Figure S1. Position of the nucleus during the protonemal cell cycle

(A) Imaging of GFP-tubulin (green) and HistoneH2B-mRFP (red) in the cell cycle of a protonemal apical cell. The mitotic spindle (58 min) and the phragmoplast (83 min) were detected during mitosis. (B) A kymograph showing the position of the nucleus relative to the cell tip. During most of the interphase, the nucleus moves forward, such that it is retained in the centre of the cell. Bars, 50 μm.
Figure S2. RNAi knockdown of kinesins of the ARK subfamily

(A) Phylogenetic analysis of the kinesin-ARK subfamily of kinesins from *Physcomitrella patens* (Pp), *Arabidopsis thaliana* (At), and *Selaginella moellendorfii* (Sm). The numbers near the branches represent local bootstrap probability. The local bootstrap values (1,000 replicates) are also shown near the branches. The horizontal branch length is proportional to the estimated evolutionary distance. Kinesin-ARKs from *Selaginella moellendorfii* were provisionally named based on the accession numbers in the Phytozome database. A bar indicates an amino acid substitution per site. *P. patens* expresses another ARK-like kinesin (Miki et al. 2014; Shen et al. 2012) However, this protein was excluded from our analysis because only the motor domain was identified. (B) Illustration of the structure of kinesins from the ARK subfamily. They contain the kinesin motor domain, the coiled-coil domain, and the armadillo repeat (marked with ‘A’). The Simple Modular Architecture Research Tool (SMART) software (EMBL) was used in the identification of each domain. (C) Three RNAi constructs were used for the downregulation of kinesin-ARKs. Grey and red bars indicate cDNAs and double-stranded RNAs, respectively. The (%) values indicate nucleotide sequence homology. (D) Quantitative real-time RT-PCR (qRT-PCR) confirmed a successful knockdown of kinesins ARK-a and -b. The values, relative to the control cell clone (set to 1.0), are plotted as mean ± SEM from 2 independent experiments.
Figure S3.  Amino acid sequence alignment of 4 *P. patens* kinesin-ARKs

The Clustal Omega program (EMBL) was used.
Figure S4. Protonemal growth, mitosis duration, or gametophore formation is not perturbed by kinesin-ARK RNAi

(A) The tip growth velocity of caulonemal apical cells was measured by time-lapse imaging. The mean values of the growth rate obtained in 3–7 independent experiments are shown with SEM. No statistically significant differences were observed for RNAi-treated cells (line #7, p = 0.40; line #9, p = 0.93 (t-test)). (B) Nuclear envelope breakdown (NEBD)-to-anaphase duration was not significantly altered following RNAi (mean ± SEM). Control, n = 52; kinesin-ARK RNAi 1st construct, line #7, n = 19, p = 0.88 (t-test); line #8, n = 9, p = 0.16; 2nd construct, line #7, n = 31, p = 0.72; line #9, n = 33, p = 0.25; 3rd construct, line #2, n = 10, p = 0.57; line #3, n = 18, p = 0.83. (C) Each clone was cultured for 3 weeks in the BCDAT agar medium with 1 μM β-estradiol. The PpXMAP215 RNAi line, which shows a severe growth defect of protonemata, was used as a control (Nakaoka et al. 2012). Bar, 1 cm.
Figure S5. The spindle positioning defect after kinesin-ARK RNAi

The relative position of the mitotic spindle in the apical cell was measured. The representative control and kinesin-RNAi cells (1st construct, line #8, and 2nd construct, line #9) have been displayed. The quantitative data has also been plotted (error bars represent SEM). Control, n = 29; kinesin-ARK RNAi 1st construct, line #7 n = 11; line #8, n = 4, 2nd construct, line #7, n = 14; line #9, n = 15; 3rd construct, line #2, n = 5; line #3, n = 10). The relative spindle position in kinesin-ARK RNAi cells is significantly different from that in the control cells (t-test). 1st construct, line #7, p = 0.001; line #8, p = 0.003, 2nd construct, line #7, p = 0.0002; line #9, p < 0.0001, and 3rd construct, line #2, p < 0.0001; line #3, p = 0.0007. Bar, 100 μm.
Figure S6. Localisation of kinesin ARK-Citrine on growing and shrinking MTs

This figure represents the time-lapse images of cells expressing mCherry-tubulin and kinesin ARK-b-Citrine by oblique illumination fluorescence microscopy. Localisation of kinesin ARK-b-Citrine along the cytoplasmic MT was observed. See also Movie 9. Bar, 10 (upper) or 5 μm (bottom).
Figure S7. Kinesin-ARK RNAi does not affect the growth rate or abundance of endoplasmic MTs

(A) The MT growth rate was not significantly different between control and kinesin-ARK RNAi cells near the cell surface. GFP-tubulin was imaged every 3 s by spinning-disc confocal microscopy, and the kymographs were analysed. Mean ± SEM is plotted (25 MTs of 5 cells for each line). The p-values based on Student’s t-test were, 0.24 for the 1st construct, line #7; 0.49 for line #8; 0.85 for the 2nd construct, line #7; 0.76 for line #9; 0.83 for the 3rd construct, line #2; 0.69 for line #3. (B) Mean fluorescent intensity of MTs in the endoplasmic region. MTs were stained with the anti-tubulin antibody. The locations, 25 μm and 50 μm from the tip, were measured (mean ± SEM, each n = 5). The representative MT network of a control cell, observed by spinning-disc confocal microscopy is also displayed. Bar, 50 μm.