Supplementary Figures

Figure S1

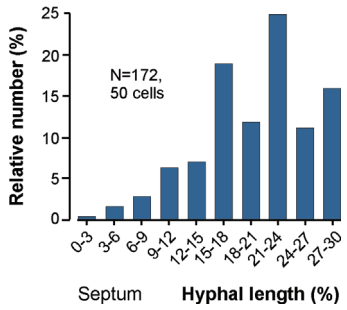

Figure S1. Bar chart showing the distribution of MTOCs (labeled with the putative gamma-tubulin ring complex protein Grc1-GFP_3) near the septum.

Figure S2

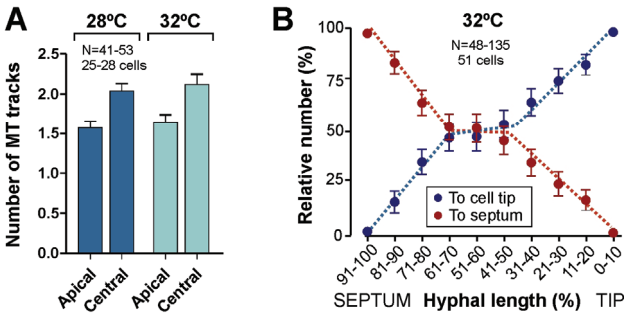

Figure S2. (A) Bar chart showing the number of MT tracks (single MTs or MT bundles) at the tip (apical) and in the middle (central) of hyphal cells at 28°C and 32°C. No significant difference was found between 28°C and 32°C. (B) Graph indicating the orientation of Peb1-YFP motility in hyphal cells at 32°C. No change in MT organization is evident at higher temperature.

Figure S3

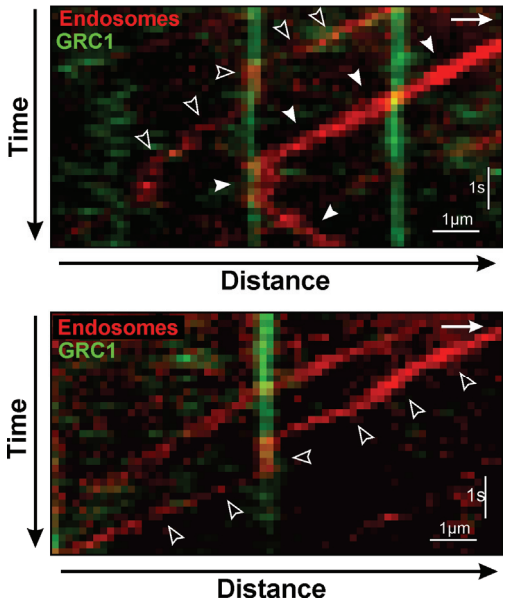

Figure S3. Kymograph showing retrograde EE motility (red) and the position of Grc1-GFP$_3$-labelled MTOCs. Note that EEs often pause at the MTOC (open arrowheads) or turn direction (closed arrowheads). Bars represent micrometers and seconds.

Figure S4

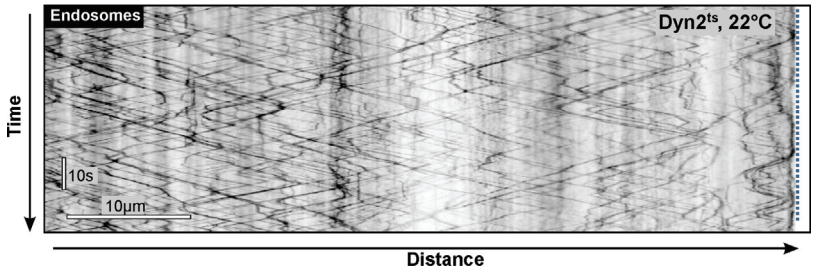

Figure S4. Bi-directional of EEs in temperature-sensitive dynein mutants at permissive temperature. Bar represents micrometers; time is given in seconds and milliseconds.

Figure S5

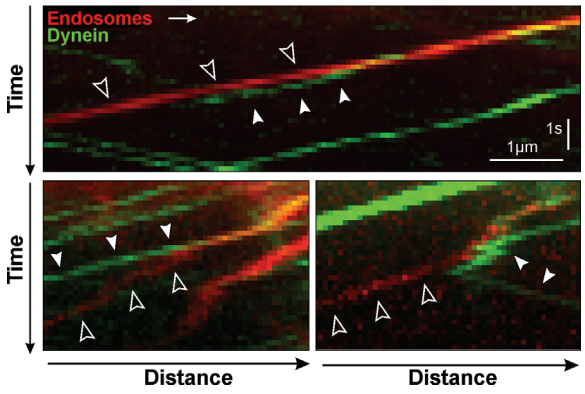

Figure S5. Kymographs showing motility of dynein (green) and EEs (red). Note that EEs travel retrograde (open arrowheads) after dynein unbinds (closed arrowheads). Bar represents micrometers; time is given in seconds and milliseconds.

Figure S6

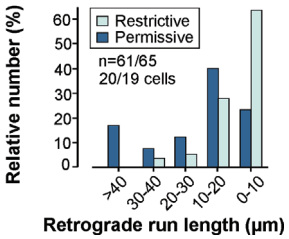

Figure S6. Run-length of EEs in Kin3ts mutants at permissive (22ºC) and restrictive (32ºC) temperature. Note that both data sets are significantly different (*P*: < 0.0001, Mann-Whitney test).

Figure S7

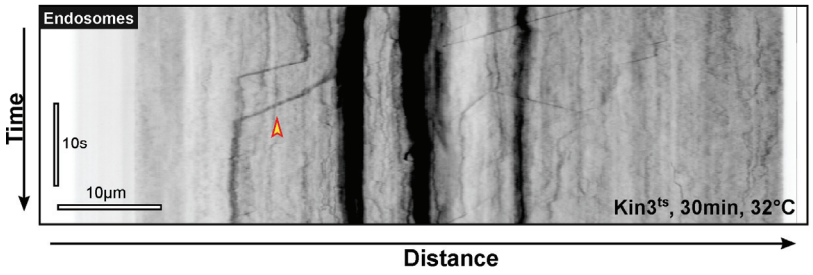

Figure S7. Distribution of EEs in kinesin-3^{ts} mutants after 30 minutes at restrictive conditions. Clusters of GFPRab5a-labeled EEs are now found predominantly in the middle part of the hyphal cell and very little motility occurs (arrowhead). Note that the phenotype of the cells varied and that some cells showed more motility. Bar represents micrometers; time is given in seconds and milliseconds.

Figure S8

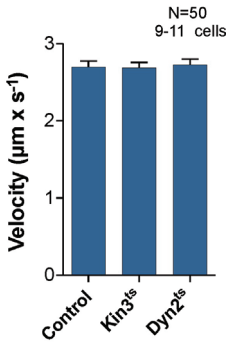

Figure S8. Velocity of EEs in control cells (Control), and temperature-sensitive kinesin-3 (Kin3^{ts}) and dynein mutants (Dyn2^{ts}) at restrictive temperature (32°C). No significant difference was found.

Supplementary Movie legends

Movie S1. MT orientation, determined by motility of the EB1-homolouge Peb1-YFP in a hyphal cell. Peb1-YFP motility indicates growth of MT plus-ends. Bar represents micrometers; time is given in seconds and milliseconds.

Movie S2. Cytoplasmic MTOCs visualized by expression of a gamma-tubulin ring complex protein. Note that the brightest spots represent nuclear spindle pole bodies (SPB) whereas the faint spots are most likely microtubule organizing centers that mark the minus-ends of MTs (MTOCs). Bar represents micrometers; time is given in seconds and milliseconds.

Movie S3. Microtubules in a hyphal cell. Sections are 3D-reconstructions of deconvolved Z-stacks. Note that cells were mildly fixed to avoid MT motility. Bar represents micrometers.

Movie S4. Long-range retrograde motility of EEs after photo-activation of paGFP-Rab5a. The position of laser activation is indicated by blue arrowhead in first frame. The organelle migrates towards the septum at the rear cell end. Note the short pause near the hyphal tip (yellow arrowhead). Bar represents micrometers; time is given in seconds and milliseconds.

Movie S5. Motility of EEs in temperature-sensitive dynein mutants.
At restrictive temperature (32°C for 30 minutes), EEs accumulate near the hyphal apex, which is due to the unbalanced activity of kinesin-3 (Lenz et al, 2006). Retrograde motility within the apical region is abolished, whereas long-

range motility is found in sub-apical regions. Note that MTs are uni-polar in the apical region with minus-ends being directed towards the cell center. Bar represents micrometers; time is given in seconds and milliseconds.

Movie S6. Dynein and retrograde EE trafficking.
EEs (red, labeled by mCherry-Rab5a) travel into a photo-bleached area. Dynein signals (green, labeled by GFP_3-Dyn2) travel independent of EEs (indicated by “1”) or co-localize with retrograde EEs (indicated by “2”). In addition, retrograde EEs move without co-localizing (indicated by “3”). Note that the microscopic setup allows visualization of individual dynein motors (Schuster et al, 2011a; Schuster et al, 2011b), suggesting that these retrograde EEs move independently of dynein. The distance to the hyphal tip to the right is indicated (arrowhead, -20 µm). Bar represents micrometers; time is given in seconds and milliseconds.

Movie S7. Co-visualization of retrograde moving dynein (green) and EEs (red). The organelle stops moving after dynein unbinds. Bar represents micrometers; time is given in seconds and milliseconds.

Movie S8. Co-visualization of retrograde moving dynein (green) and EEs (red). Initially, dynein co-localizes with the organelles, but later falls back and detaches while the EE continues retrograde motility. Bar represents micrometers; time is given in seconds and milliseconds.

Movie S9. EE motility in a conditional dynein-Δkinesin-3 double mutant.

Cells were grown in glucose-containing medium, which down regulates the *crg*-promoter, under which the dynein heavy chain gene *dyn1* was expressed. Depletion of dynein in the kinesin-3 null mutant abolished all EE motility. This strongly suggests that dynein and kinesin-3 are the only motors involved in EE motility. Synein expression was repressed by growth of hyphal cells in glucose-containing medium for 12-14 hours. Bar represents micrometers; time is given in seconds and milliseconds.

Movie S10. Retrograde motility of kinesin-3.

To reduce signal interference, all sub-apical parts of a hyphal cell of strain AB33Kin3G were photo-bleached by a 405nm laser. Kinesin-3 could be a passive cargo on retrograde EEs. However, the orientation of MTs allows and active role of kinesin-3 in transports towards the septum. Bar represents micrometers; time is given in seconds and milliseconds.

Movie S11. Motility of temperature-sensitive $Kin3^{ts}$ and EEs at restrictive temperature.

In the temperature-sensitive mutant strain AB33ΔKin3_$Kin3^{ts}$G_tagRRab5a, the EEs form a cluster behind the hyphal tip. The mutant motor protein kinesin-3^{ts} still binds to these organelles. The apical region, where MTs have a uni-polar orientation, is devoid of organelles, which is due to the activity of dynein. In sub-apical areas MTs have a bi-polar orientation. Dynein is able to support short-range motility (see left of the EE cluster). Cells were shifted to 32°C for 5-10 minutes. Bar represents micrometers; the time is given in seconds and milliseconds.