Egan et al., http://www.jcb.org/cgi/content/full/jcb.201112101/DC1

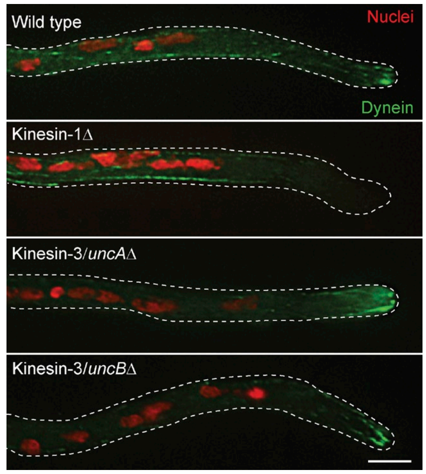


Figure S1. **Localization of dynein to microtubule plus ends requires kinesin-1 but not kinesin-3s.** Micrographs showing the localization of dynein-GFP and histone H1–mCherry-labeled nuclei in wild-type, kinesin-1Δ, kinesin-3/*uncA*Δ, and kinesin-3/*uncB*Δ hyphae. In strains lacking kinesin-1, dynein comets are absent from the microtubule plus ends and instead decorate the length of the microtubules. Dashed lines indicate the outline of the hyphae. Bar, 5 µm.

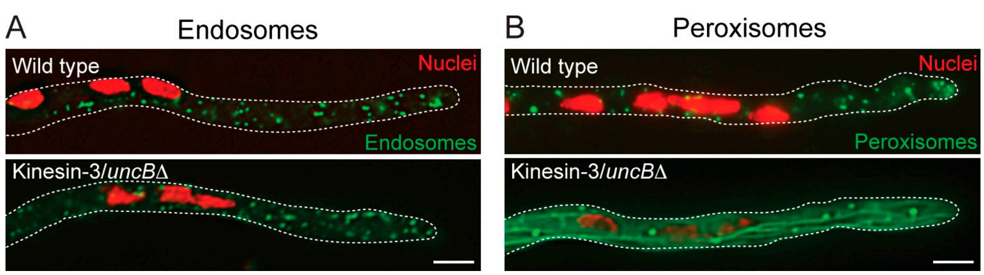


Figure S2. **Endosome and peroxisome transport is unaffected by deletion of kinesin-3/*uncB*.** (A and B) Micrographs showing the localization of GFP-Rab5/RabA–labeled endosomes (A) and Pex11/PexK-GFP–labeled peroxisomes (B) in wild-type (top images) and kinesin-3/*uncB*Δ (bottom images) hyphae. Endosomes and peroxisomes are normally distributed throughout the length of the hyphae. GFP–α-tubulin microtubules are also visible in the kinesin-3/*uncB*Δ PexK-GFP image. Dashed lines indicate the outline of the hyphae. Bars, 5 µm.

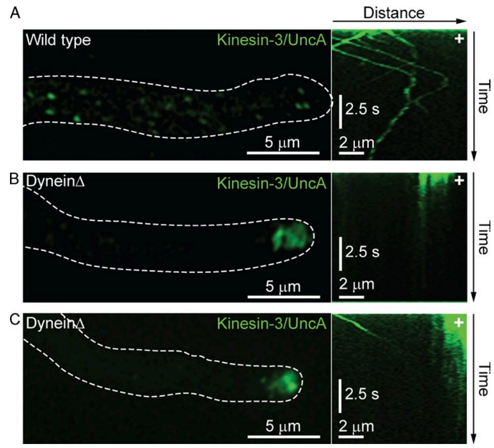


Figure S3. **Kinesin-3/UncA accumulates in the hyphal tip in the absence of dynein.** (A–C) In wild-type hyphae (A), kinesin-3/UncA-GFP particles are uniformly distributed (left images) and move bidirectionally, as indicated by kymographs generated from time-lapse videos (right images). In dyneinΔ hyphae (B and C), kinesin-3/UncA particles are mislocalized to the hyphal tip (left images) and are largely immotile, as shown in a representative kymograph (B, right), except for rare plus-end–directed movements (C, right). Dashed lines indicate the outline of the hyphae. The locations of microtubule plus ends in the hyphal tip are indicated by white plus signs.

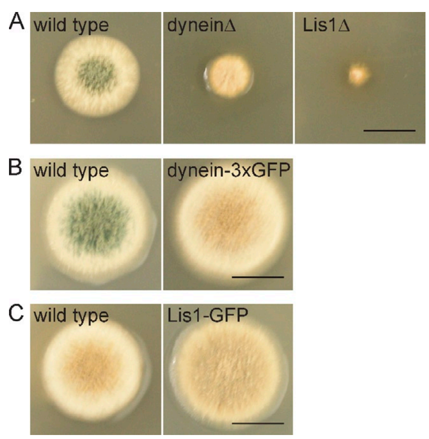


Figure S4. **Tagging dynein or Lis1 with GFP does not affect function.** (A) Targeted deletion of the sole genomic copy of dynein or Lis1 severely affects hyphal growth and colony size, leading to a *nud* phenotype. (B) Strains expressing the dynein heavy chain tagged with three tandem copies of GFP at its C terminus grow similarly to wild type. (C) Strains expressing Lis1 tagged with GFP at its C terminus grow similarly to wild type. Bars, 1 cm.

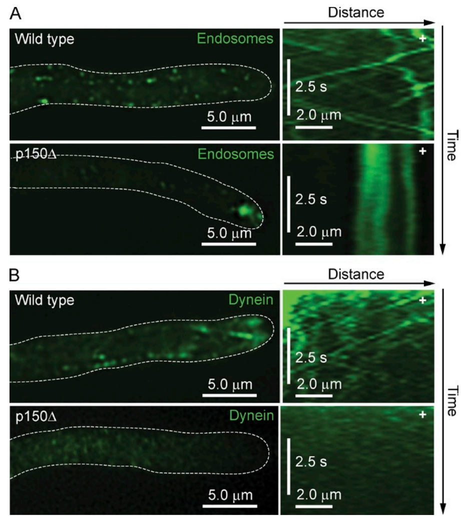
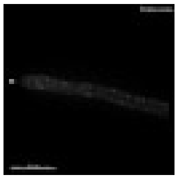
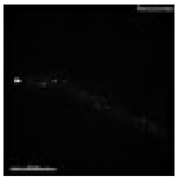


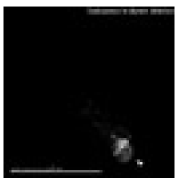
Figure S5. **Dynactin is required for endosome and dynein motility.** (A and B) Micrographs (left images) and representative kymographs (right images) showing the localizations and motilities of GFP-Rab5/RabA endosomes (A) and dynein-3xGFP (B) in wild-type (top images) and p150Δ (bottom images) hyphae. GFP-Rab5/RabA endosomes in wild-type hyphae (A, top left) are uniformly distributed and move bidirectionally (A, top right), whereas in p150Δ, they accumulate in the hyphal tip (A, bottom left) and are nonmotile (A, bottom right). In wild-type hyphae, dynein-3xGFP (B, top left) localizes to microtubule plus ends and to discrete puncta decorating the microtubule length, which move bidirectionally (B, top right). In p150Δ hyphae (B, bottom left), dynein-3xGFP appears diffuse in the cytoplasm and is nonmotile (B, bottom right). Dashed lines indicate the outline of the hyphae. The locations of microtubule plus ends in the hyphal tip are indicated by white plus signs.



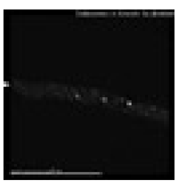
Video 1. **GFP-Rab5/RabA–labeled endosome dynamics in a wild-type hypha.** Long-distance bidirectional transport of GFP-Rab5/RabA endosomes in a wild-type hypha. Images were analyzed by time-lapse wide-field fluorescence microscopy using a custom-built microscope (DeltaVision OMX). Frames were taken every 162 ms for 32 s. Frame rate is 10 frames per second. The arrowhead points to the hyphal tip.



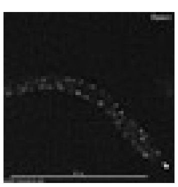
Video 2. **Pex11/PexK-GFP–labeled peroxisome dynamics in a wild-type hypha.** Sporadic anterograde and retrograde peroxisome movement in a wild-type hypha. Images were analyzed by time-lapse wide-field fluorescence microscopy using a custom-built microscope (DeltaVision OMX). Frames were taken every 162 ms for 15.9 s. Frame rate is 10 frames per second. The arrowhead points to the hyphal tip.



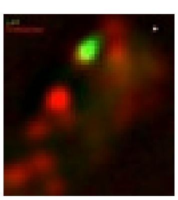
Video 3. **GFP-Rab5/RabA–labeled endosome dynamics in a dyneinΔ hypha.** Nonmotile GFP-Rab5/RabA endosomes accumulate in the hyphal tip of a dynein deletion mutant. Images were analyzed by time-lapse wide-field fluorescence microscopy using a custom-built microscope (DeltaVision OMX). Frames were taken every 142 ms for 28 s. Frame rate is 10 frames per second. The arrowhead points to the hyphal tip.



Video 4. **GFP-Rab5/RabA–labeled endosome dynamics in a kinesin-3/*uncA*Δ hypha.** GFP-Rab5/RabA endosomes are nonmotile in the absence of kinesin-3/*uncA*. Images were analyzed by time-lapse wide-field fluorescence microscopy using a custom-built microscope (DeltaVision OMX). Frames were taken every 142 ms for 28 s. Frame rate is 10 frames per second. The arrowhead points to the hyphal tip.



Video 5. **Dynein-3xGFP dynamics in a wild-type hypha.** Dynein-3xGFP particles moving bidirectionally throughout the length of a wild-type hypha. Images were analyzed by time-lapse wide-field fluorescence microscopy using a custom-built microscope (DeltaVision OMX). Frames were taken every 94 ms for 9.3 s. Frame rate is 10 frames per second. The arrowhead points to the hyphal tip.



Video 6. **Lis1-GFP and mCherry-Rab5/RabA–labeled endosomes in a wild-type hypha.** Lis1-GFP and mCherry-Rab5/RabA–labeled endosome dynamics in a wild-type hypha. Images were analyzed by time-lapse wide-field fluorescence microscopy using a custom-built microscope (DeltaVision OMX). Frames were taken every 130 ms for 4.7 s. Frame rate is 10 frames per second. The arrowhead points to the hyphal tip.

Table S1. ***A. nidulans* strains used in this study**

Strain	Genotype	Source
LO1704	[*hhoA*::GFP::*Afribo*]; *riboB2*; *pabaA1*; *pyroA4; pyrG89*; Δ*nkuA*::*argB, fawn*	B. Oakley
LO1915	[*hhoA*::mCherry::*AfpyrG*]; *wA*::[GFP::*tubA*::*AfpyroA*]; *riboB2*; *pyroA4; pyrG89*; Δ*nkuA*::*argB*	B. Oakley
MAD2056	*argB2*::[*argB*::*alcA*(p)::GFP::*rabA*]; *yA2*; *pantoB100*	X. Xiang; Zhang et al., 2010
TFJ 20.6	*argB2*::[*argB*::*alcA*(p)::GFP::*rabA*]; *yA2*; *pantoB100*	M. Peñalva
11942	*trpC*::[*pexK*::GFP::*Afpyro*]; *pyroA*	M. Hynes; Hynes et al., 2008
LZ12	[GFP::*nudA*]; *pyroA*; *pyrG89*; Δ*nkuA*::*argB*	X. Xiang; Zhuang et al., 2007
RPA197	[Δ*kinA*::*Afpyro*]; [*hhoA*::mCherry::*AfpyrG*]; [*pexK*::GFP::*Afpyro*]; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar/argB*	This study
RPA199	[Δ*uncA*::*AfpyrG*]; [*hhoA*::mCherry::*AfpyrG*]; [*pexK*::GFP::*Afpyro*]; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar/argB*	This study
RPA218	[*hhoA*::mCherry::*AfpyrG*]; [*pexK*::GFP::*Afpyro*]; *pyroA*; *pyrG89*; Δ*nkuA*::*bar/argB*	This study
RPA230	[Δ*uncB*::*bar*]; [*hhoA*::mCherry::*AfpyrG*]; [*pexK*::GFP::*Afpyro*]; *wA*::[GFP::*tubA*::*AfpyroA*]; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar/argB*	This study
RPA261	[GFP::*nudA*]; [Δ*kinA*::*Afpyro*]; [*hhoA*::mCherry::*AfpyrG*]; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar*	This study
RPA262	[GFP::*nudA*]; [Δ*uncB*::*bar*]; [*hhoA*::mCherry::*AfpyrG*]; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar*	This study
RPA263	[GFP::*nudA*]; [Δ*uncA*::*AfpyrG*]; [*hhoA*::mCherry::*AfpyrG*]; *pyroA4*; *pyrG89*; Δ*nkuA*::*argB*	This study
RPA265	[GFP::*nudA*]; [*hhoA*::mCherry::*AfpyrG*]; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar*	This study
RPA269	[Δ*uncB*::*bar*]; [*hhoA*::mCherry::*AfpyrG*]; *argB2*::[*argB*::*alcA*(p)::GFP::*rabA*]; maybe Δ*nkuA*	This study
RPA282	[*hhoA*::mCherry::*AfpyrG*]; *argB2*::[*argB*::*alcA*(p)::GFP::*rabA*]; *pyroA4*	This study
RPA313	[EB1/*AN2862*::mCherry::*Afribo*]; [*hhoA*::mCherry::*AfpyrG*]; *wA*::[GFP::*tubA*::*AfpyroA*]; *riboB2*; *pyroA4*; *pyrG89*; Δ*nkuA*::*argB*	This study
RPA349	[*nudA*::3xGFP::*AfpyrG*]; [*hhoA*::GFP::*Afribo*]; *riboB2*; *pabaA1*; *pyroA4*; *pyrG89*; *fwA*; Δ*nkuA*::*argB*	This study
RPA362	[Δ*kinA*::*Afpyro*]; [EB1/*AN2862*::mCherry::*Afribo*]; [*hhoA*::mCherry::*AfpyrG*]; *wA*::[GFP::*tubA*::*AfpyroA*]; *riboB2*; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar/argB*	This study
RPA363	[Δ*uncA*::*AfpyrG*]; [EB1/*AN2862*::mCherry::*Afribo*]; [*hhoA*::mCherry::*AfpyrG*]; *wA*::[GFP::*tubA*::*AfpyroA*]; *riboB2*; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar*	This study
RPA365	[Δ*uncB*::*bar*]; [EB1/*AN2862*::mCherry::*Afribo*]; [*hhoA*::mCherry::*AfpyrG*]; *wA*::[GFP::*tubA*::*AfpyroA*]; *riboB2*; *pyroA4*; *pyrG89*; Δ*nkuA*::*bar/argB*	This study
RPA368	[Δ*nudA*::*bar*]; [*hhoA*::mCherry::*AfpyrG*]; [*pexK*::*GFP*::*AfpyroA*]; *pyrG89*; *pyroA4*; Δ*nkuA*::*argB*	This study
RPA395	*argB2*::[*argB*::*alcA*(p)::GFP::*rabA*]; [*hhoA*::mCherry::*AfpyrG*]; *yA1*; Δ*nkuA*::*bar*	This study
RPA401	[*nudA*::3xGFP::*AfpyrG*]; *argB2*::[*argB*::*alcA*(p)::mCherry::*rabA*]; *pyrG89*; *pyroA4*; Δ*nkuA*::*bar*	This study
RPA413	[Δ*nudA*::*bar*]; [EB1/*AN2862*::mCherry::*Afribo*]; [*hhoA*::mCherry::*AfpyrG*]; *wA*::[GFP::*tubA*::*AfpyroA*]; *riboB2*; *pyroA4*; *pyrG89*; Δ*nkuA*::*argB*	This study
RPA432	[Δ*kinA*::*Afpyro*]; [*hhoA*::mCherry::*AfpyrG*]; *argB2*::[*argB*::*alcA*(p)::GFP::*rabA*]; *pyroA4*, Δ*nkuA*::*bar*	This study
RPA442	[Δ*uncA*::*AfpyrG*]; [*hhoA*::mCherry::*AfpyrG*]; *argB2*::[*argB*::*alcA*(p)::GFP::*rabA*]; Δ*nkuA*::*bar*	This study
RPA443	[Δ*nudA*::*bar*]; [*hhoA*::mCherry::*AfpyrG*]; *argB2*::[*alcA*(p)::GFP::*rabA*]; Δ*nkuA*::*argB*	This study
RPA453	[Δ*nudF*::*AfpyrG*]; *argB2*::[*argB*::*alcA*(p)::GFP::*rabA*]; *pyrG89*; Δ*nkuA*::*argB*	This study
RPA464	[TagGFP2::*rabA*::*AfpyrG*]; *yA1*; *pyrG89*; *pyroA4; pabaA1*; Δ*nkuA*::*argB*	This study
RPA480	[TagGFP2::*rabA*::*AfpyrG*]; [Δ*nudF*::*Afpyro*]; *yA1*; *pyrG89*; *pyroA4; pabaA1*; Δ*nkuA*::*argB*	This study
RPA503	[Δ*nudF*::*Afpyro*]; [*nudA*::3xGFP::*AfpyrG*]; *wA*::[GFP::*tubA*::*AfpyroA*]; *pyrG89*; *pyroA4*; Δ*nkuA*::*bar*	This study
RPA508	*argB2*::[*argB*::*alcA*(p)::mCherry::*rabA*]; [*nudF*::GFP::*AfpyrG*]; *yA1*; *pyrG89*; *pyroA4; pabaA1*; Δ*nkuA*::*bar*	This study
RPA524	[Δ*nudF*::*AfpyrG*]; [*nudA*::3xGFP::*AfpyrG*]; *argB2*::[*argB*::*alcA*(p)::mCherry::*rabA*]; *pyrG89*; *pyroA4*; Δ*nkuA*::*bar*	This study
RPA527	[*nudA*::3xGFP::*AfpyrG*]; *yA*::[*gpdA*(p)::mCherry::FLAG::PTS1::*Afpyro*]; *pyrG89*; *pyroA4*; Δ*nkuA*::*argB*	This study
RPA592	[Δ*nudF*::*bar*]; *yA*::[*gpdA*(p)::mCherry::FLAG::PTS1::*Afpyro*]; [*nudA*::3xGFP:: *AfpyrG*]; *pyrG89; pyroA4:* Δ*nkuA*::*argB*	This study
RPA597	[*uncA*::TagGFP2::*Afribo*]; *riboB2*; *pyrG89*; *pyroA4*; Δ*nkuA*::*argB*	This study
RPA660	[Δ*nudM*::*Afpyro*]; [TagGFP2::*rabA*::*AfpyrG*]; *yA1*; *pabaA1*; *pyrG89*; *pyroA4*; Δ*nkuA*::*argB*	This study
RPA661	[Δ*nudM*::*Afpyro*]; *argB2*::[*argB*::*alcA*(p)::mCherry::*rabA*]; [*nudA*::3xGFP::*AfpyrG*]; *pyrG89*; *pyroA4*; Δ*nkuA*::*bar*	This study
RPA662	[Δ*nudA*::*AfpyrG*]; [*uncA*::TagGFP2::*Afribo*]; *riboB2*; *pyrG89*; *pyroA4*; Δ*nkuA*::*argB*	This study
RPA663	[p25/*AN5022*::TagGFP2::*AfpyrG*]; *argB2*::[*argB*::*alcA*(p)::mCherry::*rabA*]; *pyrG89*; *pyroA4*; Δ*nkuA*::*bar*	This study

All promoters are the endogenous promoter, unless otherwise stated.