

Movie S1. Motility of EEs in *U. maydis* cells inside planta. The organelles were labelled with the endosomal small GTPase GFP-Rab5a. Time is given in seconds:milliseconds; the bar represents micrometers.

Movie S2. Block of dynein motility in the presence of a kinesin-1 mutant protein that contains a point mutation in the motor domain, conferring tight binding to the microtubule. Expressing this mutant protein in a wildtype hyphal cell anchors dynein (labelled by a triple GFP-tag fused to the dynein heavy chain protein Dyn2). Endogenous wildtype kinesin1 is also present but cannot overcome the blockage. Time is given in seconds:milliseconds; the bar represents micrometers.

Movie S3. Motility of EEs in cells that contain the synthetic motor K1^rPX under the control of the *crg* promoter. In the presence of glucose, the promoter is repressed and K1^rPX is not made (OFF, no K1^rPX) and EEs are moving. After shift to arabinose-containing medium for 5 hours, the synthetic protein is expressed and tightly anchors the EEs to the microtubules. Time is given in seconds:milliseconds; the bar represents micrometers.

Movie S4. Co-observation of EEs (marked by mCherry-Rab5a; Rab5a, red in merged image) and GFP-labelled kinesin3 (Kin3; green in merged image). Most EEs are immobilized. An EE-independent Kin3 signal travels towards the hyphal apex (open arrowhead; apex indicated by closed arrowhead). Note that the apical part of the hyphal cell was photo-bleached to avoid interference with cytoplasmic background. Time is given in seconds:milliseconds; the bar represents micrometers.

Movie S5. Motility of EEs in infectious hyphae in the presence and absence of K1rPX. Strong expression of the synthetic anchor under the *crg*-promoter (*crgK1rPX*) blocked EE motility during early infection (*crgK1rPX*, 2 dpi). The presence of glucose, derived from invertase-dependent digestion of glucose provided by the plant repressed the promoter and EE motility restored at later stages of infection (*crgK1rPX*, 6 dpi). The reverse is found when K1rPX is expressed under the control of the plant-specific *mi*-promoter (*migK1rPX*). The promoter is repressed during early plant infection and EEs move normally (*migK1rPX*, 2 dpi). At later stages the promoter is induced and K1rPX is expressed. Consequently, EEs are anchored at microtubules and their motility is almost abolished. Time is given in seconds:milliseconds; the bar represents micrometers.

Movie 1. Bidirectional motility of Kin3-GFP on fluorescently labelled MTs in *U. maydis* hyphal cells. Time is given in seconds:milliseconds. Bar represents 10 micrometers. Note that Kin3 motors use almost all of the MT tracks.

Movie S1. Motility of mCherry-Rab5a labelled early endosomes in a *kin3* null mutant (Δ Kin3), a *kin3* null mutant complemented with Kin3-GFP (+Kin3) and a *kin3* null mutant complemented with Kin3 Δ PH-GFP (+Kin3 Δ PH). Note Kin3 Δ PH-GFP is able to mediate some motility, indicating that the truncated motor is still able to bind to the organelle. Time is given in seconds:milliseconds; the bar indicates micrometers.

Movie S2. Structural model of the PH domain of Kin3 from *Ustilago maydis*. Red: Amino acids that are identical between human Kif1A and *U. maydis* Kin3; green: Amino acids found in Kin3, Kif1A and in 6 additional PH domains from various non-motor proteins (see Material and Methods of main text). For details on methods and accession numbers see the Method section in the main text.

Movie S3. Motility of YFP^N-Kin3-YFP^C. Fluorescence is only detectable when the two halves of the yellow fluorescent protein interacting. This strongly indicates that the tail and the motor head are in close proximity while the motor moves its cargo along microtubules. Note that the cells show no growth defect indicating that the YFP^N-Kin3-YFP^C protein is biological functional. Time is given in seconds:milliseconds; the bar indicates micrometers.