Supplemental Figure 1 Characterization of purified and recombinant proteins

Relevant fractions related the final stage of the purification protocol (Bingham et al., 1998; Toba and Toyoshima, 2004) were analyzed by SDS-PAGE. (A) Coomassie brilliant blue-stained gel showing the purity of the proteins used in the study (lane 1), and dynein- and dynactin-enriched pool before injection onto the anion exchange column (lane 2). The heavy chain, the intermediate chain and light intermediate chains of cytoplasmic dynein are identified. The major component of dynactin, p150, is also identified in dynein before purification. Note that purified dynein lacks p150. (B) Western blotting of dynein fractions was performed with an anti-LIS1 antibody (left) and an anti-NDEL1 antibody (right), which were the same fractions as above. Crude preparations of dynein before column purification contain LIS1 and NDEL1 (lane 2), while purified dynein contains neither LIS1 nor NDEL1 (lane 1). (C) Co-sedimentation experiments of LIS1/NDEL1 with dynein by sucrose gradient centrifugation. In the series of experiments, dynein and LIS1 and/or NDEL1 were mixed to an approximately 1:10 molar ratio. When LIS1 and/or NDEL1 were in excess, the calculated binding molar ratio of LIS1/dynein and that of NDEL1/dynein were 1.2 and 0.6, respectively, suggesting that NDEL1 has lower binding efficiency than LIS1. In addition, LIS1 and NDEL1 bind to dynein in a fashion approaching saturation. Interestingly, in the presence of both proteins, LIS1 and NDEL1 bound dynein in nearly equal molar ratios (dynein heavy chain/LIS1/NDEL1 = 1.0:0.9:0.8), showing that binding of NDEL1 to
dynein was increased by LIS1. The fraction numbers and sucrose gradient are indicated at the top of the panels. Gels were silver-stained. Protein combinations are indicated at right side.

Effects of removal of GST from LIS1-GST and NDEL1-GST on the in vitro motor properties of cytoplasmic dynein: (D) Characterization of recombinant proteins. Thrombin cleavage efficiently removed the GST-tag. Note: Tag-less LIS1 was efficiently obtained, whereas NDEL1 has internal thrombin site, resulting in partial truncation by thrombin cleavage (gray arrowheads and asterisk), which may result in inaccuracies in the concentration of full length NDEL1. Molecular weight is indicated at the right side. (E) Examination of gliding velocity of cytoplasmic dynein in the presence of tag-less recombinant proteins. Molecular ratio is indicated at the bottom. GST-less LIS1 reduced the velocity of cytoplasmic dynein in a dose dependent fashion, just as GST-tagged LIS1 did. Similar to GST-tagged NDEL1, GST-less NDEL1 did not change the velocity of cytoplasmic dynein appreciably, but GST-less NDEL1 released the inhibition by GST-less LIS1 on the motility of cytoplasmic dynein. The $P$ value was calculated using a Student’s $t$-test ($*: P<0.05$).

Supplemental Figure 2 Characterization of dynein distribution in Lis1−/− MEF (mouse embryonic fibroblast) cells or Ndel1−/− MEF cells

LIS1 and NDEL1 cooperatively regulate dynein function. To address how both proteins regulate dynein, we examined the distribution of cytoplasmic dynein or LIS1 protein in MEF cells. We established Lis1cko/cko, Ndel1cko/cko and wild type MEF cell lines
from E13.5 embryos isolated from pregnant dams of homozygous matings at 13 days post-fertilization. Each gene was inactivated by Cre-mediated recombination. (A) Cre-mediated recombination efficiently disrupted each gene, resulting in the disappearance of protein signal by Western blot analysis. (B) Immunostaining also revealed the loss of LIS1 or NDEL1 signal in Lis1-/- or Ndell-/- cells, respectively, compared to wild type cells. Using these MEF cells, we examined subcellular distribution of proteins. (C) Dynein intermediate chain (DIC) distribution in Lis1-/- MEF cells and Lis1+/+ MEF cells. Disruption of Lis1 by DsRed-Cre expression resulted in perinuclear clutering of cytoplasmic dynein, whereas expression of DsRed-Cre in wild type cells did not display any change. (D) Quantitation of protein distribution based on the fluorescence intensity. A linear line from the perinuclear area to the cell margin was divided into ten compartments. Then, we calculated a normalized specific intensity ratio; each fluorescence intensity measurement was corrected by subtraction of background fluorescence intensity (specific intensity) was divided by the sum of specific intensities. (E) Profiles of normalized specific intensity ratio of DIC in Lis1+/+ MEF cells (blue line) and Lis1-/- MEF cells (red line). Quantitation clearly supports the interpretation that perinuclear clustering accompanied by peripheral depletion of cytoplasmic dynein was observed in Lis1-/- MEF cells. The genotype of MEF cells are indicated within the graph. (F) DIC distribution in Ndell-/- MEF cells and Ndell+/+ MEF cells. Disruption of Ndell by DsRed-Cre expression also resulted in perinuclear cluter of cytoplasmic dynein, whereas expression of DsRed-Cre in wild type cells did not display any change. (G) Profiles of normalized specific intensity ratio of DIC in Ndell+/+ MEF cells (blue
line) and Ndell-/- MEF cells (red line). Quantitation clearly supports the interpretation that perinuclear clustering accompanied by peripheral depletion of cytoplasmic dynein was observed in Ndell-/- MEF cells. Genotypes of MEF cells are indicated within the graph. (H) LIS1 distribution in Ndell-/- MEF cells and Ndell+/+ MEF cells. Disruption of Ndell by DsRed-Cre expression resulted in homogenous distribution of LIS1 instead of perinuclear accumulation of LIS1 in the wild type MEF cells, whereas expression of DsRed-Cre in wild type cells did not display any change. (I) Profiles of normalized specific intensity ratio of LIS1 in Ndell+/+ MEF cells (blue line) and Ndell-/- MEF cells (red line). Quantitation clearly supports disappearance of the perinuclear gradient of LIS1 in Ndell-/- MEF cells. Genotypes of MEF cells are indicated within the graph.

Supplemental Figure 3 Schematic presentation of FRAP (Fluorescence Recovery After Photobleaching) analysis using DRG (Dorsal Root Ganglia)

(A) First, we evaluated isolated DRG neurons by immunostaining using a neuron specific marker (an anti-neurofilament antibody) and a Schwann cell specific marker (an anti-S100 antibody), since Schwann cells are co-purified during preparation of DRG neurons. Immunostaining revealed that 90% of total cells are neurofilament positive, indicating that the majority of cells in the preparation are DRG neurons instead of Schwann cells.

(B) DRGs from postnatal mice were dissociated using a previously described method (Lindsay, 1988). DRGs were transfected with the indicated vectors immediately
after dissection. FRAP analysis was applied to extended axons 48-72 hrs after start of culture. Media was changed to L15 media (Invitrogen) before observation.

FRAP was carried out with the LSM 510 META confocal microscope (Carl Zeiss, Inc). Open box-shaped regions covering an axon were bleached 80-100 μm in length using the line-scan function at 488/405 nm, and recovery of fluorescence was monitored 10 μm in length at the proximal (blue box) or distal side (red box) using the time-series function at 4 second intervals for up to 180 seconds. Anterograde flux was defined by the recovery of fluorescence at the proximal region, whereas retrograde flux was defined by the recovery at the distal region. A spacer region between two observatory areas ensured prevention of overlapping recovery from the opposite side. The proximal region is in a completely different position from the cell body.

(C) Schematic presentation of FRAP analysis in presence of an anti-NDEL1 antibody and AMPPNP. To compare the retrograde movements of LIS1 and dynein in the proximal and distal region, we examined fluorescence recovery of these proteins in the presence of AMPPNP to inhibit anterograde movement. The proximal region is in a completely different position from the cell body. AMPPNP nearly completely suppressed anterograde movement (Table 1), so any fluorescence recovery is attributable to retrograde movement. Observation of recovery was performed in the same area of bleaching.

FRAP analysis of EGFP control: We first examined fluorescence recovery of control EGFP, and very weak recovery was observed within the same time period used to examine FRAP using EGFP-LIS1, EGFP-NDEL1, EGFP-DIC1 or EGFP-TUBB3,
suggesting that the contribution of free diffusion of fluorescence protein is neglectable. FRAP profiles (D), time lapse image of recovery in the proximal region of axon (E), in the distal region of axon (F) and in the cell body (G) are shown. Passed time after bleach is indicated at the left. Note that much longer time is required for recovery in the axon (E, F). We performed FRAP analysis to several axonal segments covering the entire axon using control GFP, and found that diffusion was highly inhibited within the entire axon.

Subcellular distributions of GFP-tagged proteins: To address whether GFP-tagged proteins behave as the same with endogenous proteins, we examined subcellular distribution by immunocytochemistry and sucrose density gradient separation. (H) dynein intermediate chain 1 (DIC1), (I) LIS1, (J) tubulin β, (K) NDEL1 Upper left panels: GFP-tagged protein and immunostaning of endogenous protein of DRG neurons. Upper right panel: sucrose density gradient separation of DRG extract. Concentration of sucrose is indicated above. Sedimentation co-efficient is indicated bottom. Note: upper bands indicate tagged protein. Lower left panels: GFP-tagged protein and immunostaning of endogenous protein of MEF cells. White dotted line indicates the cell margin. Lower right panel: sucrose density gradient separation of MEF extract. Sucrose density is indicated above. Sedimentation co-efficient is indicated bottom. Note: upper bands indicate tagged protein.

**Supplemental Figure 4 FRAP analysis of protein transport**

Kinetics of protein transport of each protein after FRAP, using the following expressed
proteins: (A) EGFP-kinesin light chain 1 (KLC1); (B) EGFP- DIC1; (C) EGFP-TUBB3; (D) EGFP-LIS1; and (E) EGFP-NDEL1. Blue circles indicate anterograde flux. Red squares indicate retrograde flux.

**Supplemental Figure 5 FRAP analysis in the presence of AMPPNP**

DRGs were permeabilized by 8 μM of digitonin before observation. FRAP was carried out in the presence of 4 mM AMPPNP and an ATP regeneration system, and flux of the following proteins was measured: (A) EGFP-KLC 1; (B) EGFP- DIC1; (C) EGFP-TUBB3; (D) EGFP-LIS1; and (E) EGFP-NDEL1. AMPPNP selectively suppressed anterograde flux.

**Supplemental Figure 6 FRAP analysis in the presence of EHNA**

FRAP was carried out in the presence of 1 mM EHNA with an ATP regeneration system and flux of the following proteins was measured: (A) EGFP-KLC 1; (B) EGFP-TUBB3; (C) EGFP-LIS1; and (D) EGFP-NDEL1. EHNA selectively suppressed retrograde flux. (E) Dynein recovery in the presence of EHNA. Note: EHNA inhibited the movement of cytoplasmic dynein in both directions.

**Supplemental Figure 7 Characterization of blocking antibodies against LIS1 and NDEL1**

To examine whether blocking antibodies against LIS1 or NDEL1 inhibit the binding of each protein to cytoplasmic dynein, we performed a precipitation assay. GST-tagged
LIS1 (upper panels) or NDEL1 (lower panels) was incubated with purified cytoplasmic dynein, followed by precipitation using GST-sepharose or LIS1/NDEL1 antibodies. Binding of dynein with LIS1 or NDEL1 was detected by Western blot using an anti-DIC1 antibody. Compared to the GST-sepharose, dynein binding was clearly diminished by LIS1/NDEL1 antibodies. Note: an anti-LIS1 or an anti-NDEL1 antibody efficiently precipitated each recombinant protein with GST-sepharose, suggesting that reduction of dynein precipitation mirrored the inhibition of dynein-LIS1/NDEL1 binding by each antibody.

**Supplemental Figure 8 FRAP analysis in the presence of an anti-LIS1 antibody**

DRGs were permeabilized by 8 μM of digitonin before observation. FRAP was carried out in the presence of 8 mg/ml an anti-LIS1 antibody and an ATP regeneration system. To make an anti-LIS1 antibody, we immunized New Zealand white rabbits with a GST-conjugated recombinant LIS1 (1-114 AAs) expressed in bacteria and purified by GST-Sepharose (GE Healthcare, Piscataway, NJ). Flux of the following proteins were examined: (A) EGFP-LIS1; (B) EGFP-KLC 1; (C) EGFP-TUBB3; and (D) EGFP-NDEL1. Blue circles indicate antegrade flux. Red squares indicate retrograde flux.

**Supplemental Figure 9 FRAP analysis in the presence of an anti-NDEL1 antibody**

DRGs were permeabilized by 8 μM of digitonin before observation. FRAP was carried out in the presence of 8 mg/ml an anti-NDEL1 antibody with ATP regeneration system, and flux of the following proteins was measured: (A) EGFP-NDEL1, (B) EGFP-KLC 1
and (C) EGFP-TUBB3.

**Supplemental Figure 10 Examination of LIS1 concentration in DRG**

After double transfection of EGFP-LIS1 and mCherry control vector, LIS1 concentration was estimated based on the fluorescence intensity of EGFP-LIS1 using mCherry as a control. Fluorescence images of EGFP-LIS1 (A), mCherry (B) and relative proportions (C) are shown. Axons were divided into 10 equal spaces for measurements of the fluorescence intensity as indicated. Bleached regions in Figure 2C under the presence of AMPPNP and an anti-NDEL1 antibody are shown by red bars (C).
Supplemental Figure 1D-E  Yamada and Toba et al.

D

E
Supplemental Figure 2A-B  Yamada and Toba et al.

A

MEF cells

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B

Wild-type      | LIS1 -/- | Wild-type | NDEL1 -/-

anti-LIS1  
Red-CRE    
DAPI
C   Localization of endogenous DIC

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Each normalized specific intensity
= (Fluorescence intensity of each compartment-background intensity) /
(SUM of (Fluorescence specific of each compartment-background intensity))

E  Localization of endogenous DIC

![Graph showing localization of endogenous DIC](image)
Supplemental Figure 2F  

Yamada and Toba et al.

F  Localization of endogenous DIC

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G Localization of endogenous DIC

Normalized specific intensity

Nucleus

Margin

NEF-NDEL1 +/+ (n=21)
NEF-NDEL1 -/- (n=20)
H Localization of endogenous LIS1

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Localization of endogenous LIS1

Normalized specific intensity

Nucleus vs. Margin

MEF-NDEL1 +/+ (n=20)
MEF-NDEL1 -/- (n=20)
A Characterization of the mDRG cells

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Supplemental Figure 3B-C Yamada and Toba et al.

B

Soma

Bleaching area
80-100 μm

10 μm Observation area

Spacer region

10 μm Observation area

Recovery by anterograde flux

Recovery by retrograde flux

C

Soma

Bleaching and observation area

10 μm

Bleaching and observation area

10 μm

Recovery in proximal region under presence of AMPPNP

Recovery in distal region under presence of AMPPNP
Supplemental Figure 5D-E  Yamada and Toba et al.

D
Anterograde flux of EGFP-LIS1

Retrograde flux of EGFP-LIS1

E
Anterograde flux of EGFP-NDEL1

Retrograde flux of EGFP-NDEL1
**Supplemental Figure 7  Yamada and Toba et al.**

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<td>Dynein</td>
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**Blotting:**
- α-LIS1 antibody

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**Blotting:**
- α-NDEL1 antibody
- α-DIC antibody
Supplemental Figure 9A-C  Yamada and Toba et al.

A  
Anterograde flux of EGFP-NDEL1

FRAP

Time (sec) 0 45 90 135 180

with normal rabbit serum
- -
with anti-NDEL1

Retrograde flux of EGFP-NDEL1

FRAP

Time (sec) 0 45 90 135 180

with normal rabbit serum
- -
with anti-NDEL1

B  
Anterograde flux of EGFP-KLC1

FRAP

Time (sec) 0 45 90 135 180

with normal rabbit serum
- -
with anti-NDEL1

C  
Anterograde flux of EGFP-TUBB3

FRAP

Time (sec) 0 45 90 135 180

with normal rabbit serum
- -
with anti-NDEL1

Retrograde flux of EGFP-TUBB3

FRAP

Time (sec) 0 45 90 135 180

with normal rabbit serum
- -
with anti-NDEL1
Supplemental Figure 10 A-B  Yamada and Toba et al.

A

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EGFP-LIS1

B

mCherry

C

Ratio of EGFP-LIS1/mCherry

Soma proximal region for photobleaching  distal region for photobleaching