fluoromount-G® and imaged using a confocal laser-scanning microscope (Olympus® FV1000, 60x oil-immersion objective Olympus UPLSAP0 - NA 1.35).

NMJ plate counting

Gastrocnemius muscles from wild type and RNX3-Dynlrb1+/− mice were sectioned at a thickness of 50 μm. Staining of the neuromuscular junction was achieved as previously described by incubation with Alexa 555-α-bungarotoxin (1:1000 dilution). Sections were then imaged using a confocal laser-scanning microscope (Olympus FV1000, 60x oil-immersion objective Olympus UPLSAP0 - NA 1.35) and the number of positive NMJ-plate per area were manually counted and normalized by the area. Statistical analysis was performed with t-test using the statistical software GraphPad Prism.

Sensory skin innervation

Hind paw skin of wild type and RNX3-Dynlrb1-/− mice was sectioned at a thickness of 20 μm and immunostained as previously described with an α-CGRP and α-TUJ1 antibodies. The nuclear counterstaining DAPI was added to highlight all cells in the tissue. Sections were imaged using a confocal laser-scanning microscope (Olympus FV1000, 60x oil-immersion objective Olympus UPLSAP0 - NA 1.35). The number of nerve endings was manually counted and normalized by the area of the skin. Statistical analysis was performed with t-test using the statistical software GraphPad Prism.

DRG neuron count

L4 DRGs from wild type and RNX3-Dynlrb1+/− mice were serially sectioned at a thickness of 20 μm in set of 3. One set for each DRG was then processed for immunostaining as previously described for α-NFH, α-CGRP and α-TUJ1 antibody (Terenzio et al., 2018b). NFH and CGRP positive neurons were manually counted in blind. Statistical analysis was performed with t-test using the statistical software GraphPad Prism.

Gene expression analysis by Q-PCR

Wild type DRG ganglia were dissociated as described above and neurons were plated in F12 medium (ThermoFisher Scientific) supplemented with 10 μM cytosine β-D-arabinofuranoside hydrochloride (AraC, Sigma-Aldrich, C6645) to inhibit glial proliferation. AAV9-shControl and AAV9-shDynlrb1 viruses were added to the neurons 4 h after plating. 7 days after infection, cells were washed in PBS and total RNA was extracted using the Oligotex mRNA Mini Kit (Qiagen). RNA purity and concentration was determined, and 300 ng of total RNA was then used to synthesize cDNA using SuperScript III (Invitrogen). Q-PCR was performed on a Viia7 System (Applied Biosystems) using PerfeCta SYBR Green (Quanta Biosciences, Gaithersburg, USA). Forward/Reverse primers were designed to span exon-exon junction, and the RNA was treated with DNase H to avoid false-positives. Amplicon specificity was verified by melting curve analysis. All Q-PCR reactions were conducted in technical triplicates and the results were averaged for each sample, normalized to 18S levels and analyzed using the comparative ΔΔCt method (Livak and Schmittgen, 2001). The primers (Mus musculus) that were used are the following:

18S forward: AAACGGGTACACTCAGAG
18S reverse: CCTCCAATGATCTCGTTA
Dynlrb1 forward: CAAACCTGATCCACACTCATAC
Dynlrb1 reverse: TCTGATCTCACATCGAGGAATAGTC

RNA sequencing

Wild type and Dynlrb1+/− DRG ganglia were dissociated as described above and cells were plated on coverslips in F12 medium (ThermoFisher Scientific). Cells were washed in PBS at 12, 24 and 48 h after plating and total RNA was extracted using the Oligotex mRNA Mini Kit (Qiagen). RNA purity, integrity (RIN > 7) and concentration was determined. Three biological repeats were analyzed for each time point for each genotype.

RNA-sequencing libraries were prepared using the TrueSeq Stranded RNA kit (100ng). Libraries were indexed and sequenced by HiSeq4000 with 75 bp paired-end reads and at least 50M reads were obtained for each sample. Quality control (QC) was performed on base qualities and nucleotide composition of sequences, mismatch rate, mapping rate to the whole genome, repeats, chromosomes, key transcriptomic regions (exons, introns, UTRs, genes), insert sizes, AT/CG dropout, transcript coverage and GC bias to identify problems in library preparation or sequencing. Reads were aligned to the latest mouse mm10 reference genome (GRCm38.75) using the STAR spliced read aligner (ver 2.4.0). Average input read counts were 66.6M and average percentage of uniquely aligned reads were 86.3%. Total counts of read-fragments aligned to known gene regions within the mouse (mm10) ensemble (GRCm38.80) transcript reference annotation are used as the basis for quantification of gene expression. Fragment counts were derived using HTSeq program (ver 0.6.0). Genes with minimum of 5 counts for at least half of samples were selected. Filtered count data were normalized and examined for sequencing bias by PCA, and PC1 and PC2 of sequencing QC (described above) were regressed out. Differentially expressed transcripts were determined by Bioconductor package EdgeR (ver 3.14.0) at FDR < 0.1. Scripts used in the RNA sequencing analyses are available at https://github.com/icnn/RNAseq-PIPELINE.git. Raw and processed data were deposited within the Gene Expression Omnibus (GEO) repository (www.ncbi.nlm.nih.gov/geo), accession number (GSE131455).

The gene list from the 48h time point was filtered according to the FDR (FDR < 0.1, Table S1) and uploaded to the Ingenuity Pathway Analysis software suite (QIAGEN Bioinformatics). The 10 best high ranking pathways according to their score (−log(p-value)) were further evaluated and the WNT – β-catenin pathway selected for validation.
Intrathecal injection of AAV9 in mice

Intrathecal (IT) injections of adeno associated virus serotype 9 (AAV9) of 5 μl total volume were performed at the lumbar segment L4 using a sterile 10 μl Hamilton microsyringe fitted with a 30 gauge needle. For all constructs, we obtained titers in the range of 10^12–10^13 viral genomes/ml, which were used undiluted for intrathecal injections.

Behavioral profiling

All assays were performed during the “dark” active phase of the diurnal cycle under dim illumination (∼10 lx); the ventilation system in the test rooms provided a ∼65 dB white noise background. Every daily session of testing started with a 2 h habituation period to the test rooms. A recovery period of at least 1 day was provided between the different behavioral assays.

Home-cage locomotion test

Wild type and RNX3-Dynlrb1<sup>-/-</sup> mice were monitored over 72 h using the InfraMot system (TSE Systems, Germany) in order to investigate possible alterations of basal motor activity and/or circadian rhythms. The spatial displacement of body-heat images (infra-red radiation) over time was tracked (Panayotis et al., 2018). The first 24 h were considered as a habituation phase, while the mouse activity of following 48 h (2 consecutive dark/light cycles) was calculated. Statistical analysis was carried out with a one-way ANOVA followed by Tukey post-hoc multiple comparison test using the statistical software GraphPad Prism.

Open field

The motility of wild type and RNX3-Dynlrb1<sup>-/-</sup> mice was assessed in the open-field arena under 120lx (Panayotis et al., 2018). The total distance moved (cm), the mean speeds (cm/s) were recorded using VideoMot2 (TSE System, Germany) or Ethovision XT11 (Noldus Information Technology, the Netherlands). The data were analyzed with the COLORcation software (Dagan et al., 2016). Statistical analysis was carried out with a one-way ANOVA followed by Tukey post-hoc multiple comparison test or two-way ANOVA (for speed and distance) using the statistical software GraphPad Prism.

Accelerating rotarod

Wild type and RNX3-Dynlrb1<sup>-/-</sup> mice and wild type mice intrathecally injected with AAV9-shControl or AAV9-shDynlrb1 were tested with the ROTOR-ROD system (83x91x61 · SD. Instruments, San Diego) for their balance/coordination ability (Crawley, 2008; Marvaldi et al., 2015). Mice were subjected to 3 trials, with 5 min inter-trial intervals. Rotarod acceleration was set at 20 rpm in 240 s. Latency to fall (s) was recorded and the average of the 3 trials was considered. Statistical analysis was carried out with two-way ANOVA using the statistical software GraphPad Prism.

Catwalk Gait analysis

Wild type and RNX3-Dynlrb1<sup>-/-</sup> mice and wild type mice intrathecally injected with AAV9-shControl or AAV9-shDynlrb1 were assessed for their motor/balance coordination using the CatWalk as previously described (Perry et al., 2012). Motivation was achieved by placing the home cage at the end of the runway. The test was repeated 3-5 times for each mouse. Data were collected and analyzed using the Catwalk Ethovision XT11 software (Noldus Information Technology, The Netherlands). The parameters reported for each animal are the paw stance, defined as the duration in sec of the contact of the paw with the glass surface, and the step sequence regularity index, defined as the number of normal step sequence patterns relative to the total number of paw placements. Statistical analysis was carried out with a one-way ANOVA followed by Tukey post-hoc multiple comparison test (Wild type and RNX3-Dynlrb1<sup>-/-</sup> mice) or t-test (AAV injected mice) using the statistical software GraphPad Prism.

Statistical analysis

Analysis of multiple groups was made using the ANOVA method. The choice between one- or two-way ANOVA was based on the requirements for identification of specific factors’ contribution to statistical differences between groups and were followed by the Tukey and the Sidak post hoc analysis tests respectively. For 2-groups analyses, unpaired Student’s t test was used. All analyses were performed using GraphPad Prism version 7.00 for MacOS (GraphPad Software, La Jolla, California, USA, https://www.graphpad.com/). The results are expressed as the mean ± standard error of the mean (SEM). All statistical parameters for specific analyses are reported in the figure legends of the paper. Statistically significant P-values are shown as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.

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Author contributions

M.F. and M.T. designed the study. M.T., A.D.P., I.R., L.M., P.D.M., and R.K. performed experiments and data analyses. R.K. carried out RNA sequencing. M.F., E.M.C.F., G.C., and G.S. supervised research. M.F. and M.T. wrote the initial manuscript draft. All authors revised the manuscript and approved the final version.

Declaration of Competing Interest

The authors declare that they have no conflict of interests.

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References

Ben-Yaakov, K., Dagan, S.Y., Segal-Ruder, Y., Shalem, O., Vuppalanchi, D., Willis, D.E., Yudin, D., Rishal, I., Rother, F., Bader, M., Blesch, A., Pilpel, Y., Twiss, J.L., Fainzilber, M., 2012. Axonal transcription factors signal retrogradely in lesioned peripheral nerve. EMBO J. 31, 1350–1363.
Bilsland, I.G., Sabai, E., Kelly, G., Golding, M., Greensmith, L., Sichavi, G., 2010. Deficits in axonal transport precede ALS symptoms in vivo. Proc. Natl. Acad. Sci. U. S. A. 107, 20523–20528.
Bowman, A.B., Patel-King, R.S., Benashski, S.E., McCaffery, J.M., Goldstein, L.S., King,
Islam, M.A., Sharif, S.R., Lee, H., Seog, D.-H., Moon, I.S., 2015b. N-acetyl-D-glucosamine kinase.

Ilieva, H.S., Yamanaka, K., Malkmus, S., Kakinohana, O., Yaksh, T., Marsala, M., Terenzio, M., Koley, S., Samra, N., Rishal, I., Zhao, Q., Sahoo, P.K., Urisman, A., Marvaldi, L., Thongrong, S., Kozowska, A., Irschick, R., Pritz, C.O., Bäumer, B., Ronchi, G., Haase, B., Klimaschewski, L., 2015. Enhanced axon outgrowth and improved long-distance axon regeneration in sprouty2 deficient mice. Dev. Neurobiol. 75, 217–231.

Moore, J.K., Sep, D., Cooper, J.A., 2009. Neurodegeneration mutations in dysbindin impair dynein-dependent nuclear migration. PNAS 106, 5147–5152.

Münch, C., Sedelmier, R., Meyer, H., Vomberg, A., Kardur, A., Prudol, J., Peraus, G., Hanemann, C.O., Stumm, G., Ludolph, A.C., 2004. Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. Neurology 63, 724–726.

Naso, M.F., Tomkowski, B., Perry, W.L., Stroh, W.R., 2017. Adeno-associated virus (AAV) as a vector for gene therapy. BioDrugs 31, 317–334.

Olenick, M.A., Holzwarth, E.L., 2019. Dynein activators and adaptors at a glance. J. Cell Sci. 132 jcs22713.

Priestley, J.V., Averill, S., King, V.R., Ball, S., Peters, J., Toda, T., Yamamoto, A., Priestley, J.V., Averill, S., King, V.R., Ball, S., Peters, J., Toda, T., Yamamoto, A., Hara, K., Ushio, K., 1995. The Flp recombinase of the 2-microns plasmid of Saccharomyces cerevisiae. Biochim. Biophys. Acta. Academic Press, pp. 234–247.

Reck-Peterson, S.L., Redwine, W.B., Vale, R.D., Carter, A.P., 2018. The cytoplasmic dynein transport machinery and its many cargoes. Nat. Rev. Mol. Cell Biol. 19, 382.

Rhee, S.G., Green, M.S., 2002. The roadblock light chain binds a novel region of the cytoplasmic dynein intermediate chain. J. Biol. Chem. 277, 32939–32946.

Sadowski, P.D., 1995. The Flp recombinase of the 2-microns plasmid of Saccharomyces cerevisiae. Proceed. Nat. Acad. Sci. U.S.A. 92, 5097–5101.

Shcherbina, V., Geraisy, W., Huebner, E.A., Woolf, C.J., Yaron, A., Burlingame, A.L., Perry, R.B.-T., Doron-Mandel, E., Iavnilovitch, E., Rishal, I., Dagan, S.Y., Tsoory, M., Priestley, J.V., Averill, S., King, V.R., Ball, S., Peters, J., Toda, T., Yamamoto, A., Hara, K., Ushio, K., 1995. The Flp recombinase of the 2-microns plasmid of Saccharomyces cerevisiae. Biochim. Biophys. Acta. Academic Press, pp. 234–247.
Teuling, E., van Dis, V., Wulf, P.S., Haasdijk, E.D., Akhmanova, A., Hoogenraad, C.C., Jaarsma, D., 2008. A novel mouse model with impaired dynein/dynactin function develops amyotrophic lateral sclerosis (ALS)-like features in motor neurons and improves lifespan in SOD1-ALS mice. Hum. Mol. Genet. 17, 2849–2862.

Tsurumi, Y., Hamada, Y., Katoh, Y., Nakayama, K., 2019. Interactions of the dynein-2 intermediate chain WDR34 with the light chains are required for ciliary retrograde protein trafficking. Mol. Biol. Cell 30, 658–670.

Wanschers, B., van de Vorstenbosch, R., Wijers, M., Wieringa, B., King, S.M., Fransen, J., 2008. Rab6 family proteins interact with the dynein light chain protein DYNLRB1. Cell Motil. Cytoskeleton 65, 183–196. https://doi.org/10.1002/cm.20254.

Xie, Y., Zhou, B., Lin, M.-Y., Wang, S., Foust, K.D., Sheng, Z.-H., 2015. Endolysosomal deficits augment mitochondria pathology in spinal motor neurons of asymptomatic fALS mice. Neuron 87, 355–370.

Yu, H., Su, Y., Shin, J., Zhong, C., Guo, J.U., Weng, Y.-L., Gao, F., Geschwind, D.H., Coppola, G., Ming, G., Song, H., 2015. Tet3 regulates synaptic transmission and homeostatic plasticity via DNA oxidation and repair. Nat. Neurosci. 18, 836–843.