Figures and figure supplements

The human cytoplasmic dynein interactome reveals novel activators of motility

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Figure 1. Validation of BioID-tagged dynein and dynactin subunits. (A) A cartoon of the dynein/dynactin/activator complex based on cryo-EM structural studies (Chowdhury et al., 2015; Urnavicius et al., 2015) with proteins drawn to scale. (B) BioID-3×FLAG or IC2-BioID-3×FLAG were immunoprecipitated from stable HEK-293 cell lines using α-FLAG antibodies and eluted using FLAG peptide. A Sypro Red stained SDS-PAGE gel of the immunoprecipitates is shown. (C) MS/MS analysis of the immunoprecipitates from (B). Core dynein subunit dNSAF (distributed normalized spectral abundance factor) (Zhang et al., 2015) values are displayed as a gray scale heat map. (D) Immunoprecipitations were performed as in (B) with mild (M) or harsh (H) detergent conditions (see Materials and methods). Harsh detergent conditions disrupt IC2 incorporation into the dynein/dynactin complex.
Figure 1 continued

as shown by Western blots with α-HC and α-p150 (dynactin subunit) antibodies. (E) IC2-BioID-3×FLAG was immunoprecipitated from a stable HEK-293 cell line using α-FLAG antibodies and fractionated by gel filtration FPLC chromatography. Fractions were analyzed by Western blotting with α-FLAG and α-HC antibodies. The signal intensity for IC2-BioID-3×FLAG (magenta) and HC (gray) in each fraction is plotted as a fraction of the summed intensity of all fractions. The elution volumes of molecular weight standards are indicated (dashed lines). (F) BioID-3×FLAG or p62-BioID-3×FLAG were immunoprecipitated from stable HEK-293 cell lines using α-FLAG antibodies. Immunoprecipitations were performed with mild (M) or harsh (H) detergent concentrations. Harsh detergent conditions disrupt p62 incorporation into the dynein/dynactin complex. (G) p62-BioID-3×FLAG was immunoprecipitated from a stable HEK-293 cell line using α-FLAG antibodies and analyzed as described in (E) with α-FLAG and α-p150 antibodies. The signal intensities for p62-BioID-3×FLAG (magenta) and p150 (gray) are plotted as a fraction of the summed intensity of all fractions. The elution volumes of molecular weight standards are indicated (dashed lines).

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Figure 1—figure supplement 1. Schematic of the dynein/dynactin/activator complex. Dynein subunits are uniquely colored except for the light chains (green). Dynactin subunits other than Arp1 (light gray) and actin (dark gray) are colored light blue. The dynactin Arp1 filament (light gray) is indicated with a single label. A coiled coil activator (e.g. BICD2) is depicted and colored yellow. The corresponding gene names, common names, and abbreviations are listed below.

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Figure 2. BioID with the dynein IC reports on activated dynein/dynactin/activator complexes in living human cells. 
(A) BioID experimental design. For each stably expressed BioID-tagged subunit reported in this study, quadruplicate samples were prepared, analyzed, and compared to a quadruplicate BioID only control. Fold enrichment was calculated as the ratio of dNSAF between the BioID-tagged subunit and the BioID control. (B) Biotinylated proteins were isolated from cells stably expressing either IC2-BioID or BioID by streptavidin affinity purification. A Sypro Red stained SDS-PAGE gel is shown. (C) MS/MS analysis of the immunoprecipitates from (B). Core dynein subunit dNSAF (Zhang et al., 2015) values are displayed as a heat map. (D) A volcano plot showing enrichment versus significance of proteins identified in IC2-BioID experiments relative to control (BioID alone) experiments. A quadrant (dashed magenta line) bounded by a p-value of 0.05 and 3-fold enrichment contained dynein (dark blue) and dynactin (light blue) subunits, as well as the known activators BICD2, HOOK1, and HOOK3, and the candidate activator BICD1 (yellow). 
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Figure 3. BioID reports on the spatial organization of the dynein/dynactin/activator complex. (A and B) Dynein (IC1, IC2, LIC1, LIC2, RB1, TCTEX1) and dynactin (p62) subunits tagged with BioID-3xFLAG were immunoprecipitated (for 16 hr in A or 2 hr in B) from stable HEK-293 cell lines using α-FLAG antibodies. All subunits incorporated into the dynein/dynactin complex based on Western blots with α-HC and α-p150 antibodies. (C) BioID experiments were performed with cells expressing the indicated dynein and dynactin subunits (magenta and magenta arrows). Other dynein and dynactin subunits enriched in the BioID experiments are shaded light gray (≥3 fold) or dark gray (>3 fold), p<0.05 (Student’s two-tailed t-test). DOI: 10.7554/eLife.28257.006
Figure 3—figure supplement 1. Enriched and significant hits from dynein and dynactin BioID datasets were used to construct a protein-protein interaction network. Large spheres represent BioID-tagged subunits (color coded according to a schematic of the dynein/dynactin complex, bottom right). Hits (small spheres) specific to a subunit family, color-coded according to their respective subunits: LCs (green), ICs (magenta) and LICs (orange). Gray spheres (‘two datasets’) and white spheres (‘three or more datasets’) represent hits enriched in two or three separate datasets, respectively. The protein names corresponding to white spheres are listed, right. Dynein and dynactin subunits, and activators are numbered; other hits are indicated with letters. Asterisk denotes hits detected in three different subunit classes (e.g. LICs/ICs/p62). Lines connecting spheres (edges) are color coded according to their respective datasets. For this figure enrichment is $\geq 3$ fold, significance is $p<0.05$, Student’s two-tailed t-test; and average spectral counts are $2$.

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Figure 4. A secondary screen identifies candidate activators of dynein/dynactin motility. (A) A schematic of our secondary screen. (B) Location of predicted coiled coils (rectangles) in known and candidate dynein/dynactin activators. (C, D) Candidate and known (BICD2 and HOOK3) activators tagged with 3×FLAG were immunoprecipitated with α-FLAG antibodies from HEK-293 cells. Western blots with α-HC and α-p150 antibodies were used to determine which proteins co-immunoprecipitated dynein and dynactin. (E—H) The candidate NIN (1–693) and NINL (1–702) activators, as well as the known BICD2 (1–422) and HOOK3 (1–552) activators were tagged with GFP and 3×FLAG and were immunoprecipitated with α-FLAG antibodies from HEK-293 cells. The motility of immunoprecipitated dynein/dynactin/activator complexes was monitored by GFP fluorescence using TIRF microscopy. Kymographs (left) and velocity histograms (right) with mean velocity (± S.D.) shown, n is greater than 102. Data shown is analyzed from one technical replicate, although two technical replicates were collected for each activator and displayed similar trends.

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Candidate and known (BICD2 and HOOK3) activators were tagged with 3×FLAG and immunoprecipitated with α-FLAG antibodies from HEK-293 cells. Western blots with α-HC and α-p150 antibodies were used to determine which proteins co-immunoprecipitated dynein and dynactin.

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Figure 4—figure supplement 2. (A) The amino acid sequences for HOOK1, HOOK2, HOOK3 and two HOOK-related proteins (daple and girdin) were downloaded from Uniprot and aligned using Clustal Omega. A region containing the HOOK3 (1–552) C-terminal truncation point is displayed and the residue in each protein equivalent to HOOK3 (1–552) is indicated. (B) As a control for the experiments in Figure 4E–H, GFP-3×FLAG was immunoprecipitated with an α-FLAG antibody from HEK-293 cells. No moving GFP signal was detected on microtubules. (C) The percentage of processive, diffusive and non-motile runs (see Materials and methods) in single-molecule motility assays (as described in Figure 4E–H) was analyzed for NIN (1–693), NINL (1–702), girdin (1–542) and daple (1–545) and compared to the known activators BICD2 (1–422) and HOOK3 (1–552).

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**Figure 5.** NIN and NINL are novel activators of dynein/dynactin motility. 

(A) A schematic of the components added to the single-molecule motility assay. Dynein (IC2-TMR, yellow star), dynactin (p62-Halo-Atto647, red star) and GFP-tagged (green spheres) activators (BICD2) or candidate activators (NIN and NINL) were purified separately, mixed, and the motility of the complex along microtubules was monitored by nearly simultaneous three-color TIRF microscopy. 

(B-E) Time-lapse images of each imaging channel (left) and velocity histograms (right) with mean velocity (± S. D.) are shown. 

NIN had a slower velocity in this assay compared to Figure 4G. This could be due to the lack of post-translational modifications in proteins expressed in *E. coli*; future work will be required to understand this. Data shown is analyzed from one technical replicate, although two technical replicates were collected for each activator and displayed similar trends. 

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Figure 5—figure supplement 1. Purification of dynein, dynactin and activators. (A) Dynein (IC2-SNAP-3×FLAG), dynactin (p62-Halo-3×FLAG) and the activators BICD2 (25–400), NIN (1-693) and NINL (1-702) (GFP-activator-Strep tag) were separately purified and used for the motility assays shown in Figure 5. An SDS-PAGE gel stained with Sypro Red shows the purification of each component. (B) The percentage of processive, diffusive and non-motile events in single-molecule motility assays (as described in E–G) was analyzed.

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Figure 6. Dynein activators have distinct proteomes. (A) Location of predicted coiled coils in dynein activators, with minimal activating regions shown (orange rectangles). (B) Enriched and significant hits from N- and C-terminal datasets of six activators were used to construct two separate protein-protein interaction networks. Hits specific to an activator family (color-coded according to their respective activators), and hits shared between activator families (HOOK/BICD, purple; BICD/NIN, yellow; NIN/HOOK, cyan) are shown. White spheres (‘3-family’) represent hits enriched in at least one activator from each family. For this figure enrichment is ≥3 fold, significance is p<0.05, Student’s two-tailed t-test; and average spectral counts are ≥2. The location of dynein and dynactin subunits and select hits discussed in the text are indicated. We note that we identified BICD1 in our BICD2

Figure 6 continued on next page
datasets and vice versa (Supplementary files 1 and 4). The same was true for HOOK1 and HOOK3, but not for NiN and NiNL (Supplementary files 1 and 4). HOOK proteins have been shown to heterodimerize (Xu et al., 2008), whereas heterodimerization between BICD proteins has not been reported. (C) The number of total, unique (occurring in a single activator N- or C-terminal dataset), and shared (occurring in multiple activator N- or C-terminal datasets) hits for individual activator N- and C-termini are shown.

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Figure 6—figure supplement 1. An interaction map of the N- and C-terminal activator datasets combined. Enriched and significant hits from the combined N- and C-terminal datasets of six activators were used to construct a single protein-protein interaction network. Hits (small spheres) specific to an activator family (color-coded according to their respective activators), and hits shared between activator families (HOOK/BICD, purple; BICD/NIN, yellow; NIN/HOOK, cyan) are shown. White spheres (‘3-family’) represent hits enriched in at least one activator (NT or CT) from each family. Lines connecting spheres (edges) are color coded according to activator family and termini (NT = thin, CT = thick). The regions encompassing activator family CT-specific overlap are indicated with their respective n. The number of unique hits for each dataset is represented as a gray circle that is scaled according to the number of hits and the number of hits is shown. For this figure enrichment is ≥3 fold, significance is p<0.05, Student’s two-tailed t-test; and average spectral counts are ≥2. The location of dynein and dynactin subunits and select hits discussed in the text are indicated.

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Figure 6—figure supplement 2. KIF1C is a novel HOOK3-interacting protein. sfGFP-3×FLAG and full length (FL) HOOK3, HOOK3 (1–552), and HOOK3 (553–718) all tagged with sfGFP and 3×FLAG were immunoprecipitated with α-FLAG antibodies from transiently transfected HEK-293T cells. Western blots with α-HC, α-FAM160A2, α-KIF1C, and α-FLAG antibodies were used to determine which proteins co-immunoprecipitated with each HOOK3 construct.

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