Supplemental Materials.

Supplemental Figures and Tables

Supplemental Table S1. Identification of BICD2-N binding subunits of dynein and dynactin by mass spectrometry. All proteins identified with a significant score and not present in the background controls are listed.

A. Mass spectrometry analysis of purified bovine dynactin

Score	NCBI GI number	Description	Da	Coverage (%)	Unique Peptides
3890	**gi|149642611**	dynactin 1 (p150Glued)	137458	45.2	41
1478	**gi|5031569**	ARP1	42701	54.5	16
1185	**gi|77736063**	dynactin 2 (p50)	44495	35.7	14
1177	**gi|119914141**	cytoplasmic dynein heavy chain	548197	5.8	19
933	**gi|28603770**	capping protein beta	34176	27.6	12
822	**gi|61316470**	capping protein alpha 2	33073	55.6	9
712	**gi|73953656**	dynactin p62	54023	29.3	9
262	**gi|115497348**	cytoplasmic dynein intermediate chain 2	68734	9.5	3
178	**gi|119892302**	kinesin family member 21A	187179	1.5	2
151	**gi|115497064**	dynactin 3 (p22)	21292	15.1	3
130	**gi|115497256**	dynactin 6 (p27)	21061	14.2	2
68	**gi|164420721**	dynactin 5 (p24)	20698	8.2	2
63	**gi|76640631**	dynein light intermediate chain 2	54392	2.4	1

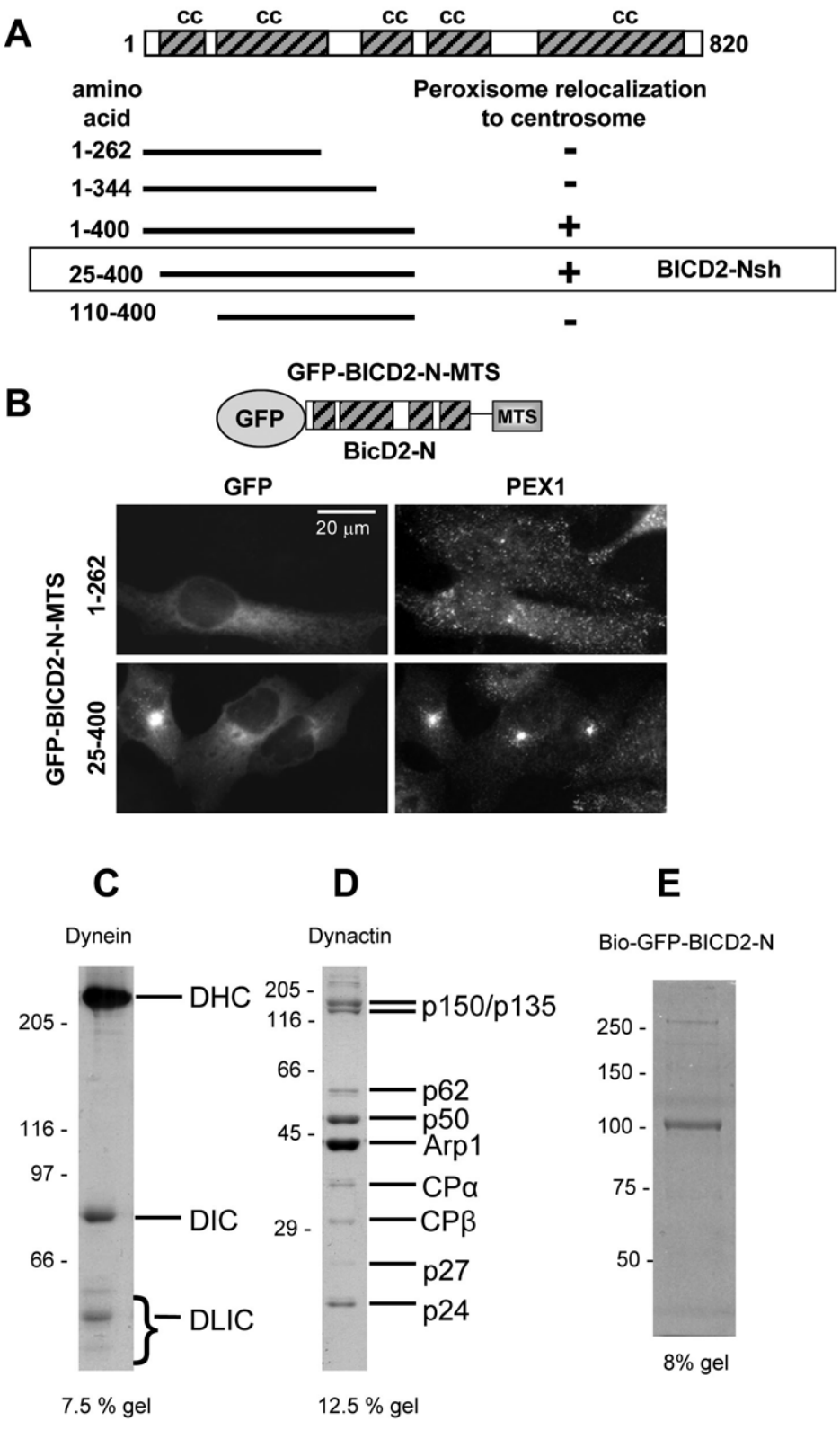
B. Mass spectrometry analysis of purified bovine dynein

Score	NCBI GI number	Description	Da	Coverage (%)	Unique Peptides
16600	**gi|119914141**	cytoplasmic dynein heavy chain	548197	50.5	199
1593	**gi|114051407**	cytoplasmic dynein light intermediate chain 1	56800	49.6	22
956	**gi|76640631**	cytoplasmic dynein light intermediate chain 2	54392	32.3	14
840	**gi|11276091**	cytoplasmic dynein intermediate chain 1	73222	22.7	10
732	**gi|74004544**	cytoplasmic dynein intermediate chain 2	69215	27.7	9
321	**gi|18777767**	cytoplasmic dynein light chain roadblock type 1	10983	74.0	4
108	**gi|5730085**	cytoplasmic dynein, light chain Tctex	12672	14.2	1
75	**gi|157074188**	ARP1	42382	2.7	1
47	**gi|77736063**	dynactin 2 (p50)	44495	2.2	1

C. Mass spectrometry analysis of Bio-GFP-BICD2-N-dynein-dynactin complex after cross-linking with low doses of Bis[sulfosuccinimidyl] glutarate and isolated by pull-down with streptavidin beads in denaturing conditions

Score	NCBI GI number	Description	Da	Coverage (%)	Unique Peptides
8026	**gi|119914141**	Cytoplasmic dynein heavy chain	548197	29.3	124
718	**gi|149642611**	Dynactin 1 (p150Glued)	137458	8.4	9
513	**gi|18139547**	BICD2	93562	8.9	7
366	**gi|114051407**	Cytoplasmic dynein light intermediate chain 1	56800	12.6	7
365	**gi|76640631**	Cytoplasmic dynein light intermediate chain 2	54392	15.2	6

Splinter et al., Suppl. Figure S1



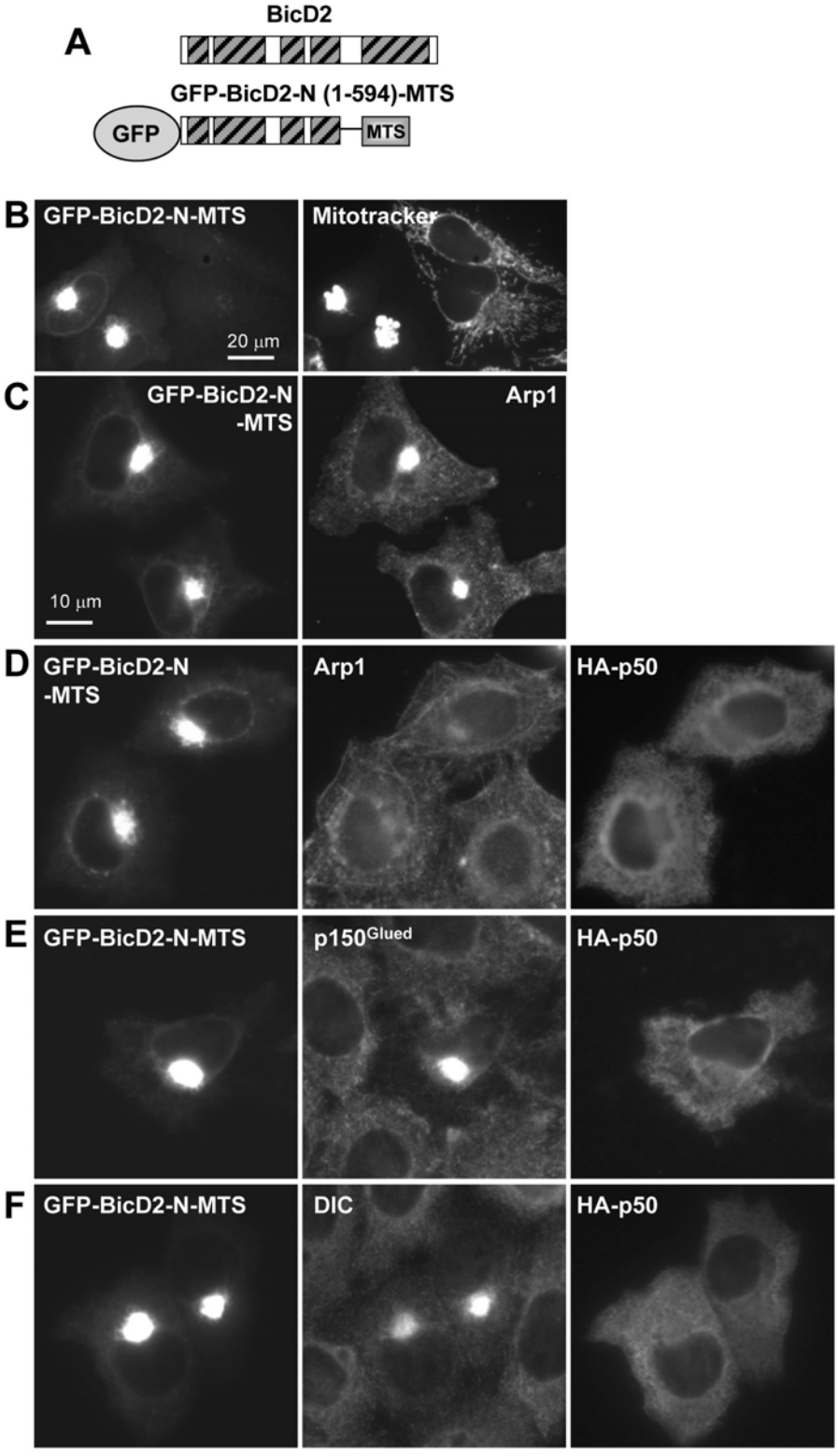
Supplemental Figure S1. Mapping of the minimal dynein-interacting domain of BICD2 and characterization of the purified protein complexes.

(A,B) Mapping of the minimal dynein-interacting domain of BICD2 using peroxisome/mitochondria relocalization assay. In this assay, BICD2-N fragments are targeted to the cytosolic side of the peroxisomes and mitochondria using the *Listeria monocytogenes* ActA-derived membrane-targeting sequence (MTS) and the distribution of the organelles is assessed by immunofluorescent staining (Hoogenraad *et al.*, 2003). When dynein motors are recruited to the organelles, these organelles form a tight pericentrosomal cluster. **(A)** A scheme of BICD2 fragments used and a summary of their effect on peroxisome localization when fused to MTS. The shortest construct, which potently relocalized is indicated by a box. **(B)** A scheme of GFP-BICD2-N-MTS constructs and representative images showing HeLa cells transfected with different BICD2-N-MTS fusions and stained for the peroxisome marker PEX1. Peroxisomes are relocated to the pericentrosomal region by the GFP-BICD2-N-MTs fusion containing amino acids 25-400 of BICD2, but not by the fusion containing amino acids 1-262.

(C,D) Coomassie-stained gels of dynein (7.5% acrylamide) **(C)** and dynactin (12.5% acrylamide) **(D)** complexes are shown with individual subunits identified.

(E) Coomassie-stained gel showing BICD2-N purified from HEK293 cells.

Splinter et al., Suppl. Figure S2

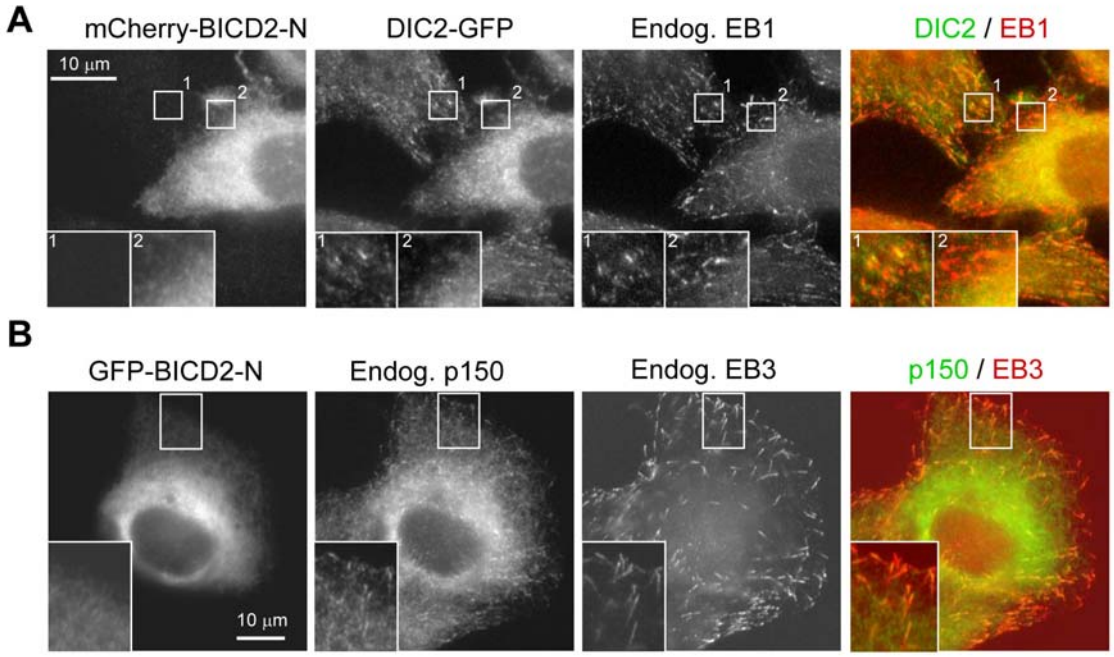


Supplemental Figure S2. Overexpression of p50/dynamitin removes Arp1 but not dynein or p150Glued from the mitochondrial cluster induced by BICD2-N expression.

(A) A scheme of GFP-BICD2-N-MTS construct.

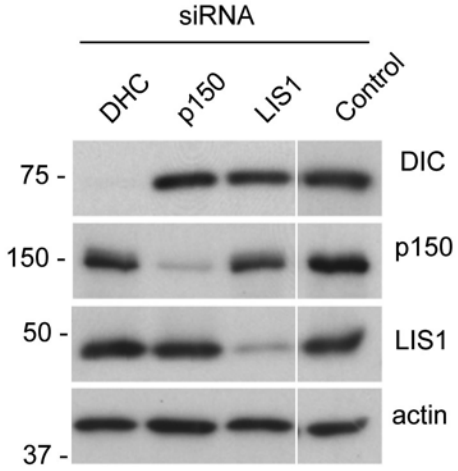
(B-F) HeLa cells were transfected either with GFP-BICD2-N-MTS alone **(B,C)** or together with HA-p50/dynamitin **(D-F)**, fixed with cold methanol (for dynein staining), with cold methanol followed by 4% paraformaldehyde (for dynactin subunits) or with 4% paraformaldehyde in culturing medium (to visualize mitochondria). Cells were stained with the indicated antibodies against dynein or dynactin subunits, HA tag, or MitoTracker Red CMXRos.

Splinter et al., Suppl. Figure S3



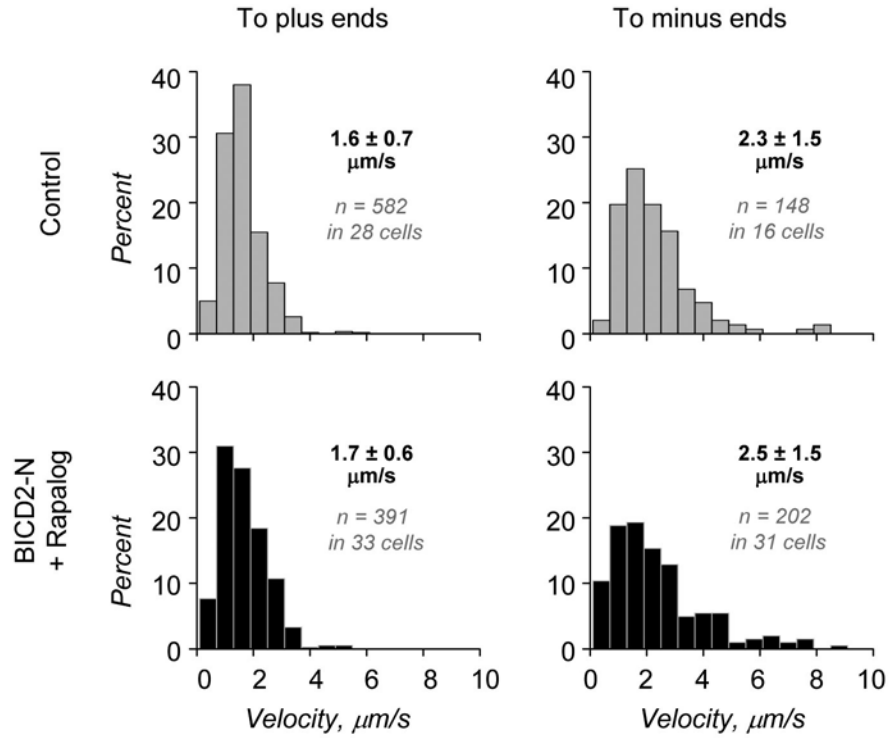
Supplemental Figure S3. BICD2-N displaces dynein but not dynactin from MT tips.
(A) HeLa cells stably expressing DIC2-GFP (green) were transfected with mCherry-BICD2-N and stained for endogenous EB1, a marker of growing MT plus ends (red). Insets show enlargements of the boxed areas indicated by numbers. Note that DIC2-GFP is diffuse in mCherry-BICD2-N expressing cell but is present at the EB1-positive MT plus ends in surrounding cells.
(B) HeLa cells were transfected with GFP-BICD2-N and stained for endogenous $p150^{Glued}$ (green) and EB3 (red). EB3 is an EB1 family member, which similar to EB1 marks growing MT plus ends. Insets show enlargements of the boxed areas. $p150^{Glued}$ is still detectable at the EB3-positive MT tips in the GFP-BICD2-N-expressing cell.

Splinter et al., Suppl. Figure S4



Supplemental Figure S4. Characterization of DHC, p150^{Glued} and LIS1 siRNAs. HeLa cells were transfected with the indicated siRNAs and Western blots were performed with the indicated antibodies 3 days after transfection.

Splinter et al., Suppl. Figure S5



Supplemental Figure S5. Rab6A vesicle movement velocities in the absence of BICD2-N and after BICD2-N recruitment.

Distributions of movement velocities to MT plus and minus ends in MRC5-SV cells expressing FKBP2-GFP-Rab6A alone or together with HA-BICD2-N-FRB, after rapalog addition.