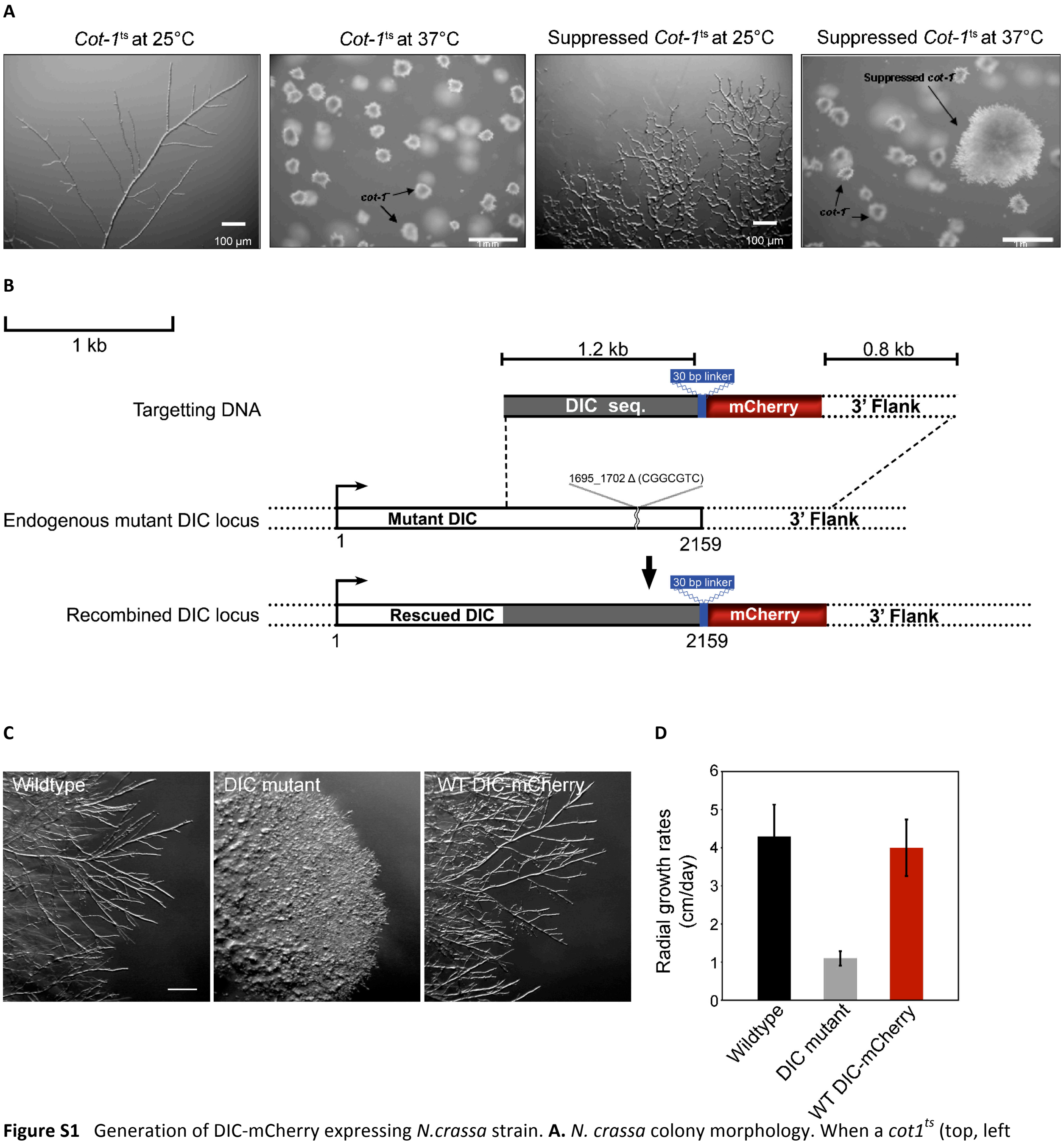

Figure S1 Generation of DIC-mCherry expressing *N.crassa* strain. **A.** *N. crassa* colony morphology. When a $cot1^{ts}$ (top, left panel) strain is grown at permissive temperature (25°C), it exhibits straight hyphal growth morphology. At restrictive temperature (top, right panel; 37°C) $cot1^{ts}$ strains exhibit compact colony growth. $cot1^{ts}$ suppression mediated by *ropy* mutations lead to curly hyphal growth morphologies at permissive temperatures (bottom, left panel) and enlarged colony morphology at restrictive temperatures (bottom, right panel).

B. A schematic representation of the targeting DNA consisting of mCherry coding sequence flanked by upstream (1.8kb) and downstream (0.8 kb) homologous sequences to the genomic locus of DIC. A DIC mutant strain ($ro\text{-}6^{7bp\ \Delta\ 1695}$) exhibiting ropy hyphal growth morphology was used for transformation. Homologous recombination of the targeting DNA into the $ro\text{-}6^{7bp\ \Delta\ 1695}$ locus resulted in the rescue of the *ropy* phenotype and restoration of wildtype hyphal growth morphology.

C. Comparison of colony morphologies between wildtype, DIC mutant $ro\text{-}6^{7bp\ \Delta\ 1695}$ and wildtype DIC-mCherry strains. Note that the wildtype DIC-mCherry strain exhibits growth morphology is indistinguishable from the wildtype strain.

D. Bar graph showing radial growth rates of wildtype, DIC mutant $ro\text{-}6^{7bp\ \Delta\ 1695}$, WT - DIC-mCherry strains. Data are shown as mean ± S.D.

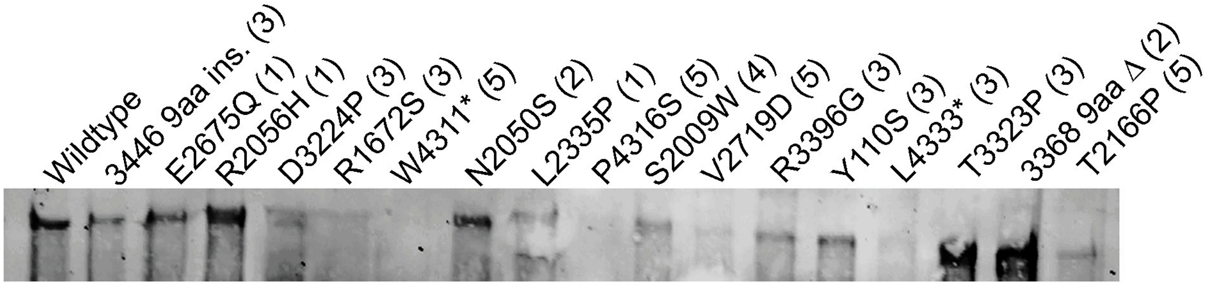

Figure S2 Protein levels in representative DHC mutant strains. Western blot analysis of DHC expression/protein levels in 17 of the 34 mutant strains, including at least one representative from every class. The class of each strain is indicated in parentheses after the designated strain name.

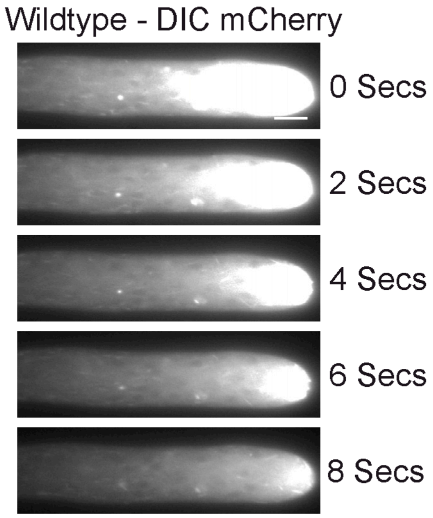

Figure S3 An 8-second time series showing photobleaching of bright hyphal tip dynein fluorescence in a wildtype strain. Bars: 10 µm.

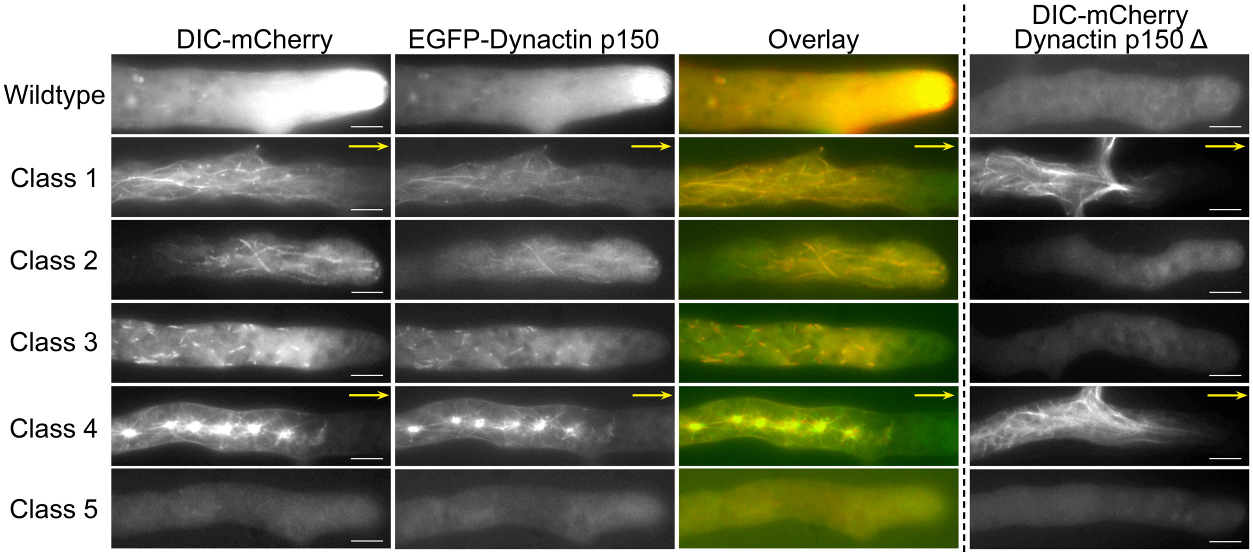

Figure S4 Colocalization of dynein and dynactin in wildtype and DHC mutant strains. **A.** Epifluorescence images of growing hyphae in strains expressing DIC-mCherry and EGFP- dynactin p150. Left panels: dynein; middle panels: dynactin, right panels: overlay. **B.** Epifluorescence images of growing hyphae in strains expressing DIC-mCherry in dynactin p150 null background. Bars: 10 µm in all panels.

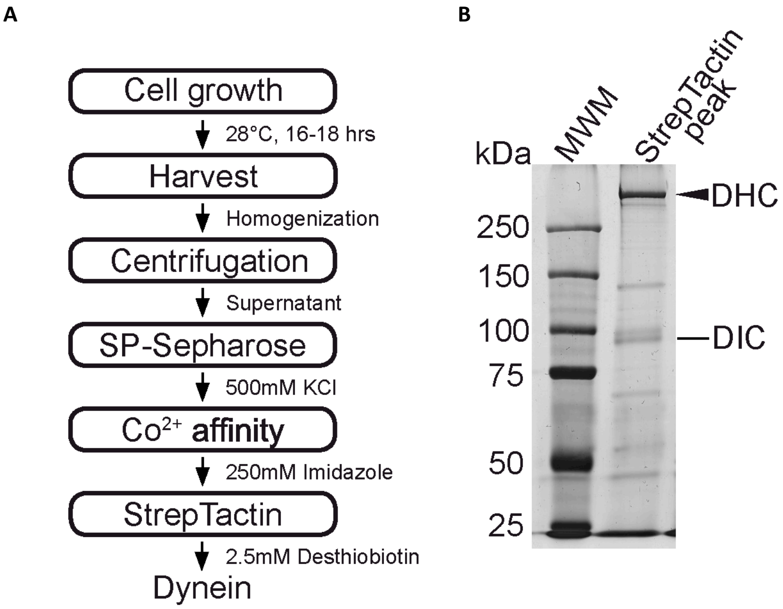

Figure S5 **A.** Dynein purification scheme for *N.crassa.* **B.** Representative silver-stained gel image showing the final elution fraction from the StrepTrap column. The arrow points to the DHC band and the bar indicates the band corresponding to the tagged DIC.

Files S1-S9

Supporting Movies

Representative live cell movies of FM 4-64 labeled vesicle trafficking in hyphal tips from each of the strains analyzed. Each movie was of 30 seconds duration and is sped up six-fold. Each movie is 86 μm x 65 μm in size.

Files S1-S9 are available for download at http://www.genetics.org/content/suppl/2012/05/29/genetics.112.141580.DC1 as compressed .avi files.

Table S1 Summary of vesicle transport dynamics.

	Wildtype	DHC Δ	p150 Δ	Nkin mut.	Class 1	Class 2	Class 3	Class 4	Class 5
Motility index	21.5	5.8	8.3	4.1	10.4	4.9	9.4	7.4	6.2
Overall movements									
Mean movements/ µm hyphal length	2.9	1.2	1.8	0.8	1.5	1.2	2.4	1.7	1.1
n	557	185	286	162	199	110	247	200	170
Mean velocity (µm/sec ± S.D.)	1.64 ± 0.59	1.46 ± 0.62*	1.34 ± 0.49*	1.35 ± 0.52*	1.51 ± 0.59*	1.24 ± 0.41*	1.33 ± 0.45*	1.33 ± 0.41*	1.31 ± 0.48*
Mean distance (µm ± S.D.)	4.26 ± 0.54	3.03 ± 1.71*	3.19 ± 1.44*	3.49 ± 2.07*	4.48 ± 2.59	3.21 ± 1.64*	2.85 ± 1.15*	3.17 ± 1.33*	3.96 ± 2.33
Inward movements									
Percent of total movements	48.7 %	47.1 %	48.9 %	48.8 %	41.7 %	52.7 %	53.8 %	41.5 %	47.1 %
Mean velocity (µm/sec ± S.D.)	1.60 ± 0.55	1.36 ± 0.53*	1.27 ± 0.51*	1.36 ± 0.53*	1.43 ± 0.54*	1.23 ± 0.45*	1.29 ± 0.48*	1.24 ± 0.40*	1.28 ± 0.53*
Mean distance (µm ± S.D.)	4.16 ± 2.47	2.70 ± 1.27*	2.78 ± 1.12*	3.23 ± 1.88*	3.90 ± 2.62	2.86 ± 1.38*	2.69 ± 1.02*	2.66 ± 0.96*	3.77 ± 2.40
Outward movements									
Percent of total movements	51.3 %	52.9 %	51.1 %	51.2 %	58.3 %	47.3 %	46.2 %	58.5 %	52.9 %
Mean velocity (µm/sec ± S.D.)	1.67 ± 0.61	1.55 ± 0.69	1.41 ± 0.47*	1.35 ± 0.51*	1.56 ± 0.62	1.24 ± 0.36*	1.38 ± 0.42*	1.40 ± 0.41*	1.35 ± 0.44*
Mean distance (µm ± S.D.)	4.36 ± 2.60	3.32 ± 1.98*	3.59 ± 1.59*	3.75 ± 2.22*	4.89 ± 2.49	3.57 ± 1.83*	3.05 ± 1.27*	3.53 ± 1.45*	4.13 ± 2.26
Vesicle accumulation									
Hyphal tip vesicle accumulation	60.0 %	92.3 %	27.3 %	100 %	72.7 %	86.7 %	90.0 %	78.6 %	100 %
Hyphal body vesicle accumulation	13.3 %	7.7 %	90.9 %	0 %	9.1 %	6.7 %	20.0 %	21.4 %	0 %

* = statistically significant difference from wild-type value as determined by students t-test ($P < 0.05$).

Table S2 Summary of dynein mislocalization phenotypes.

Mislocalization phenotype	Domain	Mutation
Class 1 (Distal - long linear tracks)	AAA1 (Turn p-loop)	G1946R
	AAA1 (H4)	R2056H
	AAA2 (PS1 Insert)	S2333R
	AAA2 (PS I Insert)	L2335P
	AAA3 (β3/Walker B)	E2675Q
	AAA5 (Strut C1)	3739 6aa Δ (RRSNLI)
Class 2 (Apical - long linear tracks)	AAA1(Sensor 1 (β4))	N2050S
	AAA3 (β4 before sensor 1)	C2722R
	MT Stalk (H8 - CC1)	3268 9aa Δ (SLEIQAALE)
	AAA6 (H1)	V4049D
Class 3 (Comet tails)	Tail	Y110S
	Tail	W1308G
	Linker Subdomain 3 (H11)	R1672S
	MT Stalk (H8 - CC1)	D3224P
	MT-binding domain (H1) o)MTBD	T3323P
	MT-Stalk (CC2)	L3332P
	MT-binding domain (H7)	R3396G
	MT-stalk (H9 - CC2)	3446 9aa ins.
	C-terminus (H1)	L4333*
Class 4 (Aggregates)	AAA1 (H3)	S2009W
	MT-BS (H8 stalk CC1)	G3215D
	AAA5 (Strut C1)	3756 7aa Δ (QLEKKLL)
Class 5 (Disperse)	AAA1 (H0)	L1933P
	AAA1 (AAA1 H1 and B2)	G1961R
	AAA1 (H4/ β4 R finger)	K2065E
	AAA1 (H8)	T2166P
	AAA3 (β4 before sensor 1)	V2719D
	AAA5 (H0)	I3591P
	AAA6 (H5)	G4146A
	AAA6 (H7)	E4168K
	AAA6 (between H8-H9)	I4232N
	AAA6 (between h11 and h12)	D4296E; 4297 3aa Δ (LVV)
	AAA6 (H12)	W4311*
	AAA6 (between H12-H13)	P4316S

AAA- AAA domains of dynein, MT – microtubule, H – helix, β – β strand, CC- coiled-coil, aa - amino acid, Δ - deletion, ins - insertion, * - nonsense mutation.

Table S3 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; kin-1	FGSC	Nkin mutant strain (FGSC # 9939)
mat a; ro-1::hygr	This study	DHC Δ mutant strain
mat a; ro-3::hygr	FGSC	Dynactin p150 Δ mutant strain (FGSC # 14726)
rid(RIP1) mat A his-3$^+$*::Pccg-1-Bml*$^+$*-sgfp*$^+$	FGSC	Tubulin-GFP strain (FGSC # 9520)
mat A his-3$^+$*::Pccg-1-hH1*$^+$*-sgfp*$^+$	FGSC	Histone h1-GFP strain (FGSC # 9518)
mat a ro-6 $^{7bp\ \Delta\ 1695}$	This study	DIC mutant strain
mat A ro-6$^+$*::mCherry*$^+$	This study	WT DIC-mCherry strain
mat A ro-6$^+$*::mCherry*$^+$*; kin-1*	This study	Nkin mutant - DIC-mCherry strain
mat A ro-6$^+$*::mCherry*$^+$*; Δ ro-3*	This study	Dynactin p150 Δ mutant - DIC-mCherry strain
mat A; ro-3$^+$*::egfp*$^+$	This study	WT EGFP-Dynactin p150 strain
mat A ro-6$^+$*::hs*$^+$	This study	WT DIC-6xHis-Strep-tag II strain