Supplemental Movies

Movie 1:
Traffic of GFP-NKIN2 labeled endosomes in a branch of a leading hypha of *N. crassa.* Wide-field fluorescence microscopy. The time is displayed in the movie in seconds.

Movie 2:
Dynamics of GFP-NKIN2 labeled spots in a leading hypha of *N. crassa.* Wide-field fluorescence microscopy. The time is displayed in the movie in seconds.

Movie 3:
Bi-directional traffic of GFP-YPT52 labeled early endosomes in a germling of *N. crassa* wild type (FGSC 9717). Wide-field fluorescence microscopy. The time is displayed in the movie in seconds.

Movie 4:
Wide-field fluorescence of GFP-YPT52 labeled early endosomes in *N. crassa* Δ*nkin2* germling. The time is displayed in the movie in seconds.

Movie 5:
Motility defects of GFP-YPT52 labeled early endosomes in a *N. crassa ro-10* germling. Wide-field fluorescence microscopy. The time is displayed in the movie in seconds.

Supplemental Figures

Fig. S1
Deletion of *nkin2*. Scheme of the deletion procedure and corresponding Southern blots. DNA was digested with *Eco*NI and *Bam*HI. The probes are indicated above.

Fig. S2
Dendrogram of EE-specific Rab5 GTPases from *Homo sapiens*, *Mus musculus*, *Saccharomyces cerevisiae*, *Ustilago maydis*, *Aspergillus nidulans* and *Neurospora crassa*. The tree was constructed by CLC Sequence Viewer 6 (Neighbor Joining algorithm with bootstrap 1000 analysis).

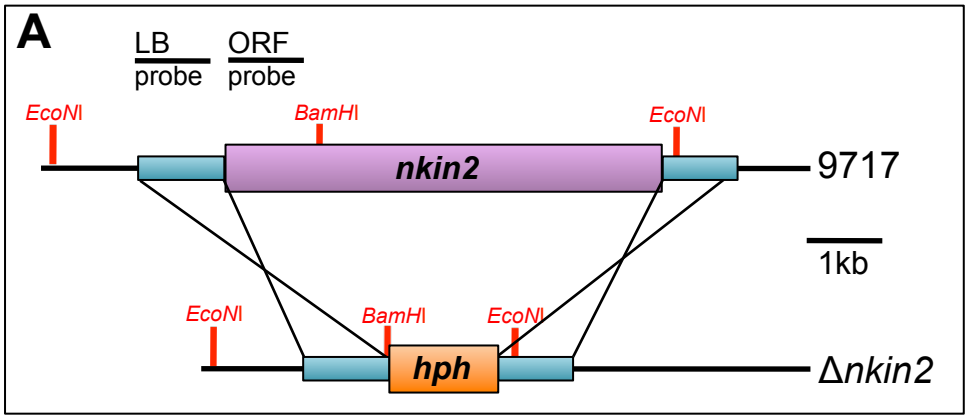
Fig. S3
Analysis of septation **(A, B)** and vacuole distribution **(C)** in wild type and Δ*nkin2*. **(D)** GFP-NKIN2^{rigor} in Δ*nkin2* binds specifically to a MT-subpopulation, which traverses several septa in one hypha (arrowheads). Scale bar, 100 µm in A, B, 20 µm in C and 5 µm in D.

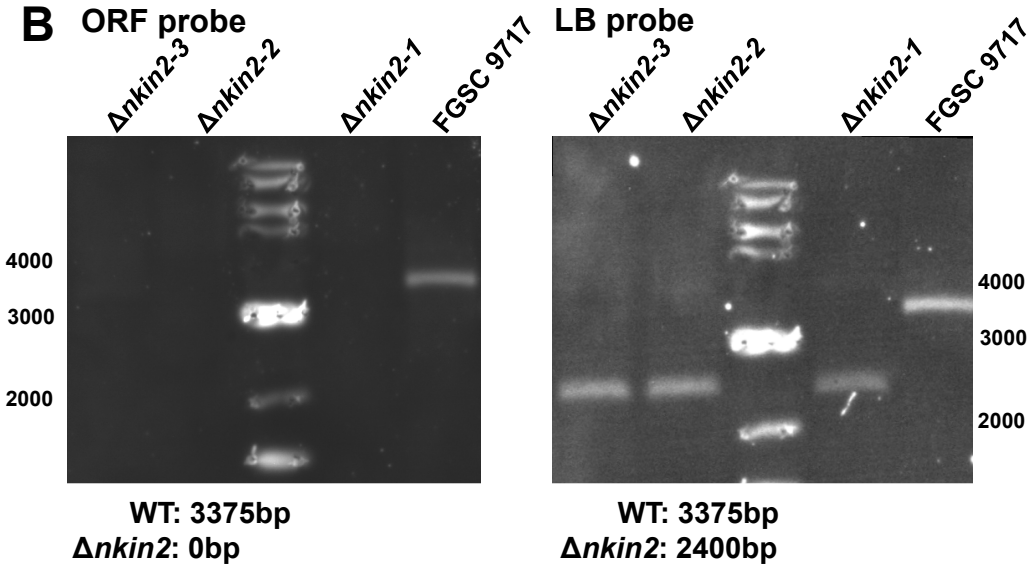
Fig. S4
Analysis of mitochondrial distribution and migration. Mitochondrial behavior was not affected in *nkin2*-deletion strains in comparison to wild type. Mitochondria of germinated hyphae were stained with MitoTracker Red. Scale bar, 5 µm.

Fig. S5
Co-localization of GFP-microtubules and mitochondria stained by MitoTracker Red in wild type confirms some linkage of mitochondria to microtubules (arrowheads), but also some mitochondria, which are not associated to microtubules (asterisks). Scale bar, 5 µm.

Fig. S6
(A) GFP-NKIN3 and **(B)** GFP-NKIN3^{rigor} localize in the cytoplasm and vacuoles. Vacuoles were stained by CMAC in subapical regions of the wild type. Scale bars, 10 µm.

Fig. S1





WT: 3375bp
Δ*nkin2*: 0bp

WT: 3375bp
Δ*nkin2*: 2400bp

Fig. S2

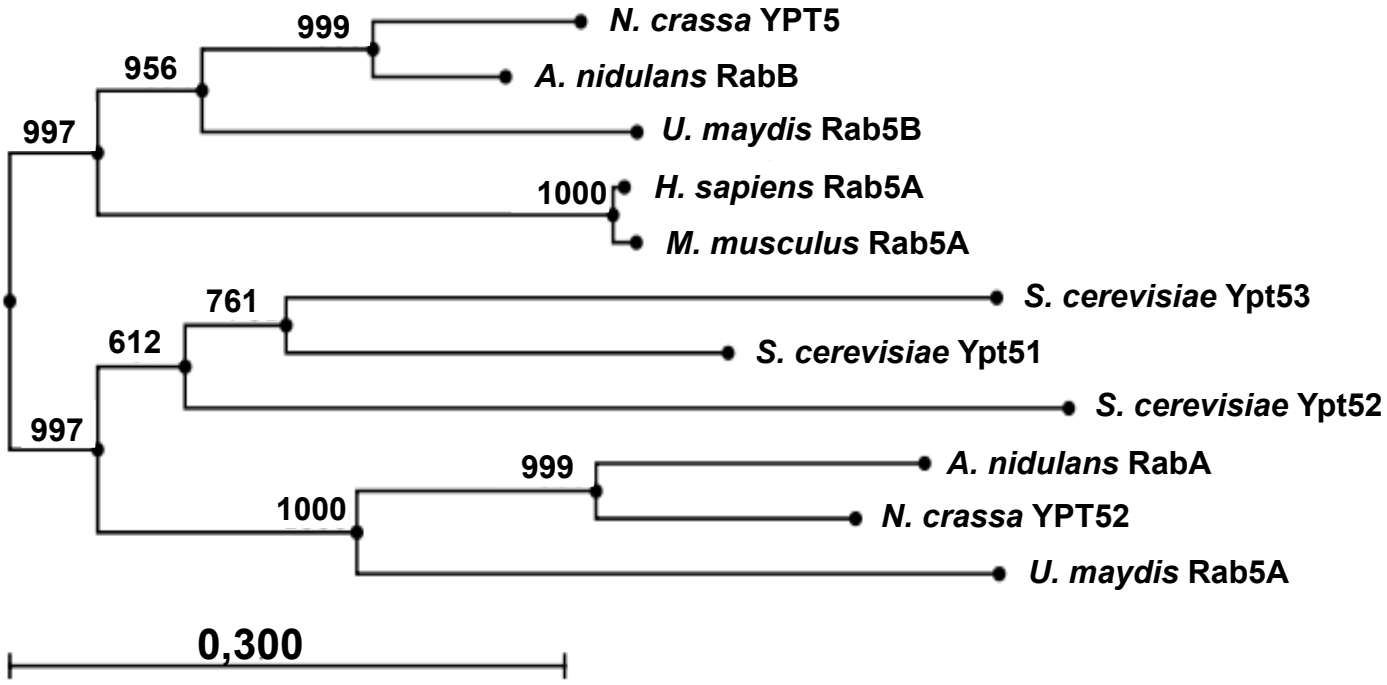

999
956
997
1000
761
612
997
999
1000
N. crassa YPT5
A. nidulans RabB
U. maydis Rab5B
H. sapiens Rab5A
M. musculus Rab5A
S. cerevisiae Ypt53
S. cerevisiae Ypt51
S. cerevisiae Ypt52
A. nidulans RabA
N. crassa YPT52
U. maydis Rab5A
0,300

Fig. S3

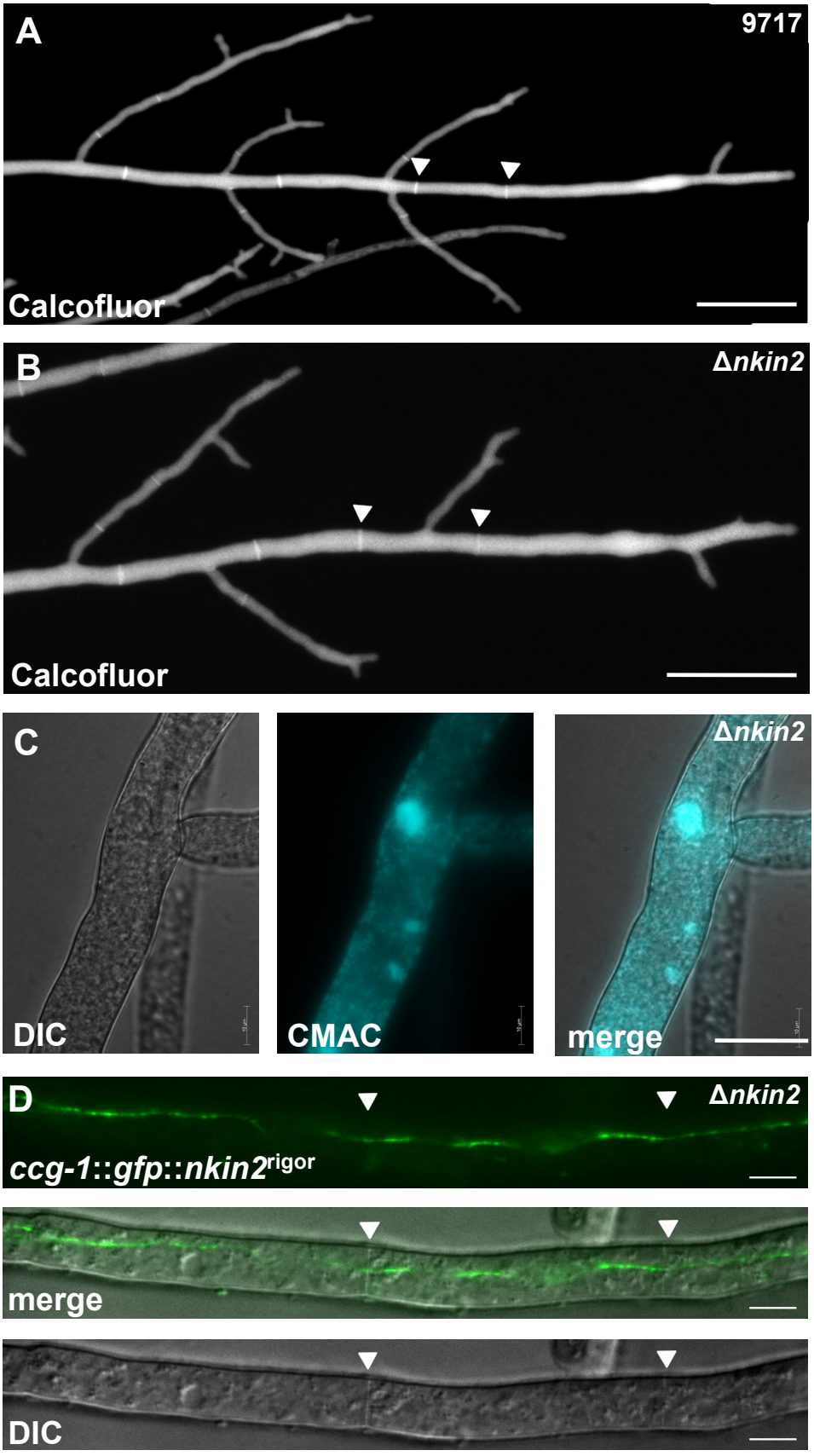

A
9717
Calcofluor
B
Δnkin2
Calcofluor
C
DIC
CMAC
Δnkin2
merge
D
Δnkin2
ccg-1::gfp::nkin2rigor
merge
DIC

Fig. S4

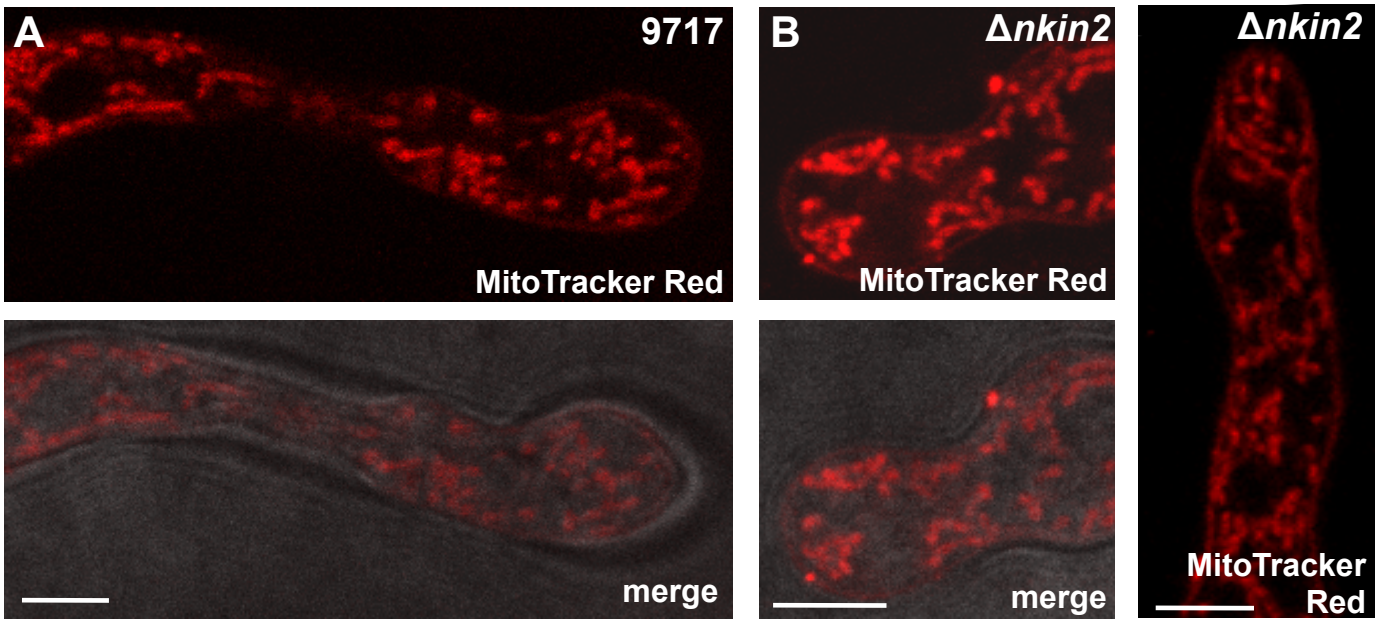

A
9717
MitoTracker Red
merge
B
Δnkin2
MitoTracker Red
merge
Δnkin2
MitoTracker
Red

Fig. S5

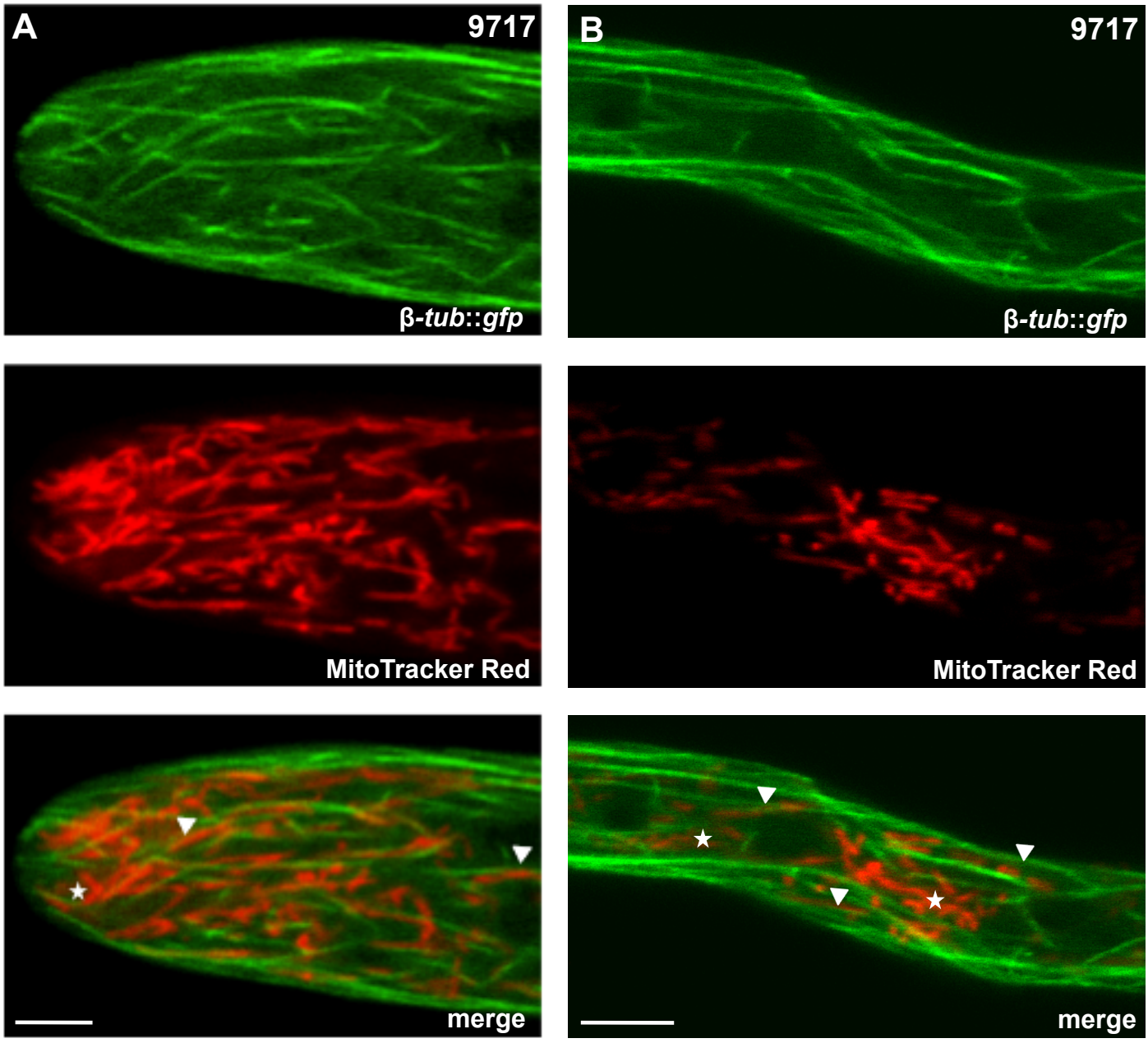
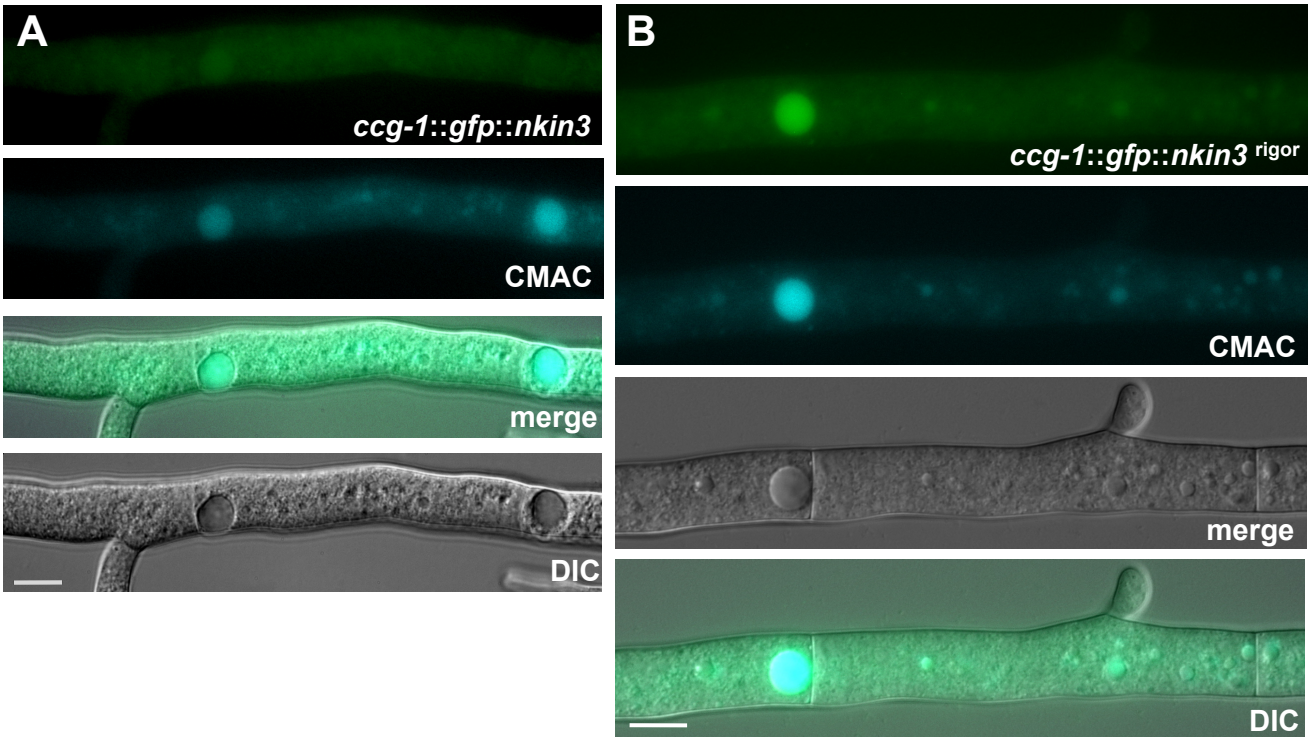

A
9717
β-tub::gfp
B
9717
β-tub::gfp
MitoTracker Red
MitoTracker Red
merge
merge

Fig. S6


A
ccg-1::gfp::nkin3
CMAC
merge
DIC
B
ccg-1::gfp::nkin3 rigor
CMAC
merge
DIC