Figure S1 Sequence homology between *N.crassa* DHC and *M.musculus* DHC. The Pustell plot was generated using a PAM 250 scoring matrix, a minimum similarity score of 60%, and a window size of 12 and a hash value of 2. Accession numbers for *N.crassa* DHC (XP_962616.1) and *M.musculus* (NP_084514.2).

Figure S2 Quantification of spherical structures. Mean fluorescence intensity (arbitrary units) and the distance between hyphal tips and the spherical structures were measured for wildtype and *Loa* strains. Error bars indicate standard deviation.

Figure S3 Kymograph analyses of vesicle transport in different strains. Representative kymographs of FM 4-64 positive vesicle transport are shown. Kymographs are oriented with the hyphal tip to the right, distance on the x axis and time on the y axis. Motor-based movements are indicated by the higher velocity diagonal lines that are most abundant in the wildtype kymograph (solid white double-sided arrow). Cytoplasmic streaming causes the general back and forth drift of vesicles during the kymographs (solid black double-sided arrow in wildtype).

Figure S4 Representative gel slices showing purified dynein from wildtype and *Loa* strains

Figure S5 Representative gel showing the amount of DHC that co-pellets with microtubules under varied conditions.

Videos SV1 and SV2 Representative live cell movies of FM 4-64 labeled vesicle trafficking in hyphal tips from wildtype (SV1) and Loa (SV2) strains are included as supplemental information. Each movie is 86 μm x 65 μm in size and is sped up six-fold for playback.

Table S I. Primary *Neurospora crassa* strains used in this study

Genotype	Source	Description
mat a	FGSC	WT strain (FGSC # 4200)
mat A	FGSC	WT strain (FGSC # 2489)
mat A; C102(t); qde-2::Bml^R; delta mus-52::bar+	This study	Cot-1 temperature sensitive
mat a; ro-1^{K770*}; qde-2::Bml^R; delta mus-52::bar+	This study	DHC K770Stop
mat a; ro-1^{F607Y}; qde-2::Bml^R; delta mus-52::bar+	This study	DHC Loa
mat A ro-6^{+}::mCherry^{+}	This study	WT DIC-mCherry
mat A; ro-3^{+}::EGFP^{+}	This study	WT EGFP-dynactin p150
rid(RIP1) mat A his-3^{+}::Pccg-1-Bml^{+}-sgfp^{+}	FGSC	Tubulin-GFP (FGSC # 9520)
mat a his-3^{+}::Pccg-1-Bml^{+}-sgfp^{+} ro-6^{+}::mcherry^{+}	This study	WT, DIC-mCherry - Tub-GFP
his-3^{+}::Pccg-1::tdimer2(12)::cax^{+}	FGSC	WT RFP-Cax (FGSC # 10157)
mat A ro-6^{+}::hs^{+}	This study	WT DIC-6xHis-Strep-tag II strain

Figure S1

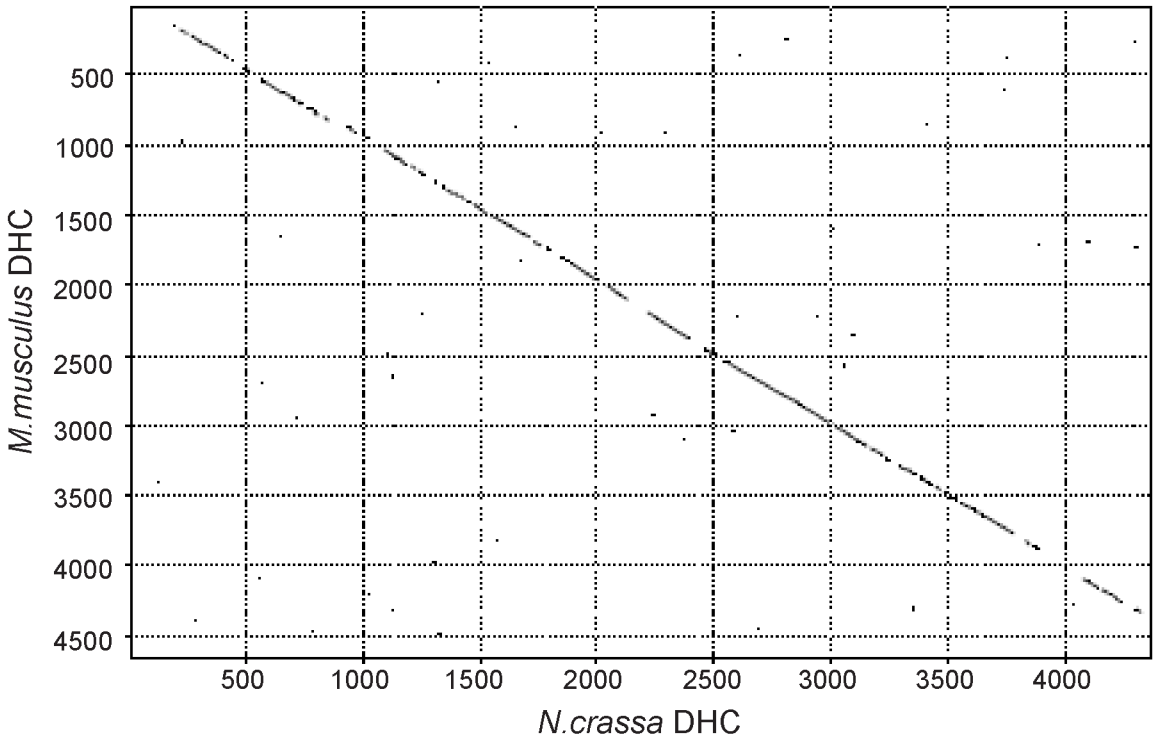
M.musculus DHC
500
1000
1500
2000
2500
3000
3500
4000
4500
500
1000
1500
2000
2500
3000
3500
4000
N.crassa DHC

Figure S2

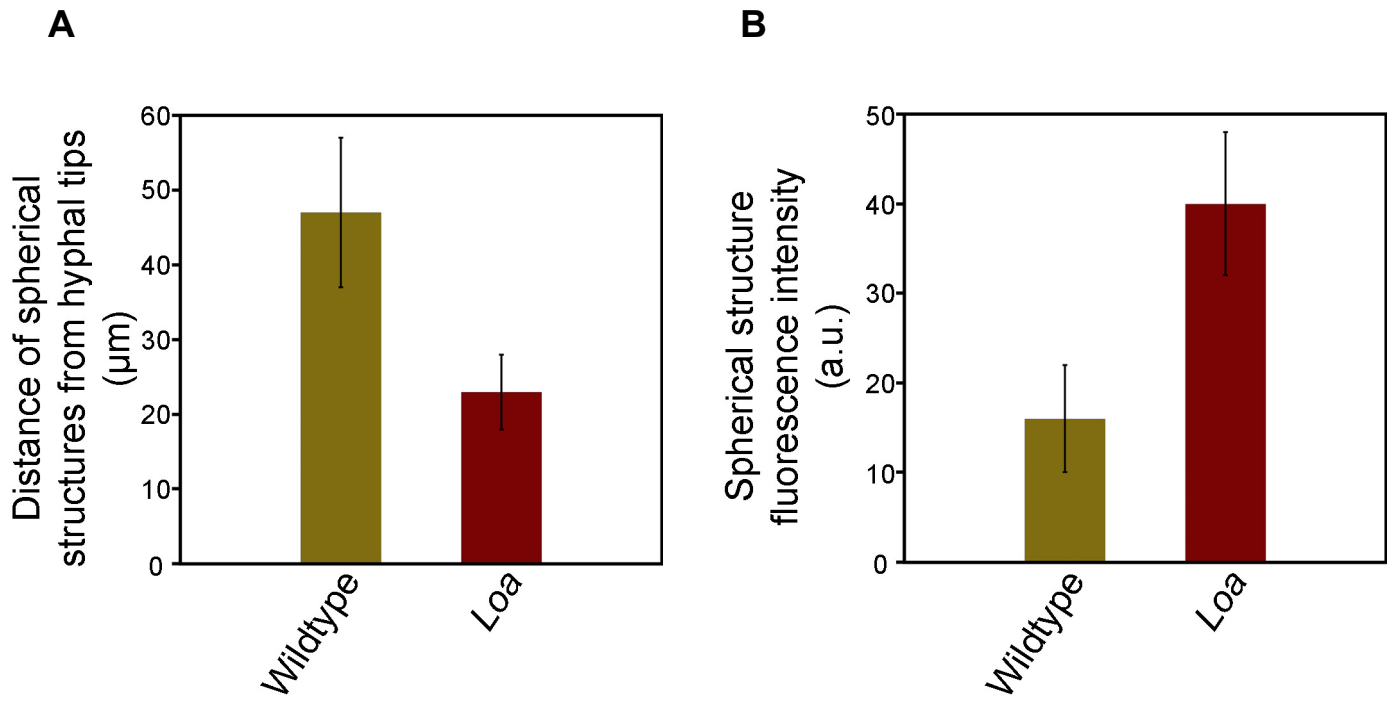
A
Distance of spherical structures from hyphal tips (µm)
60
50
40
30
20
10
0
Wildtype
Loa
B
Spherical structure fluorescence intensity (a.u.)
50
40
30
20
10
0
Wildtype
Loa

Figure S3

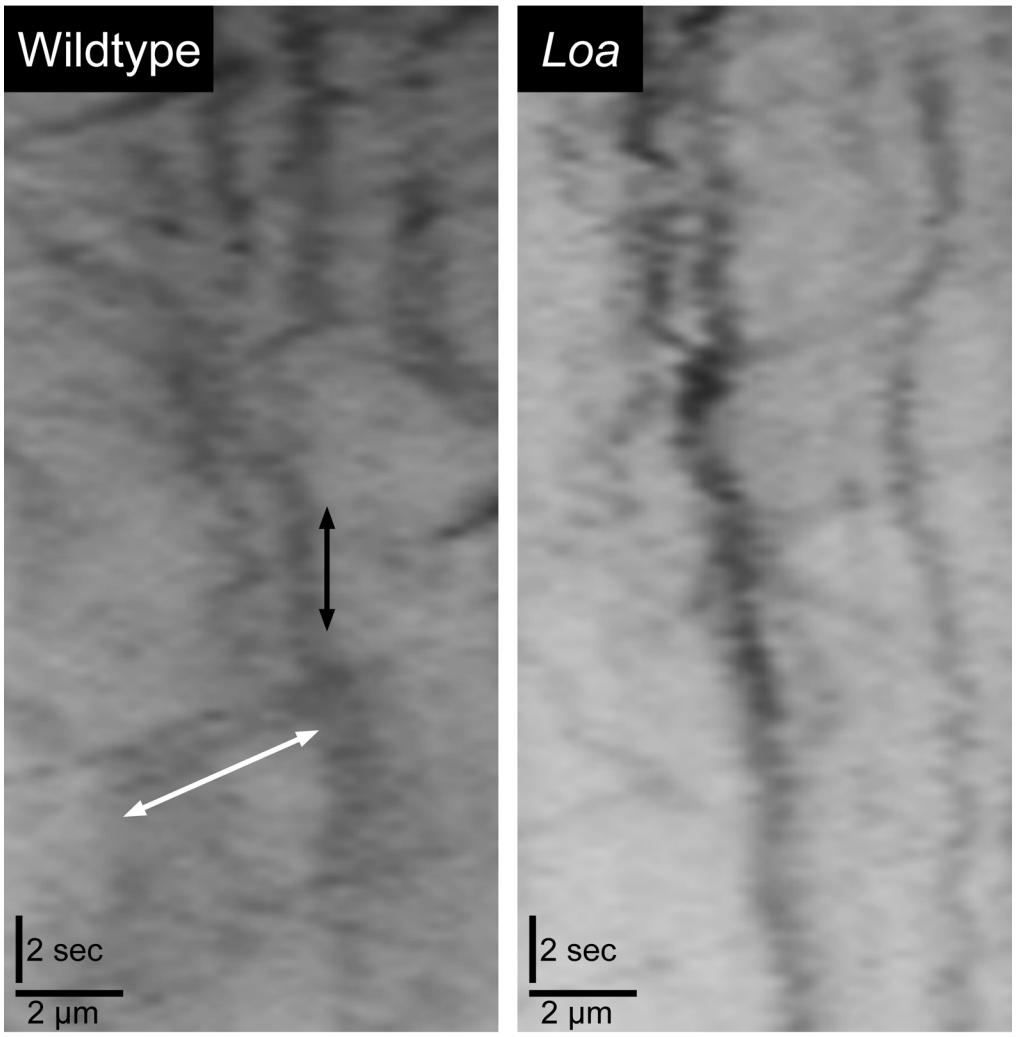

Figure S4

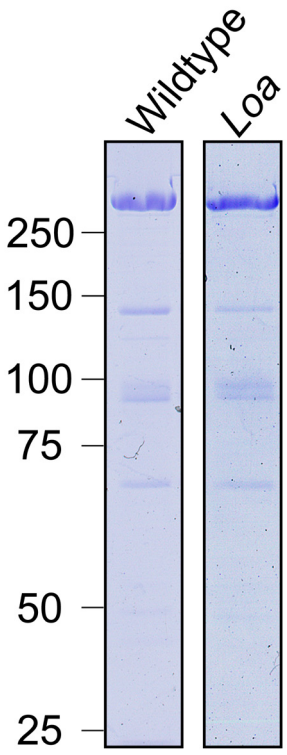

Figure S5