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Lis1 promotes the formation of activated cytoplasmic dynein-1 complexes

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Cytoplasmic dynein-1 is a molecular motor that drives nearly all minus-end-directed microtubule-based transport in human cells, performing functions ranging from retrograde axonal transport to mitotic spindle assembly^{1,2}. Activated dynein complexes consist of one or two dynein dimers, the dynactin complex, and an “activating adaptor”, with faster velocity seen when two dynein dimers are present^{3–6}. Little is known about the assembly process of this massive ~4MDa complex. Here, using purified recombinant human proteins, we uncover a role for the dynein-binding protein Lis1 in promoting the formation of activated dynein/ dynactin complexes containing two dynein dimers. Complexes activated by

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ZMH, JPG, MED, AEL and SRP designed the experiments. ZMH, JPG, MED and RWB performed the experiments. ZMH, JPG, MED, AEL and SRP wrote the manuscript. All authors interpreted the data and reviewed and edited the manuscript.

Competing Interests statement

The authors have no competing interests.

proteins representing three families of activating adaptors: BicD2, Hook3, and Nln1 all show enhanced motile properties in the presence of Lis1. Activated dynein complexes do not require sustained Lis1 binding for fast velocity. Using cryo-electron microscopy we show that human Lis1 binds to dynein at two sites on dynein's motor domain. Our work suggests that Lis1 binding at these sites functions in multiple stages of assembling the motile dynein/dynactin/activating adaptor complex.

Cytoplasmic dynein-1 (dynein) is responsible for the long-distance transport of nearly all cargos that move towards the minus ends of microtubules². Mutations in components of the dynein machinery cause neurodevelopmental and neurodegenerative diseases⁷. Activated human dynein is a large multi-subunit complex composed of one or two dynein dimers (each dynein dimer contains two motor subunits and two copies each of five additional subunits), the dynactin complex (composed of 23 polypeptides) and a dimeric, coiled-coil-containing activating adaptor (Fig. 1a)^{2-6,8}. The dynein motor subunit, or heavy chain, is an ATPase containing six AAA+ domains and a microtubule-binding domain that emerges from a long coiled-coil "stalk" (Fig. 1b).

Though yeast dynein is capable of moving processively on its own *in vitro*⁹, mammalian dynein is largely immotile in the absence of dynactin and an activating adaptor^{3,4,10}. However, dynein activation *in vivo* is likely conserved across eukaryotes as dynactin subunits and a candidate activating adaptor (Num1) are required for dynein function in yeast¹¹. Activating adaptors also link dynein/dynactin to its cargos^{2,8}. Nearly a dozen activating adaptors have been described; they share little sequence identity, but contain a long stretch of predicted coiled-coil that spans the ~40 nm length of dynactin^{2,8}. All activated dynein complexes that have been investigated structurally can bind two dynein dimers^{5,6} (Fig. 1a).

Mammalian dynein in the absence of these other components adopts a conformation known as "Phi"^{12,13}. Phi dynein is autoinhibited and cannot interact with microtubules productively¹². The current model for dynein activation proposes that Phi dynein must first adopt an "Open" conformation and ultimately a "Parallel" conformation that is observed when it is bound to dynactin and an activating adaptor (Fig. 1a)^{5,6}. Little is known about how dynein switches between the autoinhibited Phi conformation and the Open and Parallel conformations that lead to the assembly of the motile activated dynein complex.

Genetic studies in model organisms place the dynein-binding protein Lis1 in the dynein pathway¹⁴⁻¹⁶. Given that deletion or mutations in Lis1 phenocopy deletion or mutation of dynein or dynactin subunits in these organisms¹⁴⁻¹⁶, Lis1 is considered a positive regulator of dynein's cellular activities. Activities that require Lis1 range from organelle trafficking (e.g.¹⁷⁻²⁰) to nuclear migration/positioning (e.g.^{15,21-23}) to RNA localization (e.g.²⁴). The *LIS1* gene is mutated in the neurodevelopmental disease type-1 lissencephaly²⁵, and was first directly linked to dynein through genetic studies in *Aspergillus nidulans*¹⁵. Lis1 is a dimer of β -propellers^{26,27} and yeast Lis1 binds dynein at two distinct sites on the dynein motor domain: dynein's ATPase ring at AAA3 and AAA4 ("site_{ring}") and dynein's stalk ("site_{stalk}") (Fig. 1b)²⁸⁻³⁰. In yeast, binding of Lis1 to dynein at site_{ring} causes tight microtubule binding and decreases velocity^{29,30}, whereas binding at both sites leads to weak

microtubule binding and increases velocity³⁰. Lis1 also increases the binding of mammalian dynein to microtubules^{31,32} and increases the velocity of mammalian dynein/ dynactin complexes containing the BicD2 activating adaptor^{33,34}. How Lis1 exerts these effects on mammalian dynein is unknown. It is also unknown if Lis1 has the same effects on dynein/ dynactin bound to other activating adaptors.

To determine how Lis1 regulates activated human dynein complexes, we purified full-length recombinant human dynein in the presence of its accessory chains (“dynein”)³ and human Lis1 from insect cells, dynactin from human HEK293T cells³⁵, and the human activating adaptors BicD2, Hook3, and Ninl from *E. coli* (Extended Data Fig. 1a). Since some activating adaptors are known to be autoinhibited³⁶, we used well-characterized carboxy-terminal truncations of BicD2, Hook3 and Ninl^{3,4,35} (Fig. 1c). We made two truncations of BicD2: BicD2-S (aa 25–398), which activates dynein in vitro^{3,4} and BicD2-L (aa 1–598), which activates dynein in cells^{18,37}.

We first determined the effects of Lis1 on the microtubule binding properties of dynein alone and dynein/ dynactin bound to different activating adaptors using a single-molecule assay³⁰. Lis1 increased the microtubule binding density of dynein alone (Fig. 1d and Extended Data Fig. 1b), consistent with studies of yeast^{29,30} and mammalian^{31,32} dynein. Lis1 also increased the microtubule binding density of dynein/ dynactin complexes bound by the activating adaptors BicD2-S, BicD2-L, Hook3, and Ninl (Fig. 1e and Extended Data Fig. 1b).

Next, we examined how Lis1 affected the motile properties of activated dynein complexes. While Lis1 inhibits the motility of human dynein alone in microtubule gliding assays^{31,33,38,39}, in agreement with some previous studies^{33,34} we found that Lis1 increased the velocity of dynein/ dynactin / BicD2-S complexes (Fig. 1f, Extended Data Fig. 1c, and Supplementary video 1). We also found that Lis1 increased the velocity of dynein/ dynactin activated by BicD2-L and Ninl in our standard motility assay buffer (Fig. 1f, Extended Data Fig. 1c, and Supplementary Videos 2–4). Lis1 also increased the percentage of processive runs for dynein/ dynactin activated by BicD2-S, BicD2-L and Ninl (Extended Data Fig. 1d). While Hook3-activated dynein complexes were not affected by Lis1 in these conditions (Fig. 1f), increasing the ionic strength of our assay buffer led to increased velocity and an increase in processive runs of these complexes in the presence of Lis1 (Fig. 1g,h and Supplementary Video 5). We interpret this difference in sensitivity to the ionic strength of our assay conditions as an indication that Hook3 may have a higher affinity for dynactin, the dynein tails, or the dynein light intermediate chains compared to BicD2 and Ninl. These data show that Lis1 increases both microtubule binding and motility of dynein/ dynactin complexes bound by activating adaptors from three different families and allude to a role for Lis1 in activated dynein/ dynactin complex formation.

We next asked if Lis1 had a similar effect on dynein velocity in cells using a well-established peroxisome relocation assay (Fig. 1i)^{37,40}. We co-transfected human U2OS cells with: 1) the rapamycin binding protein FRB fused to BicD2-S and 2) another rapamycin binding protein FKBP fused to mEmerald and the peroxisome targeting protein Pex3 (Fig. 1i). In U2OS cells peroxisomes rarely move, but upon the addition of rapalog, which causes

FRB and FKBP to interact, we observed many processive runs. This is an indication that BicD2-S recruits and activates dynein/ dynactin (Supplementary Videos 6–9)⁴⁰. We observed a significant decrease in peroxisome velocity when Lis1 expression was knocked down with siRNA (Fig. 1j, Extended Data Fig. 1e–g), suggesting that Lis1 also increases the velocity of dynein complexes in a cellular environment.

We next sought to determine where Lis1 binds human dynein. Experiments with yeast proteins showed that Lis1 binds dynein at two sites on the dynein motor domain (site_{ring} and site_{stalk}^{29,30}), although previous studies with mammalian protein fragments reported interactions with other regions of dynein^{41,42}. We used cryo-electron microscopy (cryo-EM) to identify the Lis1 binding sites on human dynein. We purified monomeric human dynein motor domains and mixed them with dimeric human Lis1 in the presence of ATP-vanadate. This ATP analog was previously shown to promote an interaction between mammalian dynein and Lis1³² and causes dynein's linker to adopt a bent position⁴³ that would prevent the linker from sterically interfering with Lis1 binding at site_{ring}. We generated two-dimensional (2D) class averages of the dynein/ Lis1 complex that showed high-resolution features in both dynein and Lis1 (Fig. 2a).

To determine whether the binding sites for Lis1 are similar in human and yeast dynein, we compared our experimental class averages with calculated 2D projections of a model of human dynein bound to Lis1 (Fig. 2b). To make this model we combined the structure of human dynein-2 bound to ATP-vanadate (PDB: 4RH7⁴⁴) with a homology model of human Lis1 bound to dynein at the two binding sites observed with the yeast proteins (PDB: 5VLJ³⁰) (Fig. 2b). To highlight the densities corresponding to Lis1, we also calculated 2D projections of human dynein-2 alone (Fig. 2c). The correspondence between our data and the model with two Lis1s bound (Fig. 2a,b) suggests that the yeast and human Lis1 binding sites are in similar regions of the dynein motor domain on the ring at AAA3/4 and on the stalk. The strong preferred orientation adopted by the sample prevented us from obtaining a 3D reconstruction and mapping the exact sites of interaction on either human dynein or Lis1. The stoichiometry of this complex is 1 dynein monomer to 1.2 Lis1 dimers \pm 0.3 (Extended Data Fig. 2a), suggesting that the majority of dynein monomers are bound to a single Lis1 dimer.

Mutation of five amino acids on the dynein binding face of yeast Lis1 disrupts the interaction between dynein²⁸. We made the equivalent mutations in human Lis1 ("Lis1–5A"; Fig. 2d). To determine if Lis1–5A could enhance the velocity of activated dynein complexes we focused on complexes activated by BicD2-S, as Lis1 had the greatest effect on these complexes (Fig. 1g). We found that 300 nM Lis1–5A still enhanced the velocity of dynein/ dynactin/ BicD2-S complexes. We hypothesized that Lis1–5A might still weakly interact with dynein. Thus, we lowered the concentration of Lis1 and Lis1–5A to 24 nM; under these conditions wild-type Lis1 more potently increased dynein velocity compared to Lis1–5A (Fig. 2e and Extended Data Fig. 2b). We also found that Lis1–5A was less potent at enhancing processive runs of dynein/ dynactin/ Hook3 complexes compared to wild-type Lis1 in a higher ionic strength buffer (Fig. 2f) and the velocity of these runs was no longer increased (Extended Data Fig. 2c and 2d).

Since Lis1 is a dimer, we wondered whether Lis1's effects on activated dynein/ dynactin complexes required dimerization. To test this, we purified human Lis1 lacking its amino-terminal high affinity LisH dimerization domain²⁶, which we refer to as Lis1 N. Similar to the equivalent yeast construct, which is a monomer by gel filtration analysis²⁹, the human construct is mostly monomeric (Extended Data Fig. 2e). However, with the human construct a small amount of dimer is also observed (Extended Data Fig. 2e), likely due to an interaction between Lis1's two β -propellers, as seen by Cryo-EM here and previously³⁰. Lis1 N still increased dynein/ dynactin/ BicD2-S velocity at the same molar ratio of dynein to Lis1 β -propellers (Fig. 2g), indicating that the high affinity LisH dimerization domain is not required for Lis1 to increase dynein's velocity.

As activated dynein complexes containing two dynein dimers are faster than those containing a single one⁵, we hypothesized that Lis1 may play a role in promoting the recruitment of a second dynein dimer to the dynein/ dynactin complex. To determine if Lis1 enhances the formation of dynein/ dynactin complexes *in vitro* we measured the formation of activated dynein complexes by mixing dynein and dynactin with an excess of BicD2-S conjugated to magnetic beads. We then quantified the percentage of dynein bound to the BicD2-S beads (Fig. 3a). The presence of Lis1 increased the percentage of dynein bound to BicD2-S beads (Fig. 3b) and dynactin was required for this effect (Fig. 3c).

To directly test if Lis1 promotes the recruitment of a second dynein dimer to the activated complex we performed two-color single-molecule assays. To do this we added equimolar amounts of dynein labeled with either TMR or Alexa647 to dynactin and BicD2-S and quantified the percentage of moving two-color complexes. If all moving dynein complexes contained two dynein dimers, 50% of events would show co-localization (Fig. 3d). The presence of Lis1 significantly increased the number of moving two-color dynein/ dynactin/ BicD2-S complexes (Fig. 3e,f). Two-color complexes moved faster in both the presence and absence of Lis1 (Extended Data Fig. 3a). One-color complexes in the presence of Lis1 also moved faster, presumably because half of these events contained two dynein dimers labeled with the same color (Extended Data Fig. 3a). We conclude that Lis1 promotes the recruitment of a second dynein dimer to activated dynein complexes.

We wondered if Lis1 must remain bound to moving activated dynein complexes to sustain fast velocity. To address this, we sought to determine if TMR-labeled Lis1 co-migrated with moving dynein/ dynactin/ BicD2-S complexes tagged with Alexa647. Most of our earlier experiments used 300 nM Lis1, a concentration that is too high to visualize single Lis1 molecules. Therefore, we lowered the Lis1 concentration to 50 nM, which is still well above the concentration where a maximal increase in dynein velocity by Lis1 is observed (Fig. 4a and Extended Data Fig. 2b). Single-molecule motility assays with TMR-Lis1 and Alexa647-dynein/ dynactin/ BicD2-S complexes showed that only $16.8 \pm 1.9\%$ of dynein runs co-migrated with Lis1, with co-migrating runs moving slower than those with no detectable Lis1 (Fig. 4b,c). Occasionally the disappearance of the TMR-Lis1 signal coincided with an increase in speed of a dynein/ dynactin/ BicD2-S run (Fig. 4d), similar to a previous report³⁴. As shown previously³², we found that human Lis1 in the absence of dynein does not bind to microtubules (Extended Data Fig. 4a), suggesting that the effects of Lis1 we observe on dynein activity and complex assembly are not due to an interaction between Lis1

and microtubules. We also found that Lis1–5A increased the velocity of activated dynein complexes significantly less than wild-type Lis1 (Extended Data Fig. 4b) and we observed no colocalization of TMR-Lis1–5A with Alexa647-dynein (Extended Data Fig. 4c). These results suggest that the presence of Lis1 is not required for sustained fast velocity of activated dynein complexes. Our results showing that activated dynein complexes move faster when Lis1 is no longer co-migrating are consistent with contemporaneous work⁴⁵, but differ from other reports^{33,34}, perhaps due to differences in assay conditions or protein source.

We next explored which step(s) Lis1 affects in the dynein complex assembly pathway (Fig. 1a). Because the site_{ring} Lis1 binding site on dynein is not accessible in Phi dynein (Fig. 5a), we wondered if Lis1 had higher affinity for a dynein mutant that does not form the Phi particle (K1610E and R1567E¹², “Open dynein”). Indeed, we found that Lis1 had a higher affinity for Open dynein (Fig. 5b). We hypothesize that binding of Lis1 to dynein at site_{ring} alters the equilibrium between Phi and Open dynein to favor the Open conformation.

We next considered two possibilities: first, that Lis1’s only role is to stabilize dynein’s open conformation; and second, that in addition to this role Lis1 also promotes activated complex formation. To do this we examined Open dynein complexes in the absence of Lis1 and found that Open dynein/ dynactin/ BicD2-S complexes were more likely to form and moved faster compared to complexes containing wild-type dynein (Fig. 5c,d)¹². We then asked if Lis1 altered the motile properties of complexes containing Open dynein. We found that Lis1 further increased complex formation and the velocity of Open dynein/ dynactin/ BicD2-S complexes (Fig. 5d). Lis1 also increased the percentage of complexes containing two dynein dimers when Open dynein was used (Fig. 5e). Previous studies showed that the Open dynein mutant does not form Phi particles¹². Assuming this is the case in our experiments, the ability of Lis1 to further activate Open dynein suggests that Lis1’s effect on complex formation may have additional roles beyond altering the equilibrium between Phi and Open dynein.

Together our work suggests that Lis1 promotes the formation of human dynein/ dynactin/ activating adaptor complexes containing two dynein dimers. Experiments in human cells¹⁸, *Drosophila* embryos²⁴, *Xenopus* extracts³⁹, *Aspergillus nidulans*⁴⁶, and yeast⁴⁷ showed that Lis1 is required for the interaction of dynein and dynactin with each other and/or with their cargos. Our work offers a biochemical explanation for this requirement for Lis1. Our data suggest that Lis1 promotes complex formation by favoring a conformation of dynein that drives association with dynactin and an activating adaptor. First, Lis1 may promote the Open dynein conformation (Fig. 5f, step 1). We propose this based on our data showing that Open dynein has a higher affinity for Lis1 and because the structure of Phi dynein is incompatible with Lis1 binding at site_{ring}. Recent work in *Aspergillus nidulans*⁴⁶ and *Saccharomyces cerevisiae*⁴⁷ also supports this. Second, Lis1 may favor a conformation of dynein that is primed to assemble the fully activated complex (Fig. 5f, step 2). Our data showing that Open dynein is further activated by Lis1 supports this. Third, Lis1 favors the formation of dynein complexes containing two dynein dimers, which move faster (Fig. 5f, step 3). Our single-molecule experiments measuring the velocity of activated dynein complexes in the presence of Lis1 and our experiments showing that Lis1 promotes the recruitment of two dynein

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dimers support this. Because we also observe these effects with Lis1 N the underlying mechanism does not rely on the high affinity Lis1H dimerization domain, although interactions between the Lis1 β -propeptides could play a role. Finally, once a fully activated dynein/ dynactin/ activating adaptor complex is formed, Lis1 dissociates from moving complexes (Fig. 5f, step 4). This component of our model is based on our data showing that most moving dynein complexes do not remain bound to Lis1 and those that do, move slower. Contemporaneous work made complementary findings that supports steps 3 and 4 of our model⁴⁵.

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How does our current work and other recent studies^{45–47} relate to previous mechanistic studies of yeast Lis1, which showed how allosteric effects of Lis1 binding to dynein's motor domain controlled dynein's microtubule binding affinity^{28–30}? This earlier work was done in the absence of dynactin and an activating adaptor because yeast dynein is a processive motor on its own⁹, allowing the dissection of dynein function in a minimal system (however, dynactin and a candidate activating adaptor, Num1, are required for yeast dynein function *in vivo*). Here we show that human Lis1 binds to two sites on human dynein's motor domain, which are similar to the yeast binding sites^{28–30}. Vertebrate Lis1 increases dynein's affinity for microtubules and slows microtubule gliding velocity^{31–33,39}, again mirroring findings in yeast. Thus, the Lis1 binding sites on dynein and some of the consequences of these interactions on dynein's mechanochemical cycle are conserved.

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Lis1's ability to regulate dynein's mechanochemistry may be important for its role in assembling activated dynein complexes. For example, dynein idling on the microtubule (caused by tight microtubule binding induced by Lis1), could be well-suited for aiding in the challenging kinetics of loading two dynein motors on to dynactin before transport begins. In support of this, Lis1 has a role in localizing dynein to microtubule plus ends^{21,48} or initiating transport from microtubule plus ends^{19,20,49}, where tight microtubule binding and reduced motility is likely important for maintaining dynein at these sites. Binding of Lis1 to dynein at microtubule plus ends could promote the Open dynein conformation, lead to tight microtubule binding of dynein, and ultimately favor the formation of activated dynein/ dynactin/ activator complexes containing two dynein dimers. Lis1 binding to dynein may have additional allosteric effects that promote the formation of the full activated dynein/ dynactin complex, perhaps influencing the conformation of the dynein tails that interact with dynactin and activating adaptors. A contemporaneous study suggests that Lis1's regulation of yeast dynein involves an interaction between Lis1 and microtubules⁴⁷. However, much of the past work with yeast proteins^{28–30} cannot be accounted for by this model. Furthermore, we and others³² have shown that mammalian Lis1 does not interact with microtubules.

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Finally, we have shown that proteins representing three distinct families of activating adaptors, BicD2, Hook3, and Ninl, all move faster in the presence of Lis1. This raises the possibility that activated dynein complexes in cells contain two dynein dimers. We hypothesize that related activating adaptors or candidate activating adaptors will also use Lis1 to form activated complexes. In humans there are additional BicD, Hook and Ninl family members, as well as a number of known and candidate activating adaptors^{2,8}. Thus, we predict that Lis1 will play a role in the cell biological processes that additional dynein activating adaptors facilitate.

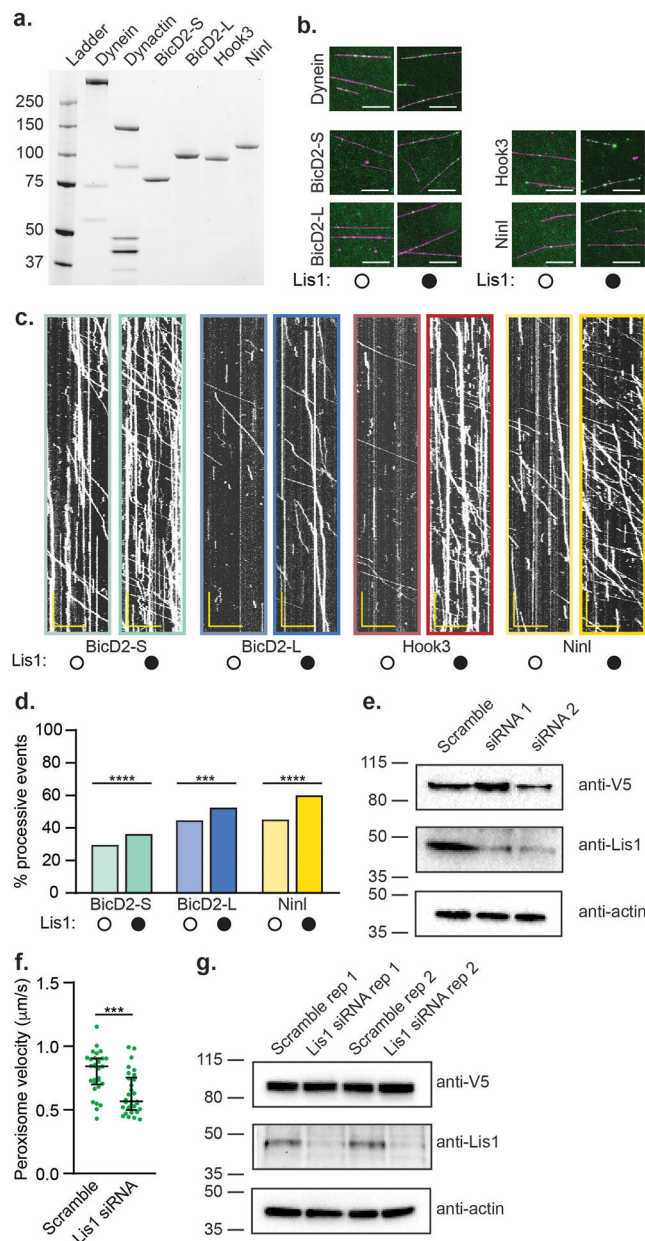
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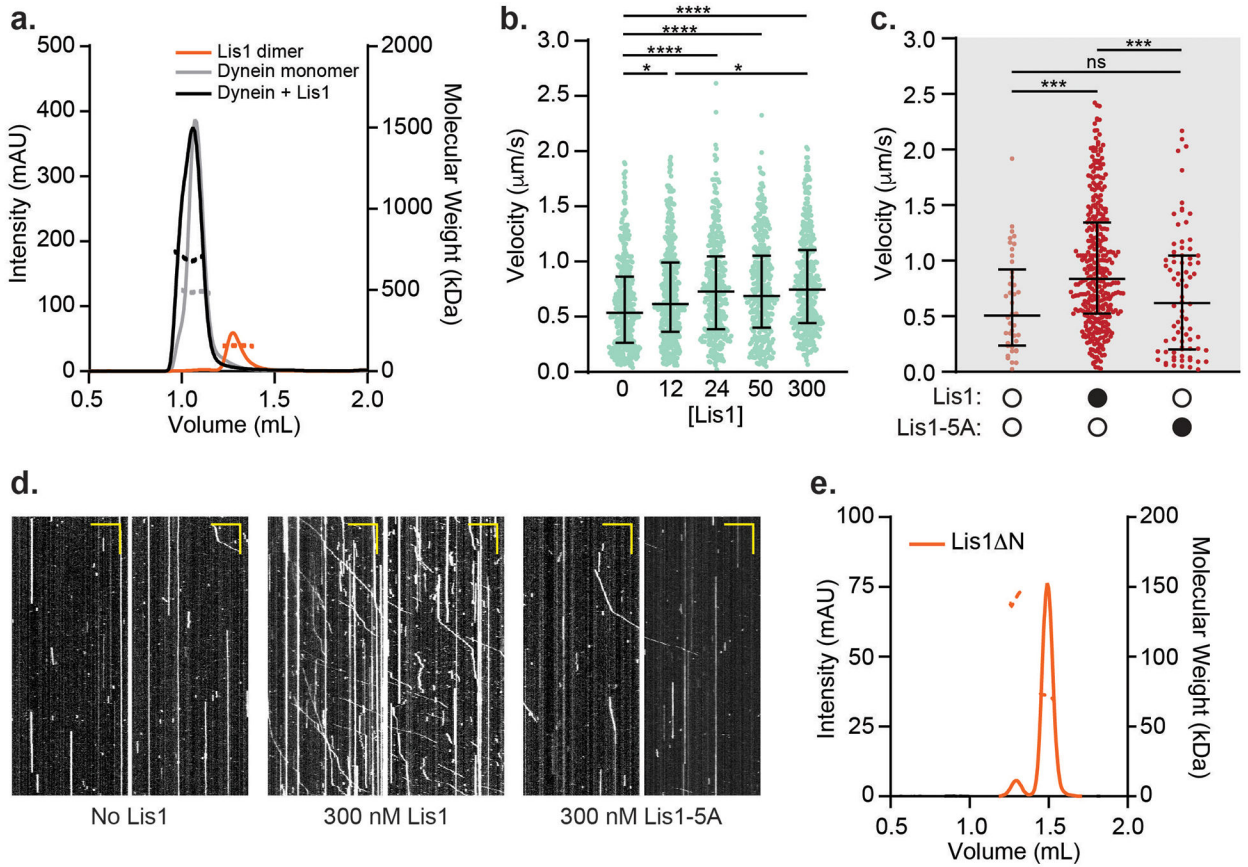
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Extended Data Fig. 1. Effect of Lis1 on the motility and microtubule binding of activated dynein complexes.

a. SDS-PAGE gel stained with Sypro Red of human dynein, dynactin and the activating adaptors BicD2-S (aa 25–398), BicD2-L (aa 1–598), Hook3 (aa 1–552), and Ninl (aa 1–702) used here. The dynein heavy chain was tagged with the SNAP tag, the dynactin subunit p62 with the HaloTag, and each activating adaptor with the HaloTag. The dynein light chains are too small to be seen on this low percentage gel. SDS-PAGE gels were run after all protein purifications. **b.** Example microscopy images for microtubule binding density data in the absence (white circles) or presence (black circles) of 300 nM Lis1 presented in Figure 1 d and e. Microtubules in magenta and dynein or activating adaptor foci in green. Scale bars are 10 μ m. **c.** Example kymographs of dynein/ dynactin/ activating adaptor complexes in the

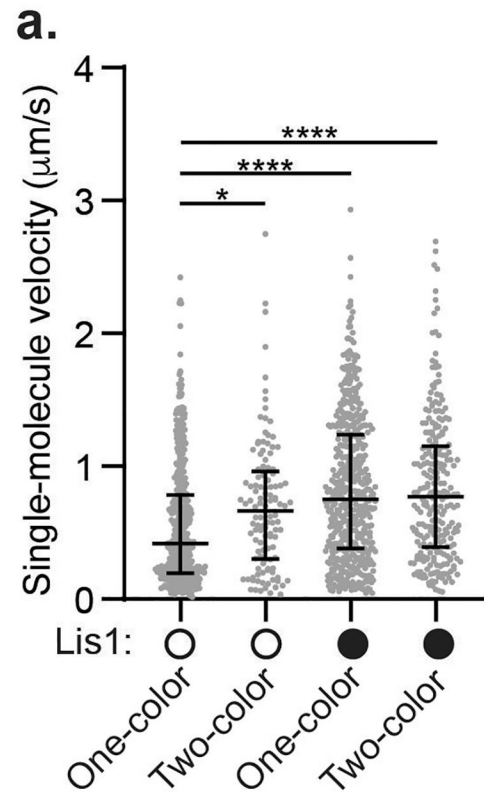
absence (white circles) or presence (black circles) of 300 nM Lis1. Scale bars are 10 μm (x) and 20 sec (y). **d.** Percent processive runs of dynein/ dynactin/ activating adaptor complexes in standard motility buffer in the absence (white circles) or presence (black circles) of 300 nM Lis1. Statistical analysis was performed on data pooled from all replicates with a chi-squared test. **e.** Immunoblots of cell lysates from human U2OS cells co-transfected with PEX3-mEmerald-FKBP and BicD2-S-V5-FRB constructs, as well as either scramble siRNA or Lis1 siRNA 1 or 2. Blots were performed for each biorep with similar results. **f.** Peroxisome velocity in human U2OS cells with scrambled or Lis1 siRNA pool knockdown. The median and interquartile range are shown. At least 7 peroxisome motility events were measured per cell. **g.** Immunoblots of cell lysates from human U2OS cells co-transfected with PEX3-mEmerald-FKBP and BicD2-S-V5-FRB constructs and scramble or Lis1 siRNA pool. Two bio-replicates (1 and 2) are shown. An anti-V5 antibody detects BicD2-S-V5-FRB, an anti-Lis1 antibody assesses the efficiency of Lis1 knockdown, and an anti-actin antibody serves as a loading control for immunoblots shown in e and g. Statistical data and unprocessed gel and blot images are available as source data for Extended Data Figure 1.



Extended Data Fig. 2. Characterization of the dynein binding interface and dimerization domain of Lis1.

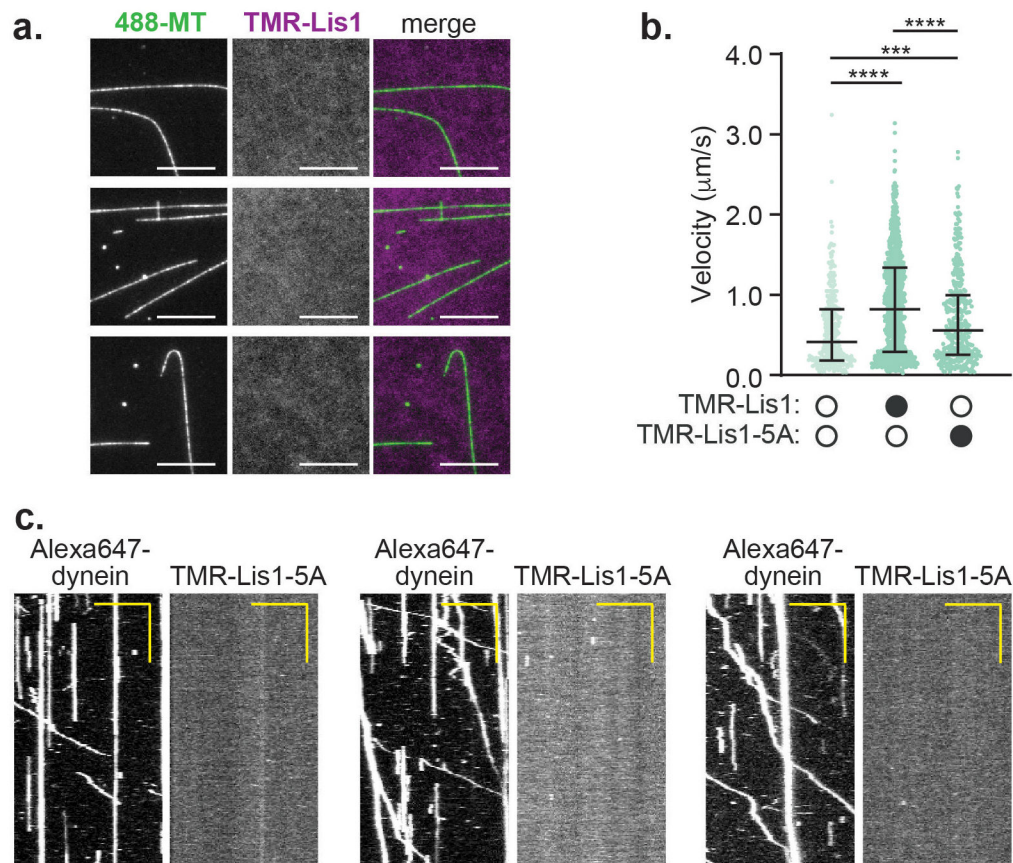
a. Example SEC-MALS traces with Lis1 dimer (orange), dynein monomer (grey), and dynein monomer with Lis1 dimer (black). The intensity of the UV signal (solid line) and the molecular weight fit (dashed line) are shown. Dimeric Halo-tagged-Lis1 is expected to be 161.4 kDa and monomeric dynein is expected to be 380.4 kDa. In this experiment we observe Halo-tagged-Lis1 to be 157.6 kDa, monomeric dynein to be 489.5 kDa and the Lis1-dynein complex to be 700.1 kDa. The high apparent molecular weight of monomeric dynein may be due to a self-association species that appears as a shoulder in the UV trace. The experiment was repeated in triplicate yielding similar results, giving a stoichiometry of 1.2 ± 0.3 Lis1 dimers per dynein monomer. Based on this data we cannot rule out that some dynein monomers are bound to two Lis1 dimers (which has been reported to occur³⁴), but our data suggest that most dynein monomers bind a single Lis1 dimer, and that Lis1 does not tether two dynein monomers. **b.** Single-molecule velocity of dynein/ dynactin/ BicD2-S complexes with increasing concentrations of Lis1. The median and interquartile range are shown. **c.** Single-molecule velocity of dynein/ dynactin/ Hook3 complexes in the presence of a higher ionic strength buffer in the absence (white circles) or presence (black circles) of 300 nM Lis1 or Lis1-5A. The data in the presence and absence of WT Lis1 was also presented in Fig. 1h. The median and interquartile range are shown. **d.** Example kymographs of dynein/ dynactin/ Hook3 complexes in a higher ionic strength buffer in the absence or presence of 300 nM Lis1 or Lis1-5A. Scale bars are 10 μm (x) and 20 sec (y). Data is

quantified in Extended Data Fig. 2C. **e.** Example SEC-MALS trace of Lis1 N (orange). The intensity of the UV signal (solid line) and the molecular weight fit (dashed line) are shown. Monomeric Halo-tagged-Lis1 N is expected to be 71.5kDa. In this experiment we observe Halo-tagged-Lis1 N to have a monomer peak at 72.0 kDa and a dimer peak at 141.2 kDa. The experiment was repeated in triplicate yielding similar results. Statistical data is available as source data for Extended Data Figure 2.



Extended Data Fig. 3. Quantification of the velocity of one-color and two-color activated dynein complexes in the presence of absence of Lis1.

a. Single-molecule velocity of dynein/ dynactin/ BicD2-S complexes in the absence (white circles) or presence (black circles) of 300 nM Lis1 with colocalized dynein (two color) or without observed colocalization (one color). The median and interquartile range are shown. Statistical data is available as source data for Extended Data Figure 3.



Extended Data Fig. 4. Characterization of Lis1 binding to microtubules and activated dynein complexes.

a. Example microscopy images for imaging 50 nM TMR-Lis1 (magenta in merge) in the presence of microtubules (green in merge). Lis1 does not colocalize with microtubules. Scale bars are 10 μm . The experiment was repeated in triplicate yielding similar results. **b.** Single-molecule velocity of dynein/ dynactin/ BicD2-S complexes in the absence (white circles) or presence (black circles) of 50 nM TMR-Lis1 or TMR-Lis1-5A. The median and interquartile range are shown. **c.** Representative kymographs of Alexa647-dynein/ dynactin/ BicD2-S complexes in the presence of 50 nM TMR-Lis1-5A. Scale bars are 10 μm (x) and 20 sec (y). Statistical data is available as source data for Extended Data Figure 4.

Supplementary Material

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References

1. Raaijmakers JA & Medema RH Function and regulation of dynein in mitotic chromosome segregation. *Chromosoma* 123, 407–422 (2014). [PubMed: 24871939]
2. Reck-Peterson SL, Redwine WB, Vale RD & Carter AP The cytoplasmic dynein transport machinery and its many cargoes. *Nature Reviews Molecular Cell Biology* 19, 1–17 (2018).
3. Schlager MA, Hoang HT, Urnavicius L, Bullock SL & Carter AP In vitro reconstitution of a highly processive recombinant human dynein complex. *EMBO J* 33, 1855–1868 (2014). [PubMed: 24986880]
4. Mckenny RJ, Huynh W, Tanenbaum ME, Bhabha G & Vale RD Activation of cytoplasmic dynein motility by dynactin-cargo adapter complexes. *Science* 345, 337–341 (2014). [PubMed: 25035494]
5. Urnavicius L et al. Cryo-EM shows how dynactin recruits two dyneins for faster movement. *Nature* 554, 202–206 (2018). [PubMed: 29420470]
6. Grotjahn DA et al. Cryo-electron tomography reveals that dynactin recruits a team of dyneins for processive motility. *Nat Struct Mol Biol* 25, 203–207 (2018). [PubMed: 29416113]
7. Lipka J, Kuijpers M, Jaworski J & Hoogenraad CC Mutations in cytoplasmic dynein and its regulators cause malformations of cortical development and neurodegenerative diseases. *Biochem Soc Trans* 41, 1605–1612 (2013). [PubMed: 24256262]
8. Olenick MA & Holzbaur ELF Dynein activators and adaptors at a glance. *J Cell Sci* 132, jcs227132 (2019).
9. Reck-Peterson SL et al. Single-molecule analysis of dynein processivity and stepping behavior. *Cell* 126, 335–348 (2006). [PubMed: 16873064]
10. Trokter M, Mücke N & Surrey T Reconstitution of the human cytoplasmic dynein complex. *Proc Natl Acad Sci USA* 109, 20895–20900 (2012). [PubMed: 23213255]
11. Moore JK, Stuchell-Brereton MD & Cooper JA Function of dynein in budding yeast: mitotic spindle positioning in a polarized cell. *Cell Motil Cytoskeleton* 66, 546–555 (2009). [PubMed: 19402153]
12. Zhang K et al. Cryo-EM Reveals How Human Cytoplasmic Dynein Is Auto-inhibited and Activated. *Cell* 169, 1303–1314 (2017). [PubMed: 28602352]
13. Torisawa T et al. Autoinhibition and cooperative activation mechanisms of cytoplasmic dynein. *Nat Cell Biol* 16, 1118–1124 (2014). [PubMed: 25266423]
14. Geiser JR et al. *Saccharomyces cerevisiae* genes required in the absence of the CIN8-encoded spindle motor act in functionally diverse mitotic pathways. *Mol Biol Cell* 8, 1035–1050 (1997). [PubMed: 9201714]
15. Xiang X, Osmani AH, Osmani SA, Xin M & Morris NR NudF, a nuclear migration gene in *Aspergillus nidulans*, is similar to the human LIS-1 gene required for neuronal migration. *Mol Biol Cell* 6, 297–310 (1995). [PubMed: 7612965]
16. Liu Z, Xie T & Steward R Lis1, the *Drosophila* homolog of a human lissencephaly disease gene, is required for germline cell division and oocyte differentiation. *Development* 126, 4477–4488 (1999). [PubMed: 10498683]
17. Lam C, Vergnolle MAS, Thorpe L, Woodman PG & Allan VJ Functional interplay between LIS1, NDE1 and NDEL1 in dynein-dependent organelle positioning. *J Cell Sci* 123, 202–212 (2010). [PubMed: 20048338]
18. Splinter D et al. BICD2, dynactin, and LIS1 cooperate in regulating dynein recruitment to cellular structures. *Mol Biol Cell* 23, 4226–4241 (2012). [PubMed: 22956769]
19. Lenz JH, Schuchardt I, Straube A & Steinberg G A dynein loading zone for retrograde endosome motility at microtubule plus-ends. *EMBO J* 25, 2275–2286 (2006). [PubMed: 16688221]
20. Moughamian AJ, Osborn GE, Lazarus JE, Maday S & Holzbaur ELF Ordered Recruitment of Dynactin to the Microtubule Plus-End is Required for Efficient Initiation of Retrograde Axonal Transport. *Journal of Neuroscience* 33, 13190–13203 (2013). [PubMed: 23926272]

21. Lee W-L, Oberle JR & Cooper JA The role of the lissencephaly protein Pac1 during nuclear migration in budding yeast. *J Cell Biol* 160, 355–364 (2003). [PubMed: 12566428]
22. Tsai J-W, Chen Y, Kriegstein AR & Vallee RB LIS1 RNA interference blocks neural stem cell division, morphogenesis, and motility at multiple stages. *J Cell Biol* 170, 935–945 (2005). [PubMed: 16144905]
23. Tanaka T et al. Lis1 and doublecortin function with dynein to mediate coupling of the nucleus to the centrosome in neuronal migration. *J Cell Biol* 165, 709–721 (2004). [PubMed: 15173193]
24. Dix CI et al. Lissencephaly-1 promotes the recruitment of dynein and dynactin to transported mRNAs. *J Cell Biol* 202, 479–494 (2013). [PubMed: 23918939]
25. Reiner O et al. Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. *Nature* 364, 717–721 (1993). [PubMed: 8355785]
26. Kim MH et al. The structure of the N-terminal domain of the product of the lissencephaly gene Lis1 and its functional implications. *Structure* 12, 987–998 (2004). [PubMed: 15274919]
27. Tarricone C et al. Coupling PAF signaling to dynein regulation: structure of LIS1 in complex with PAF-acetylhydrolase. *Neuron* 44, 809–821 (2004). [PubMed: 15572112]
28. Toropova K et al. Lis1 regulates dynein by sterically blocking its mechanochemical cycle. *Elife* 3, e03372 (2014).
29. Huang JJ, Roberts AJA, Leschziner AEA & Reck-Peterson SLS Lis1 acts as a ‘clutch’ between the ATPase and microtubule-binding domains of the dynein motor. *150*, 975–986 (2012).
30. DeSantis ME et al. Lis1 Has Two Opposing Modes of Regulating Cytoplasmic Dynein. *Cell* 170, 1197–1208 (2017). [PubMed: 28886386]
31. Yamada M et al. LIS1 and NDEL1 coordinate the plus-end-directed transport of cytoplasmic dynein. *EMBO J* 27, 2471–2483 (2008). [PubMed: 18784752]
32. Mckenney RJ, Vershinin M, Kunwar A, Vallee RB & Gross SP LIS1 and NudE induce a persistent dynein force-producing state. *141*, 304–314 (2010).
33. Baumbach J et al. Lissencephaly-1 is a context-dependent regulator of the human dynein complex. *Elife* 6, e21768 (2017). [PubMed: 28406398]
34. Gutierrez PA, Ackermann BE, Vershinin M & Mckenney RJ Differential effects of the dynein-regulatory factor Lissencephaly-1 on processive dynein-dynactin motility. *J Biol Chem* 292, 12245–12255 (2017). [PubMed: 28576829]
35. Redwine WB et al. The human cytoplasmic dynein interactome reveals novel activators of motility. *Elife* 6, e28257 (2017). [PubMed: 28718761]
36. Hoogenraad CC et al. Mammalian Golgi-associated Bicaudal-D2 functions in the dynein-dynactin pathway by interacting with these complexes. *EMBO J* 20, 4041–4054 (2001). [PubMed: 11483508]
37. Hoogenraad CC et al. Bicaudal D induces selective dynein-mediated microtubule minus end-directed transport. *EMBO J* 22, 6004–6015 (2003). [PubMed: 14609947]
38. Torisawa T et al. Functional dissection of LIS1 and NDEL1 towards understanding the molecular mechanisms of cytoplasmic dynein regulation. *J Biol Chem* 286, 1959–1965 (2011). [PubMed: 21036906]
39. Wang S et al. Nudel/NudE and Lis1 promote dynein and dynactin interaction in the context of spindle morphogenesis. *Mol Biol Cell* 24, 3522–3533 (2013). [PubMed: 24025714]
40. Kapitein LC et al. Probing Intracellular Motor Protein Activity Using an Inducible Cargo Trafficking Assay. *Biophys J* 99, 2143–2152 (2010). [PubMed: 20923648]
41. Tai C-Y, Dujardin DL, Faulkner NE & Vallee RB Role of dynein, dynactin, and CLIP-170 interactions in LIS1 kinetochore function. *J Cell Biol* 156, 959–968 (2002). [PubMed: 11889140]
42. Sasaki S et al. A LIS1/NUDEL/cytoplasmic dynein heavy chain complex in the developing and adult nervous system. *Neuron* 28, 681–696 (2000). [PubMed: 11163259]
43. Schmidt H & Carter AP Review: Structure and mechanism of the dynein motor ATPase. *Biopolymers* 105, 557–567 (2016). [PubMed: 27062277]
44. Schmidt H, Zalyte R, Urnavicius L & Carter AP Structure of human cytoplasmic dynein-2 primed for its power stroke. *Nature* 518, 435–438 (2015). [PubMed: 25470043]

45. Elshenaway M et al. Lis1 activates dynein motility by pairing it with dynactin. *bioRxiv* 10.1101/685826 (2019).
46. Qiu R, Zhang J & Xiang X LIS1 regulates cargo-adapter-mediated activation of dynein by overcoming its autoinhibition in vivo. *J Cell Biol* 218, 3630–3646 (2019). [PubMed: 31562232]
47. Marzo MG, Griswold JM & Markus SM Pac1/LIS1 promotes an uninhibited conformation of dynein that coordinates its localization and activity. *bioRxiv* 10.1101/684290 (2019).
48. Sheeman B et al. Determinants of *S. cerevisiae* dynein localization and activation: implications for the mechanism of spindle positioning. *Curr Biol* 13, 364–372 (2003). [PubMed: 12620184]
49. Egan MJ, Tan K & Reck-Peterson SL Lis1 is an initiation factor for dynein-driven organelle transport. *J Cell Biol* 197, 971–982 (2012). [PubMed: 22711696]

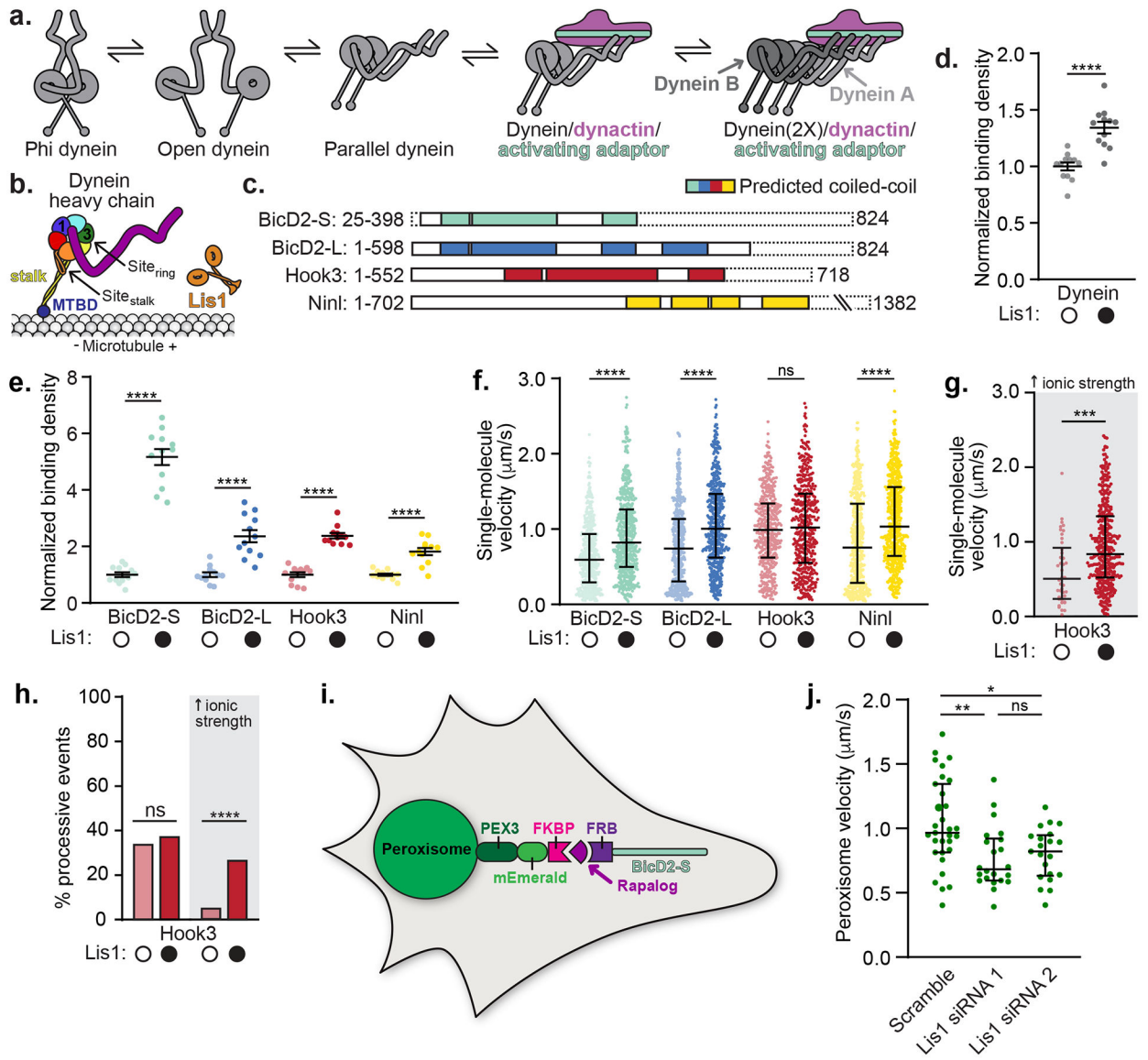


Fig. 1. Lis1 increases microtubule binding and velocity of activated dynein complexes.
a. Current model for dynein activation. Dynein is autoinhibited in the Phi conformation, opens, and then adopts a parallel conformation in the activated dynein complex, which can contain two dynein dimers (A and B). **b.** Schematic of the AAA+ ATPase dynein heavy chain. The two Lis1 binding sites, “Site_{ring}” and “Site_{stalk}” are shown. Microtubule binding domain (MTBD). **c.** Activating adaptor constructs used in this study. Dashed lines highlight the regions that were truncated. **d, e.** Binding density (mean ± s.e.m.) of full-length recombinant human dynein with its associated intermediate, light intermediate and light chains (d) or dynein/ dynactin/ activating adaptor complexes (e) on microtubules in the absence (white circle) or presence (black circle) of 300 nM Lis1. Data was normalized to a density of 1.0 in the absence of Lis1. **f.** Single-molecule velocity of dynein/ dynactin/ activating adaptor complexes in the absence (white circles) or presence (black circles) of 300 nM Lis1. The median and interquartile range are shown. **g.** Single-molecule velocity of

dynein/ dynactin/ Hook3 complexes in a higher ionic strength buffer (67.5 mM compared to 37.5 mM in our standard buffer) in the absence (white circle) or presence (black circle) of 300 nM Lis1. The median and interquartile range are shown. **h.** Percent processive runs of dynein/ dynactin/ Hook3 complexes in standard and higher (grey panel) ionic strength motility buffer in the absence (white circles) or presence (black circles) of 300 nM Lis1. Statistical analysis was performed on data pooled from all replicates with a chi-squared test. **i.** Peroxisome relocation assay. The peroxisomal protein, Pex3, is fused to mEmerald and FKBP and BicD2-S is fused to FRB. Rapalog induces the association of FKBP and FRB. **j.** Peroxisome velocity in human U2OS cells with scrambled or Lis1 siRNA knockdown with two independent siRNAs. The median and interquartile range are shown. Statistical data is available as source data for Fig. 1.

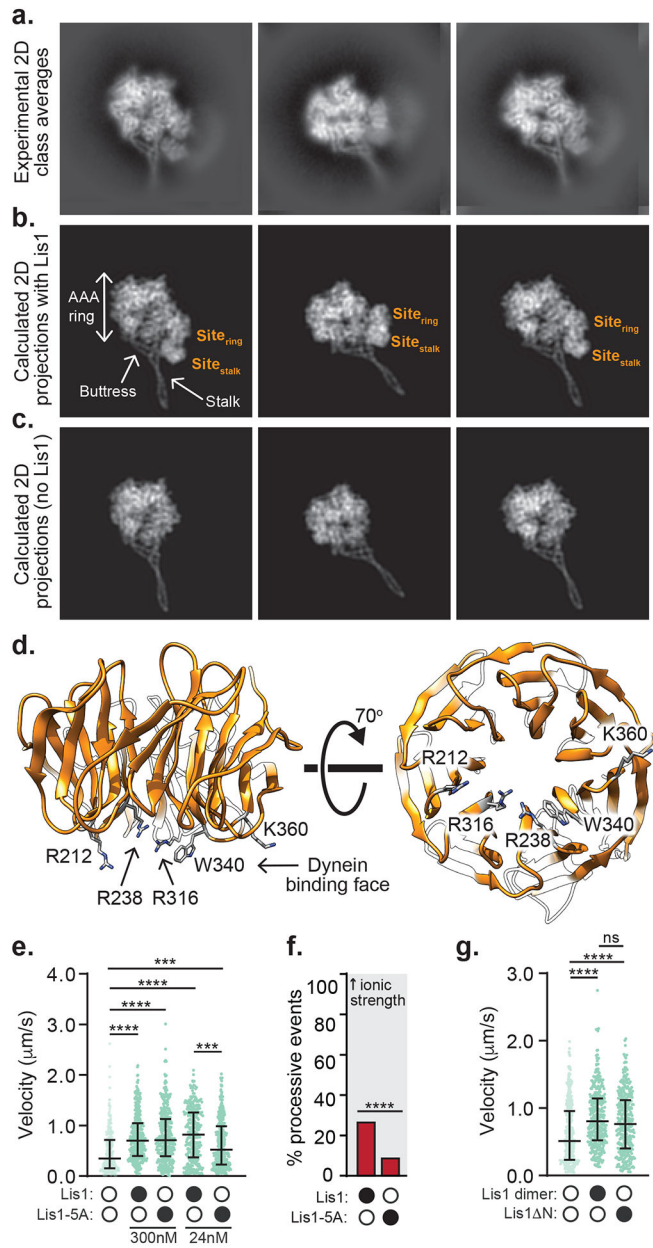


Fig. 2. Human Lis1 binds the human dynein motor domain at AAA3/4 and the stalk.
a. 2D class averages of human dynein monomers bound to human Lis1 dimers in the presence of ATP-vanadate. **b.** Best-matching projections of a model combining human dynein-2 bound to ATP-vanadate (PDB: 4RH7) with homology models of human Lis1 at the locations where Lis1 binds to yeast dynein in the presence of ATP-vanadate (PDB: 5VLJ). The two Lis1's ("Site_{ring}" and "Site_{stalk}") identified in yeast dynein, dynein's AAA ring, stalk and buttness are labeled. **c.** Projections of 4RH7 alone in the same orientations as those shown in (b). **d.** Homology model of human Lis1 (from SWISS-MODEL) showing the five residues mutated to alanine in "Lis1-5A". **e.** Single-molecule velocity of dynein/ dynactin/ BicD2-S complexes in the absence (white circles) or presence (black circles) of Lis1 or Lis1-5A. The median and interquartile range are shown. **f.** Percent processive runs of

dynein/ dynactin/ Hook3 complexes in a higher ionic strength buffer in the absence (white circles) or presence (black circles) of 300 nM Lis1 or Lis1-5A. Data in the presence of 300 nM Lis1 was also presented in Fig. 1h. Statistical analysis was performed on data pooled from all replicates with a chi-squared test. **g.** Single-molecule velocity of dynein/ dynactin/ BicD2-S complexes in the absence (white circles) or presence (black circles) of 300 nM Lis1 dimer or 600 nM Lis1 N. Since Lis1 N is largely monomeric (Extended data Figure 2e), 300 nM Lis1 dimer is roughly equivalent to 600 nM Lis1 N. The median and interquartile range are shown. Statistical data is available as source data for Fig. 2.

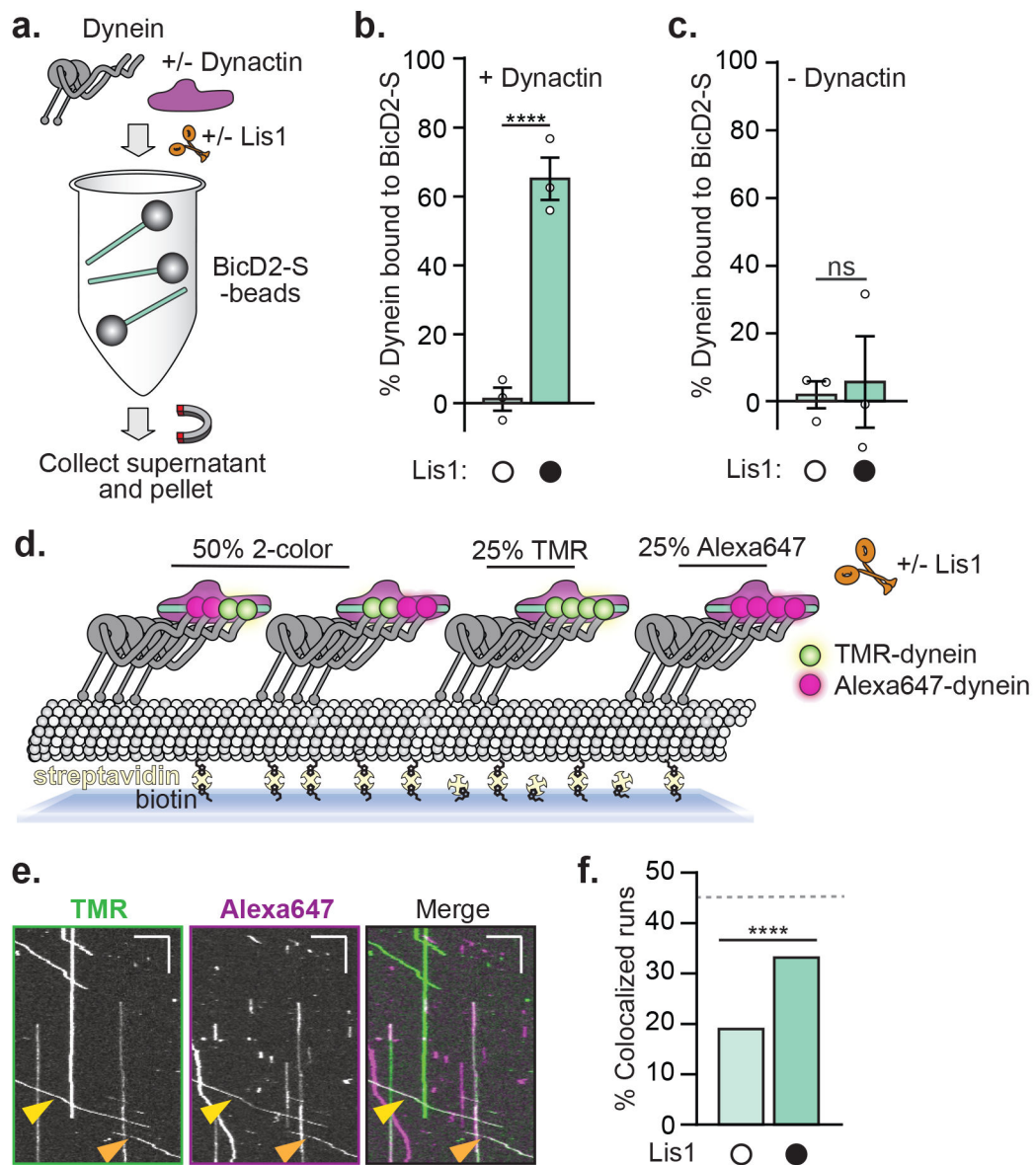


Fig. 3. Lis1 recruits a second dynein dimer to dynein/ dynactin/ BicD2-S complexes.

a. Schematic of the dynein/ dynactin complex formation assay. **b, c.** Percent dynein bound to BicD2-S-coupled beads (mean \pm s.e.m.) in the presence (b) or absence (c) of dynactin and in the absence (white circle) or presence (black circle) of 150 nM Lis1. **d.** Schematic depicting the maximum probability of forming various dynein/ dynactin/ BicD2-S complexes containing two dynein dimers. The maximum probability of colocalization was 45% (grey dashed line shown in f and g) given our labeling efficiency (see Methods). **e.** Representative kymographs showing the colocalization of TMR- and Alexa647-labeled dynein in moving dynein/ dynactin/ BicD2-S complexes in the presence of 300 nM Lis1. Each channel is shown separately (left and middle panels) and the merged TMR- and Alexa647-channels in pseudocolor (right panel). Scale bars are 10 μ m (x) and 20 sec (y). Data is quantified in Fig. 3f. **f.** Percent two-color dynein/ dynactin/ BicD2-S runs in the absence (white circle) or

presence (black circle) of 300 nM Lis1. Statistical analysis was performed using a chi-squared test. Statistical data is available as source data for Fig. 3.

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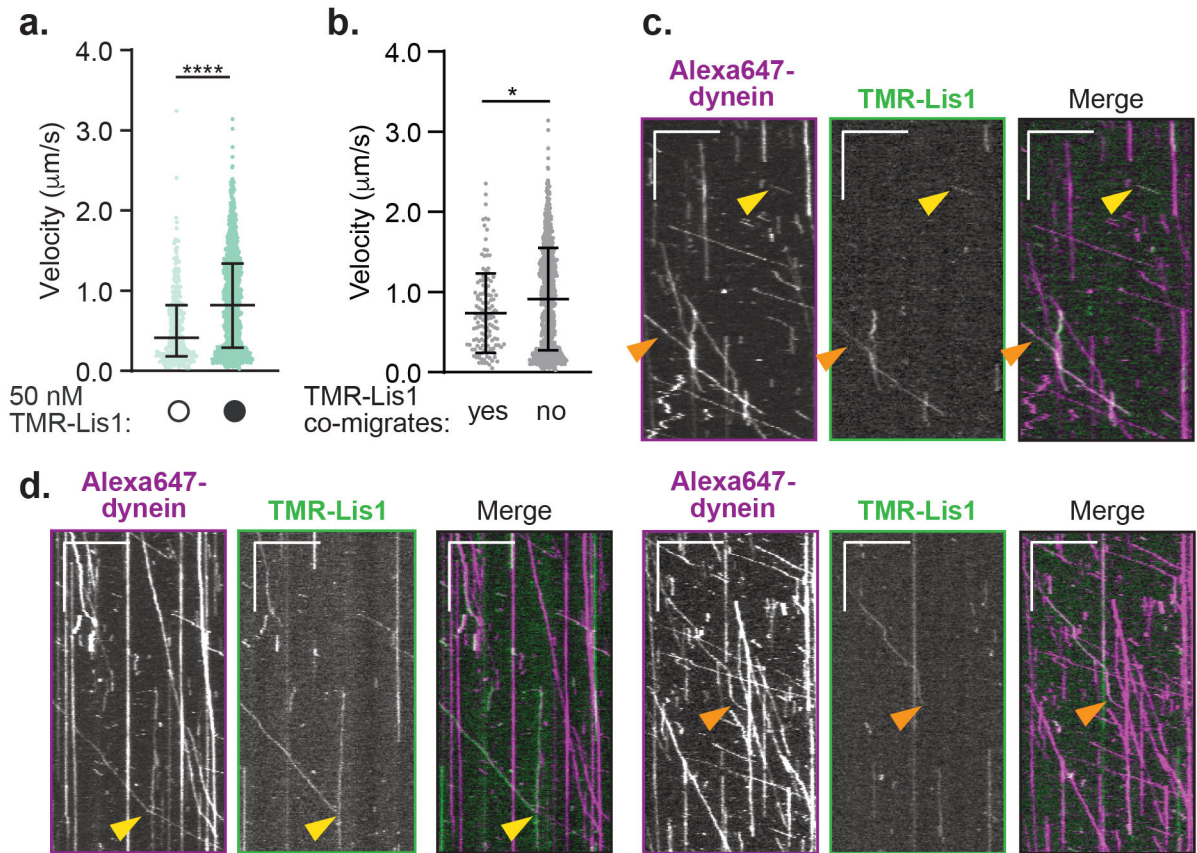


Fig. 4. Lis1 is not required to sustain fast velocity of activated dynein complexes.

a. Single-molecule velocity of dynein/ dynactin/ BicD2-S complexes in the absence (white circle) or presence (black circle) of 50 nM TMR-Lis1. The median and interquartile range are shown. **b.** Single-molecule velocity of Alexa647-dynein/ dynactin/ BicD2-S complexes in the presence of 50 nM TMR-Lis1 either co-migrating with TMR-Lis1 (yes) or not (no). The median and interquartile range are shown. **c.** Representative kymographs of the Alexa647-dynein and TMR-Lis1 channels (left and middle panels) and the merged images in pseudocolor (right panel). Arrowheads indicate colocalized runs. Scale bars are 10 μm (x) and 20 sec (y). Data is quantified in Fig. 4b. **d.** Kymographs showing examples of dynein's velocity changing upon loss of the TMR-Lis1 signal. The Alexa647-dynein and TMR-Lis1 channels are shown (left and middle panels) and the merged images in pseudocolor (right panel). Arrowheads indicate instances of velocity change. Four such events were observed. Scale bars are 10 μm (x) and 20 sec (y). Data is quantified in Fig. 4b. Statistical data is available as source data for Fig. 4.

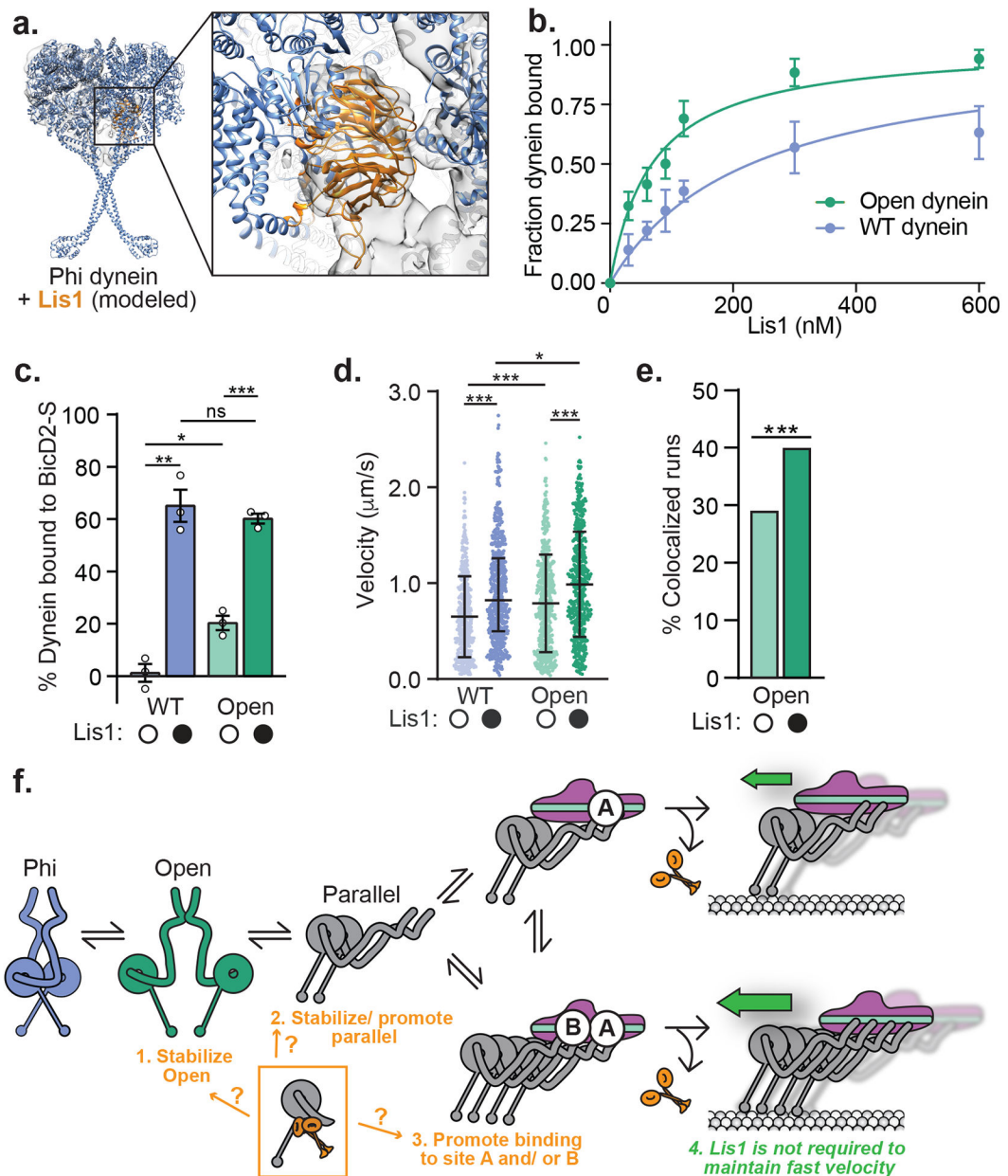


Fig. 5. Lis1 preferentially binds to Open dynein and enhances the formation of complexes containing two Open dynein dimers.

a. One of the dynein protomers in the Phi conformation (PDB: 5NVU) was aligned to the structure of yeast dynein (AAA3-Walker B) bound to Lis1 in the presence of ATP-vanadate (PDB: 5VLJ). The inset shows the cryo-EM map for the yeast structure with Lis1 docked at Site_{ring} and highlights the steric incompatibility between the Phi conformation and binding of Lis1 at this site. **b.** Determination of the binding affinity of Lis1 for wild-type (WT) dynein (blue, $K_d = 144 \text{ nM} \pm 25$) and Open dynein (green, $K_d = 80 \text{ nM} \pm 8.1$). **c.** Percent (mean \pm s.e.m.) of WT dynein (blue) and Open dynein (green) bound to BicD2-S conjugated to beads in the absence (white circles) or presence (black circles) of 150 nM Lis1. Data with WT dynein in the presence and absence of Lis1 is also presented in Fig. 3b. Statistical

analysis was performed using a two-tailed unpaired t test with Bonferroni corrected significance levels for two comparisons. **d.** Single-molecule velocity of dynein/ dynactin/ BicD2-S complexes with WT dynein (blue) and Open dynein (green) in the absence (white circles) or presence (black circles) of 300 nM Lis1. The median and interquartile range are shown. Data with WT dynein with and without Lis1 was also presented in Fig. 1f. **e.** Percent two-color colocalized runs with activated dynein complexes with Open dynein in the absence (white circle) or presence (black circle) of 300 nM Lis1. Statistical analysis was performed using a chi-squared test. The labeling efficiency for both TMR- and Alexa-647 dynein in this experiment was 100%. **f.** Model for the roles of Lis1 in forming activated dynein complexes. Statistical data is available as source data for Fig. 5.