Zhang et al., http://www.jcb.org/cgi/content/full/jcb.201011022/DC1

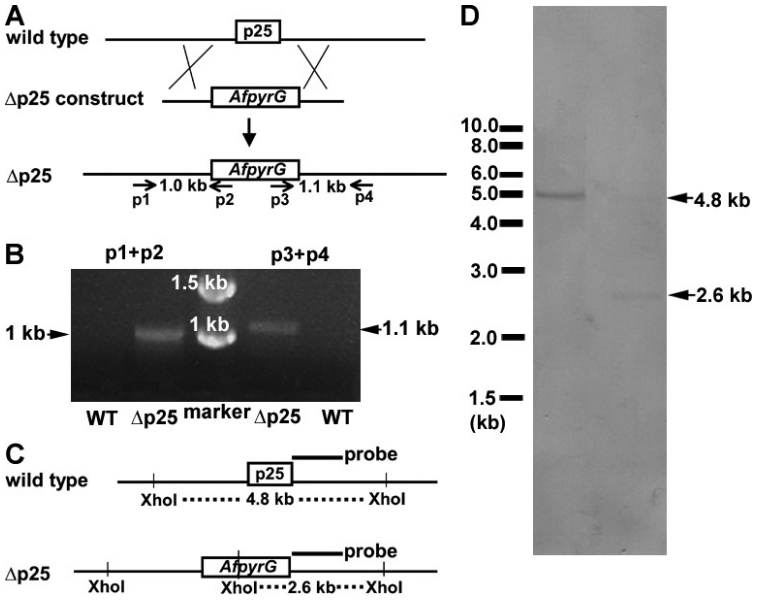


Figure S1. **Site-specific integration of the Δp25 construct into the genome of the Δp25 mutant as indicated by PCR and Southern analyses.** (A) A diagram showing the Δp25 linear construct with the *AfpyrG* marker flanked by the 5′ and 3′ flanking sequences of the p25 gene. Homologous recombination events that occurred between this construct and the wild-type genome (wild type) are indicated by crosses. The resulting Δp25 locus is shown at the bottom. The positions of the primers used for PCR analyses are indicated by arrows. Note that these primers are located outside of the flanking sequences of the Δp25 construct and thus will produce the expected products of 1.0 kb and 1.1 kb only when the construct integrated into the p25 locus. (B) Result of a PCR analysis on genomic DNAs from the Δp25 mutant and a wild-type strain (WT). (C) A diagram showing the sizes of expected signals on a Southern blot using XhoI-digested genomic DNAs from wild type and the Δp25 mutant when the 3′ flanking region of the Δp25 construct is used as a probe. A 4.8-kb band is expected from the wild type based on the genomic sequence of the region. However, because a XhoI site is within the *AfpyrG* marker, a 2.6 kb signal is expected from the Δp25 mutant. (D) The result of the Southern analysis shows expected signals both in the wild type (WT) and the Δp25 mutant samples.

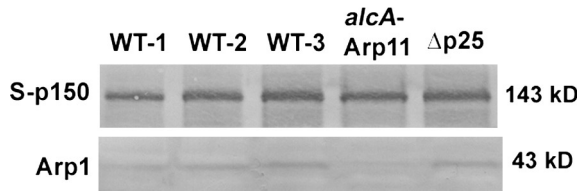


Figure S2. **S-tagged p150 (S-p150) pulled down a normal amount of Arp1 in extract from Δp25 cells but not from *alcA*-Arp11 cells.** To test whether Δp25 affects p150–Arp1 interaction, the *alcA*-Arp11 mutant was used as a positive control, as we showed previously that loss of Arp11 negatively affected the amount of Arp1 pulled down by the S-tagged p150 (Zhang et al., 2008). In addition, different loadings of the wild-type sample on the same gel were done, which strengthens our conclusion that loss of Arp11 significantly lowered the amount of Arp1 pulled down by S-p150 (Zhang et al., 2008). However, loss of p25 does not apparently affect the amount of Arp1 pulled down by S-p150. WT-1, 20 µl; WT-2, 30 µl; WT-3, 40 µl. For both the *alcA*-Arp11 and Δp25 mutants, 40 µl of eluate was loaded.

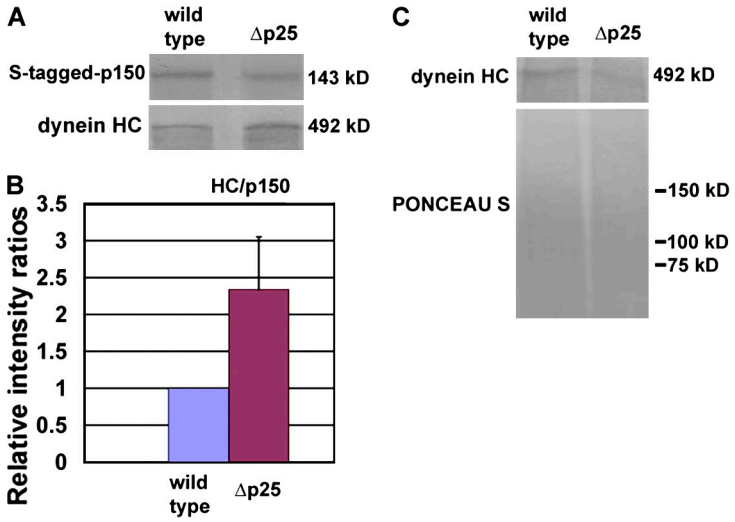
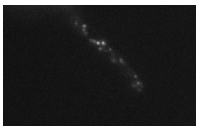
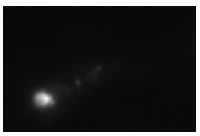


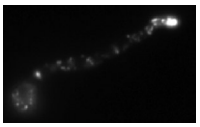
Figure S3. **Dynein–dynactin interaction is not negatively affected by loss of p25.** (A) In the extracts of the Δp25 mutant, S-p150 pulled down dynein heavy chains, and the amount of pulled-down dynein was even higher than that in the extract of wild-type cells. (B) A quantitative analysis of the Western results. All the values were relative to wild-type values, which were set as 1. Mean and standard deviation values (error bars) were based on results from three independent pull-down experiments ($P < 0.05$). (C) Western analyses on total extracts indicate that the relatively high level of pulled-down dynein in the Δp25 mustant is not caused by a relatively high level of total dynein in the mutant. A similar phenomenon was also observed during our previous study on Arp11 (Zhang et al., 2008). Although we currently do not understand the mechanism behind this phenomenon, the fact that nuclear distribution appears normal in the Δp25 mutant suggests that this enhancement in dynein–dynactin interaction does not significantly affect the normal function of dynein.



Video 1. **The mCherry-RabA fusion protein is expressed in a wild-type strain, which allows early endosomes to be labeled.** By using an inverted fluorescence microscope (IX70; Olympus), early endosomes were observed to undergo bidirectional movements. 30 frames were taken with a 0.1-s exposure time and a 0.3-s interval between frames. The movie has been sped up 5 times.

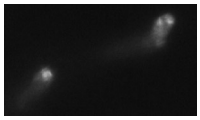


Video 2. **Early endosomes labeled by mCherry-RabA in a Δp25 hypha are largely accumulated at the hyphal tip.** Note that an early endosome moves toward the hyphal tip. An inverted fluorescence microscope (Olympus IX70) was used for capturing images. 30 frames were taken with a 0.1-s exposure time and a 0.3-s interval between frames. The movie has been sped up 5 times.

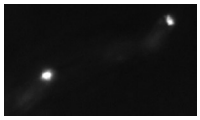


Video 3. **Early endosomes labeled by mCherry-RabA in Δp25 hypha are largely accumulated at the hyphal tip.** This is a different hypha than that in Video 2. Note that many early endosomes along the hypha do not undergo directional movements. An inverted fluorescence microscope (IX70; Olympus) was used for capturing images. 12 frames were taken with a 0.1-s exposure time and a 1-s interval between frames. The movie has been sped up 10 times.

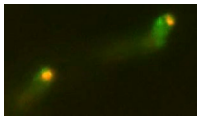
Video 4. **Early endosomes labeled by mCherry-RabA in a Δ*nudA* (dynein heavy chain) hypha are largely accumulated at the hyphal tip.** An inverted fluorescence microscope (IX70; Olympus) was used for capturing images. 12 frames were taken with a 0.1-s exposure time and a 1-s interval between frames. The whiteness of the images was enhanced to show the nonmobile mCherry-RabA positive dots in the hypha. The movie has been sped up 10 times.



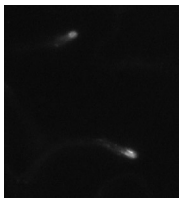
Video 5. **GFP-dynein HC (GFP-HC) signals in the Δ*nudF* mutant.** The GFP-HC fusion was introduced into the Δ*nudF* mutant by a genetic cross. An inverted fluorescence microscope (IX70; Olympus) was used for capturing images. 12 frames were taken with a 0.1-s exposure time and a 1-s interval between frames. The movie has been sped up 10 times.



Video 6. **mCherry-RabA signals in the same Δ*nudF* cells shown in Video 5.** The mCherry-RabA fusion was introduced into the Δ*nudF* mutant containing GFP-HC by a genetic cross. An inverted fluorescence microscope (IX70; Olympus) was used for capturing images. 12 frames were taken with a 0.1-s exposure time and a 1-s interval between frames. The movie has been sped up 10 times.



Video 7. **Merge of the GFP-HC and mCherry-RabA signals in the same Δ*nudF* mutant cells as shown in Videos 5 and 6.** Note that both GFP-HC and mCherry-RabA localize to the hyphal tip region, and the signals largely but not fully overlap with each other. An inverted fluorescence microscope (IX70; Olympus) was used for capturing images. 12 frames were taken with a 0.1-s exposure time and a 1-s interval between frames. The movie has been sped up 10 times.



Video 8. **A cloud-like structure at the hyphal tip formed by p150-GFP in *alcA-nudF* cells.** The p150–GFP fusion was introduced into the *alcA-nudF* mutant by a genetic cross. An inverted fluorescence microscope (IX70; Olympus) was used for capturing images. 12 frames were taken with a 0.1-s exposure time and a 1-s interval between frames. The movie has been sped up 10 times.

Reference

Zhang, J., L. Wang, L. Zhuang, L. Huo, S. Musa, S. Li, and X. Xiang. 2008. Arp11 affects dynein-dynactin interaction and is essential for dynein function in *Aspergillus nidulans*. *Traffic*. 9:1073–1087. doi:10.1111/j.1600-0854.2008.00748.x