Kinesin-13 and Kinesin-8 Function during Cell Growth and Division in the Moss Physcomitrella patens

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Kinesin-13 and Kinesin-8 are well-known microtubule (MT) depolymerases that regulate MT length and chromosome movement in animal mitosis. While much is unknown about plant Kinesin-8, Arabidopsis (Arabidopsis thaliana) and rice (Oryza sativa) Kinesin-13 have been shown to depolymerize MTs in vitro. However, the mitotic function of both kinesins has yet to be determined in plants. Here, we generated complete null mutants of Kinesin-13 and Kinesin-8 in moss (Physcomitrella patens). Both kinesins were found to be nonessential for viability, but the Kinesin-13 knockout (KO) line had increased mitotic duration and reduced spindle length, whereas the Kinesin-8 KO line did not display obvious mitotic defects. Surprisingly, spindle MT poleward flux, which is mediated by Kinesin-13 in animals, was retained in the absence of Kinesin-13. MT depolymerase activity was not detectable for either kinesin in vitro, while MT catastrophe-inducing activity (Kinesin-13) or MT gliding activity (Kinesin-8) was observed. Interestingly, both KO lines showed waviness in their protonema filaments, which correlated with positional instability of the MT foci in their tip cells. Taken together, the results suggest that plant Kinesin-13 and Kinesin-8 have diverged in both mitotic function and molecular activity, acquiring roles in regulating MT foci positioning for directed tip growth.

INTRODUCTION

Microtubule (MT)-based motor proteins, kinesins, form a large superfamily in animal and plant species (61 genes in Arabidopsis [Arabidopsis thaliana], 78 in Physcomitrella patens, 45 in Homo sapiens, and 25 in Drosophila melanogaster; Reddy and Day, 2001; Miki et al., 2005; Shen et al., 2012). Kinesins show various activities in association with MTs and play pivotal roles in eukaryotic cells, such as cargo transport, MT organization, MT dynamics regulation, and force generation (Walczak and Heald, 2008; Hirokawa et al., 2009). Comprehensive functional analyses in several animal model systems, such as fly and human cell lines, frog egg extracts, and mouse, together with biochemical characterization of each kinesin motor have provided insights into how MT-based intracellular processes are driven and regulated during cell proliferation, differentiation, and animal development. By contrast, the cellular and developmental functions of plant kinesins are less clear, partly due to the difficulty in making and characterizing the phenotypes of complete knockout (KO) lines of paralogous kinesins that likely function redundantly. The use of high-resolution live microscopy, which was particularly critical for assessing kinesin functions during mitosis in animals, has also been limited in plants.

Within the kinesin superfamily, Kinesin-13 and Kinesin-8 commonly show a unique activity in vitro: MT depolymerization (Desai et al., 1999; Howard and Hyman, 2007; Walczak et al., 2013). In vivo, Kinesin-13 is able to depolymerize relatively stable MTs from both ends, while MT catastrophe-inducing activity is limited to the plus end (Rogers et al., 2004; Mennella et al., 2005). The best-studied Kinesin-13, human KIF2C/MCAK, accumulates at MT ends either by diffusion or through recruitment by other MT-associated proteins (Lee et al., 2008). At the ends, it binds to and stabilizes protofilament bends, which promotes strain on the association between protofilaments within the MT lattice (Moore et al., 2002; Ovechkina et al., 2002; Ogawa et al., 2017). In the mitotic spindle, KIF2C/MCAK is localized to the kinetochore and likely triggers depolymerization of MT plus ends that are erroneously attached to the kinetochore (Kline-Smith et al., 2004; Walczak et al., 2013). Another Kinesin-13 (human KIF2A, fly KLP10A) localizes at the pole region and depolymerizes MTs, including relatively stable kinetochore-bound MTs, from the minus end, driving poleward movement of MTs (called spindle MT poleward flux) and chromosome segregation at anaphase (Rogers et al., 2004; Ganem et al., 2005; Walczak et al., 2013). Depletion of Kinesin-13 causes various mitotic errors, such as spindle elongation, spindle monopolization, erroneous kinetochore–MT attachment, and chromosome lagging at anaphase (Walczak et al., 2013).

While Kinesin-13 does not show motility on MTs, Kinesin-8 possesses both MT-depolymerizing activity and processive plus end-directed motility and thus preferentially destabilizes MT plus ends (Howard and Hyman, 2007; Walczak et al., 2013). During mitosis, Kinesin-8 concentrates at the outer kinetochore region and prevents excessive elongation of kinetochore MTs and stabilizes this kinetochore–MT attachment to promote chromosome alignment to the spindle equator (May et al., 2007; Stumpf et al., 2008, 2012; Edzuka and Goshima, 2019). As a whole, Kinesin-13 and Kinesin-8 MT depolymerization activity is generally required for proper MT length regulation and correct chromosome movement during mitosis of various animal cell types (Walczak et al., 2013).
IN A NUTSHELL

Background: Microtubules (MTs) are dynamic macromolecular protein filaments of the cytoskeletal network that can undergo assembly and disassembly repeatedly. Regulation of MT dynamics is essential to its function in many different cellular processes, such as cell growth and cell division. Kinesin-13 and Kinesin-8 are well-known MT depolymerases in animal cells, reported to induce severe mitotic defects upon protein depletion. While little is known about plant Kinesin-8, rice and Arabidopsis Kinesin-13 have been shown to have MT depolymerising activity. However, complete depletion of plant Kinesin-13 and -8 have yet to be studied, with their function during mitosis largely unknown.

Question: What are Kinesin-13 and Kinesin-8 doing in plants, or more specifically, in mitosis?

Findings: Complete knockout (KO) plants of Kinesin-13 and -8 in the moss Physcomitrella patens were generated, indicating that both kinesins are not essential for viability. While the Kinesin-8 KO line showed no observable mitotic defects, the Kinesin-13 KO line showed mild mitotic defects, such as reduced spindle length, increased mitotic duration, and retrograde nuclear movement during prophase. However, neither of the kinesin KO lines showed the dramatic mitotic phenotypes observed in animal cells. Moreover, MT depolymerisation activity could not be verified for either kinesin. In in vitro assays, the Kinesin-13 motor domain instead showed mild MT catastrophe-inducing activity, and the Kinesin-8 motor domain showed MT gliding activity. Interestingly, both kinesin KO lines had wavy protonema filaments, which correlated with unstable positioning of the MT foci at the tip of these filaments. These results suggest that plant Kinesin-13 and -8 have acquired different intrinsic molecular activity and functions.

Next steps: Depletion of either Kinesin-13 or Kinesin-8 does not result in aberrant mitosis, suggesting that a unique mechanism underlies plant mitotic spindle formation. It would be interesting to study how plant cells regulate spindle dynamics without both kinesins.

RESULTS

Kinesin-13 Affects Protonema Growth, but Not Gametophore Morphology

To investigate Kinesin-13’s role in P. patens, all three paralogous Kinesin-13 genes (Kinesin-13a, Kinesin-13b, and Kinesin-13c;
Figure 1B; Supplemental Data Set 1) were sequentially deleted by homologous recombination-mediated gene replacement in the moss lines expressing GFP-tubulin and histoneH2B-monomeric red fluorescent protein (mRFP; Supplemental Figures 1A and 1B). Kinesin-13 single and double KO moss colonies did not have observable developmental defects. Moreover, Kinesin-13 triple KO lines (hereafter Kinesin-13 KO) were successfully generated, indicating that Kinesin-13 genes are not essential in moss. There

Figure 1. Conservation of Kinesin-13 and Kinesin-8 in the Moss *P. patens*.

(A) Protein domains of Kinesin-13 (represented with Kinesin-13b) and Kinesin-8 (represented with Kinesin-8II) of moss, compared with *D. melanogaster* KLP10A/Kinesin-13 and *S. cerevisiae* Kip3/Kinesin-8. Domains of *Drosophila* and budding yeast proteins were obtained from UniProt, whereas moss protein domains were predicted using InterPro.

(B) Kinesin-13 and Kinesin-8 phylogeny across the moss *P. patens*, the Brassica *Arabidopsis*, the liverwort *Marchantia polymorpha*, the rice *O. sativa* subsp japonica, the fruitfly *D. melanogaster*, mammals *Mus musculus* and *H. sapiens*, and also for Kinesin-8 in the budding yeast *S. cerevisiae* and fission yeast *Schizosaccharomyces pombe*. After amino acid sequences were aligned with MAFFT, gapped regions were removed manually using MacClade. The phylogenetic tree was constructed using neighbor-joining methods using MEGA software. Reliability was assessed with 1000 bootstrapping trials. Protein sequences are presented in Supplemental Data Set 1. Horizontal branch length is proportional to the estimated evolutionary distance. Bar = 0.1 amino acid substitution per site.
was an overall reduction in colony size in the Kinesin-13 KO compared to the control (Figures 2A and 2B). However, the overall morphology of the protonema colonies, gametophore (leafy shoots encasing gametangia), and rhizoids (root-like filamentous cells differentiated from gametophore basal cells; Cove, 2005; Menand et al., 2007; Kofuji and Hasebe, 2014) were indistinguishable from the control (Figures 2A to 2C), which differs from the case of the rice Kinesin-13A mutant, which has small and round grains with shortened panicles and internodes of the whole rice plant (Kitagawa et al., 2010).

To further investigate the colony growth phenotype in Kinesin-13 KO moss, early-stage moss colonies regenerated from single protoplasts cultured for 8 d were analyzed for nonapical cell length and protonema filament branching pattern (Figures 2D to 2G). Nonapical cells, which undergo little cell expansion after cell division, were found to be shorter in the Kinesin-13 KO moss caulonema cells (Figures 2E and 2F), consistent with reduced cell length in rice Kinesin-13A mutants (Deng et al., 2015). The branching pattern was analyzed by measuring the parameters of branching distance (distances from the tip of the protonema filament to the first three branching sites), branch filament length, and branch angle (Figure 2E). While the first branching distance (distance from the tip of the protonema filament to the nearest branching site) increased in the Kinesin-13 KO line, other branching pattern parameters were not observably different from that of the control (Figure 2G).

Kinesin-13 Facilitates Spindle MT Organization and Chromosome Alignment, but Does Not Drive Spindle MT Flux

The protonema tissue propagates by concerted asymmetric cell division and tip growth in the apical stem cells (Rounds and Bezanilla, 2013). Therefore, a reduction in colony size in the Kinesin-13 KO moss could be attributed to a defect in either or both events. To study mitosis in the Kinesin-13 KO moss, localization of moss Kinesin-13s to the mitotic spindle was first confirmed. As previously reported (Miki et al., 2014), moss Kinesin-13s showed spindle localization, most enriched at the spindle equator, with the level of expression varying among the three paralogues; they did not show punctate signals at the spindle pole or kinetochore like animal Kinesin-13 (Supplemental Figure 2A). Next, time-lapse imaging of moss protonema cells revealed that MT-dependent nuclear movement in prophase was abnormal in the Kinesin-13 KO line. In the control, nuclear movement is minimal or mildly apically directed as cells undergo nuclear envelope breakdown (NEBD). By contrast, in the KO line, the nucleus displayed severe retrograde movement leading up into NEBD and often continued moving basally even during spindle establishment (Figures 3A and 3B). This retrograde nuclear movement was also observed in the Kinesin-13ac double KO lines to a lesser degree, but not in the single or Kinesin-13ab double KO lines (Figure 3B). Additionally, overexpression of Kinesin-13b(full-length)-Cerulean under the EF1α promoter complemented the retrograde nuclear movement (Figures 3C and 3D). However, mutant Kinesin-13b constructs in which motor activity (Kinesin-13bKO-Cerulean; Dawson et al., 2007), conserved MT depolymerization motifs (Kinesin-13bKDVKEC-Cerulean; Shipley et al., 2004), and a conserved MT binding domain (Kinesin-13bloop12-Cerulean; Soppina and Verhey, 2014) were compromised could not restore the retrograde nuclear movement (Figure 3D). Overall, these results suggest that Kinesin-13s contribute to nuclear movement redundantly in a motor-dependent manner.

The severe retrograde nuclear/spindle movement during prophase likely resulted in cross cell wall–positioning defects in the Kinesin-13 KO moss. Indeed, analysis of subapical and apical cell length at anaphase onset showed that subapical cell length was reduced in the Kinesin-13 KO moss (Figures 3E and 3F). This is associated with a reduction in nonapical cell length of early-stage moss colonies and suggests that moss Kinesin-13 has a role in cell length maintenance.

Consistent with the retrograde nuclear/spindle movement, high-resolution time-lapse imaging showed that Kinesin-13 KO moss also has a disparity of the nucleus-surrounding MT array during prophase (Figure 4A; Supplemental Movie 1; Supplemental Movie Legends). In the control, shortly before NEBD, MTs associated asymmetrically to the nucleus, with more MTs gathering on the apical side (Doonian et al., 1985; Nakaoka et al., 2012). By contrast, this apically directed MT asymmetry was altered in the KO line, with the GFP-tubulin intensity ratio of apical-to-basal hemispheres of the nucleus decreasing from 1.2 in the control to 1.0 (Figures 4B and 4C), suggesting that Kinesin-13s are important for MT organization during prophase.

Upon NEBD, MTs assemble into a bipolar spindle. However, spindle assembly required more time than control cells as anaphase onset was delayed, with the majority of the delay due to slow spindle MT organization as prometaphase was delayed but metaphase was unaffected (Figure 4D). Despite the drastic nuclear movements and mitotic delay, MTs reorganized into the phragmoplast, which is the MT-based machinery required for cell plate formation, and cytokinesis was completed in 15 of 15 cells, indicating that Kinesin-13s are dispensable in the later stages of cell division.

Unexpected from previous studies in animals and the predicted MT depolymerization activity of Kinesin-13 (Walczak et al., 2013), the metaphase spindle was shorter, rather than longer, in the KO cells (Figure 4E). In animal cells, MT depolymerization at the spindle pole by Kinesin-13 is important for poleward flux of spindle MTs, where MTs flow from the spindle equator to the pole regions through the continuous addition and removal of tubulin heterodimers at the plus ends and minus ends, respectively (Rogers et al., 2005). To investigate whether Kinesin-13 depletions affects poleward MT flux in moss, GFP-tubulin at the equator of the mitotic spindle was bleached and the movement of the photobleached strip was monitored. Surprisingly, the strip migrated toward the poles as in control cells, indicating that MT poleward flux took place in spite of complete Kinesin-13s depletion (Figures 4F and 4G; Supplemental Movie 2; Supplemental Movie Legends). Thus, Kinesin-13 contributes to mitosis in an unconventional manner in moss.

Kinesin-13 Regulates Straight Growth of the Protonema Filament by Controlling the Position of MT Focal Points

To study the colony growth defect of the Kinesin-13 KO line in detail, long-term time-lapse imaging of protonema filament growth was performed. Protonema filaments were wavy with the protonema cell tip periodically changing growth direction in the Kinesin-13 KO line (Figure 5A; Supplemental Movie 3;
Figure 2. Kinesin-13 and Kinesin-8 KO Mosses Are Morphologically Normal, but Kinesin-13 KO Moss Shows Retarded Growth and Reduced Cell Length.

(A) and (B) Colony size comparison between control (GFP-tubulin/histoneH2B-mRFP) and Kinesin-13 KO (GPH0438##30, left) or Kinesin-8 KO (GPH0433##9, right) moss. Colonies were cultured from single protoplasts for 27 to 28 d on BCDAT. At least two independent experiments each with at least two plates of colonies were performed. The average colony area for each line on each plate was obtained. Actual areas were then divided by the average area of the control sample to get relative colony size. In the Kinesin-13 KO experiment, KO moss had a relative size of 0.55 ± 0.04 (mean ± SE; n = 7), whereas control moss had a relative size of 1.00 ± 0.12 (mean ± SE; n = 6). In the Kinesin-8 KO experiment, KO moss had a relative size of 1.01 ± 0.05 (mean ± SE; n = 8), whereas control moss had a relative size of 1.00 ± 0.08 (mean ± SE; n = 8). Points represent individual colonies; results are from one of at least two independent experiments. Bar = 5 mm.

(C) Gametophore and rhizoids of control moss (GFP-tubulin/histoneH2B-mRFP) and Kinesin-13 KO (GPH0438##6) or Kinesin-8 KO (GPH0433##7) moss. Gametophores were isolated from colonies from small colony subcultures cultured on BCDAT for 27 to 28 d. Bar = 1 mm.

(D) Day-8 moss colonies cultured from protoplast of control (GFP-tubulin/histoneH2B-mRFP) and Kinesin-13 KO (GPH0433#30) under bright-field light (top) and with calcofluor staining (bottom). Yellow dashed boxes, inset region. Bars = 500 μm; inset bar = 50 μm.

(E) Cartoon depicting the measurements taken for nonapical cell length in (F) and branching phenotype analysis in (G).

(F) Nonapical cell lengths of caulonema filaments were measured using calcofluor-stained colonies as in (D, bottom) for control (GFP-tubulin/histoneH2B-mRFP) and Kinesin-13 KO (GPH0433#30). Nonapical cell length was reduced in Kinesin-13 KO moss to 79.9 ± 5.5 μm (mean ± SE; n = 43), compared with control moss of 113.7 ± 1.9 μm (mean ± SE; n = 132). Points represent individual cells; results are pooled from two independent experiments where two independent lines were analyzed.
Supplemental Movie Legends). The Kinesin-13 KO line was wavier, with a bend frequency of 0.024 ± 0.002 μm⁻¹ (mean ± se; n = 26) compared to the control (0.006 ± 0.001 μm⁻¹, mean ± se; n = 28; Figure 5B). Interestingly, the Kinesin-13ac double KO line showed a milder wavy phenotype, while the single and Kinesin-13ab double KO lines did not (Supplemental Figure 3A). Additionally, ectopic expression of full-length Kinesin-13b rescued the waviness phenotype (Figure 5B, Supplemental Figure 3B). Thus, Kinesin-13s are required for straight tip growth.

Directionality of protonema tip growth in moss has been stipulated to be dependent on MTs (Dooran et al., 1988). At the apex of the protonema tip cell, plus ends of MTs converge into a focus known as MT foci (Hiwatashi et al., 2014). This occupies about the protonema tip cell, plus ends of MTs converge into a focus known as MT foci (Hiwatashi et al., 2014). Tip growth defects including abnormal tip branching, retarded growth, and isotropic growth are the phenotypes observed where its organization at the tip is impaired (actin-related proteins, tarded growth, and isotropic growth are the phenotypes observed dependent manner (Wu and Bezanilla, 2018; Yamada and Goshima, 2018). MT foci behavior in the Kinesin-13 KO line was investigated with spinning disc confocal microscopy where MT foci of the Kinesin-13 KO moss were unstable and fluctuated frequently (Figures 5C and 5D; Supplemental Movie 4; Supplemental Movie Legends). Interestingly, in 19 of 20 bending events observed, the displacement of MT foci occurred prior to cell bending, indicating that MT foci dictated protonema growth direction (Supplemental Figure 4). These results suggest that Kinesin-13s regulate anisotropic growth of protonema filaments by positional maintenance of MT foci at the cell tip.

Kinesin-13 Is an Interphase MT Plus End Tracking Protein

To investigate Kinesin-13’s localization during interphase, endogenously tagged Kinesin-13-Citrine lines (Miki et al., 2014) were observed with spinning disc confocal microscopy. Consistent with the depletion data, Kinesin-13s localized to MT foci (Figure 6A; Supplemental Figure 2B). To determine whether Kinesin-13 also associates with individual MTs in the endoplasm, we used oblique illumination fluorescence microscopy that enables observation of single MTs near the cell cortex with reduced effect of chloroplast autofluorescence (Jonsson et al., 2015; Nakaoka et al., 2015). In the interphase MT array, Kinesin-13s accumulated at the ends of growing MTs and disappeared from the ends when MTs switched to the shrink phase (Figures 6B and 6C; Supplemental Figure 2C; Supplemental Movie 5). Since MT minus ends are stabilized and exhibit little to no dynamics in this cell type (Leong et al., 2018), we concluded that Kinesin-13 localizes to the plus ends of growing MTs. The plus end tracking behavior is reminiscent of human KIF2C/MCAK and Drosophila KLP10A, which are recruited by End Binding 1 (EB1) to growing plus ends (Mennella et al., 2005; Lee et al., 2008).

MT Shrinkage Rate and Rescue Frequency Increase, While MT Growth Rate and Catastrophe Frequency Reduce, upon Kinesin-13 Depletion

Since Kinesin-13s tracked growing MT plus ends, the effect of Kinesin-13 depletion on MT plus end dynamics during interphase was analyzed using time-lapse oblique illumination imaging of GFP-tubulin. MT shrinkage rate increased upon Kinesin-13 depletion, from 0.25 ± 0.01 μm/s (mean ± se; 5 MTs per cell analyzed, n = 25 cells) in the control to 0.43 ± 0.02 μm/s (mean ± se; 5 MTs per cell analyzed, n = 25 cells) in the KO line (Figure 6D). The catastrophe frequency was reduced from 9.3 ± 1.2 × 10⁻³/s (mean ± se; n = 33) in the control to 2.2 ± 0.5 × 10⁻³/s (mean ± se; n = 28) in the KO line (Figure 6E), while the rescue frequency was increased from 14 ± 3 × 10⁻³/s (mean ± se; n = 25) in the control to 25 ± 6 × 10⁻³/s (mean ± se; n = 23) in the KO line (Figure 6F). To analyze MT growth rate, Kinesin-13 KO moss expressing EB1-Citrine (Supplemental Figure 1C), a tracker of growing MT plus ends, was imaged with oblique illumination fluorescence microscopy. MT growth rate based on EB1-Citrine comet movement was reduced from 0.147 ± 0.013 μm/s (mean ± se; 10 MTs per cell analyzed, n = 5 cells) in the control to 0.093 ± 0.003 μm/s (mean ± se; 10 MTs per cell analyzed, n = 5 cells) in the KO line (Figure 6G). These results suggest that Kinesin-13 plays a role in regulating MT dynamics in the interphase MT network.

Altered MT Dynamics Parameters May Underlie MT Length Phenotypes in Kinesin-13 KO

Depletion of MT depolymerases or catastrophe-promoting factors causes cytoplasmic MT lengthening and spindle expansion (Howard and Hyman, 2007; Goshima and Scholey, 2010). For example, in fission yeast cells lacking catastrophe-promoting factors, cytoplasmic MTs are more frequently polymerized beyond the limits of the cell, resulting in MT bending and curling (West et al., 2001). However, shorter metaphase spindle formation (Figure 4E) and the observation that MT foci often are unable to reach the apex of the cell tip in Kinesin-13 KO lines (Supplemental Figure 5; Supplemental Movie 4; Supplemental Movie Legends) appear to contradict this general rule. We reasoned that a decrease in MT growth rate and increase in shrinkage rate might limit overall MT length, despite a significant reduction in catastrophe frequency. To evaluate this possibility, we built a probability model...
Figure 3. *Kinesin-13abc* Triple KO Shows Retrograde Nuclear Movement, While *Kinesin-13ac* Double KO Shows Both Retrograde and Anterograde Nuclear Movement as Cells Enter Mitosis.

(A) Snapshots of control (GFP-tubulin/histoneH2B-mRFP) and *Kinesin-13 KO* (GPH0438#30) moss during prophase nuclear/spindle movement. *Kinesin-13 KO* moss shows retrograde nuclear/spindle movement. Apical side, right, positive value for analysis in (B) and (D); basal side, left, negative values for analysis in (B) and (D); yellow dotted line, nucleus position at NEBD. NEBD, 0 min. Bar = 50 µm.

(B) Nuclear movement velocity during prophase of control (GFP-tubulin/histoneH2B-mRFP; 0.68 ± 0.10 µm/min, mean ± SE; n = 8), *Kinesin-13a* single KO (GPH0411#43; 0.85 ± 0.10 µm/min, mean ± SE; n = 8), *Kinesin-13b* single KO (GPH0412#11; 0.43 ± 0.03 µm/min, mean ± SE; n = 7), *Kinesin-13ab* double KO (GPH0419#33; 0.62 ± 0.04 µm/min, mean ± SE; n = 11), *Kinesin-13ac* double KO (GPH0420#125; −0.03 ± 0.13 µm/min, mean ± SE; n = 16), and *Kinesin-13abc* triple KO (GPH0438#30; −0.69 ± 0.08 µm/min, mean ± SE; n = 11). *Kinesin-13abc* triple KO shows a clear retrograde nuclear movement, whereas *Kinesin-13ac* double KO shows intermediate retrograde nuclear movement. Apically directed/anterograde movement, positive values; basally directed/retrograde movement, negative values. Points represent individual mitotic events. Results are from one of three independent experiments where two independent lines were analyzed.

(C) Protein domains of *Kinesin-13b* and mutant proteins for rescue experiments. Point mutations on *Kinesin-13b*-Cerulean, which was introduced under the EF1α promoter in the moss used for rescue experiments are illustrated. a.a., amino acids.

(D) Nuclear movement velocity during prophase of control (GFP-tubulin/histoneH2B-mRFP; 0.94 ± 0.10 µm/min, mean ± SE; n = 17), *Kinesin-13abc* triple KO (GPH0438#30; −1.43 ± 0.22 µm/min, mean ± SE; n = 29), Cerulean/*Kinesin-13abc* triple KO (GPH0903#1; −0.99 ± 0.25 µm/min, mean ± SE; n = 16), *Kinesin-13b(Fl)-Cerulean/*Kinesin-13abc* triple KO (GPH0899#10; 1.04 ± 0.09 µm/min, mean ± SE; n = 13), *Kinesin-13b(RIG)-Cerulean/*Kinesin-13abc* triple KO (GPH0902#2; −0.58 ± 0.20 µm/min, mean ± SE; n = 17), *Kinesin-13b(KVD/KEC)-Cerulean/*Kinesin-13abc* triple KO (GPH0900#4; −0.94 ± 0.08 µm/min,
fixed by the parameters of MT growth rate, shrinkage rate, catastrophe frequency, and rescue frequency and ran a simulation in which 4000 MTs exhibited dynamic instability for 4 min (Figure 6H; Table 1). With control parameters, a normal distribution was obtained where 50% of MTs ranged from −12.4 to 23.4 μm in length and the longest 1000 MTs ranged from 23.4 to 59.8 μm in length. By contrast, with MT dynamics parameters of Kinesin-13 KO cells, a narrower normal distribution was obtained, with 50% of MTs having lengths of 11.5 to 22.6 μm and the longest 1000 MTs ranging from 22.6 to 29.4 μm. Thus, the formation of shorter metaphase spindles and apex-displaced MT foci is a theoretically possible outcome, and actually a more likely outcome, associated with depletion of moss Kinesin-13 that affects both MT catastrophe frequency and growth/shrinkage rate.

**Kinesin-13 Motor Domain Induces Catastrophe in Vitro**

To investigate the direct effect of moss Kinesin-13 on MT dynamics, recombinant Kinesin-13bmotor-monomeric GFP (mGFP) was expressed and purified from a bacterial expression system (Figure 7A; Supplemental Figure 5A; full-length Kinesin-13 could not be obtained in either bacteria or insect cell culture expression system). The purified Kinesin-13bmotor-mGFP was subjected to binding-release experiments to confirm ATP hydrolysis activity: it bound to MTs in the presence of a nonhydrolyzable ATP analog (AMPPNP) and dissociated from MTs upon ATP addition (Supplemental Figure 5B). The ATPase-active protein was added to GMPCPP-stabilized MTs, but it did not show active MT depolymerization like that of animal Kinesin-13 protein (Drosophila KLP10A; Figure 7B; Rogers et al., 2004; M oriwaki and Goshima, 2016). We considered the possibility of moss Kinesin-13 requiring a binding partner like MID1 for Arabidopsis Kinesin-13 (Oda and Fukuda, 2013). A BLAST search showed that moss does not have MID1 homologues; so, moss Kinesin-13 was tested for MT depolymerization activity in the presence of Arabidopsis MID1, but it also did not depolymerize MTs (Figure 7B). Overall, the purified Kinesin-13bmotor-mGFP construct did not exhibit MT depolymerase activity under these experimental conditions.

The purified Kinesin-13bmotor-mGFP was also subjected to an in vitro MT polymerization assay at concentrations of 0, 0.15, 0.3, 0.6, and 1.5 μM. While the growth rate was somewhat reduced with a higher Kinesin-13bmotor concentration (Figure 7D), the shrinkage rate and rescue frequency were not obviously affected by Kinesin-13bmotor-mGFP addition (Figures 7E and 7F). Interestingly, in the presence of Kinesin-13bmotor-mGFP, the catastrophe frequency was reproducibly increased (Figure 7G). This result is consistent with in vivo data in which MT catastrophe frequency was lower in the Kinesin-13 KO line. By contrast, the recombinant protein did not reproduce the plus end accumulation seen in vivo, indicating that the truncated region and/or a separate factor may be required for plus end recruitment.

**Chromosome Segregation and Cell Division Proceed Normally in the Absence of Kinesin-8**

The mitotic phenotype associated with Kinesin-13 deletion was fairly mild. We reasoned that another MT depolymerase instead might have a major role in MT depolymerization in mitosis of protonema filaments, and we decided to investigate Kinesin-8, which shows strong depolymerization activity during mitosis of yeast (Hildebrandt and Hoyt, 2000; Unsworth et al., 2008). To study Kinesin-8 function in the moss, all three paralogous genes phylogenetically classified into the moss Kinesin-8 subfamily (Kinesin-8a, Kinesin-8b, and Kinesin-8l; Figure 1B; Supplemental Data Set 1; Shen et al., 2012; Miki et al., 2014) were knocked out (Supplemental Figure 1D). Moss colonies, gametophores, and rhizoids were normal in the Kinesin-8 KO line (Figures 2A to 2C). High-resolution mitosis imaging did not show any defect in prophase MT organization, spindle formation, chromosome alignment, anaphase chromosome segregation, and cytokinesis (9.1 ± 0.2 min from NEBD to anaphase onset; n = 10; Figures 4A, 4D, and 4E). We concluded that Kinesin-8s are dispensable for mitotic cell division in moss protonema filaments.

**Kinesin-8 Controls the Positioning of MT Foci for Straight Tip Growth**

Interestingly, the Kinesin-8 KO line also had wavy protonema filaments with bends occurring at smaller magnitudes, with a bend frequency of 0.022 ± 0.005 μm−1 (mean ± se; n = 8; Figures 5A and 5B; Supplemental Movie 3; Supplemental Movie Legends). Tracking of the MT foci at tip cells showed that it fluctuates more frequently in the Kinesin-8 KO line than in the control or Kinesin-13 KO line (Figures 5C and 5D; Supplemental Movie 4; Supplemental Movie Legends), consistent with its smaller magnitudes of bends.

**Kinesin-8lmotor Glides MTs, but Does Not Show a MT Depolymerization Activity in Vitro**

To analyze the intrinsic activity of moss Kinesin-8, the recombinant Kinesin-8lmotor-GFP protein was expressed and purified from a bacterial expression system (Figure 8A; Supplemental Figure 5C) and was subjected to a MT depolymerization assay. Next, 200 nM Kinesin-8lmotor-GFP was added to GMPCPP-stabilized MTs, but it could not depolymerize them, while 200 nM budding yeast (*Saccharomyces cerevisiae*) Kinesin-8/Klp3

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**Figure 3.** (continued).

mean ± se; n = 10), and Kinesin-13bΔloop2-Cerulean/Kinesin-13abc triple KO (GPH0901#1; −1.05 ± 0.20 μm/min, mean ± se; n = 27). Apically directed movement, positive values; basally directed movement, negative values. Points represent individual mitotic events. Results are from one of two independent experiments where at least two independent lines were analyzed.

(E) Cartoon depicting how subapical and apical cell lengths were measured for (F).

(F) Subapical cell length was reduced in the Kinesin-13 KO line (GPH0438#30; 70.9 ± 3.6 μm [mean ± se; n = 26; P-value < 0.05, Welch’s two-sample t test]) compared with the control (GFP-tubulin/histoneH2B-mRFP; 106.2 ± 12.4 μm [mean ± se; n = 11]). Each point represents individual mitotic events. Results shown are from one of two independent experiments where two independent lines were analyzed.
Figure 4. Kinesin-13 KO Moss Shows Defects in Nuclear-Proximal MT Array, Mitotic Duration, and Spindle Length, but Shows No Difference in Spindle Flux Rate.

(A) Mitosis of control (GFP-tubulin/histoneH2B-mRFP), Kinesin-13 KO (GPH0438#6), and Kinesin-8 KO (GPH0433#9) moss. Kinesin-13 KO showed reduced metaphase spindle length, retrograde nuclear movement during prophase, increased mitotic duration, and loss of apical bias of nuclear MTs. Kinesin-8 KO did not show mitotic defects. NEBD, 0 min; left, basal side; right, apical side. Bar = 10 μm.

(B) and (C) Apical/basal GFP-intensity ratio of GFP-tubulin around the nucleus 1 min before NEBD was measured as the ratio of GFP-tubulin intensities between apical and basal hemispheric circumference. In (C), control (GFP-tubulin/histoneH2B-mRFP), 1.17 ± 0.04 (mean ± SE; n = 9; P-value < 0.05, Welch’s two sample t test); Kinesin-13 KO (GPH0438#6, 30), 1.00 ± 0.07 (mean ± SE; n = 10). Points represent individual mitotic events.

(D) Mitotic duration of control (GFP-tubulin/histoneH2B-mRFP), Kinesin-13 KO (GPH0438#6, 30), and Kinesin-8 KO (GPH0433#7, 9) moss as measured from NEBD to anaphase onset. Control, 9.8 ± 0.3 min (mean ± SE; n = 11); Kinesin-13 KO, 11.8 ± 0.4 min (mean ± SE, n = 15; P-value < 0.001, Welch’s two sample t test); Kinesin-8 KO, 9.1 ± 0.2 min (mean ± SE; n = 10; P-value < 0.05, Welch’s two sample t test). The duration of prometaphase (from NEBD to chromosome alignment) and metaphase (chromosome alignment to anaphase onset) was also measured and shown. Data shown were pooled from two independent experiments.

(E) Spindle length was measured at metaphase (defined as 1 min before anaphase onset) by obtaining the distance between midpoints of apical and basal spindle widths. Control (GFP-tubulin/histoneH2B-mRFP), 13.0 ± 0.3 μm (mean ± SE, n = 4); Kinesin-13 KO (GPH0438#30), 11.2 ± 0.3 μm (mean ± SE, n = 10; P-value < 0.01, Welch’s two-sample t test); Kinesin-8 KO (GPH0433#9), 12.3 ± 0.5 μm (mean ± SE; n = 10). Points represent individual mitotic events.

(F) Spindle poleward flux of control (GFP-tubulin/histoneH2B-mRFP) and Kinesin-13 KO (GPH0438#30) moss was examined in photobleaching experiments where GFP-tubulin signals on a strip along the metaphase plate was bleached. The bleached regions separating toward the poles are indicative of
could depolymerize the MTs (Figure 8B). Kinesin-8[imotor]GFP was then tested for MT gliding activity. Protein immobilized on silanized coverglass showed ability to glide GMPCPP-stabilized MTs in an ATP-dependent manner, but it also could not depolymerize those MTs (Figures 8C and 8D).

**DISCUSSION**

The KO lines generated in this study showed some characteristic phenotypes unreported in previous plant kinesin mutants, such as wavy cell growth accompanying MT foci positional fluctuation (Kinesin-13 and Kinesin-8) and prophase MT disorganization (Kinesin-13). Intriguingly, several processes driven by these motors in many animal and yeast species were normal in their absence in moss (*Physcomitrella patens*), such as spindle MT flux and chromosome segregation. Moreover, the hallmark activity of these kinesins, MT depolymerization, was not detected in vitro. Overall, this study provides a comprehensive view on the roles of Kinesin-13 and Kinesin-8 in a single plant species. Furthermore, our results reinforce the emerging view that the kinesin superfamily is well conserved in plants but that members of this superfamily have diverged in their function (Gicking et al., 2018; Nebenführ and Dixit, 2018).

**Are Plant Kinesin-13 and Kinesin-8 MT Depolymerases?**

Kinesin-13 is a well-known MT depolymerase in animals. Arabidopsis and rice Kinesin-13s have also been shown to depolymerize stabilized MTs (Oda and Fukuda, 2013; Deng et al., 2015). However, moss Kinesin-13 only exhibited catastrophe-inducing activity in vitro and could not depolymerize GMPCPP-stabilized MTs. This in vitro result is consistent with the reduced catastrophe frequency seen for interphase MTs in the Kinesin-13 KO moss. Nevertheless, negative results obtained in vitro are not necessarily conclusive: inappropriate expression systems or unsuitable biochemical environments could prevent full activity of the protein. In this study, a motor-only construct was used due to technical constraints. Thus, it is possible that another domain(s) on the Kinesin-13 protein is required for MT depolymerization activity. One such element may be the coiled coil, which in animal Kinesin-13 dimerizes the protein and increases MT depolymerization activity (Hertzer et al., 2006). However, the coiled coil region required for dimerization is located immediately upstream/downstream of the motor domain in animal Kinesin-13 (Maney et al., 2001), but it is located further down the C terminus in moss Kinesin-13 (Figure 1A); it is unclear whether dimerization of moss Kinesin-13 could enhance the activity in a similar manner to animal homologues. Furthermore, animal Kinesin-13 monomers are capable of depolymerizing MTs in vitro (Maney et al., 2001; Hertzer et al., 2006). Moss and also Arabidopsis Kinesin-13s lack the neck domain that is important for strong MT depolymerization activity in animals (Ovechkina et al., 2002); based on this feature, it was indeed originally speculated that plant Kinesin-13 might not have MT-depolymerizing activity (Lu et al., 2005). Thus, although we cannot rule out the possibility that moss Kinesin-13 has MT-depolymerizing activity, possibly with the aid of a specific binding partner, it is enticing to speculate that it has diverged structurally and functionally from animal Kinesin-13.

In cells, there is even less evidence to support Kinesin-13 as a MT depolymerase. Upon Kinesin-13 KO, interphase MTs show reduced MT growth rate and increased shrinkage rate. Such results instead point to Kinesin-13 being a MT growth promoter. However, MT growth-promoting activity was not observed in vitro. This may be due to the use of the motor-only construct with which we could not recapitate the plus end enrichment of Kinesin-13. Alternatively, considering the decrease and increase in catastrophe and rescue frequency of interphase MTs, it is possible the Kinesin-13 regulates the growth and shrinkage rate indirectly via tubulin cycling: reduced catastrophe would result in reduced availability of tubulin in the free tubulin pool, which might affect MT growth and shrinkage rates, as was proposed in studies of Arabidopsis Armadillo Repeat-containing Kinesin (ARK) proteins (Eng and Wasteney, 2014) and more recently for the plant-specific MT nucleator MACETERA-TOR4 (MACET4; Schmidt and Smertenko, 2019).

Similar to observations of Kinesin-13, we could not observe MT depolymerization of the Kinesin-8 motor in our assay, which differs from human and yeast Kinesin-8. This might be due to our use of a truncated construct (—440 amino acids), as we failed to purify the longer fragment (—640 amino acids). However, we recently found that *Drosophila* Kinesin-8 (full length) shows plus end-directed motility and induces MT catastrophe at the plus end, but that it is not able to depolymerize stable MTs in vitro (Edzuka and Goshima, 2019). Moss Kinesin-8 might have similar activities.

**Kinesin-13 and Kinesin-8 for Mitosis**

We could not detect any aberrant phenotypes in Kinesin-8 KO lines during mitotic cell division, such as chromosome misalignment or mitotic delay, which are common phenotypes observed in yeast and animal cells, suggesting that Kinesin-8 has lost mitotic functions in moss. By contrast, some, but not all, known mitotic functions of Kinesin-13 (Walczak et al., 2013) were observed in moss. In animal mitosis, centrosomal MTs (astral MTs) are overly developed during prophase in the absence of Kinesin-13 (Goshima and Vale, 2003; Rogers et al., 2004). Similarly, disorganized MTs were observed around the nucleus, despite the loss of centrosomes in moss (and all other land plants). MTs surrounding the nucleus may act as MTOCs equivalent to animal centrosomes. During prometaphase, the kinetochore–MT attachment appears to be less efficient, since prometaphase duration was slightly prolonged in the Kinesin-13 KO moss; whether this is due to overall...
changes in MT dynamics or the lack of error correction, as is the case of KIF2C/MCAK depletion in animal cells, remains elusive. Spindle monopolarization, as reported in centrosome-containing animal cells (Goshima and Vale, 2003), was not detected. At metaphase, Kinesin-13 in animal cells acts as a MT depolymerase at the pole, driving MT poleward flux and halting spindle extension. Surprisingly, we did not obtain data that moss Kinesin-13 plays such a role: Kinesin-13 does not localize to the spindle pole, MT flux was detected in the Kinesin-13 KO moss. Figure 5. Kinesin-13 and Kinesin-8 KO Moss Have Wavy Protonema Filaments Associated with Unstable MT Foci Positioning. (A) Protonema filaments of control (GFP-tubulin/histoneH2B-mRFP), Kinesin-13 KO (GPH0438#30), and Kinesin-8 KO (GPH0433#9) moss. Bar = 50 μm. (B) Waviness of protonema filaments measured as frequency of wavy bend (18° < angle < 90°) of protonema filaments over measured lengths. Control (GFP-tubulin/histoneH2B-mRFP), 0.006 ± 0.001 μm⁻¹ (mean ± se; n = 28 filaments); Kinesin-13 KO (GPH0438#30), 0.024 ± 0.002 μm⁻¹ (mean ± se; n = 26 filaments; P-value < 0.0001); Kinesin-8 KO (GPH0433#7), 0.022 ± 0.005 μm⁻¹ (mean ± se; n = 8 filaments; P-value < 0.01); Cerulean/Kinesin-13 KO (GPH0903#1), 0.022 ± 0.004 μm⁻¹ (mean ± se; n = 12); Kinesin-13b/full-length-Cerulean/Kinesin-13 KO (GPH0899#10), 0.004 ± 0.001 μm⁻¹ (mean ± se; n = 8; P-value < 0.0001). Points represent individual protonema filaments, and results shown are from one experiment of at least four independent experiments. Tukey’s multiple comparison test was used for statistics (Supplemental Data Set 3). (C) MT foci at the tip of the caulonema cell of the control (GFP-tubulin/histoneH2B-mRFP), Kinesin-13 KO (GPH0438#30), and Kinesin-8 KO (GPH0433#9) moss. Images were acquired with z-sections at 0.3-μm intervals over a 20-μm range, and maximum z-projections are displayed. Bottom panels show overlaid time series. Colors in time series indicate different time points as labeled in top panels. Bar = 10 μm. (D) MT foci positions were tracked using the FIJI MOSAIC plug-in 2D/3D particle tracker (Sbalzarini and Koumoutsakos, 2005) in time-lapse imaging data as in (C). (x, y) trajectories of three representative MT foci (shown in different colors) for each line are displayed. Each point represents subsequent positions at each time point, at 3-min intervals for 3 h. Same lines as in (B) are represented.
Figure 6. Kinesin-13 Localizes to the Interphase MT Network and Depletion of Kinesin-13 Results in Increased Shrinkage Rate, Reduced Catastrophe Frequency, Increased Rescue Frequency, and Reduced Growth Rate.

(A) MT foci of Kinesin-13c-Citrine/mCherry-tubulin (GPH0100#15) moss. Images were acquired at 0.3-μm intervals over a 10-μm range; shown is maximum z-projection. Bar = 5 μm.

(B) Interphase MT network of Kinesin-13c-Citrine/mCherry-tubulin (GPH0100#15) moss. Images were acquired by oblique illumination fluorescence split-view microscopy to avoid chloroplast autofluorescence. Bar = 2 μm.

(C) Kymograph of MT growth taken from imaging as in (B), taken every 3 s. Vertical bar = 2 min; horizontal bar = 5 μm.

(D) Interphase MT plus end shrinkage rate of control (GFP-tubulin/histoneH2B-mRFP, 0.245 ± 0.012 μm/s [mean ± se; n = 25 cells]) and Kinesin-13 KO (GPH0438#30, 0.429 ± 0.021 μm/s [mean ± se; n = 25 cells]; P-value < 0.0001, Welch’s two-sample t test) moss. Points represent individual cells; results shown are from one experiment of two independent experiments.

(E) Interphase MT catastrophe frequency of control (GFP-tubulin/histoneH2B-mRFP, 9.3 ± 1.2 × 10⁻³/s [mean ± se; n = 33 cells]) and Kinesin-13 KO (GPH0438#30, 2.2 ± 0.5 × 10⁻³/s [mean ± se; n = 28 cells]; P-value < 0.0001, Welch’s two-sample t test). Points represent individual cells; results shown are from two independent experiments.

(F) Interphase MT rescue frequency of control (GFP-tubulin/histoneH2B-mRFP, 14 ± 3 × 10⁻³/s [mean ± se; n = 25 cells]) and Kinesin-13 KO (GPH0438#30, 25 ± 6 × 10⁻³/s [mean ± se; n = 23 cells]). Points represent individual cells; results shown are from two independent experiments.

(G) Interphase MT plus end growth rate of control (EB1-Citrine/mCherry-tubulin, GPH0379#2, 0.147 ± 0.013 μm/s [mean ± se; n = 5 cells, 50 MTs]) and Kinesin-13 KO moss (GPH0577#11, 0.093 ± 0.003 μm/s [mean ± se; n = 5 cells, 50 MTs; P-value < 0.05, Welch’s two-sample t test]). Points represent individual cells.

(H) Simulation of MT growth of 4000 MTs in 4 min based on a probability model established using MT dynamics parameters from in vivo interphase MT dynamics analyses (D) to (G) (see Methods and Table 1). Control MT dynamics parameters yielded approximately normal distributions of MT lengths and tended to have a larger population of MTs with longer lengths, with the longest 25% of MTs ranging between 23.4 to 59.8 μm in length. For MTs under Kinesin-13 KO conditions, the distribution of MT length was narrower, with 50% of all MTs being from 11.5 to 22.6 μm in length, whereas the longest 25% of MTs ranged from 22.6 to 29.4 μm in length. Histogram bin width = 0.5 μm.
KO line, and the spindle was shorter, rather than longer, in the complete absence of Kinesin-13. MT dynamics is a major contributor to spindle length regulation in animal somatic cells (Goshima and Scholey, 2010); therefore, shortening might be due to the reduced MT growth rate observed in the endoplasm, consistent with Kinesin-13 localizing to the spindle equator where MT plus ends are enriched. Chromosome segregation during anaphase A was normal, further supporting the notion that Kinesin-13 does not act as a MT depolymerase at the pole. These data indicate that the moss mitotic spindle possesses a mechanism that drives spindle MT poleward flux independently of Kinesin-13.

**Kinesin-13 and Kinesin-8 for Tip Growth**

The most prominent phenotype observed both in the **Kinesin-13** and **Kinesin-8** KO lines was the tip growth defect. Recent studies suggest the importance of MT-converging centers, the MT foci, in protonema tip growth in moss, where F-actin, which is absolutely essential for tip growth, is concentrated near the MT foci. In several mutants of MT-associated motors in which the tip grows more slowly, the MT foci are not persistently formed (Hiwatashi et al., 2014; Wu and Bezanilla, 2018; Yamada and Goshima, 2018). The transient MT foci produced bursts of MT concentration at random locations along the tip region of the apical cell, causing the bending of the protonema filament at abrupt angles (Hiwatashi et al., 2014). Such phenotypes are similar to the tip growth defects seen when moss is treated with MT-disruptive drugs (Doonan et al., 1988). In contrast to those mutants, we persistently observed single MT foci in the **Kinesin-13** or **Kinesin-8** KO. However, their positions were unstable, exhibiting the waviness of rather regular amplitude and frequencies. This suggests that Kinesin-13 and Kinesin-8 play a role in MT foci positional guidance, rather than MT foci formation/maintenance, which ensures straight growth of the protonema filament. While straight tip growth with limited MT foci fluctuations would allow the fastest propagation of moss, wavy growth would be an advantageous mechanism to facilitate innovative exploration of the environment. Our study highlights the regulation of MT plus end dynamics by MT-associated proteins as a possible intracellular mechanism to modulate cell growth in response to environmental cues, reminiscent of axon guidance in neurons (Sabry et al., 1991; Tanaka et al., 1995; Menon and Gupton, 2016).

**METHODS**

**Molecular Cloning and Gene Targeting Experiments**

All transgenic moss lines, plasmids, and primers used in this study are listed in Supplemental Data Set 2; all lines originated from the Physcomitrella patens Gransden 2004 strain. Moss culture, transformation, and transgenic line selection were performed as described previously (Yamada et al., 2016). In brief, moss cells were cultured on BCDAT or BCD media under 24 h of light illumination (FL40SD [NEC], 2500 luxes), and transformation was performed by the standard polyethylene glycol–mediated method.

**KO Moss Generation**

**Kinesin-13** KO (GPH0438) and **Kinesin-8** KO (GPH0433) were generated in the moss strain expressing GFP-tubulin and histoneH2B-mRFP (Nakaoka et al., 2012) sequentially replacing the targeted genes with antibiotic resistance using homologous recombination (HR). To do this, 1 kb of genomic DNA sequences upstream/downstream of the stop codon of the target genes were cloned around an antibiotic resistance cassette and then transformed into the moss. To knock out **Kinesin-13** genes in the moss expressing EB1-Citrine and mCherry-tubulin (Kosetsu et al., 2013), **Kinesin-13b** was first removed by antibiotic resistance–mediated HR as described before. For **Kinesin-3a** and **Kinesin-13c**, CRISPR-mediated gene removal was utilized. Next, 20-bp guide RNAs (gRNAs) targeting the start and end of the gene were designed using CRISPOR (http://crispor.tefor.net/; Supplemental Figure 1A). gRNAs were then integrated into a BsaI site of a vector carrying the U6 promoter and RNA scaffold (pCasGuide/ pUC18; Lopez-Obando et al., 2016; Collonnier et al., 2017), and then the CRISPR cassettes were cloned into a vector carrying nourseothricin resistance (pSY034) with InFusion (Takara) to assemble multiple gRNA cassettes into a plasmid (pSY062) following methods described previously (Leong et al., 2018). Equal amounts of this circular multicassette plasmid and Streptococcus pyogenes Cas9 expression vector (pGenius; Collonnier et al., 2017) were transformed into the **Kinesin-13b** KO/EB1-Citrine/ mCherry-tubulin background. Confirmation of **Kinesin-13** and **Kinesin-8** KO lines (Supplemental Figures 1B to 1D) were performed by PCR as described previously by Yamada et al. (2016) and Leong et al. (2018).

**Endogenous C-Terminal Citrine Tagging**

C-Terminal endogenous Kinesin-13 and Kinesin-8 Citrine tagging lines from Miki et al. (2014) were used. In these lines, Kinesin-13 and Kinesin-8 were tagged endogenously with Citrine at the C terminus via HR where C-terminal sequence and downstream sequence of the stop codon of the target gene (both 1-kb lengths) flanking an antibiotic resistance cassette was used as the HR template. Confirmation of this line was by PCR using primers designed to target the C-terminal region and outside the 3′ untranslated region of the target gene. To make rescue plasmids, the **Kinesin-13** sequence was cloned from a cDNA library and ligated into the pENTRY/TOPO vector. The **Kinesin-13** mutant plasmids (Kinesin-13b<sup>PRQ</sup>-Cerulean, Kinesin-13b<sup>WVDNEC</sup>-Cerulean, and Kinesin-13b<sup>loop+12</sup>-Cerulean) were generated by mutagenesis with the full-length Kinesin-13 plasmid, and primers are listed in Supplemental Data Set 2. The cloned Kinesin-13 sequences were ligated into the pMN603 vector by a Gateway LR reaction, which contains the EFlα promoter, Cerulean gene, nourseothricin-resistance cassette, and PTA1 sequences designated for HR-mediated integration (Miki et al., 2016; Yoshida et al., 2019).

**Imaging Sample Preparation**

**Agar Pad Sample**

Moss samples used in time-lapse imaging were prepared either in six-well glass-bottomed plates or 35-mm glass-bottomed dishes as described previously by Yamada et al. (2016). Briefly, protonema cells were plated onto glasses coated with BCD agar medium and cultured for 4 to 6 d.

**Microdevice Sample**

Samples used for oblique illumination fluorescence microscopy were prepared in BCD liquid medium in 15-μm-height polydimethylsiloxane
microfluidic chambers mostly following methods previously described by Leong et al. (2018) and Kozgunova and Goshima (2019). Briefly, protonema cells were homogenized in BCD liquid media, filtered through a sheet of 50-µm nylon mesh, and injected into microfluidic chambers attached to 24 m × 32 mm micro coverglasses (No. 1-S, Matsunami) and cultured for 4 to 6 d.
Figure 8. Recombinant Kinesin-8 Motor Does Not Depolymerize MTs but Shows MT Gliding Activity.

(A) Protein domains of Kinesin-8II (FL) and recombinant Kinesin-8IImotor-GFP (recombinant) construct. Protein domains were identified using InterPro. His-tag for affinity purification was attached to the C terminus of the recombinant protein. a.a., amino acids.

(B) In vitro MT depolymerization assay using GMPCPP-stabilized MT seeds was performed using purified ScKip3, recombinant Kinesin-8IImotor-GFP, and also under buffer only conditions. Only ScKip3 showed MT depolymerization activity. The slight reduction in intensity in the bottom panels is due to photobleaching during imaging. All proteins were used at 200 nM. Bar = 10 μm.

(C) ATP-dependent MT gliding velocity of Kinesin-8IImotor-GFP. 1 mM ATP, 0.68 ± 0.03 μm/min (mean ± se; n = 124 MTs); 5 mM ATP, 1.18 ± 0.02 μm/min (mean ± se; n = 121 MTs, P-value < 0.0001, Welch’s two-sample t test).

(D) In vitro MT gliding assay using GMPCPP-stabilized MTs on Kinesin-8IImotor-GFP, which was immobilized on glass, at 0, 1, and 5 mM ATP. Red dotted line in the top panel indicates the segmented line used to draw kymographs (bottom panels). Gliding activity of Kinesin-8IImotor-GFP was verified in three independent experiments. Vertical bar = 45 s; horizontal bar = 2 μm.
Calcofluor-Stained Sample

Eight-day-old moss colonies regenerated from single protoplasts were mounted on 35-mm glass-bottomed dishes with BCD agar medium. The moss colonies were stained with 100 μL of 0.1 mg/mL calcofluor solution diluted with water and covered with a coverslip. The samples were then imaged with an Eclipse TE2000-E inverted microscope (Nikon).

Moss Colony Assay

A protoplast regeneration assay was performed following the method described by Vidali et al. (2007) and Yamada et al. (2016), with some modifications. In brief, sonicated moss on cellophane-lined BCDAT plate was digested by 8% (w/v) mannitol solution supplemented with 2% (w/v) driselase. The generated protoplasts were washed three times with 8% (w/v) mannitol solution, and 1.5 × 10^6 mL^-1 of cells was resuspended with 7 mL of protoplast regeneration liquid. After overnight incubation in darkness, the protoplasts were centrifuged at 510g for 2 min at 25°C and resuspended in 4 mL of Protoplast Regeneration Media (PRM) solution in which CaCl2 was added after autoclave. Next, 0.5 to 1 mL of 4 mL of protoplast solution was spread on a cellophane-lined PRM plate. The protoplasts were cultured for 4 d and transferred to a BCDAT plate, followed by 4 d of culture. The 8-d-old moss colonies were observed with a stereomicroscope.

Live in Vivo Imaging

Spinning disc confocal microscopy using a 100× 1.45-NA lens and ImageX camera (Hamamatsu Photonics) was performed with a Nikon Ti inverted microscope as described previously by Yamada et al. (2016). The z-series were taken using a Nano-Z Series nanopositioner combined with a Nano-Drive controller (Mad City Labs), where z-stack imaging was performed at 0.3 μm. Oblique illumination fluorescence microscopy was performed with a Ti microscope (Nikon) with a total internal reflection fluorescence (TIRF) unit, a 100× 1.49-NA lens, GEMINI split view (Hamamatsu Photonics), and electron multiplication charge-coupled device camera (Evole, Roper). The microscopes were controlled by a Micro-manager or NIS-Elements (Nikon). Low-magnification epifluorescence imaging was performed using an Eclipse TE2000-E inverted microscope (Nikon) with 10×/0.3 LN1C Plain Fluo lens and Zyla 5.5 sCMOS camera (Andor), controlled with iQ3 (Andor). Photobleaching experiments were performed using an LSM780-DUO-NLO confocal microscopy system (Zeiss) using a Plan-Apochromat 63×/1.40 oil differential interference contrast lens controlled using Zen (Zeiss) with a 489-nm diode laser (time-lapse imaging taken at 2 mW and bleaching at 100 mW). Moss expressing GFP-tubulin (GPH0438#30 for Kinesin-13b, which is the most highly expressed protein of the three paralogous Kinesin-17 genes, was cloned from moss cDNA and transgenically linked to mGFP and 6×His at the C terminus (plasmid pG0885; Supplemental Data Set 2). Kinesin-13b–mGFP expression was induced in SOLBL21 E. coli with the same protocol as Kinesin-13b–mGFP. Instead of the binding-release experiment, elutions of KL10a were subjected to buffer exchange to 1× MB880 with 75 mM KCl and 0.1 mM ATP using a PD-25 column (GE Healthcare). Drosophila KL10a (plasmid pG0885) was purified refactoring (Moriwaki and Goshima, 2016) and was purified with the same protocel as Kinesin-13b–mGFP. Instead of the binding-release experiment, elutions of KL10a were subjected to buffer exchange to 1× MB880 with 75 mM KCl and 0.1 mM ATP using a PD-25 column (GE Healthcare). AtMID1 was purified following Oda and Fukuda (2013), with some modifications. Briefly, GST-AtMID1 expression was induced in SOLBL21 E. coli using 0.2 mM IPTG and cultured for 20 h at 18°C. Harvested cells were lysed using the Advanced Digital Sonifier D450 (Branson) in lysis buffer (25 mM MOPS, pH 7.0, 250 mM KCl, 2 mM MgCl2, 5% [w/v] Suc, 30 mM imidazole, and 0.1 mM ATP) supplemented with 5 mM β-mercaptoethanol and protease inhibitors (0.5 mM PMSF and peptide inhibitor cocktails). After rotation with Ni-NTA–coated beads (0.5-ml bed volume) for 2 h at 4°C, proteins were eluted with 500 μL of elution buffer (25 mM MOPS, pH 7.0, 250 mM KCl, 2 mM MgCl2, 400 mM imidazole, 5% [w/v] Suc, 1 mM ATP, and 5 mM β-mercaptoethanol). Elution was repeated five to seven times. Eluates were used immediately. For the in vitro MT depolymerization assay, the buffer for the elution was exchanged to 1× standard assay buffer (SAB: 25 mM MOPS, pH 7.0, 75 mM KCl, 2 mM MgCl2, and 1 mM EGTA) supplemented with 1 mM ATP to remove imidazole using a PD-25 column (GE Healthcare). Drosophila KL10a (plasmid pG0885) was purified refactoring (Moriwaki and Goshima, 2016) and was purified with the same protocel as Kinesin-13b–mGFP. Instead of the binding-release experiment, elutions of KL10a were subjected to buffer exchange to 1× MB880 with 75 mM KCl and 0.1 mM ATP using a PD-25 column (GE Healthcare). AtMID1 was purified following Oda and Fukuda (2013), with some modifications. Briefly, GST-AtMID1 expression was induced in SOLBL21 E. coli using 0.2 mM IPTG and cultured for 20 h at 18°C. Harvested cells were lysed using the Advanced Digital Sonifier D450 (Branson) in lysis buffer (10 mM HEPES, pH 7.4, 1 mM EGTA, 1 mM MgCl2, and 0.1 mM ATP) supplemented with 2 mM DTT and protease inhibitors (0.5 mM PMSF and peptide inhibitor cocktails). After rotation with glutathione Sepharose 4B beads (GE Healthcare; 0.5-ml bed volume) for 2 h at 4°C, proteins were eluted with 500 μL of elution buffer (100 mM HEPES, pH 7.4, 100 mM KCl, and 30 mM reduced glutathione). Elution was repeated five to seven times. Buffer was then exchanged using a PD-10 column (GE Healthcare). Budding yeast Kip3 was purified following Kip3 purification (Gupta et al., 2006) and Drosophila KL10a purification (Edzuka and Goshima, 2016). In brief, ScKip3-mGFP–6×His (pED273) was expressed in S211 moth cells at 27°C for 72 h. Cells were lysed with 1% (v/v) Triton X-100 in lysis buffer (60 mM MOPS, pH 7.0, 250 mM NaCl, 2 mM MgCl2, 1 mM EGTA, 20 mM imidazole, and 0.1 mM ATP) supplemented with 2 mM β-mercaptoethanol and protease inhibitors (0.5 mM PMSF and peptide inhibitor cocktails) for 30 min at 25°C. After rotation with Ni-NTA–coated beads (0.5-ml bed volume) for 2 h at 4°C, proteins were eluted with 500 μL of elution buffer (100 mM HEPES, pH 7.4, 100 mM KCl, and 30 mM reduced glutathione). Elution was repeated five to seven times. Buffer was then exchanged using a PD-10 column (GE Healthcare).
of elution buffer (25 mM MOPS, pH 7.0, 250 mM KCl, 2 mM MgCl₂, 400 mM imidazole, 5% [w/v] Suc, 1 mM ATP, and 5 mM β-mercaptoethanol). Buffer was then exchanged to 1× SAB supplemented with 1 mM ATP to remove imidazole using a PD-25 column (GE Healthcare).

In Vitro MT Depolymerization

The in vitro MT depolymerization assay in Moriwaki and Goshima (2016) and Gei et al. (2010) was followed, with some modifications. DmKLP10A, Kinesin-13b\textsubscript{motor}-mGFP, and AtMIDD1 were mixed with 30% Alexa Fluor 568–labeled GMPCPP-MT seeds immobilized on silanized coverglass in assay buffer (1× MRB80, 1 mM ATP, 50 mM Glc, 0.5 μg/μL κ-casein, and 0.1% [w/v] methylcellulose) supplemented with an oxygen scavenger system. Similarly, Kinesin-8IImotor-GFP and ScKip3 were also introduced to immobilized methylcellulose supplemented with an oxygen scavenger system. GMPCPP-MT seeds immobilized on silanized coverglass in assay buffer (50 nM Glc, 1 mM GTP, 0.8 mM EGTA). Next, 10 μL of reaction mix (1× SAB, 0.1% [w/v] methylcellulose, 50 mM Glc, 0.5 μg/μL κ-casein, 1 mM ATP, and 75 mM KCl, supplemented with oxygen scavenger system) were used at 200 nM concentrations, except AtMIDD1, which was used at 100 nM following Oda and Fukuda (2013). TIRF imaging was taken every 3 s for 10 min at 25°C.

In Vitro MT Polymerization Assay

We largely followed methods described previously (Li et al., 2012; Moriwaki and Goshima, 2016; Leong et al., 2018) for the MT polymerization assay. MT growth was initiated by flowing 20 μM porcine brain tubulin (containing 10% Alexa Fluor 647–labeled tubulin) and 0, 0.15, 0.3, 0.6, and 1.5 μM purified Kinesin-13b\textsubscript{motor}-mGFP in assay buffer (1× MRB80, 75 mM KCl, 1 mM ATP, 50 mM Glc, 1 mM GTP, 0.8 μg/μL κ-casein, and 0.1% [w/v] methylcellulose) supplemented with an oxygen scavenger system. TIRF imaging of Alexa Fluor 568–labeled GMPCPP-MT seeds and Alexa Fluor 647–labeled free tubulin was taken every 3 s for 10 min at 25°C. Kymographs were drawn for 25 trackable dynamic MTs and were analyzed for catastrophe events and rescue events. Catastrophe frequency was then defined by the number of catastrophe events per growth time (minutes), whereas rescue frequency was defined by the number of rescue events per shrink time (minutes). Growth and shrinkage rates were taken from the corresponding slopes from the same kymographs. Three independent experiments, each with a different batch of purified proteins were performed.

In Vitro MT Gliding Assay

For the gliding assay with purified Kinesin-8IImotor-GFP, methods described in Miki et al. (2015) were referenced. Briefly, 10 μL of the freshly purified recombinant protein was introduced into the flow chamber and incubated at room temperature for 2 min in the dark and then washed with 20 μL of SAB (25 mM MOPS, pH 7.0, 75 mM KCl, 2 mM MgCl₂, and 1 mM EGTA). Next, 10 μL of reaction mix (1× SAB, 0.1% [w/v] methylcellulose, 50 mM Glc, 0.5 μg/μL κ-casein, 50 mM GMPCPP-MT seeds, and 75 mM KCl, supplemented with oxygen scavenger system and varying concentrations of ATP) was introduced into the flow chamber, and it was sealed with candle wax. TIRF imaging of Alexa Fluor 647–labeled GMPCPP-MT seeds in vitro MT gliding assays with Kinesin-8IImotor-GFP supplemented with various concentrations of ATP was taken every 3 s for 10 min at 23 to 25°C. Kymographs were drawn for ~30 to 50 trackable dynamic MTs per sample, and the slopes of the kymographs were taken as gliding velocity.

Data Analysis

Phylogenetic Analysis

Protein sequences used for phylogenetic analysis are presented in Supplemental Data Set 1. After amino acid sequences were aligned with MAFFT, gapped regions were removed manually using MacClade. The phylogenetic tree was constructed using neighbor-joining methods using the MEGA software. Reliability was assessed with 1000 bootstrapping trials.

Moss Colony Growth Rate

GPH0438#30 (Kinesin-13 KO) and GPH0002#5 (control) protoplasts were made following Yamada et al. (2016), with some modifications. In brief, moss was incubated with 2% (w/v) driselase solution (in 8% [w/v] mannitol), washed thrice with 8% (w/v) mannitol, incubated overnight in protoplast liquid medium, and then plated in PRM/T medium on cellophane-lined PRM plates, cultured at 25°C under continuous light. On day 2, moss-lined cellophane was transferred to BCDAT plates. Approximately day 7 when individual colonies were larger, they were picked and inoculated on BCDAT plates and then cultured at 25°C under continuous light. On day 27 to 28, images of plates with grown colonies were taken with the in-built camera of Xperia X Performance (23 MP Type 1/2.3’ Exmor RS sensor, 24-mm equivalent lens with f/2.0 aperture). Images were converted to 8-bit, automatically thresholded, binarized, and then analyzed with image processing software FIJI. Colonies were automatically outlined with the wand tool. Data in Figure 2B are pooled from three independent experiments.

Nonapical Cell Length

Low-magnification calcifiouir-stained images of moss colonies were used to measure nonapical cell length (Figure 2E), where only caulonema cells were measured. To distinguish between caulonema and chloronema cells, protonema filaments were first judged by eye in bright-field images of the same colonies, in which chloroplast density was used as an indicator of cell state (caulonema, chloroplast sparse; chloronema, or chloroplast dense).

Branching Distance, Branch Filament Length, and Branching Angle

Low-magnification images of 8-d-old moss colonies regenerated from single protoplasts were used to analyze branching pattern parameters. Branching parameters were manually measured as shown in the cartoon in Figure 2E.

Nuclear Movement Velocity

Samples from epifluorescence imaging of moss protonema filaments undergoing mitosis were analyzed. Kymographs were drawn on the filaments, and nuclear movement velocity was obtained from the slopes of nuclear movement in these kymographs where positive and negative values were assigned to apical and basal directions, respectively.

Subapical Cell Length

Samples from epifluorescence imaging of moss protonema filaments undergoing mitosis were analyzed. Kymographs were drawn on the filaments, and the length from the middle of the spindle at anaphase to the cell tip and to the previous cell plate was measured as apical and subapical cell length, respectively (Figure 3E, cartoon).

GFP-Tubulin Intensity around the Nucleus

Spinning disc confocal fluorescence time-lapse imaging of GFP-tubulin and histoneH2B-mRFP moss taken every 1 min was used for analysis. Segmented line built-in tool in FIJI was used to mark the hemispheric circumference around the nucleus on the apical and basal side at 1 min before NEBD (Figure 4B, cartoon), and the mean pixel intensity was measured. The apical/basal GFP-intensity ratio was defined as the ratio of the mean pixel intensity of the apical hemisphere over that of the basal hemisphere.

Mitotic Duration

Mitotic images taken every 1 min with a spinning disc confocal microscope (Nikon Ti; EM charge-coupled device camera ImagEM [Hamamatsu Photonics]; CSU-X1 [Yokogawa]) were used for analysis. Mitotic duration was defined as time from NEBD to anaphase onset, whereas prometaphase is the
time from NEBD to chromosome alignment, and metaphase duration is the time from chromosome alignment to anaphase onset.

**Spindle Length**

Mitosis images taken every 1 min with a spinning disc confocal microscope (Nikon Ti; electron multiplication charge-coupled device camera ImagEM [Hamamatsu Photonics]; CSU-X1 [Yokogawa]) were used for analysis. The metaphase spindle (defined as 1 min before anaphase onset) was used to analyze spindle area. Four points (the two limits of basal and apical pole widths) were marked and their \((x, y)\) positions were obtained, where spindle length was obtained from the distance between the midpoints of the basal and apical pole widths.

**Spindle MT Flux Rate**

Mitosis images were taken every 3 s with a LSM780-DUO-NLO confocal microscope. Kinesin-13 KO (GPH0438#30) and control (GFP-tubulin/histoneH2B-mRFP) mitosis were used for analysis. Cells were bleached along the equator of the mitotic spindle shortly after NEBD once the spindle shape was formed, before anaphase entry. Next, 38-pixel width slices covering the length of the spindle were cut out and arranged using the montage tool in FIJ for easy viewing of the movement of the bleached region. Their slopes were then taken as the MT flux rate.

**Protonema Filament Bend Frequency**

Epifluorescence images of moss protonema filaments cultured on BCDAT media were used for analysis in FIJ. Contrast was adjusted to make edges of protonema filaments clearer. A blind test was performed to ascertain the waviness threshold; acute angles of above 18° were determined to be wavy. Randomly chosen protonema filaments were analyzed for all the angles of bends along a length of protonema filament using the FIJ in-built angle tool. The number of bends that were wavy (18° < angle < 90°) were counted and taken over the length measured as bend frequency.

**MT Foci Trajectories**

The MT foci of *Kinesin-13* KO, *Kinesin-8* KO, and control moss expressing GFP-tubulin were imaged at × 60 magnification with a z-series every 0.3 μm for a range of 20 μm every 3 min for 3 h. The maximum z-projection of the files was analyzed using a FIJ MOSAIC plug-in (Sbalzarin and Koumoutsakos, 2005) particle tracker 2D/3D (radius, 20; cutoff, 0.001; per/abs, 0.005; link range, 5; displacement, 20; dynamics, Brownian) to automatically generate the MT foci trajectories. The linear regression of the trajectories was rotated to horizontal and normalized to start at \((x, y) = (0, 0)\) to plot the graphs in Figure 5D.

**Interphase Endoplasmic MT Plus End Dynamics**

For plus end shrinkage rate, catastrophe frequency, and rescue frequency, oblique illumination time-lapse images taken every 3 s of the interphase endoplasmic MT network of *Kinesin-13* KO (GPH0438#30) and control (GFP-tubulin/histoneH2B-mRFP) were analyzed. For MT shrinkage rate, kymographs were drawn on five randomly chosen MTs per cell (cell \(n = 25\); total MT \(n = 125\)), and the slope was taken as shrinkage rate. To analyze catastrophe and rescue frequencies, an \(\sim 5 \times 6\) μm area of a cell was randomly selected whereupon every traceable MT plus end was traced for a kymograph to count the number of catastrophe or rescue events over the observed growth or shrink time, respectively. For ease of z-averaging, areas with fewer MTs, but that were not near the cell plate, were preferred. Two independent experiments were performed and consistent results were obtained. To determine the MT growth rate, *Kinesin-13* KO moss expressing EB1-Citrine was used, where Citrine marks growing MT plus ends. Spinning disc time-lapse images taken every 3 s of the interphase endoplasmic MT array in *Kinesin-13* KO (GPH0577 no. 2, 11) and control (EB1-Citrine/mCherry-tubulin, GPH0379 no. 2) moss were analyzed. Kymographs were taken along the edge of the apical side of the tip cell of protonema filaments. Slopes of EB1-Citrine comets in kymographs were measured and taken as the growth rate.

**Statistics**

Welch’s two-sample \(t\) test (two sided) was used when samples were approximately normally distributed. Tukey multiple comparison test was used to analyze the data presented in Figure 5B. Results from statistic tests are in Supplemental Data Set 3. Significance with the following \(P\)-values are indicated as follows: *, \(P < 0.05\); **, \(P < 0.01\); ***, \(P < 0.001\); and ****, \(P < 0.0001\).

**Accession Numbers**

Sequence data from this article can be found in the Joint Genome Institute, U.S. Department of Energy/Phytozome data library under the following accession numbers: *Kinesin-13* a (Pp3c1_27370); *Kinesin-13* b (Pp3c14_9830); *Kinesin-13c* (Pp3c10_106980); *Kinesin-8* a (Pp3c1_32120); *Kinesin-8* b (Pp3c2_3070); *Kinesin-8* (Pp3c21_9290).

**Supplemental Data**

**Supplemental Figure 1.** PCR confirmation of *Kinesin-13* and *Kinesin-8* KO lines.

**Supplemental Figure 2.** Localization of *Kinesin-13* paralogues during mitosis and interphase.

**Supplemental Figure 3.** Protonema filaments of *Kinesin-13* single, double, and triple KO moss, *Kinesin-13b* full-length rescue moss, and *Kinesin-13b* mutant moss.

**Supplemental Figure 4.** MT foci and cell tip directional changes during bending of protonema filament.

**Supplemental Figure 5.** Coomassie blue staining of purified proteins used in in vitro assays.

**Supplemental Data Set 1.** Alignments used to generate the phylogenies presented in Figure 1B.

**Supplemental Data Set 2.** Materials used in this study.

**Supplemental Data Set 3.** Welch’s two-sample \(t\) test and Tukey multiple comparison test results.

**Supplemental Movie 1.** Mitosis of control, *Kinesin-13*, and *Kinesin-8* KO moss.

**Supplemental Movie 2.** Spindle poleward flux of control and *Kinesin-13* KO moss.

**Supplemental Movie 3.** Protonema filament growth of control, *Kinesin-13* and *Kinesin-8* KO moss.

**Supplemental Movie 4.** MT foci in protonema growth of control, *Kinesin-13* and *Kinesin-8* KO moss.

**Supplemental Movie 5.** Localization of *Kinesin-13* during interphase.

**Supplemental Movie Legends.** Legends for Supplemental Movies 1 to 5.

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AUTHOR CONTRIBUTIONS

M.Y. and G.G. conceived the project; S.Y.L. and M.Y. designed and performed experiments; S.Y.L., T.E., and M.Y. analyzed experimental data; T.E. performed computer simulation; S.Y.L., G.G., and M.Y. wrote the article.

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