

1 **Novel roles of Kinesin-13 and Kinesin-8 during cell growth and division in the**  
2 **moss *Physcomitrella patens***

3

4 Shu Yao Leong, Tomoya Edzuka, \*Gohta Goshima, and \*Moé Yamada

5

6 Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho,  
7 Chikusa-ku, Nagoya 464-8602, Japan

8

9 \*Corresponding authors: [yamada.moe@a.mbox.nagoya-u.ac.jp](mailto:yamada.moe@a.mbox.nagoya-u.ac.jp); [goshima@bio.nagoya-u.ac.jp](mailto:goshima@bio.nagoya-u.ac.jp)

10

11

12 **Short title:** Kinesin-13 and Kinesin-8 in mitosis and tip-growth

13

14 **One sentence summary:** This study uncovered the roles of Kinesin-13 and Kinesin-8 in regulating  
15 microtubule dynamics for mitotic spindle formation and straight tip cell growth in the moss  
16 *Physcomitrella patens*

17

18

19 The authors responsible for distribution of materials integral to the findings presented in this article in  
20 accordance with the policy described in the Instructions for Authors ([www.plantcell.org](http://www.plantcell.org)) are: Moé

21 Yamada ([yamada.moe@a.mbox.nagoya-u.ac.jp](mailto:yamada.moe@a.mbox.nagoya-u.ac.jp)) and Gohta Goshima ([goshima@bio.nagoya-u.ac.jp](mailto:goshima@bio.nagoya-u.ac.jp)).

22

23

## 24 **Abstract**

25

26 Kinesin-13 and -8 are well-known microtubule (MT) depolymerases that regulate MT length and  
27 chromosome movement in animal mitosis. While much is unknown about plant Kinesin-8, *Arabidopsis*  
28 and rice Kinesin-13 have been shown to depolymerise MTs *in vitro*. However, mitotic function of both  
29 kinesins has yet to be understood in plants. Here, we generated the complete null mutants in plants of  
30 *Kinesin-13* and -8 in the moss *Physcomitrella patens*. Both kinesins were found to be non-essential for  
31 viability, but the *Kinesin-13* knockout (KO) line had increased mitotic duration and reduced spindle  
32 length, whereas the *Kinesin-8* KO line did not display obvious mitotic defects. Surprisingly, spindle MT  
33 poleward flux, for which Kinesin-13 is responsible for in animals, was retained in the absence of  
34 Kinesin-13. Concurrently, MT depolymerase activity of either moss kinesins could not be observed,  
35 with MT catastrophe inducing (Kinesin-13) or MT gliding (Kinesin-8) activity observed *in vitro*.  
36 Interestingly, both KO lines showed waviness in their protonema filaments, which correlated with  
37 positional instability of the MT foci in their tip cells. Taken together, the results suggest that plant  
38 Kinesin-13 and -8 have diverged in both mitotic function and molecular activity, acquiring new roles in  
39 regulating MT foci positioning for directed tip-growth.

40

41 **Key words:** *Physcomitrella patens*, mitosis, microtubule foci, microtubule dynamics, tip-growth,  
42 Kinesin-13, Kinesin-8

43 **Running title:** Kinesin-13 and Kinesin-8 in mitosis and protonema tip-growth

44

45

## 46 **Introduction**

47

48 Microtubule (MT)-based motor proteins, Kinesins, form a large superfamily in animal and plant species  
49 (61 genes in *Arabidopsis thaliana*, 78 in *Physcomitrella patens*, 45 in *Homo sapiens*, and 25 in  
50 *Drosophila melanogaster*) (Reddy and Day, 2001; Miki et al., 2005; Shen et al., 2012). Kinesins show  
51 various activities in association with MTs and play pivotal roles in eukaryotic cells, such as cargo  
52 transport, MT organisation, MT dynamics regulation, and force generation (Walczak and Heald, 2008;  
53 Hirokawa et al., 2009). Comprehensive functional analysis in several animal model systems, such as fly  
54 and human cell lines, frog egg extracts, and mouse, together with biochemical characterisation of each  
55 kinesin motor have provided insights into how MT-based intracellular processes are driven and  
56 regulated during cell proliferation, differentiation, and animal development. In contrast, cellular and  
57 developmental function of plant kinesins are less clear, partly due to the difficulty in making and  
58 characterising the phenotypes of complete knockout (KO) lines of paralogous kinesins that likely

59 function redundantly. The use of high-resolution live microscopy, which was particularly critical for  
60 assessing kinesin functions during mitosis in animals, has also been limited in plants.

61 Within the kinesin superfamily, Kinesin-13 and Kinesin-8 commonly show a unique activity *in*  
62 *vitro*: MT depolymerisation (Desai et al., 1999; Howard and Hyman, 2007; Walczak et al., 2013). *In*  
63 *vivo*, Kinesin-13 is able to depolymerise relatively stable MTs from both ends while MT catastrophe  
64 inducing activity is limited to the plus-end (Rogers et al., 2004; Mennella et al., 2005). The best-studied  
65 Kinesin-13, human KIF2C/MCAK, accumulates at MT ends either by diffusion or through recruitment  
66 by other MAPs (Lee et al., 2008). At the ends, it binds to and stabilises protofilament bends, which  
67 promotes strain on the association between protofilaments within the MT lattice (Moore et al., 2002;  
68 Ovechkina et al., 2002; Ogawa et al., 2017). In the mitotic spindle, KIF2C/MCAK is localised to the  
69 kinetochore and likely triggers depolymerisation of MT plus-ends that are erroneously attached to the  
70 kinetochore (Kline-Smith et al., 2004; Walczak et al., 2013). Another Kinesin-13 (human KIF2A, fly  
71 KLP10A) localises at the pole region and depolymerises MTs, including relatively stable kinetochore-  
72 bound MTs, from the minus-end, driving poleward movement of MTs (called spindle MT poleward  
73 flux) and chromosome segregation at anaphase (Rogers et al., 2004; Ganem et al., 2005; Walczak et al.,  
74 2013). Depletion of Kinesin-13 causes various mitotic errors, such as spindle elongation, spindle  
75 monopolarisation, erroneous kinetochore-MT attachment, and chromosome lagging at anaphase  
76 (Walczak et al., 2013).

77 While Kinesin-13 does not show motility on MTs, Kinesin-8 possesses both MT depolymerising  
78 activity and processive plus-end directed motility, and thus preferentially destabilises MT plus-ends  
79 (Howard and Hyman, 2007; Walczak et al., 2013). During mitosis, Kinesin-8 concentrates at the outer  
80 kinetochore region and prevents excessive elongation of kinetochore MTs and stabilises this  
81 kinetochore-MT attachment to promote chromosome alignment to the spindle equator (Mayr et al.,  
82 2007; Stumpff et al., 2008; Stumpff et al., 2012; Edzuka and Goshima, 2019). As a whole, Kinesin-13  
83 and -8 MT depolymerisation activity is generally required for proper MT length regulation and correct  
84 chromosome movement during mitosis of various animal cell types (Walczak et al., 2013). This mitotic  
85 activity extends to cytokinesis, where Kinesin-13 and -8 also control anaphase spindle length and  
86 bundling, respectively (Gatt et al., 2005; Uehara et al., 2013). Kinesin-13 and -8 are also repurposed for  
87 interphase where mouse KIF2A (Kinesin-13) suppresses excessive axonal outgrowths (Homma et al.,  
88 2003), and KIF24 (Kinesin-13) and KIF19 (Kinesin-8) have roles in regulating primary cilia formation  
89 and cilia length, respectively (Kobayashi et al., 2011; Niwa et al., 2012).

90 Despite protein conservation, the function and activity of Kinesin-13 and -8 are not fully  
91 understood in plants. Neither mutant phenotypes nor biochemical activity have been reported for  
92 Kinesin-8. On the other hand, rice and *Arabidopsis* Kinesin-13s have been shown to preserve some  
93 degree of MT depolymerisation activity *in vitro* and *in vivo* (Oda and Fukuda, 2013; Deng et al., 2015).

94 In *Arabidopsis thaliana* xylem vessel elements, Kinesin-13A is essential to create MT deficient areas in  
95 the cortical MT network that is utilised as a scaffold for cellulose synthase movement. Cellulose is  
96 deposited only at areas with patterned MTs, thus creating cellulose -lacking regions in MT deficient  
97 areas, called pits, allowing for lateral transport of solutes and liquids in the plant. Knockdown of  
98 Kinesin-13A by RNAi results in loss of MT patterning and smaller secondary cell wall pit formation  
99 (Oda and Fukuda, 2013). Rice Kinesin-13A was shown to be important in regulating MT dynamicity  
100 and organisation of the cortical MT network in a variety of cell types (Deng et al., 2015). However,  
101 potency of the depolymerisation activity is uncertain, since plant Kinesin-13 lacks a domain required for  
102 the robust activity of animal Kinesin-13 (Ovechkina et al., 2002; Lu et al., 2005) (Figure 1A comparing  
103 animal and plant domains) and because overexpression of Kinesin-13A in non-xylem cells did not  
104 depolymerise MTs unless coexpressed with an additional binding partner MIDD1 (Oda and Fukuda,  
105 2013). On the other hand, Kinesin-13's function during mitosis is unknown as the *Kinesin-13A* mutants  
106 in *Arabidopsis* and rice did not show mitotic defects. Moreover, *Arabidopsis* Kinesin-13s have been  
107 suggested to be functionally redundant as complete null mutants were embryonic lethal (Fujikura et al.,  
108 2014).

109 In the present study, the moss *Physcomitrella patens*, a model basal plant system, was used to  
110 investigate Kinesin-13 and -8 function in general cellular processes, such as cell division. Using  
111 homologous recombination and CRISPR gene editing techniques, all three paralogues of Kinesin-13  
112 and -8 were knocked out, generating viable complete null mutants for each of the kinesin subfamilies.  
113 We demonstrated that Kinesin-13 has a mitotic role in plants with *Kinesin-13* triple KO line having  
114 longer prometaphase duration. However, spindle MT flux was still observed and shorter metaphase  
115 spindles than the control were formed in the KO lines. In contrast, *Kinesin-8* triple KO line did not  
116 display mitotic phenotypes. Unexpectedly, neither kinesin was shown to actively depolymerise MTs *in*  
117 *vitro*; Kinesin-13 motor domain was able to induce MT catastrophe, while gliding activity of the  
118 Kinesin-8 motor domain was confirmed. Notably, both KO lines had wavy protonema filaments, which  
119 correlated with the MT foci abnormally fluctuating at the cell tip. Taken together, functional analyses of  
120 *Kinesin-13* and *Kinesin-8* KO in moss revealed a divergence in mitotic function and molecular activity,  
121 while revealing a novel role in regulating MT positioning for directed tip-growth.

122  
123

## 124 **Results**

125

### 126 **Kinesin-13 affects protonema growth, but not gametophore morphology**

127 To investigate Kinesin-13's role in the moss *Physcomitrella patens*, all three paralogous *Kinesin-13*  
128 genes (*Kinesin-13a*, *-13b*, *-13c*) (Figure 1B) were sequentially deleted by homologous recombination  
129 mediated gene replacement in the moss lines expressing GFP-tubulin and histoneH2B-mRFP (Figure  
130 S1A, B). *Kinesin-13* single and double KO moss colonies did not have observable developmental  
131 defects. Moreover, *Kinesin-13* triple KO lines (hereafter *Kinesin-13* KO) were successfully generated,  
132 indicating that *Kinesin-13s* are not essential genes in moss. There was an overall reduction in colony  
133 size in the *Kinesin-13* KO when compared to the control (Figure 2A, 2B). However, the overall  
134 morphology of the protonema colonies, gametophore (leafy shoots encasing gametangia), and rhizoids  
135 (root-like filamentous cells differentiated from gametophore basal cells) (Cove, 2005; Menand et al.,  
136 2007; Kofuji and Hasebe, 2014) were indistinguishable from the control (Figure 2A, C), which differs  
137 from the case of rice *Kinesin-13A* mutant that shows small and round grains with shortened panicles  
138 and internodes of the whole rice plant (Kitagawa et al., 2010).

139 To further investigate the colony growth phenotype in the *Kinesin-13* KO moss, early stage moss  
140 colonies regenerated from single protoplasts cultured for 8 days were analysed for non-apical cell length  
141 and protonema filament branching pattern (Figure 2D–G). Non-apical cells, which undergo little cell  
142 expansion after cell division, were found to be shorter in the *Kinesin-13* KO moss caulonema cells  
143 (Figure 2E, F), consistent with reduced cell length in rice *Kinesin-13A* mutants (Deng et al., 2015). The  
144 branching pattern was analysed by measuring the parameters of branching distance (distances from tip of  
145 protonema filament to the first three branching sites), branch filament length, and branch angle (Figure  
146 2E). While the first branching distance (distance from tip of protonema filament to nearest branching  
147 site) increased in the *Kinesin-13* KO line, other branching pattern parameters were not observably  
148 different from that of the control (Figure 2G).

149

### 150 **Kinesin-13 facilitates spindle MT organisation and chromosome alignment, but does not drive 151 spindle MT flux**

152 The protonema tissue propagates by concerted asymmetric cell division and tip-growth in their apical  
153 stem cells (Rounds and Bezanilla, 2013). Therefore, a reduction in colony size in the *Kinesin-13* KO  
154 moss could be attributed to a defect in either or both events. To study mitosis in the *Kinesin-13* KO  
155 moss, localisation of moss Kinesin-13s to the mitotic spindle was first confirmed. As previously  
156 reported (Miki et al., 2014), moss Kinesin-13s showed spindle localisation, most enriched at the spindle  
157 equator, with the level of expression varying amongst the three paralogues; they did not show punctate  
158 signals at the spindle pole or kinetochore like animal Kinesin-13 (Supplemental Figure 2A). Next, time-

159 lapse imaging of moss protonema cells revealed that MT-dependent nuclear movement in prophase  
160 was abnormal in the *Kinesin-13* KO line. In the control, nuclear movement is minimal or mildly  
161 apically directed as cells undergo nuclear envelope breakdown (NEBD). In contrast, in the KO line, the  
162 nucleus displayed severe retrograde movement leading up into NEBD and often continued moving  
163 basally even during spindle establishment (Figure 3A, B). This retrograde nuclear movement was also  
164 observed in the *Kinesin-13ac* double KO lines to a lesser degree, but not in the single or *Kinesin-13ab*  
165 double KO lines (Figure 3B). Additionally, overexpression of Kinesin-13b(full-length)-Cerulean under  
166 the *EF1 $\alpha$*  promoter complemented the retrograde nuclear movement (Figure 3C, D). However, mutant  
167 Kinesin-13b constructs in which motor activity (Kinesin-13b<sup>RIG</sup>-Cerulean) (Dawson et al., 2007),  
168 conserved MT depolymerisation motifs (Kinesin-13b<sup>KVD/KEC</sup>-Cerulean) (Shipley et al., 2004), and a  
169 conserved MT binding domain (Kinesin-13b<sup>Loop12</sup>-Cerulean) (Soppina and Verhey, 2014) were  
170 compromised could not restore the retrograde nuclear movement (Figure 3D). Overall, these results  
171 suggest that Kinesin-13s contribute to nuclear movement redundantly in a motor-dependent manner.

172 The severe retrograde nuclear/spindle movement during prophase likely resulted in cross cell wall  
173 positioning defects in the *Kinesin-13* KO moss. Indeed, analysis of subapical and apical cell length at  
174 anaphase onset found that subapical cell length was reduced in the *Kinesin-13* KO moss (Figure 3E, F).  
175 This correlates with reduction in non-apical cell length of early stage moss colonies, and suggests that  
176 moss Kinesin-13 has a role in cell length maintenance.

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

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

190 Unexpected from previous studies in animals and the predicted MT depolymerisation activity of  
191 Kinesin-13, the metaphase spindle was shorter, rather than longer in the KO cells (Figure 4E). In animal  
192 cells, MT depolymerisation at the spindle pole by Kinesin-13 is important for poleward flux of spindle  
193 MTs, where tubulin is flowed from the spindle equator to the pole regions through the continuous

194 addition and removal of tubulin heterodimers at the plus- and minus-ends, respectively (Rogers et al.,  
195 2005). To investigate if Kinesin-13 depletion affects poleward MT flux in moss, GFP-tubulin at the  
196 equator of the mitotic spindle was bleached, and the movement of the photobleached strip was  
197 monitored. Surprisingly, the strip migrated towards the poles as in control cells, indicating that MT  
198 poleward flux took place in spite of complete Kinesin-13s depletion (Figure 4F, G, Movie 2). Thus,  
199 Kinesin-13 contributes to mitosis in an unconventional manner in moss.

200

### 201 **Kinesin-13 regulates straight growth of the protonema filament by controlling the position of MT** 202 **focal points**

203 To study the colony growth defect of the *Kinesin-13* KO line in detail, long-term time-lapse imaging of  
204 protonema filament growth was performed. Protonema filaments were found to be wavy with the  
205 protonema cell tip periodically changing growth direction in the *Kinesin-13* KO line (Figure 5A, Movie  
206 3). The *Kinesin-13* KO line was shown to be wavier with a bend frequency of  $0.024 \pm 0.002 \mu\text{m}^{-1}$   
207 (mean  $\pm$  SEM; N = 26) compared to the control ( $0.006 \pm 0.001 \mu\text{m}^{-1}$ , mean  $\pm$  SEM; N = 28) (Figure  
208 5B). Interestingly, the *Kinesin-13ac* double KO line showed a milder wavy phenotype, while the single  
209 and *Kinesin-13ab* double KO lines did not (Supplemental Figure 3A). Additionally, ectopic expression  
210 of full-length Kinesin-13b rescued the waviness phenotype (Figure 5B, Supplemental Figure 3B). Thus,  
211 Kinesin-13s are required for straight tip-growth.

212 Directionality of protonema tip-growth in moss has been stipulated to be dependent on MTs  
213 (Doonan et al., 1988). At the apex of the protonema tip cell, plus-ends of MTs converge into a focus  
214 known as the MT foci (Hiwatashi et al., 2014). This occupies about the same place as the focal point of  
215 the actin filament cloud in a mutually dependent manner (Wu and Bezanilla, 2018; Yamada and  
216 Goshima, 2018). Tip-growth defects including abnormal tip branching, retarded growth, and isotropic  
217 growth are the phenotypes observed amongst transgenic mutants for regulators of cytoskeletal  
218 dynamics where its organisation at the tip is impaired (actin related proteins, myo8, KINID kinesin,  
219 KCH kinesin (Rounds and Bezanilla, 2013; Hiwatashi et al., 2014; Wu and Bezanilla, 2018; Yamada  
220 and Goshima, 2018)). As such, it is possible that *Kinesin-13* depletion may result in defective MT  
221 organisation at the cell tip, causing abnormal wavy protonema growth. MT foci behaviour in the  
222 *Kinesin-13* KO line was investigated with spinning disc confocal microscopy where the MT foci of the  
223 *Kinesin-13* KO moss was unstable and fluctuated frequently (Figure 5C, D, Movie 4). Interestingly, in  
224 19 of 20 bending events observed, the displacement of the MT foci occurred prior to cell bending,  
225 indicating that the MT foci dictated protonema growth direction (Supplemental Figure 4). These results  
226 suggest that Kinesin-13s regulate anisotropic growth of protonema filaments by positional maintenance  
227 of the MT foci at the cell tip.

228

229 **Kinesin-13 is an interphase MT plus-end tracking protein**

230 To investigate Kinesin-13's localisation during interphase, endogenously tagged Kinesin-13-Citrine  
231 lines (Miki et al., 2014) was observed with spinning disc confocal microscopy. Consistent with the  
232 depletion data, Kinesin-13s localised to the MT foci (Figure 6A and Supplemental Figure 2B). To  
233 address if Kinesin-13 also associates with individual MTs in the endoplasm, we utilised oblique  
234 illumination fluorescence microscopy that enables observation of single MTs near the cell cortex with  
235 reduced effect of chloroplast autofluorescence (Jonsson et al., 2015; Nakaoka et al., 2015). In the  
236 interphase MT array, Kinesin-13s accumulated at the ends of growing MTs and disappeared from ends  
237 when MTs switched to the shrink phase (Figure 6B, C, Supplemental Figure 2C, Movie 5). Since MT  
238 minus-ends are stabilised and exhibit little to no dynamicity in this cell type (Leong et al., 2018), we  
239 concluded that Kinesin-13 localises to the plus-ends of growing MTs. The plus-end tracking behaviour  
240 is reminiscent of human KIF2C/MCAK and *Drosophila* KLP10A, which are recruited by EB1 protein  
241 to growing plus-ends (Mennella et al., 2005; Lee et al., 2008).

242

243 **MT shrink rate and rescue frequency increase while MT growth rate and catastrophe frequency**  
244 **reduce upon *Kinesin-13* depletion**

245 Since Kinesin-13s tracked growing MT plus-ends, the effect of *Kinesin-13* deletion on MT plus-end  
246 dynamics during interphase was analysed using time-lapse oblique illumination imaging of GFP-  
247 tubulin. MT shrink rate increased upon *Kinesin-13* depletion, from  $0.25 \pm 0.01 \mu\text{m/s}$  (mean  $\pm$  SEM; 5  
248 MTs per cell analysed, N = 25 cells) in the control to  $0.43 \pm 0.02 \mu\text{m/s}$  (mean  $\pm$  SEM; 5 MTs per cell  
249 analysed, N = 25 cells) in the KO line (Figure 6D). Catastrophe frequency reduced from  $9.3 \pm 1.2 \times 10^{-3}/\text{s}$   
250  $^3/\text{s}$  (mean  $\pm$  SEM; N = 33) in the control to  $2.2 \pm 0.5 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N = 28) in the KO line  
251 (Figure 6E), while rescue frequency increased from  $14 \pm 3 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N = 25) in the control  
252 to  $25 \pm 6 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N = 23) in the KO line (Figure 6F). To analyse MT growth rate,  
253 *Kinesin-13* KO moss expressing EB1-Citrine (Supplemental Figure 1C), a tracker of growing MT plus-  
254 ends, was imaged with oblique illumination fluorescence microscopy. MT growth rate based on EB1-  
255 Citrine comet movement reduced from  $0.147 \pm 0.013 \mu\text{m/s}$  (mean  $\pm$  SEM; 10 MTs per cell analysed, N  
256 = 5 cells) in the control to  $0.093 \pm 0.003 \mu\text{m/s}$  (mean  $\pm$  SEM; 10 MTs per cell analysed, N = 5 cells) in  
257 the KO lines (Figure 6G). The results suggest that Kinesin-13 plays a role in regulating MT dynamics in  
258 the interphase MT network.

259

260 **Altered MT dynamics parameters may underlie MT length phenotypes in *Kinesin-13* KO**

261 Depletion of MT depolymerases or catastrophe-promoting factors causes cytoplasmic MT lengthening  
262 and spindle expansion (Howard and Hyman, 2007; Goshima and Scholey, 2010). For example, in  
263 fission yeast cells lacking catastrophe-promoting factors, cytoplasmic MTs are more frequently



264 polymerised beyond the limits of the cell, resulting in MT bending and curling (West et al., 2001).  
265 However, shorter metaphase spindle formation (Figure 4E) and the observation that the MT foci often is  
266 unable to reach the apex of the cell tip in *Kinesin-13* KO lines (Supplemental Figure 5, Movie 4) appear  
267 to be contradictory to this general rule. We reasoned that a decrease in MT growth rate and increase in  
268 shrink rate might be limiting for overall MT length, despite significant reduction in catastrophe  
269 frequency. To evaluate this idea, we built a probability model fixed by the parameters of MT growth  
270 rate, shrink rate, catastrophe frequency, and rescue frequency, and ran a simulation in which 4,000 MTs  
271 exhibit dynamic instability for 4 min (Figure 6H, Table 1). With control parameters, a normal  
272 distribution was obtained where the 50% of MTs ranged from -12.4 to 23.4  $\mu\text{m}$  lengths, and the longest  
273 1000 MTs ranged between 23.4 to 59.8  $\mu\text{m}$  lengths. In contrast, with MT dynamics parameters of  
274 *Kinesin-13* KO cells, a narrower normal distribution was obtained, with 50% of MTs having lengths of  
275 11.5 to 22.6  $\mu\text{m}$  and the longest 1000 MTs ranged between 22.6 to 29.4  $\mu\text{m}$  lengths. Thus, the  
276 formation of shorter metaphase spindles and apex-displaced MT foci is a theoretically possible  
277 outcome, and actually a more likely outcome, associated with depletion of moss *Kinesin-13* that affects  
278 both MT catastrophe frequency and growth/shrink rate.

279

### 280 **Kinesin-13 motor domain induces catastrophe *in vitro***

281 To investigate the direct effect of moss Kinesin-13 on MT dynamics, recombinant Kinesin-13b<sup>motor</sup>-  
282 mGFP protein was expressed and purified from bacterial expression system (Figure 7A and  
283 Supplemental Figure 5A; full-length Kinesin-13 could not be obtained in either bacteria or insect culture  
284 cell expression system). The purified Kinesin-13b<sup>motor</sup>-mGFP protein was subjected to ‘binding-release’  
285 experiments to confirm ATP hydrolysis activity: it bound to MTs in the presence of non-hydrolysable  
286 ATP analogue (AMPPNP) and dissociated from MTs upon ATP addition (Supplemental Figure 5B).  
287 The ATPase-active protein was added to GMPCPP-stabilised MTs, but did not show active MT  
288 depolymerisation like that of animal Kinesin-13 protein (*Drosophila* KLP10A) (Figure 7B) (Rogers et  
289 al., 2004; Moriwaki and Goshima, 2016). We considered the possibility of moss Kinesin-13 requiring a  
290 binding partner like MIDD1 for *Arabidopsis* Kinesin-13 (Oda and Fukuda, 2013). BLAST search  
291 showed that the moss does not have MIDD1 homologues, and so moss Kinesin-13 was tested for MT  
292 depolymerisation activity in the presence of *Arabidopsis* MIDD1, but also did not depolymerise MTs  
293 (Figure 7B). Overall, the purified Kinesin-13b<sup>motor</sup>-mGFP construct did not exhibit MT depolymerase  
294 activity under the current experimental condition..

295 The purified Kinesin-13b<sup>motor</sup>-mGFP was also subjected to an *in vitro* MT polymerisation assay at  
296 concentrations of 0, 0.15, 0.3, 0.6, 1.5  $\mu\text{M}$ . While growth rate was somewhat reduced with higher  
297 Kinesin-13b<sup>motor</sup> concentration (Figure 7D), shrink rate and rescue frequency were not obviously  
298 affected by Kinesin-13b<sup>motor</sup>-mGFP addition (Figure 7E, F). Interestingly, in the presence of Kinesin-

299 13b<sup>motor</sup>-mGFP, catastrophe frequency was reproducibly increased (Figure 7G). This result is consistent  
300 with *in vivo* data in which MT catastrophe frequency decreased in the *Kinesin-13* KO line. In contrast,  
301 the recombinant protein did not reproduce the plus-end accumulation seen *in vivo*, indicating that  
302 truncated region and/or a separate factor may be required for plus-end recruitment.

303

### 304 **Chromosome segregation and cell division proceed normally in the absence of Kinesin-8**

305 Mitotic phenotype associated with Kinesin-13 deletion was fairly mild. We reasoned that another MT  
306 depolymerase instead might have a major role in MT depolymerisation in mitosis of protonema  
307 filaments, and thought to investigate Kinesin-8, which shows strong depolymerisation activity during  
308 mitosis of yeast (Hildebrandt and Hoyt, 2000; Unsworth et al., 2008). To study Kinesin-8 function in  
309 the moss, all three paralogous genes phylogenetically classed into the moss Kinesin-8 subfamily  
310 (*Kinesin-8Ia*, *-8Ib*, *-8II*) (Shen et al., 2012; Miki et al., 2014) (Figure 1B) were knocked out  
311 (Supplemental Figure 1D). Moss colonies, gametophores, and rhizoids were normal in the *Kinesin-8*  
312 KO line (Figure 2A–C). High-resolution mitosis imaging did not show any defect in prophase MT  
313 organisation, spindle formation, chromosome alignment, anaphase chromosome segregation, and  
314 cytokinesis ( $9.1 \pm 0.2$  min from NEBD to anaphase onset; N = 10) (Figure 4A, D, E). We concluded  
315 that Kinesin-8s are dispensable for mitotic cell division in moss protonema filaments.

316

### 317 **Kinesin-8 controls positioning of the MT foci for straight tip growth**

318 Interestingly, the *Kinesin-8* KO line also had wavy protonema filaments with bends occurring at smaller  
319 magnitudes with a bend frequency of  $0.022 \pm 0.005 \mu\text{m}^{-1}$  (mean  $\pm$  SEM; N = 8) (Figure 5A, B, Movie  
320 3). Tracking of the MT foci at tip cells showed that it fluctuates more frequently in the *Kinesin-8* KO  
321 line than in the control or *Kinesin-13* KO (Figure 5C, D, Movie 4), consistent with its smaller  
322 magnitudes of bends.

323

### 324 **Kinesin-8II<sup>motor</sup> glides MT but does not show a MT depolymerisation activity *in vitro***

325 To analyse intrinsic activity of moss Kinesin-8, the recombinant Kinesin-8II<sup>motor</sup>-GFP protein was  
326 expressed and purified from bacterial expression system (Figure 8A, Supplemental Figure 5C) and was  
327 subjected to a MT depolymerisation assay. 200 nM Kinesin-8II<sup>motor</sup>-GFP was added to GMPCPP-  
328 stabilised MTs but could not depolymerise them, while 200 nM *Saccharomyces cerevisiae* Kinesin-  
329 8/Kip3 could depolymerise the MTs (Figure 8B). Kinesin-8II<sup>motor</sup>-GFP was then tested for MT gliding  
330 activity. Protein immobilised on silanised cover glass showed ability to glide GMPCPP-stabilised MTs  
331 in an ATP-dependent manner, but also could not depolymerise those MTs (Figure 8C, D).

332

333

## 334 Discussion

335

336 The KO lines generated in this study showed some characteristic phenotypes unreported in previous  
337 plant kinesin mutants, such as wavy cell growth accompanying MT foci positional fluctuation (Kinesin-  
338 13, Kinesin-8) and prophase MT disorganisation (Kinesin-13). Intriguingly, several processes driven by  
339 these motors in many animal and yeast species were normal in their absence in moss, such as spindle  
340 MT flux and chromosome segregation. Moreover, the hallmark activity of these kinesins, MT  
341 depolymerisation, was not detected *in vitro*. Overall, this study provides a comprehensive view on the  
342 roles of Kinesin-13 and -8 in a single plant species. Furthermore, our results reinforce the emerging  
343 view that the kinesin superfamily is well conserved in plants but have diverged in their function  
344 (Gicking et al., 2018; Nebenfuhr and Dixit, 2018).

345

### 346 Are plant Kinesin-13 and -8 MT depolymerases?

347 Kinesin-13 is a well-known MT depolymerase in animals. *Arabidopsis* and rice Kinesin-13s have also  
348 been shown to depolymerise stabilised MTs (Oda and Fukuda, 2013; Deng et al., 2015). However,  
349 moss Kinesin-13 only exhibited catastrophe-inducing activity *in vitro* and could not depolymerise  
350 GMPCPP-stabilised MTs. This *in vitro* result is consistent with the reduced catastrophe frequency seen  
351 with interphase MTs in the *Kinesin-13* KO moss. Nevertheless, ‘negative’ results obtained *in vitro* is not  
352 necessarily conclusive: inappropriate expression systems or unsuitable biochemical environments could  
353 prevent full activity of the protein. In this study, a motor-only construct was used due to technical  
354 constraints. Thus, it is possible that other domain(s) on the *Kinesin-13* protein is required for MT  
355 depolymerisation activity. One such element may be the coiled coil, which in animal *Kinesin-13*  
356 dimerises the protein and increases MT depolymerisation activity (Hertzer et al., 2006). However, the  
357 coiled coil region required for dimerisation is located immediately upstream/downstream of the motor  
358 domain in animal Kinesin-13 (Maney et al., 2001), but is located further down the C-terminus in moss  
359 Kinesin-13 (Figure 1A); it is unclear if dimerisation of moss Kinesin-13 could enhance the activity in a  
360 similar manner to animal homologues. Furthermore, animal Kinesin-13 monomers are capable of  
361 depolymerising MTs *in vitro* (Maney et al., 2001; Hertzer et al., 2006). It is also worth noting that moss  
362 and also *Arabidopsis* Kinesin-13s lack the ‘neck’ domain that is important for strong MT  
363 depolymerisation activity in animals (Ovechkina et al., 2002); based on this feature, it was indeed  
364 originally speculated that plant Kinesin-13 might not have MT depolymerising activity (Lu et al., 2005).  
365 Thus, although it is not ruled out that moss Kinesin-13 has a MT depolymerising activity, possibly with  
366 the aid of a specific binding partner, it is enticing to say that it has diverged structurally and functionally  
367 from animal Kinesin-13.

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

378 Similar to Kinesin-13, we could not observe MT depolymerisation of the Kinesin-8 motor in our  
379 assay, which differs from human and yeast Kinesin-8. This might be due to our use of truncated  
380 construct (~440 a.a.) as we failed to purify the longer construct (~640 a.a.). However, we recently found  
381 that *Drosophila* Kinesin-8 (full-length) shows plus-end directed motility and induces MT catastrophe at  
382 the plus end, but is not able to depolymerise stable MTs *in vitro* (Edzuka and Goshima, 2019). Similar  
383 activities might be endowed to moss Kinesin-8.

384

### 385 **Kinesin-13 and -8 for mitosis**

386 We could not detect any phenotypes in *Kinesin-8* KO lines during mitotic cell division, such as  
387 chromosome alignment and mitotic delay, which are common phenotypes observed in yeast and animal  
388 cells, suggesting that Kinesin-8 has lost mitotic functions in moss. In contrast, some but not all known  
389 mitotic functions of Kinesin-13 (Walczak et al., 2013) were observed in moss. In animal mitosis,  
390 centrosomal MTs (astral MTs) are overly developed during prophase in the absence of Kinesin-13  
391 (Goshima and Vale, 2003; Rogers et al., 2004). Similarly, disorganised MTs were observed around the  
392 nucleus, despite the loss of centrosomes in moss (and all other land plants). Nucleus surrounding MTs  
393 may act as MTOCs equivalent to animal centrosomes. During prometaphase, kinetochore-MT  
394 attachment appears to be less efficient, since prometaphase duration was slightly prolonged; whether  
395 this was due to overall MT dynamics change or the lack of error correction, like the case of  
396 KIF2C/MCAK depletion in animal cells, remains elusive. Spindle monopolarisation that was observed  
397 in centrosome-containing animal cells (Goshima and Vale, 2003) was not detected. At metaphase,  
398 Kinesin-13 in animal cells acts as a MT depolymerase at the pole, driving MT poleward flux and  
399 halting spindle extension. Surprisingly, we could not obtain data that moss Kinesin-13 plays such a role:  
400 Kinesin-13 does not localised at the spindle pole, MT flux was detected in the *Kinesin-13* KO line, and  
401 the spindle was shorter, rather than longer, in the complete absence of Kinesin-13. MT dynamics is a  
402 major contributor to spindle length regulation in animal somatic cells (Goshima and Scholey, 2010);

403 therefore, shortening might be due to the reduced MT growth rate observed in the endoplasm,  
404 consistent with Kinesin-13 localising at spindle equator where MT plus-ends are enriched.  
405 Chromosome segregation during anaphase A was normal, further supporting the notion that Kinesin-13  
406 does not act as a MT depolymerase at the pole. These data indicate that the moss mitotic spindle  
407 possesses a mechanism to drive spindle MT poleward flux independent of Kinesin-13.

#### 408 409 **Kinesin-13 and -8 for tip growth**

410 The most prominent phenotype observed both in the *Kinesin-13* and *-8* KO lines was the tip-growth  
411 defect. Recent studies suggest the importance of the MT converging centre, the MT foci, in protonema  
412 tip-growth in moss, where F-actin, which is absolutely essential for tip-growth, is concentrated near the  
413 MT foci. In several mutants of MT-associated motors in which tip grows more slowly, the MT foci is  
414 not persistently formed (Hiwatashi et al., 2014; Wu and Bezanilla, 2018; Yamada and Goshima, 2018).  
415 These transient MT foci produced bursts of MT concentration at random locations along the tip region  
416 of the apical cell, causing the bending of the protonema filament at abrupt angles (Hiwatashi et al.,  
417 2014). Such phenotypes are similar to tip-growth defects seen when moss is treated with MT disruptive  
418 drugs (Doonan et al., 1988). Different from those mutants, we persistently observed a single MT focus  
419 in the *Kinesin-13* or *-8* KO. However, their positions were unstable, exhibiting the waviness of rather  
420 regular amplitude and frequencies. This suggests that Kinesin-13 and -8 play a role in MT foci  
421 positional guidance, rather than MT foci formation/maintenance, which ensures straight growth of the  
422 protonema filament. While straight tip-growth with limited MT foci fluctuations would allow fastest  
423 propagation of moss, wavy growth would be an advantageous mechanism to facilitate innovative  
424 exploration of the environment. Our study highlights the regulation of MT plus-end dynamics by MAPs  
425 as an intracellular mechanism to modulate cell growth in response to environmental cues, reminiscent  
426 of axon guidance in neurons (Sabry et al., 1991; Tanaka et al., 1995; Menon and Gupton, 2016).

#### 427 428 **Materials & Methods**

##### 429 430 **Molecular cloning and gene targeting experiments**

431 All transgenic moss lines, plasmids, and primers used in this study are listed in Table S1, S2 and S3  
432 respectively; all lines originated from the *Physcomitrella patens* Gransden 2004 strain. Moss culture,  
433 transformation, and transgenic line selection were performed as previously described (Yamada et al.,  
434 2016). In brief, moss cells were cultured on BCDAT or BCD media under 24 h light illumination, and  
435 transformation was performed by the standard PEG-mediated method.

436

437 KO moss generation *Kinesin-13* KO (GPH0438) and *Kinesin-8* KO (GPH0433) were generated in the  
438 moss strain expressing GFP-tubulin and histone-H2B-mRFP (Nakaoka et al., 2012) sequentially  
439 replacing the targeted genes with antibiotic resistance using homologous recombination (HR). To do  
440 this, 1 kb of genomic DNA sequences upstream/downstream of start/stop codons of the target genes  
441 were cloned around an antibiotic resistance cassette, and then transformed into the moss. To knock out  
442 *Kinesin-13* genes in the moss expressing EB1-Citrine and mCherry-tubulin (Kosetsu et al., 2013),  
443 *Kinesin-13b* was first removed by antibiotic resistance mediated HR as described before. For *Kinesin-*  
444 *13a* and *-13c*, CRISPR mediated gene removal was utilised. 20 bp guide RNAs (gRNAs) targeting the  
445 start and end of the gene were designed using CRISPOR (<http://crispor.tefor.net/>) (Figure S1A).  
446 gRNAs were then integrated into a BsaI site of a vector carrying U6 promoter and RNA scaffold  
447 (pCasGuide/pUC18) (Lopez-Obando et al., 2016; Collonnier et al., 2017), then the CRISPR cassettes  
448 were cloned into a vector carrying nourseothricin resistance (pSY034) with InFusion to assemble  
449 multiple gRNA cassettes into a plasmid (pSY062) following methods previously described (Leong et  
450 al., 2018). Equal amounts of this circular multicassette plasmid and *Streptococcus pyogenes* Cas9  
451 expression vector (pGenius, (Collonnier et al., 2017)) were transformed into the *Kinesin-13b* KO/EB1-  
452 Citrine/mCherry-tubulin background. Confirmation of *Kinesin-13* and *Kinesin-8* KO lines (Figure  
453 S1B–D) were carried out by PCR as previously described (Yamada et al., 2016; Leong et al., 2018).

454  
455 Endogenous C-terminal Citrine tagging C-terminal endogenous *Kinesin-13* and *-8* Citrine tagging lines  
456 from (Miki et al., 2014) were used. In these lines, *Kinesin-13* and *-8* were tagged endogenously with  
457 Citrine at the C-terminal via HR where 1 kb C-terminal sequence and the downstream sequence of stop  
458 codon of the target gene flanking an antibiotic resistance cassette was used as the HR template.  
459 Confirmation of this line was by PCR using primers designed to target the C-terminal region and  
460 outside the 3'UTR of the target gene. To make rescue plasmids, *Kinesin-13* sequence was cloned from  
461 a cDNA library and ligated into the pENTR/D-TOPO vector. The *Kinesin-13* mutant plasmids  
462 (*Kinesin-13b*<sup>RIG</sup>-Cerulean, *Kinesin-13b*<sup>KVD/KEC</sup>-Cerulean, *Kinesin-13b*<sup>Loop12</sup>-Cerulean) were generated  
463 by mutagenesis with the full-length *Kinesin-13* plasmid and primers listed in Table S2, S3. The cloned  
464 *Kinesin-13* sequences were ligated into pMN603 vector by a Gateway LR reaction, which contains  
465 EF1 $\alpha$  promoter, *Cerulean* gene, nourseothricin-resistance cassette, *PTAI* sequences designated for  
466 homologous recombination mediated integration (Miki et al., 2016; Yoshida et al., 2019).

## 467 468 **Imaging sample preparation**

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

472

473 Microdevice sample Samples used for oblique illumination fluorescence microscopy were prepared in  
474 BCD liquid medium in 15  $\mu\text{m}$  height PDMS microfluidic chambers mostly following previously  
475 described methods (Leong et al., 2018; Kozgunova and Goshima, 2019). Briefly, protonema cells were  
476 homogenised in BCD liquid media, filtered through a sheet of 50  $\mu\text{m}$  nylon-mesh, and injected into  
477 microfluidic chambers attached unto 24 m x 32 mm micro cover glass (Matsunami, No. 1-S) and  
478 cultured for 4 to 6 days.

479

480 Calcofluor stained sample 8-days-old moss colonies regenerated from single protoplasts were mounted  
481 35 mm glass bottomed dishes with BCD agar medium. The moss colonies were stained with 100  $\mu\text{L}$  of  
482 0.1 mg/mL calcofluor solution diluted with water and covered with a cover glass. The samples were  
483 then imaged with Nikon Eclipse TE2000-E inverted microscope

484

#### 485 **Moss colony assay**

486 Protoplast regeneration assay was performed following the method described in (Vidali et al., 2007)  
487 and (Yamada et al., 2016) with some modifications. In brief, sonicated moss on cellophane-lined  
488 BCDAT plate was digested by 8% mannitol solution supplemented with 2% driselase. Generated  
489 protoplasts were washed three times with 8% mannitol solution, and  $1.5 \times 10^5 \text{ mL}^{-1}$  cells were  
490 resuspended with 7ml of protoplast regeneration liquid. After overnight incubation under the dark  
491 condition, the protoplasts were centrifuged at 510 g for 2 min and resuspended in 4 ml PRM solution in  
492 which  $\text{CaCl}_2$  was added after autoclave. 0.5–1 ml out of 4 ml protoplast solution was spread on  
493 cellophane-lined PRM plate. Then, protoplasts were cultured for 4 d and transferred to BCDAT plate,  
494 followed by 4 d culture. The 8-days-old moss colonies were observed with a stereomicroscope.

495

#### 496 **Live *in vivo* imaging**

497 Spinning disc confocal microscopy using 100x 1.45-NA lens and ImagEM camera (Hamamatsu  
498 Photonics) was performed with Nikon Ti inverted microscope as previously described (Yamada et al.,  
499 2016). Z-series were taken using Nano-Z Series nanopositioner combined with a Nano-Drive controller  
500 (Mad City Labs), where z-stack imaging was performed at 0.3  $\mu\text{m}$ . Oblique illumination fluorescence  
501 microscopy was carried out with a Nikon Ti microscope with a TIRF unit, a 100x 1.49-NA lens,  
502 GEMINI split view (Hamamatsu Photonics), and EMCCD camera Evolve (Roper). Microscopes were  
503 controlled by Micromanager or NIS-Elements (Nikon). Low magnification epifluorescence imaging  
504 was carried out using Nikon Eclipse TE2000-E inverted microscope with 10x/0.3 LN1C Plain Fluo  
505 lens and Zyla 5.5 sCMOS camera (Andor), controlled with IQ3 (Andor). Photobleaching experiments  
506 were performed using an LSM780-DUO-NLO confocal microscopy system (Zeiss) using Plan-

507 Apochromat 63x/1.40 Oil DIC lens controlled using Zen (Zeiss) with 489 nm diode laser (time-lapse  
508 imaging taken at 2 mW and bleaching at 100 mW). Moss expressing GFP-tubulin (GPH0438#30 for  
509 *Kinesin-13* KO and *GFP-tubulin/histoneH2B-mRFP* for control) were used for photobleaching  
510 experiments where images were acquired every 3 s. All imaging was performed at room temperature in  
511 the dark. Moss colonies and gametophores were imaged with SMZ800N (Nikon) and ILCE-QX1  
512 camera (SONY). The stereomicroscope system was controlled with PlayMemories software (SONY).  
513

#### 514 **Computer simulations**

515 Simulations were built in R (version 3.6.0)  
516 ([https://github.com/TomoyaEdzuka/MT\\_dynamics\\_simulation](https://github.com/TomoyaEdzuka/MT_dynamics_simulation)). Parameters used in this simulation for  
517 each condition (control or *Kinesin-13* KO) are listed in Table 1. In this simulation, catastrophe and  
518 rescue frequency parameters were used to determine the probability of individual steps (1 s) undergoing  
519 a transition change or to continue a growth/shrink phase. At each phase transition (i.e.  
520 catastrophe/rescue event) new growth/shrink rates were assigned following a log normal distribution of  
521 the growth and shrink parameters. MT lengths were then simulated for 4,000 trials (i.e. 4,000 MTs) for  
522 240 steps (i.e. 4 min).  
523

#### 524 **Protein purification**

525 The motor domain of Kinesin-13b, which is the most highly expressed protein of the three paralogous  
526 *Kinesin-13* genes, was cloned from moss cDNA and transgenically linked to monomeric GFP (mGFP)  
527 and 6xHis at the C-terminus (plasmid pGG885, Table S2). Kinesin-13b<sup>motor</sup>-mGFP expression was  
528 induced in SOLBL21 *E. coli* with 0.1 mM IPTG for 20 h at 18 °C. Harvested cells were lysed using the  
529 Advanced Digital Sonifier D450 (Branson) in lysis buffer (50 mM Tris-HCl [pH 8.0], 100 mM KCl, 2  
530 mM MgCl<sub>2</sub>, 20 mM imidazole, 0.1 mM ATP) supplemented with 10 mM β-mercaptoethanol and  
531 protease inhibitors (1 mM PMSF and peptide inhibitor cocktail: 5 mg/mL Aprotinin, 5 mg/mL  
532 Chymostatin, 5 mg/mL Leupeptin, 5 mg/mL Pepstatin A). After rotation with nickel-NTA coated beads  
533 for 2 h at 4 °C, the proteins were eluted using 500 μL elution buffer (50 mM Tris-HCl [pH 8.0], 100  
534 mM KCl, 2 mM MgCl<sub>2</sub>, 300 mM Imidazole, 0.1 mM ATP). Elution was repeated 5 to 7 times. The  
535 eluates were then further purified through an AMPPNP-ATP ‘binding-release’ experiment. Eluates  
536 were first bound with 1 mM AMPPNP to 76.5 μM of 1 mM GMPCPP-stabilised MTs, and sedimented  
537 through an 80% glycerol cushion. Finally, the proteins were released from the pellet with 10 mM ATP.  
538 The motor domain and nearby coiled-coil domain (residues 200-639) of Kinesin-8II was cloned from  
539 moss cDNA and transgenically joined to GFP and 6xHis tag at the C-terminus (pTM266, Table S2),  
540 and introduced into a pET-23 *E. coli* expression vector. The recombinant protein was purified from  
541 SOLBL21 *E. coli* induced with 0.2 mM IPTG for 20 h at 18 °C. Harvested cells were lysed using the



542 Advanced Digital Sonifier D450 (Branson) in lysis buffer (25 mM MOPS [pH 7.0], 250 mM KCl, 2  
543 mM MgCl<sub>2</sub>, 5% sucrose, 30 mM imidazole, 0.1 mM ATP) supplemented with 5 mM β-  
544 mercaptoethanol and protease inhibitors (0.5 mM PMSF and peptide inhibitor cocktails). After rotation  
545 with nickel-NTA coated beads (0.5 mL bed volume) for 2 h at 4 °C, proteins were eluted with 500 μL  
546 elution buffer (25 mM MOPS [pH 7.0], 250 mM KCl, 2 mM MgCl<sub>2</sub>, 400 mM imidazole, 5% sucrose,  
547 1 mM ATP, 5 mM β-mercaptoethanol). Elution was repeated 5 to 7 times. Eluates were used  
548 immediately. For the *in vitro* MT depolymerisation assay, the buffer for the elute was exchanged to 1x  
549 Standard Assay Buffer (SAB: 25 mM MOPS [pH 7.0], 75 mM KCl, 2 mM MgCl<sub>2</sub>, 1 mM EGTA)  
550 supplemented with 1 mM ATP to remove imidazole using PD-25 column (GE Healthcare). *Drosophila*  
551 KLP10A (plasmid pGG885) was purified referencing (Moriwaki and Goshima, 2016), and was  
552 purified with the same protocol as Kinesin-13b<sup>motor</sup>-mGFP. Instead of the ‘binding-release’ experiment,  
553 elutes of KLP10A were subjected to buffer exchange to 1xMRB80 with 75 mM KCl and 0.1 mM ATP  
554 using a PD-25 column (GE Healthcare). AtMIDD1 was purified following (Oda and Fukuda, 2013),  
555 with some modifications. Briefly, GST-AtMIDD1 expression was induced in SOLBL21 *E. coli* using  
556 0.2 mM IPTG, and cultured for 20 h at 18 °C. Harvested cells were lysed using the Advanced Digital  
557 Sonifier D450 (Branson) in lysis buffer (10 mM HEPES [pH 7.4], 1 mM EGTA, 1 mM MgCl<sub>2</sub>, 150  
558 mM KCl) supplemented with 1 mM DTT and protease inhibitors (0.5 mM PMSF and peptide inhibitor  
559 cocktails). After rotation with Glutathione Sepharose 4B beads (GE Healthcare, 0.5 mL bed volume)  
560 for 2 h at 4 °C, proteins were eluted with 500 μL elution buffer (100 mM HEPES [pH 7.4], 100 mM  
561 KCl, 30 mM reduced glutathione). Elution was repeated 5 to 7 times. Buffer was then exchanged using  
562 a PD-10 column (GE Healthcare). Budding yeast Kip3 was purified following Kip3 purification (Gupta  
563 et al., 2006) and *Drosophila* KLP67A purification (Edzuka and Goshima, 2019). In brief, ScKip3-  
564 sfGFP-6xHis (pED273) was expressed in Sf21 moth cells at 27 °C for 72 h. Cells were lysed with 1%  
565 Triton X-100 in lysis buffer (50 mM MOPS [pH 7.0], 250 mM NaCl, 2 mM MgCl<sub>2</sub>, 1 mM EGTA, 20  
566 mM imidazole, 0.1 mM ATP) supplemented with 2 mM β-mercaptoethanol and protease inhibitors (0.5  
567 mM PMSF and peptide inhibitor cocktails) for 30 min at 25 °C. After rotation with nickel-NTA coated  
568 beads (0.5 mL bed volume) for 2 h at 4 °C, proteins were eluted with 500 μL elution buffer (25 mM  
569 MOPS [pH 7.0], 250 mM KCl, 2 mM MgCl<sub>2</sub>, 400 mM imidazole, 5% sucrose, 1 mM ATP, 5 mM β-  
570 mercaptoethanol). Buffer was then exchanged to 1xSAB supplemented with 1 mM ATP to remove  
571 imidazole using PD-25 column (GE Healthcare).

572

### 573 ***In vitro* MT depolymerisation**

574 The *in vitro* MT depolymerisation assay in (Moriwaki and Goshima, 2016) and (Gell et al., 2010) was  
575 followed with some modifications. DmKLP10A, Kinesin-13b<sup>motor</sup>-mGFP, AtMIDD1, and AtMIDD1  
576 and Kinesin-13b<sup>motor</sup>-mGFP were mixed with 30% Alexa Fluor-568 labelled GMPCPP-MT seeds

577 immobilised on silanised cover glass in assay buffer with (1x MRB80, 1 mM ATP, 50 mM glucose, 0.5  
578  $\mu\text{g}/\mu\text{L}$   $\kappa$ -casein, 0.1% methylcellulose) supplemented with an oxygen scavenger system. Similarly,  
579 Kinesin-8II<sup>motor</sup>-GFP and ScKip3 were also introduced to immobilised GMPCPP-MT seeds, but in a  
580 different assay buffer (1xSAB, 0.1% methylcellulose, 50 mM glucose, 0.5  $\mu\text{g}/\mu\text{L}$   $\kappa$ -casein, 1 mM ATP,  
581 75 mM KCl, supplemented with oxygen scavenger system). Proteins were used at 200 nM  
582 concentrations, except AtMIDD1 which was used at 100 nM following (Oda and Fukuda, 2013). TIRF  
583 imaging was taken every 3 s for 10 min at 25 °C.

584

### 585 ***In vitro* MT polymerisation assay**

586 We largely followed methods previously described (Li et al., 2012; Moriwaki and Goshima, 2016;  
587 Leong et al., 2018) for the MT polymerisation assay. MT growth was initiated by flowing 20  $\mu\text{M}$   
588 porcine brain tubulin (containing 10% Alexa Fluor 647-labelled tubulin) and 0, 0.15, 0.3, 0.6, and 1.5  
589  $\mu\text{M}$  purified Kinesin-13b<sup>motor</sup>-mGFP in assay buffer (1x MRB80, 75 mM KCl, 1 mM ATP, 50 mM  
590 glucose, 1 mM GTP, 0.8  $\mu\text{g}/\mu\text{L}$   $\kappa$ -casein, 0.1% methylcellulose) supplemented with an oxygen  
591 scavenger system. TIRF imaging of Alexa Fluor-568 labelled GMPCPP-MT seeds and Alexa Fluor-  
592 647 labelled free tubulin was taken every 3 s for 10 min at 25 °C. Kymographs were drawn for 25  
593 trackable dynamic MTs, and were analysed for catastrophe events and rescue events. Catastrophe  
594 frequency was then defined by the number of catastrophe events per growth time (min), whereas rescue  
595 frequency was defined by the number of rescue event per shrink time (min). Growth and shrink rates  
596 were taken from the corresponding slopes from the same kymographs. Three independent experiments,  
597 each with a different batch of purified proteins were performed.

598

### 599 ***In vitro* MT gliding assay**

600 For the gliding assay with purified Kinesin-8II<sup>motor</sup>-GFP, methods described in (Miki et al., 2015) were  
601 referenced. Briefly, 10  $\mu\text{L}$  of the freshly purified recombinant protein was introduced into the flow  
602 chamber and incubated at room temperature for 2 min in the dark, then washed with 20  $\mu\text{L}$  1x Standard  
603 Assay Buffer (SAB: 25 mM MOPS [pH 7.0], 75 mM KCl, 2 mM  $\text{MgCl}_2$ , 1 mM EGTA). Then 10  $\mu\text{L}$   
604 reaction mix (1xSAB, 0.1% methylcellulose, 50 mM glucose, 0.5  $\mu\text{g}/\mu\text{L}$   $\kappa$ -casein, 50 mM GMPCPP-  
605 MT seeds, 75 mM KCl, supplemented with oxygen scavenger system and varying concentrations of  
606 ATP) was introduced into the flow chamber, and it was sealed with candle wax. TIRF imaging of  
607 Alexa Fluor-647 labelled GMPCPP-MT seeds in *in vitro* MT gliding assays with Kinesin-8II<sup>motor</sup>-GFP  
608 at varying ATP concentrations addition was taken every 3 s for 10 min at 23–25 °C. Kymographs were  
609 drawn for 30–50 trackable dynamic MTs per sample, and the slopes of the kymographs were taken as  
610 gliding velocity.

611

612 **Data analysis**

613 Moss colony growth rate GPH0438#30 (*Kinesin-13* KO) and GPH0002#5 (control) protoplasts were  
614 made following (Yamada et al., 2016) with some modifications. In brief, moss was incubated with 2%  
615 driselase solution (in 8% mannitol), washed thrice with 8% mannitol, incubated overnight in protoplast  
616 liquid medium, and then plated in PRM/T medium on cellophane-lined PRM plates, cultured at 25 °C  
617 under continuous light. On day 2, moss-lined cellophane was transferred to BCDAT plates. Around day  
618 7 when individual colonies were larger, they were picked and inoculated on BCDAT plates, and  
619 cultured at 25 °C under continuous light. On day 27–28, images of plates with grown colonies were  
620 taken with the in-built camera of Xperia X Performance (23 MP Type 1/2.3' Exmor RS sensor, 24 mm  
621 equivalent lens with f/2.0 aperture). Images were analysed with FIJI, where images were first converted  
622 to 8-bit, automatically thresholded, and binarised. Colonies were automatically outlined with the wand  
623 tool and resulting area was obtained. Data in Figure 2B is pooled from three independent experiments.  
624

625 Non-apical cell length Low magnification calcofluor stained images of moss colonies were used  
626 to measure non-apical cell length (see Figure 2E cartoon), where only caulonema cells were  
627 measured. To distinguish between caulonema and chloronema cells, protonema filaments were  
628 first judged by sight in bright field images of the same colonies, in which chloroplast density was  
629 used as an indicator of cell state (caulonema, chloroplast sparse; chloronema, chloroplast dense).  
630

631 Branching distance, branch filament length, and branching angle Low magnification images of  
632 8-days-old moss colonies regenerated from single protoplasts were used to analyse branching  
633 pattern parameters. Branching parameters were manually measured as shown in cartoon Figure  
634 2E.  
635

636 Nuclear movement velocity Samples from epifluorescence imaging of moss protonema filaments  
637 undergoing mitosis were analysed. Kymographs were drawn on the filaments, and nuclear movement  
638 velocity was obtained from the slopes of nuclear movement in these kymographs where positive and  
639 negative values were assigned to apical and basal directions, respectively.  
640

641 Subapical cell length Samples from epifluorescence imaging of moss protonema filaments undergoing  
642 mitosis were analysed. Kymographs were drawn on the filaments, and the lengths from the middle of  
643 the spindle at anaphase to the cell tip and to the previous cell plate was measured as apical and subapical  
644 cell length, respectively (see Figure 3E cartoon).  
645

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

652

653 Mitotic duration Mitosis images taken every 1 min with spinning disc confocal microscope were used  
654 for analysis. Mitotic duration was defined as time from NEBD to anaphase onset, whereas  
655 prometaphase is the time from NEBD to chromosome alignment, and metaphase duration is the time  
656 from chromosome alignment to anaphase onset.

657

658 Spindle length Mitosis images taken every 1 min with spinning disc confocal microscope were used for  
659 analysis. The metaphase spindle (defined as 1 min before anaphase onset) was used to analyse spindle  
660 area. 4 points (the two limits of basal and apical pole widths) were marked and their (x, y) positions  
661 were obtained, where spindle length was obtained from the distance between the midpoints of the basal  
662 and apical pole widths.

663

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

670

671 Protonema filament bend frequency Epifluorescence images of moss protonema filaments cultured on  
672 BCDAT media were used for analysis in FIJI. Contrast was adjusted to make edges of protonema  
673 filaments clearer. A blind test was performed to ascertain waviness threshold, where acute angles above  
674  $18^\circ$  were determined to be 'wavy'. Randomly chosen protonema filaments were analysed for all the  
675 angles of bends along a length of protonema filament using the FIJI in-built angle tool. The number of  
676 bends that were 'wavy' ( $>18^\circ$ ) were counted, and taken over the length measured as bend frequency.

677

678 MT foci trajectories The MT foci of *Kinesin-13* KO, -8 KO, and control moss expressing GFP-tubulin  
679 were imaged at 60x magnification with z-series every 0.3  $\mu\text{m}$  for a range of 20  $\mu\text{m}$  every 3 min for 3 h.  
680 Maximum z-projection of the files were analysed using FIJI MOSAIC plug-in (Sbalzarini and

681 Koumoutsakos, 2005) particle tracker 2D/3D (radius: 20, cut-off: 0.001, per/abs: 0.005, link range: 5,  
682 displacement: 20, dynamics: Brownian) to automatically generate the MT foci trajectories. The linear  
683 regression of the trajectories was rotated to horizontal and normalised to start at  $(x, y) = (0, 0)$  in order to  
684 plot graphs in Figure 5D.

685

686 Interphase endoplasmic MT plus-end dynamics For plus-end shrink rate, catastrophe frequency, and  
687 rescue frequency, oblique illumination time-lapse images taken every 3 s of the interphase endoplasmic  
688 MT network of *Kinesin-13* KO (GPH0438#30) and control (*GFP-tubulin/histoneH2B-mRFP*) were  
689 analysed. For MT shrink rate, kymographs were drawn on five randomly chosen MTs per cell (cell N =  
690 25; total MT N = 125), and the slope was taken as shrink rate. To analyse catastrophe and rescue  
691 frequencies,  $\sim 5 \times 6 \mu\text{m}$  area in a cell was randomly selected whereupon every traceable MT plus-end  
692 was traced for a kymograph to count the number of catastrophe or rescue events over the observed  
693 growth or shrink time respectively. For ease of tracking, areas with fewer MTs but were not near the  
694 cell plate were preferred. Two independent experiments were performed and consistent. To determine  
695 MT growth rate, *Kinesin-13* KO moss expressing EB1-Citrine was used, where Citrine marks growing  
696 MT plus-ends. Spinning disc time-lapse images taken every 3 s of the interphase endoplasmic MT array  
697 in *Kinesin-13* KO (GPH0577#2, 11) and control (*EB1-Citrine/mCherry-tubulin*, GPH0379#2) moss  
698 were analysed. Kymographs were taken along the edge of the apical side of the tip cell of protonema  
699 filaments. Slopes of EB1-Citrine comets in kymographs were measured and taken as growth rate.

700

701 Statistics Welch's two-sample t-test (two-sided) was used when samples were approximately normally  
702 distributed. Tukey multiple comparison test was used for Figure 5B. Significance with the following p-  
703 values are indicated as follows: \*: < 0.05; \*\*: < 0.01; \*\*\*: < 0.001; \*\*\*\*: < 0.0001.

704

## 705 **Accession numbers**

706 *Physcomitrella patens Kinesin-13a*, Pp3c1\_27370; *Kinesin-13b*, Pp3c14\_9830; *Kinesin-13c*,  
707 Pp3c10\_106980; *Kinesin-8Ia*, Pp3c1\_32120; *Kinesin-8Ib*, Pp3c2\_3070; *Kinesin-8II*, Pp3c21\_9290.

708

## 709 **Author contributions**

710 MY and GG conceived project; SYL and MY designed and performed experiments; SYL, TE, and  
711 MY analysed experimental data; TE performed computer simulation; SYL, GG, and MY wrote paper.

712

## 713 **Acknowledgements**

714 We would like to thank Yoshihisa Oda, Momoko Nishina and Tomohiro Miki for moss lines and  
715 plasmids; Yoshikatsu Sato and Advanced Imaging Support (ABiS) platform (JP16H06280) for help of

716 photobleaching experiments; Elena Kozgunova, and Rie Inaba for technical assistance, Mitsuyasu  
717 Hasebe for reagents, and Shogo Takatani for discussions. This work was funded by JSPS KAKENHI  
718 (17H06471 to G.G. and 19K23723 to M.Y.) and by JSPS and DFG under the Joint Research Projects-  
719 LEAD with UKRI (to G.G.). The authors declare no competing interests.

720

## 721 **Figure legend**

722 Figure 1: Conservation of Kinesin-13 and Kinesin-8 in the moss *Physcomitrella patens*.

723 (A) Protein domains of Kinesin-13 (represented with Kinesin-13b) and Kinesin-8 (represented with  
724 Kinesin-8II) of moss, compared against *Drosophila melanogaster* KLP10A/Kinesin-13 and budding  
725 yeast *Saccharomyces cerevisiae* Kip3/Kinesin-8. Domains of *Drosophila* and budding yeast proteins  
726 were referenced from UniProt, whereas moss protein domains were predicted using InterPro.

727 (B, C) Kinesin-13 and -8 phylogeny across the moss *Physcomitrella patens*, the Brassicaceae  
728 *Arabidopsis thaliana*, the liverwort *Marcantia polymorpha*, the rice *Oryza sativa* subspecies *Japonica*,  
729 the fruit fly *Drosophila melanogaster*, mammals *Mus musculus* and *Homo sapiens*, and also for  
730 Kinesin-8 in budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe*.  
731 After amino acid sequences were aligned with MAFFT, gapped regions were removed manually using  
732 MacClade. The phylogenetic tree was constructed using neighbour-joining methods using the Molecular  
733 Evolutionary Genetics Analysis (MEGA) software. Reliability was assessed with 1,000 bootstrapping  
734 trials. Protein sequences are presented in supplemental dataset. Horizontal branch length is proportional  
735 to the estimated evolutionary distance. Bar, 0.1 amino acid substitution per site.

736

737 Figure 2: *Kinesin-13* and -8 KO mosses are morphologically normal, but *Kinesin-13* KO moss shows  
738 retarded growth and reduced cell length.

739 (A, B) Colony size comparison between control (*GFP-tubulin/histoneH2B-mRFP*) and *Kinesin-13* KO  
740 (GPH0438#30, left) or *Kinesin-8* KO (GPH0433#9, right) moss. Colonies were cultured from single  
741 protoplasts for 27-28 days on BCDAT where at least two independent experiments each with at least 2  
742 plates of colonies were performed. The average colony area for each line on each plate was obtained.  
743 Actual areas were then divided by the average area of the control sample to get relative colony size. In  
744 the *Kinesin-13* KO experiment, KO moss had a relative size of  $0.55 \pm 0.04$  (mean  $\pm$  SEM; N = 7)  
745 whereas control had a relative size of  $1.00 \pm 0.12$  (mean  $\pm$  SEM; N = 6). In the *Kinesin-8* KO  
746 experiment, KO moss had a relative size of  $1.01 \pm 0.05$  (mean  $\pm$  SEM; N = 8) whereas control had a  
747 relative size of  $1.00 \pm 0.08$  (mean  $\pm$  SEM; N = 8). Points represent individual colonies, results are from  
748 one of at least two independent experiment. Bar, 5 mm.

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

752 (D) Day-8 moss colonies cultured from protoplast of control (*GFP-tubulin/histoneH2B-mRFP*) and  
753 *Kinesin-13* KO (GPH0438#30) under brightfield light (top) and with calcofluor staining (bottom).  
754 Yellow dashes boxes, inset region; bars, 500  $\mu\text{m}$ ; inset bar, 50  $\mu\text{m}$ .

755 (E) Cartoon depicting the measurements taken for non-apical cell length in (F) and branching phenotype  
756 analysis in (G).

757 (F) Non-apical cell lengths of caulonema filaments were measured using calcofluor stained colonies as in  
758 (D, bottom) for control (*GFP-tubulin/histoneH2B-mRFP*) and *Kinesin-13* KO (GPH0438#30). Non-  
759 apical cell length was reduced in *Kinesin-13* KO moss to  $79.9 \pm 5.5 \mu\text{m}$  (mean  $\pm$  SEM; N = 43),  
760 compared to control moss of  $113.7 \pm 1.9 \mu\text{m}$  (mean  $\pm$  SEM; N = 132). Points represent individual cells;  
761 results are pooled from two independent experiments where two independent lines were analysed.

762 (G) Branching phenotype analysis of control (*GFP-tubulin/histoneH2B-mRFP*) and *Kinesin-13* KO  
763 (GPH0438#30). In particular, branching distance of the first branch site to cell tip (top graph, leftmost  
764 bars) was increased in *Kinesin-13* KO moss to  $338.4 \pm 12.9 \mu\text{m}$  (mean  $\pm$  SEM; N = 55), compared to  
765 control moss of  $293.1 \pm 8.8 \mu\text{m}$  (mean  $\pm$  SEM; N = 71). Points represent individual filaments; results are  
766 pooled from two independent experiments where two independent lines were analysed.

767

768 Figure 3: *Kinesin-13s* contribute to triple KO moss shows retrograde nuclear movement not seen in  
769 single KOs, but manifests in to a lesser degree in the *Kinesin-13ac* double KO line.

770 (A) Snapshots of control (*GFP-tubulin/histoneH2B-mRFP*) and *Kinesin-13* KO (GPH0438#30) moss  
771 during prophase nuclear/spindle movement. *Kinesin-13* KO moss shows retrograde nuclear/spindle  
772 movement. Apical side, right, positive value for analysis in (B) and (D); basal side, left, negative values  
773 for analysis in (B) and (D); yellow dotted line, nucleus position at NEBD; bar, 50  $\mu\text{m}$ ; NEBD, 0 min.

774 (B) Nuclear movement velocity during prophase of control (*GFP-tubulin/histoneH2B-mRFP*;  $0.68 \pm$   
775  $0.10 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 8), *Kinesin-13a* single KO (GPH0411#43;  $0.85 \pm 0.10 \mu\text{m}/\text{min}$ , mean  
776  $\pm$  SEM; N = 8), *Kinesin-13b* single KO (GPH0412#11;  $0.43 \pm 0.03 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 7),  
777 *Kinesin-13ab* double KO (GPH0419#33;  $0.62 \pm 0.04 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 11), *Kinesin-13ac*  
778 double KO (GPH0420#125;  $-0.03 \pm 0.13 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 16), and *Kinesin-13abc* triple KO  
779 (GPH0438#30;  $-0.69 \pm 0.08 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 11). *Kinesin-13abc* triple KO shows a clear  
780 retrograde nuclear movement, whereas *Kinesin-13ac* double KO shows intermediate retrograde nuclear  
781 movement. Apically directed movement, positive values; basally directed movement, negative values.

782 represent individual mitotic events. Results are from one of three independent experiments where two  
783 independent lines were analysed.

784 (C) Protein domains of Kinesin-13b and mutant proteins for rescue experiments. Point mutations on  
785 Kinesin-13b-Cerulean which was introduced under the *EF1 $\alpha$*  promoter at the *PTA1* site in the moss used  
786 for rescue experiments are illustrated.

787 (D) Nuclear movement velocity during prophase of control (*GFP-tubulin/histoneH2B-mRFP*;  $0.94 \pm$   
788  $0.10 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 17), *Kinesin-13abc* triple KO (GPH0438#30;  $-1.43 \pm 0.22 \mu\text{m}/\text{min}$ ,  
789 mean  $\pm$  SEM; N = 29), *Cerulean/Kinesin-13abc* triple KO (GPH0903#1;  $-0.99 \pm 0.25 \mu\text{m}/\text{min}$ , mean  $\pm$   
790 SEM; N = 16), *Kinesin-13b(FL)-Cerulean/Kinesin-13abc* triple KO (GPH0899#10;  $1.04 \pm 0.09$   
791  $\mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 13), *Kinesin-13b<sup>RIG</sup>-Cerulean/Kinesin-13abc* triple KO (GPH0902#2;  $-0.58$   
792  $\pm 0.20 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 17), *Kinesin-13b<sup>KVD/KEC</sup>-Cerulean/Kinesin-13abc* triple KO  
793 (GPH0900#4;  $-0.94 \pm 0.08 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 10), and *Kinesin-13b<sup>Loop2</sup>-Cerulean/Kinesin-*  
794 *13abc* triple KO (GPH0901#1;  $-1.05 \pm 0.20 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM; N = 27). Apically directed  
795 movement, positive values; basally directed movement, negative values. Points represent individual  
796 mitotic events. Results are from one of two independent experiments where at least two independent  
797 lines were analysed.

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

799 (F) Subapical cell length was reduced in the *Kinesin-13* KO line (GPH0438#30;  $70.9 \pm 3.6 \mu\text{m}$  (mean  $\pm$   
800 SEM; N = 26; p-value < 0.05)) compared to the control (*GFP-tubulin/histoneH2B-mRFP*;  $105.2 \pm 12.4$   
801  $\mu\text{m}$  (mean  $\pm$  SEM; N = 11)). Each point represents individual mitotic events. Results shown are from  
802 one of two independent experiments where two independent lines were analysed.

803

804 Figure 4: *Kinesin-13* KO moss shows defects in nuclear-proximal MT array, mitotic duration, and  
805 spindle length, but shows no difference in spindle flux rate.

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

810 (B, C) Apical:basal GFP-intensity ratio of GFP-tubulin around the nucleus 1 min before NEBD was  
811 measured as the ratio of GFP-tubulin intensities between apical and basal hemispheric circumference.  
812 Control (*GFP-tubulin/histoneH2B-mRFP*),  $1.17 \pm 0.04$  (mean  $\pm$  SEM; N=9; p-value < 0.05); *Kinesin-13*  
813 KO (GPH0438#6, 30),  $1.00 \pm 0.07$  (mean  $\pm$  SEM; N = 10). Points represent individual mitotic events.

814 (D) Mitotic duration of control (*GFP-tubulin/histoneH2B-mRFP*), *Kinesin-13* KO (GPH0438#6, 30),  
815 and *Kinesin-8* KO (GPH0433#7, 9) moss as measured from NEBD to anaphase onset. Control,  $9.8 \pm 0.3$



816 min (mean  $\pm$  SEM; N = 11); *Kinesin-13* KO,  $11.8 \pm 0.4$  min (mean  $\pm$  SEM, N = 15; p-value < 0.001).  
817 *Kinesin-8* KO,  $9.1 \pm 0.2$  min (mean  $\pm$  SEM; N = 10; p-value < 0.05). The duration of prometaphase  
818 (from NEBD to chromosome alignment) and metaphase (chromosome alignment to anaphase onset)  
819 was also measured and shown. Data shown was pooled from two independent experiments.

820 (E) Spindle length was measured at metaphase (defined as 1 min before anaphase onset) by obtaining the  
821 distance between midpoints of apical and basal spindle widths. Control (*GFP-tubulin/histoneH2B-*  
822 *mRFP*),  $13.0 \pm 0.3$   $\mu\text{m}$  (mean  $\pm$  SEM; N = 4); *Kinesin-13* KO (GPH0438#30),  $11.2 \pm 0.3$   $\mu\text{m}$  (mean  $\pm$   
823 SEM; N = 10; p-value < 0.01); *Kinesin-8* KO (GPH0433#9),  $12.3 \pm 0.5$   $\mu\text{m}$  (mean  $\pm$  SEM; N = 10).  
824 Points represent individual mitotic events.

825 (F) Spindle poleward flux of control (*GFP-tubulin/histoneH2B-mRFP*) and *Kinesin-13* KO  
826 (GPH0438#30) moss was examined in photobleaching experiments where GFP-tubulin signals on a strip  
827 along the metaphase plate was bleached. The bleached regions separating towards the poles are  
828 indicative of spindle poleward flux function. Horizontal bar, 5  $\mu\text{m}$ ; vertical bar, 12 s; yellow dashed  
829 rectangle in the top panel indicates region used to make time series (bottom panel); cyan dashed rectangle  
830 represents bleached region; red lines indicate the segmented lines drawn on the kymograph to obtain flux  
831 rate in (G).

832 (G) Quantification of spindle poleward flux experiment as shown in (F). Control,  $2.1 \pm 0.2$   $\mu\text{m}/\text{min}$   
833 (mean  $\pm$  SEM; N = 22); *Kinesin-13* KO,  $2.2 \pm 0.4$   $\mu\text{m}/\text{min}$  (mean  $\pm$  SEM; N = 19). Points represent  
834 individual mitotic events, shown are results from four independent experiments.

835

836 Figure 5: *Kinesin-13* and *-8* KO moss have wavy protonema filaments correlated with unstable MT foci  
837 positioning.

838 (A) Protonema filaments of control (*GFP-tubulin/histoneH2B-mRFP*), *Kinesin-13* KO (GPH0438#30),  
839 *Kinesin-8* KO (GPH0433#9) moss. Bar, 50  $\mu\text{m}$ .

840 (B) Waviness of protonema filaments measured as frequency of wavy bend ( $>18^\circ$ ) of protonema  
841 filaments over measured lengths. Control (*GFP-tubulin/histoneH2B-mRFP*),  $0.006 \pm 0.001$   $\mu\text{m}^{-1}$  (mean  
842  $\pm$  SEM; N = 28 filaments); *Kinesin-13* KO (GPH0438#30),  $0.024 \pm 0.002$   $\mu\text{m}^{-1}$  (mean  $\pm$  SEM; N = 26  
843 filaments; p-value < 0.0001); *Kinesin-8* KO (GPH0433#7),  $0.022 \pm 0.005$   $\mu\text{m}^{-1}$  (mean  $\pm$  SEM; N = 8  
844 filaments; p-value < 0.01); *Cerulean/Kinesin-13* KO (GPH0903#1),  $0.022 \pm 0.004$   $\mu\text{m}^{-1}$  (mean  $\pm$  SEM;  
845 N = 12); *Kinesin-13b(full-length)-Cerulean/Kinesin-13* KO (GPH0899#10),  $0.004 \pm 0.001$   $\mu\text{m}^{-1}$  (mean  
846  $\pm$  SEM; N = 8; p-value < 0.0001). Points represent individual protonema filaments, results shown are  
847 from one experiment of at least four independent experiments.

848 (C) MT foci at tip of caulonema cell of control (*GFP-tubulin/histoneH2B-mRFP*), *Kinesin-13* KO  
849 (GPH0438#30), *Kinesin-8* KO (GPH0433#9) moss. Images were acquired with z-sections at 0.3  $\mu\text{m}$

850 intervals for 20  $\mu\text{m}$  range, and maximum z-projections are displayed. Bottom panels show overlaid time  
851 series. Bar, 10  $\mu\text{m}$ ; colours in time series indicate different time points as labelled in top panels.

852 (D) MT foci positions were tracked using FIJI MOSAIC plug-in 2D/3D particle tracker (Sbalzarini and  
853 Koumoutsakos, 2005) in time-lapse imaging data as in (C). (x, y) trajectories of three representative MT  
854 foci (shown in different colours) for each line are displayed. Each point represents subsequent positions  
855 at each time point, at 3 min intervals for 3 h. Same lines as in (B) are represented.

856

857 Figure 6: *Kinesin-13* localises to the interphase MT network and depletion of *Kinesin-13* results in  
858 increased shrink rate, reduced catastrophe frequency, increased rescue frequency, and reduced growth  
859 rate.

860 (A) MT foci of *Kinesin-13c-Citrine/mCherry-tubulin* (GPH0100#15) moss. Image was acquired at 0.3  
861  $\mu\text{m}$  intervals for 10  $\mu\text{m}$  range; shown is maximum z-projection. Bar, 5  $\mu\text{m}$ .

862 (B) Interphase MT network of *Kinesin-13c-Citrine/mCherry-tubulin* (GPH0100#15) moss. Images were  
863 acquired by oblique illumination fluorescence split-view microscopy to avoid chloroplast  
864 autofluorescence. Bar, 2  $\mu\text{m}$ .

865 (C) Kymograph of MT growth taken from imaging as in (B), taken every 3 s. Vertical bar, 2 min;  
866 horizontal bar, 5  $\mu\text{m}$ .

867 (D) Interphase MT plus-end shrink rate of control (*GFP-tubulin/histoneH2B-mRFP*,  $0.245 \pm 0.012 \mu\text{m/s}$   
868 (mean  $\pm$  SEM; N = 25 cells)) and *Kinesin-13* KO (GPH0438#30,  $0.429 \pm 0.021 \mu\text{m/s}$  (mean  $\pm$  SEM; N  
869 = 25 cells; p-value < 0.0001)) moss. Points represent individual cells; results shown are from one  
870 experiment of two independent experiments.

871 (E) Interphase MT catastrophe frequency of control (*GFP-tubulin/histoneH2B-mRFP*,  $9.3 \pm 1.2 \times 10^{-3}/\text{s}$   
872 (mean  $\pm$  SEM; N = 33 cells)) and *Kinesin-13* KO (GPH0438#30,  $2.2 \pm 0.5 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N =  
873 28 cells; p-value < 0.0001)). Points represent individual cells; results shown are from two independent  
874 experiments.

875 (F) Interphase MT rescue frequency of control (*GFP-tubulin/histoneH2B-mRFP*,  $14 \pm 3 \times 10^{-3}/\text{s}$  (mean  $\pm$   
876 SEM; N = 25 cells)) and *Kinesin-13* KO (GPH0438#30,  $25 \pm 6 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N = 23 cells)).  
877 Points represent individual cells; results shown are from two independent experiments.

878 (G) Interphase MT plus-end growth rate of control (*EB1-Citrine/mCherry-tubulin*, GPH0379#2,  $0.147 \pm$   
879  $0.013 \mu\text{m/s}$  (mean  $\pm$  SEM; N = 5 cells, 50 MTs)) and *Kinesin-13* KO moss (GPH0577#11,  $0.093 \pm$   
880  $0.003 \mu\text{m/s}$  (mean  $\pm$  SEM; N = 5 cells, 50 MTs; p-value < 0.05)). Points represent individual cells.

881 (H) Simulation of MT growth of 4,000 MTs in 4 min based on a probability model established using MT  
882 dynamics parameters from *in vivo* interphase MT dynamics analyses (D–G) (refer to Materials &  
883 Methods, and Table 1). Control MT dynamics parameters yielded approximately normal distributions of

884 MT lengths and tended to have a larger population of MTs with longer lengths, with the longest 25% of  
885 MTs ranging between 23.4 to 59.8  $\mu\text{m}$  in length. For MTs under *Kinesin-13* KO conditions, the  
886 distribution of MT length was narrower, with 50% of all MTs between 11.5 to 22.6  $\mu\text{m}$  lengths, whereas  
887 the longest 25% of MTs ranged from 22.6 to 29.4  $\mu\text{m}$  in length. Histogram bin width = 0.5  $\mu\text{m}$ .

888

889 Figure 7: Recombinant Kinesin-13 does not depolymerise stabilised GMPCPP-MT seeds but shows MT  
890 catastrophe inducing activity.

891 (A) Protein domains of Kinesin-13b and recombinant Kinesin-13b<sup>motor</sup>-mGFP construct. Protein  
892 domains were determined using InterPro. His-tag for affinity purification was attached to C-terminus of  
893 the recombinant protein.

894 (B) *In vitro* MT depolymerisation assay using GMPCPP-stabilised MT seeds was performed using  
895 purified DmKLP10A, recombinant Kinesin-13b<sup>motor</sup>-mGFP construct, AtMIDD1, AtMIDD1 and  
896 Kinesin-13b<sup>motor</sup>-mGFP construct, and also under buffer only conditions. Only DmKLP10A  
897 successfully depolymerised MT seeds. The slight reduction in intensity in the bottom panels is due to  
898 photobleaching during imaging. All proteins were used at 200 nM except for AtMIDD1 which was at  
899 100 nM. Bar, 5  $\mu\text{m}$ .

900 (C) Representative kymographs of *in vitro* MT dynamics polymerisation assays with Kinesin-13b<sup>motor</sup>-  
901 mGFP construct at 0, 0.3, and 1.5  $\mu\text{M}$ . Time-lapse imaging was performed with TIRF microscopy  
902 taken every 3 s. Brightness and contrast was manually adjusted. Vertical bar, 2 min; horizontal bar; 5  
903  $\mu\text{m}$ .

904 (D-G) *In vitro* MT dynamics parameters were analysed from time-lapse imaging of *in vitro* MT  
905 dynamics polymerisation assays with Kinesin-13b<sup>motor</sup>-mGFP construct at 0, 0.15, 0.3, 0.6, and 1.5  $\mu\text{M}$   
906 taken using TIRF microscopy at every 3 s. In particular, growth rate was observed to reduce slightly,  
907 from  $9.0 \pm 0.4 \times 10^{-3} \mu\text{m/s}$  (mean  $\pm$  SEM; N = 3) in buffer only conditions, to  $7.5 \pm 0.7 \times 10^{-3} \mu\text{m/s}$  (mean  
908  $\pm$  SEM; N = 3) in 1.5  $\mu\text{M}$  protein. Catastrophe frequency was observed to reproducibly increase with  
909 high concentrations of Kinesin-13b<sup>motor</sup>-mGFP, having a catastrophe frequency of  $2.3 \pm 0.2 \times 10^{-3}/\text{s}$   
910 (mean  $\pm$  SEM; N = 3) at 1.5  $\mu\text{M}$  protein, compared to  $1.2 \pm 0.3 \times 10^{-3}/\text{s}$  in buffer only conditions. Points  
911 represent mean values from independent experiments.

912

913 Figure 8: Recombinant Kinesin-8 motor does not depolymerise MTs but shows MT gliding activity.

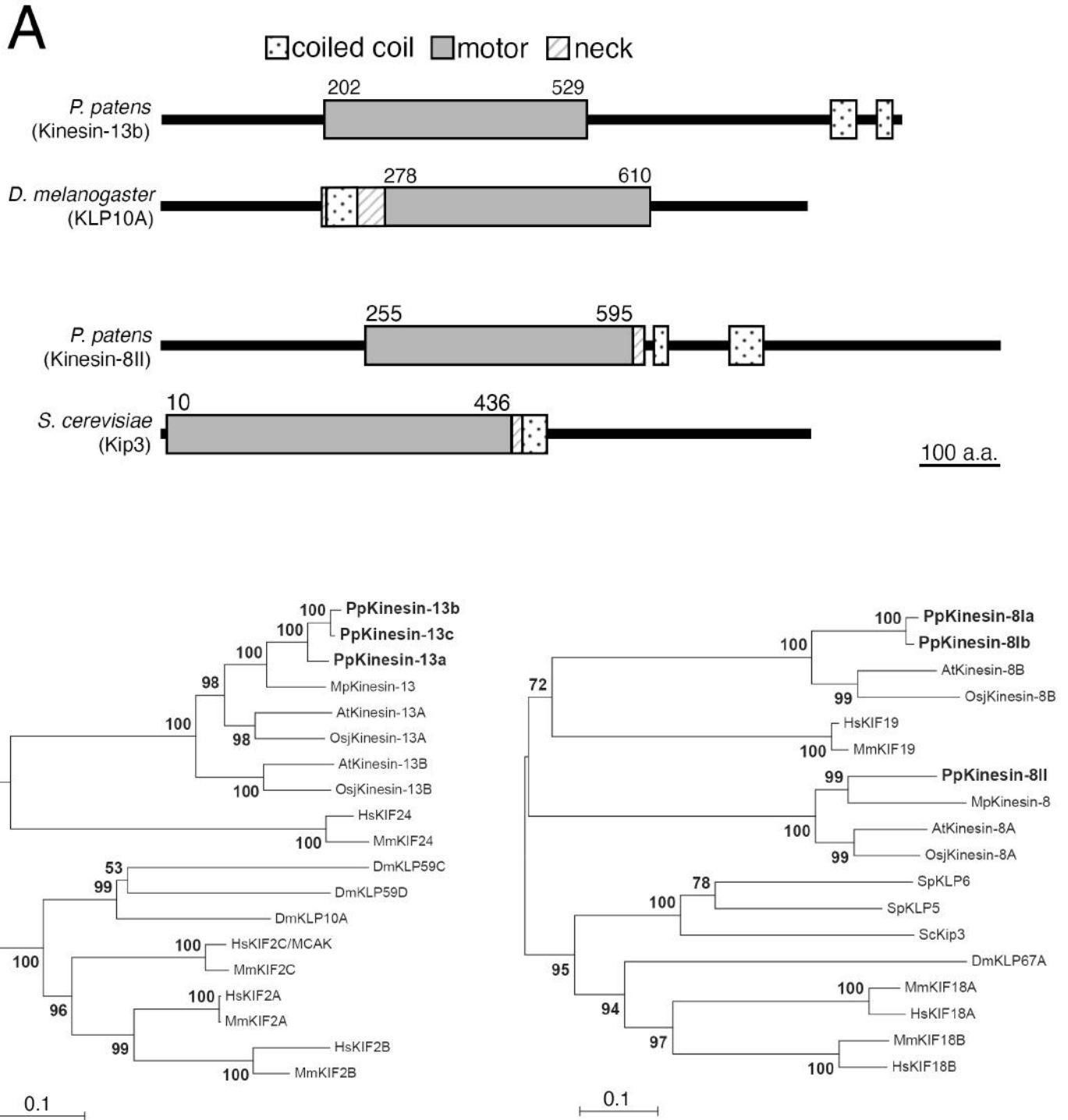
914 (A) Protein domains of Kinesin-8II and recombinant Kinesin-8II<sup>motor</sup>-GFP construct. Protein domains  
915 were identified using InterPro. His-tag for affinity purification was attached to C-terminus of the  
916 recombinant protein.

917 (B) *In vitro* MT depolymerisation assay using GMPCPP-stabilised MT seeds was performed using  
918 purified ScKip3, recombinant Kinesin-8II<sup>motor</sup>-GFP, and also under buffer only conditions. Only

919 ScKip3 showed MT depolymerisation activity. The slight reduction in intensity in bottom panels is due  
920 to photobleaching during imaging. All proteins were used at 200 nM. Bar, 10  $\mu\text{m}$ .  
921 (C) ATP-dependent MT gliding velocity of Kinesin-8II<sup>motor</sup>-GFP. 1 mM ATP,  $0.68 \pm 0.03 \mu\text{m}/\text{min}$   
922 (mean  $\pm$  SEM; N = 124 MTs); 5 mM ATP,  $1.18 \pm 0.02 \mu\text{m}/\text{min}$  (mean  $\pm$  SEM; N = 121 MTs, p-value  
923  $< 0.0001$ ).  
924 (D) *In vitro* MT gliding assay using GMPCPP-stabilised MTs on Kinesin-8II<sup>motor</sup>-GFP which was  
925 immobilised on glass, at 0, 1, and 5 mM ATP. Red dotted line in top panel indicates segmented line  
926 used to draw kymographs (bottom panels). Gliding activity of Kinesin-8II<sup>motor</sup>-GFP was verified in 3  
927 independent experiments. Vertical bar, 45 s; horizontal bar, 2  $\mu\text{m}$ .  
928

# Figure 1

bioRxiv preprint doi: <https://doi.org/10.1101/819722>; this version posted October 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

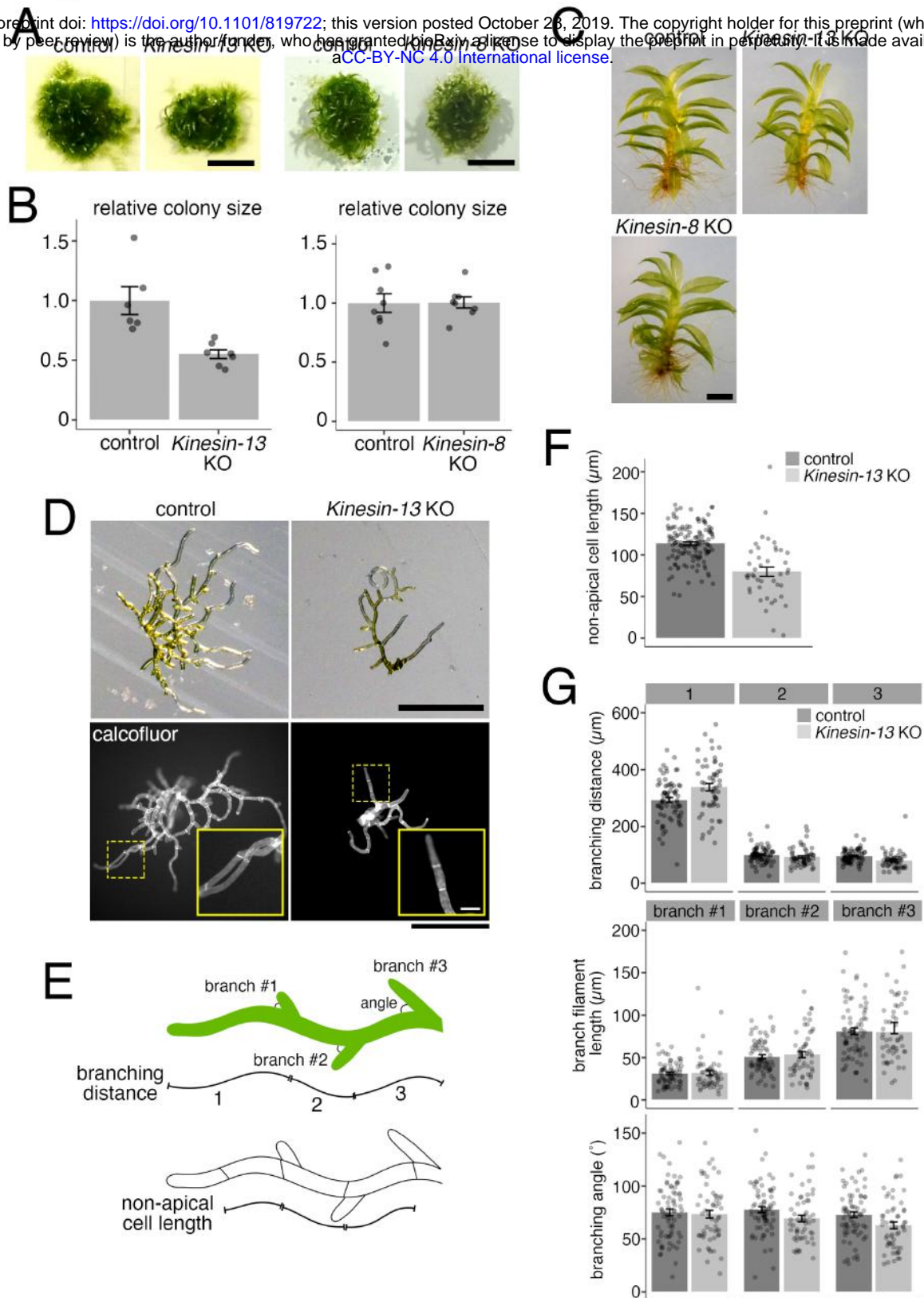


**Figure 1: Conservation of Kinesin-13 and Kinesin-8 in the moss *Physcomitrella patens*.**

**(A)** Protein domains of Kinesin-13 (represented with Kinesin-13b) and Kinesin-8 (represented with Kinesin-8II) of moss, compared against *Drosophila melanogaster* KLP10A/Kinesin-13 and budding yeast *Saccharomyces cerevisiae* Kip3/Kinesin-8. Domains of *Drosophila* and budding yeast proteins were referenced from UniProt, whereas moss protein domains were predicted using InterPro. **(B, C)** Kinesin-13 and -8 phylogeny across the moss *Physcomitrella patens*, the Brassicaceae *Arabidopsis thaliana*, the liverwort *Marcantia polymorpha*, the rice *Oryza sativa* subspecies *Japonica*, the fruit fly *Drosophila melanogaster*, mammals *Mus musculus* and *Homo sapiens*, and also for Kinesin-8 in budding yeast *Saccharomyces 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 neighbour-joining methods using the Molecular Evolutionary Genetics Analysis (MEGA) software. Reliability was assessed with 1,000 bootstrapping trials. Horizontal branch length is proportional to the estimated evolutionary distance. Protein sequences are presented in supplemental dataset. Bar, 0.1 amino acid substitution per site.

# Figure 2

bioRxiv preprint doi: <https://doi.org/10.1101/819722>; this version posted October 26, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

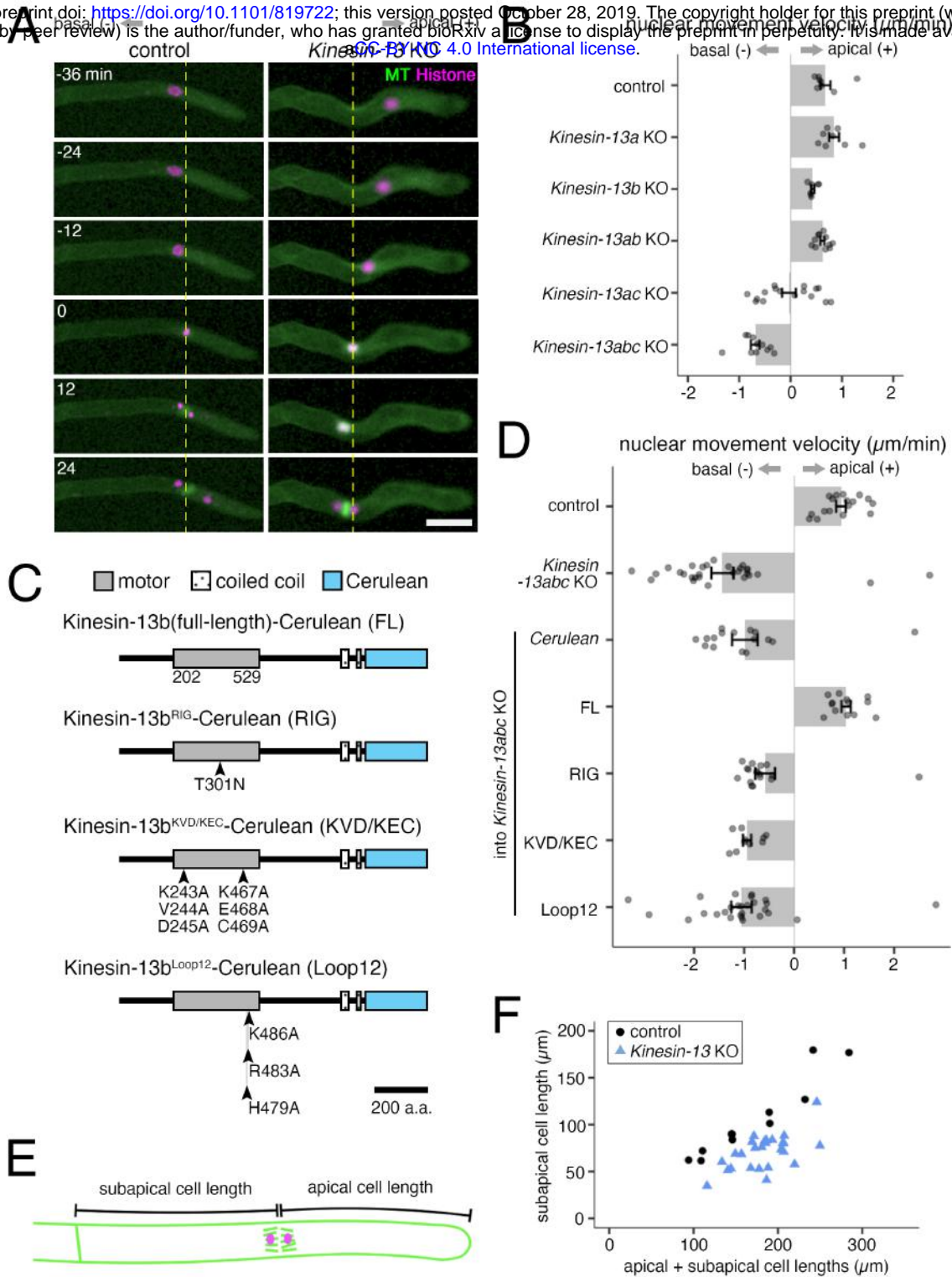


**Figure 2: *Kinesin-13* and *-8* KO mosses are morphologically normal, but *Kinesin-13* KO moss shows retarded growth and reduced cell length.**

**(A, 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 27-28 days on BCDAT where at least two independent experiments each with at least 2 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  $0.55 \pm 0.04$  (mean  $\pm$  SEM; N = 7) whereas control had a relative size of  $1.00 \pm 0.12$  (mean  $\pm$  SEM; N = 6). In the *Kinesin-8* KO experiment, KO moss had a relative size of  $1.01 \pm 0.05$  (mean  $\pm$  SEM; N = 8) whereas control had a relative size of  $1.00 \pm 0.08$  (mean  $\pm$  SEM; 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 (*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 27-28 days. Bar, 1 mm. **(D)** Day-8 moss colonies cultured from protoplast of control (*GFP-tubulin/histoneH2B-mRFP*) and *Kinesin-13* KO (GPH0438#30) under brightfield light (top) and with calcofluor staining (bottom). Yellow dashes boxes, inset region; bars, 500  $\mu\text{m}$ ; inset bar, 50  $\mu\text{m}$ . **(E)** Cartoon depicting the measurements taken for non-apical cell length in (F) and branching phenotype analysis in (G). **(F)** Non-apical 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 (GPH0438#30). Non-apical cell length was reduced in *Kinesin-13* KO moss to  $79.9 \pm 5.5$   $\mu\text{m}$  (mean  $\pm$  SEM; N = 43), compared to control moss of  $113.7 \pm 1.9$   $\mu\text{m}$  (mean  $\pm$  SEM; N = 132). Points represent individual cells; results are pooled from two independent experiments where two independent lines were analysed. **(G)** Branching phenotype analysis of control (*GFP-tubulin/histoneH2B-mRFP*) and *Kinesin-13* KO (GPH0438#30). In particular, branching distance of the first branch site to cell tip (top graph, leftmost bars) was increased in *Kinesin-13* KO moss to  $338.4 \pm 12.9$   $\mu\text{m}$  (mean  $\pm$  SEM; N = 55), compared to control moss of  $293.1 \pm 8.8$   $\mu\text{m}$  (mean  $\pm$  SEM; N = 71). Points represent individual filaments; results are pooled from two independent experiments where two independent lines were analysed.

# Figure 3

bioRxiv preprint doi: <https://doi.org/10.1101/819722>; this version posted October 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.



**Figure 3: *Kinesin-13s* contribute to triple KO moss shows retrograde nuclear movement not seen in single KOs, but manifests in a lesser degree in the *Kinesin-13ac* double KO line.**

**(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; bar, 50  $\mu\text{m}$ ; NEBD, 0 min. **(B)** Nuclear movement velocity during prophase of control (*GFP-tubulin/histoneH2B-mRFP*);  $0.68 \pm 0.10 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 8$ ), *Kinesin-13a* single KO (GPH0411#43;  $0.85 \pm 0.10 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 8$ ), *Kinesin-13b* single KO (GPH0412#11;  $0.43 \pm 0.03 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 7$ ), *Kinesin-13ab* double KO (GPH0419#33;  $0.62 \pm 0.04 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 11$ ), *Kinesin-13ac* double KO (GPH0420#125;  $-0.03 \pm 0.13 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 16$ ), and *Kinesin-13abc* triple KO (GPH0438#30;  $-0.69 \pm 0.08 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 11$ ). *Kinesin-13abc* triple KO shows a clear retrograde nuclear movement, whereas *Kinesin-13ac* double KO shows intermediate retrograde nuclear movement. Apically directed movement, positive values; basally directed movement, negative values. represent individual mitotic events. Results are from one of three independent experiments where two independent lines were analysed. **(C)** Protein domains of Kinesin-13b and mutant proteins for rescue experiments. Point mutations on Kinesin-13b-Cerulean which was introduced under the *EF1 $\alpha$*  promoter at the *PTA1* site in the moss used for rescue experiments are illustrated. **(D)** Nuclear movement velocity during prophase of control (*GFP-tubulin/histoneH2B-mRFP*);  $0.94 \pm 0.10 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 17$ ), *Kinesin-13abc* triple KO (GPH0438#30;  $-1.43 \pm 0.22 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 29$ ), *Cerulean/Kinesin-13abc* triple KO (GPH0903#1;  $-0.99 \pm 0.25 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 16$ ), *Kinesin-13b(FL)-Cerulean/Kinesin-13abc* triple KO (GPH0899#10;  $1.04 \pm 0.09 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 13$ ), *Kinesin-13b<sup>RIG</sup>-Cerulean/Kinesin-13abc* triple KO (GPH0902#2;  $-0.58 \pm 0.20 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 17$ ), *Kinesin-13b<sup>KVD/KEC</sup>-Cerulean/Kinesin-13abc* triple KO (GPH0900#4;  $-0.94 \pm 0.08 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 10$ ), and *Kinesin-13b<sup>Loop12</sup>-Cerulean/Kinesin-13abc* triple KO (GPH0901#1;  $-1.05 \pm 0.20 \mu\text{m}/\text{min}$ , mean  $\pm$  SEM;  $N = 27$ ). 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 analysed. **(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;  $70.9 \pm 3.6 \mu\text{m}$  (mean  $\pm$  SEM;  $N = 26$ ;  $p$ -value  $< 0.05$ )) compared to the control (*GFP-tubulin/histoneH2B-mRFP*;  $105.2 \pm 12.4 \mu\text{m}$  (mean  $\pm$  SEM;  $N = 11$ )). Each point represents individual mitotic events. Results shown are from one of two independent experiments where two independent lines were analysed.

# Figure 4

bioRxiv preprint doi: <https://doi.org/10.1101/819722>; this version posted October 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

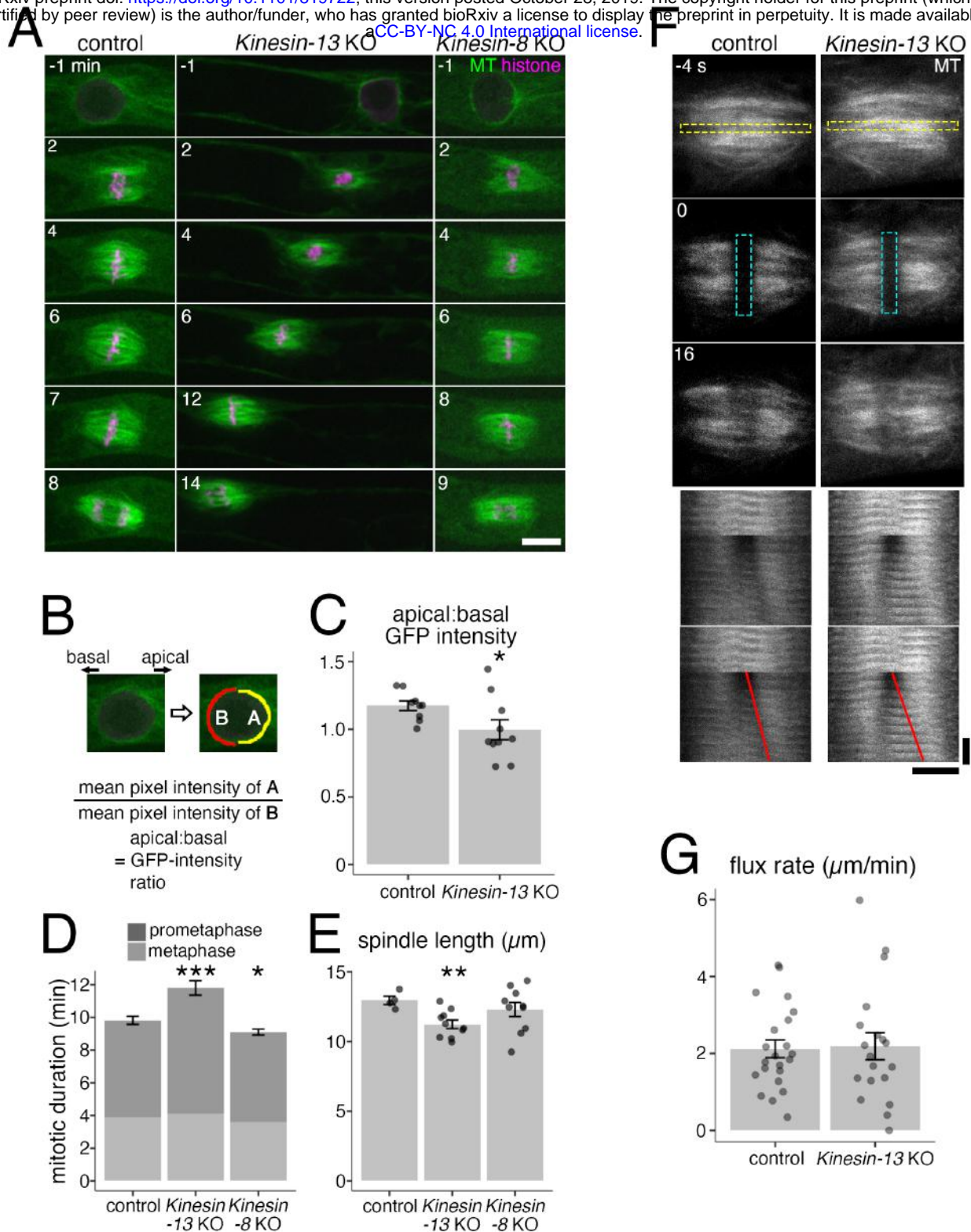


Figure 4: *Kinesin-13* KO moss shows defects in nuclear-proximal MT array, mitotic duration, and spindle length, but shows no difference in spindle flux rate.

**(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. Bar, 10  $\mu\text{m}$ ; NEBD, 0 min; left, basal side; right, apical side. **(B, 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. Control (*GFP-tubulin/histoneH2B-mRFP*),  $1.17 \pm 0.04$  (mean  $\pm$  SEM; N=9; p-value < 0.05); *Kinesin-13* KO (GPH0438#6, 30),  $1.00 \pm 0.07$  (mean  $\pm$  SEM; 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,  $9.8 \pm 0.3$  min (mean  $\pm$  SEM; N = 11); *Kinesin-13* KO,  $11.8 \pm 0.4$  min (mean  $\pm$  SEM, N = 15; p-value < 0.001). *Kinesin-8* KO,  $9.1 \pm 0.2$  min (mean  $\pm$  SEM; N = 10; p-value < 0.05). The duration of prometaphase (from NEBD to chromosome alignment) and metaphase (chromosome alignment to anaphase onset) was also measured and shown. Data shown was 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*),  $13.0 \pm 0.3$   $\mu\text{m}$  (mean  $\pm$  SEM; N = 4); *Kinesin-13* KO (GPH0438#30),  $11.2 \pm 0.3$   $\mu\text{m}$  (mean  $\pm$  SEM; N = 10; p-value < 0.01); *Kinesin-8* KO (GPH0433#9),  $12.3 \pm 0.5$   $\mu\text{m}$  (mean  $\pm$  SEM; 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 towards the poles are indicative of spindle poleward flux function. Horizontal bar, 5  $\mu\text{m}$ ; vertical bar, 12 s; yellow dashed rectangle in the top panel indicates region used to make time series (bottom panel); cyan dashed rectangle represents bleached region; red lines indicate the segmented lines drawn on the kymograph to obtain flux rate in (G). **(G)** Quantification of spindle poleward flux experiment as shown in (F). Control,  $2.1 \pm 0.2$   $\mu\text{m}/\text{min}$  (mean  $\pm$  SEM; N = 22); *Kinesin-13* KO,  $2.2 \pm 0.4$   $\mu\text{m}/\text{min}$  (mean  $\pm$  SEM; N = 19). Points represent individual mitotic events, shown are results from four independent experiments.



# Figure 5

bioRxiv preprint doi: <https://doi.org/10.1101/819722>; this version posted October 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

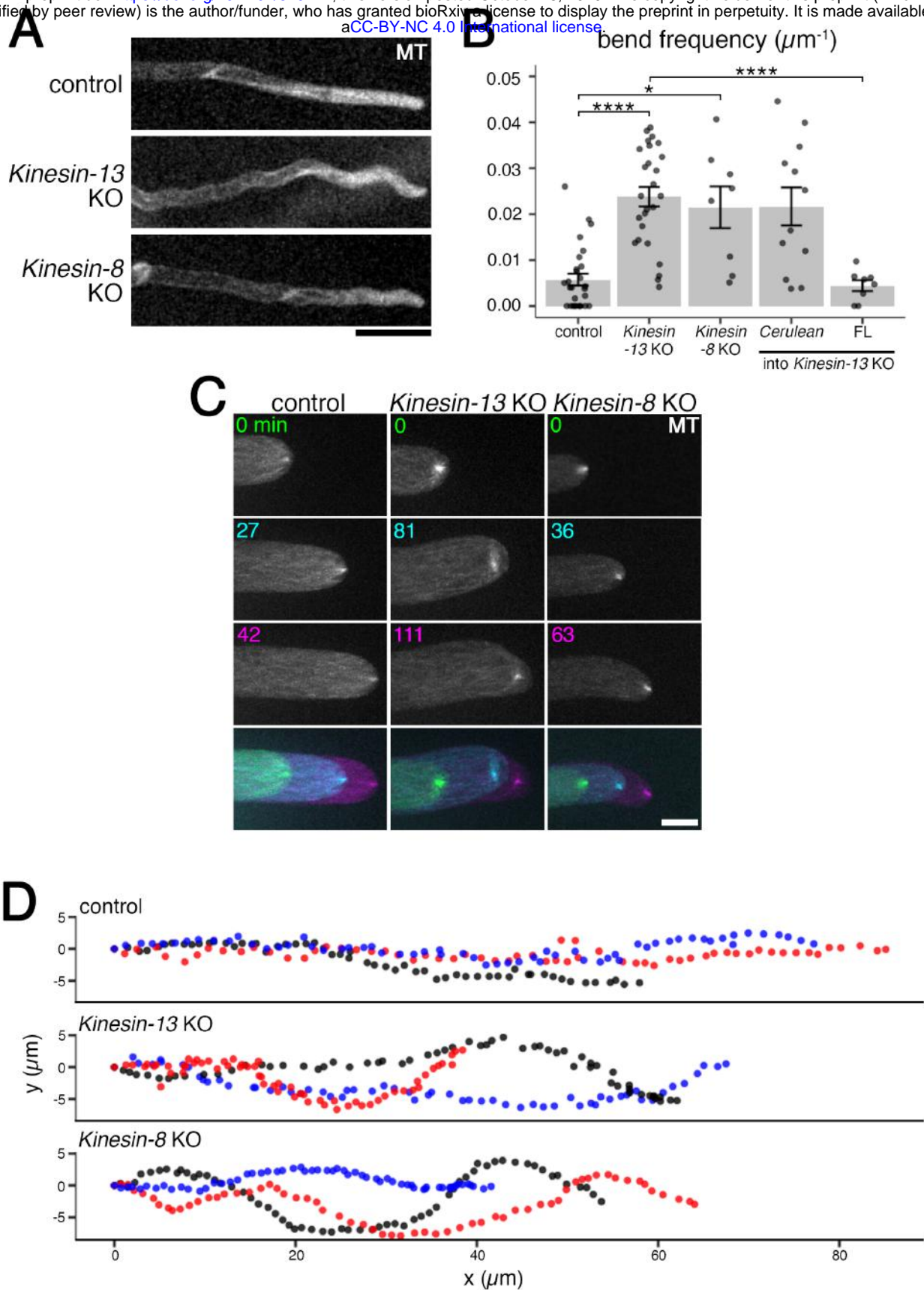
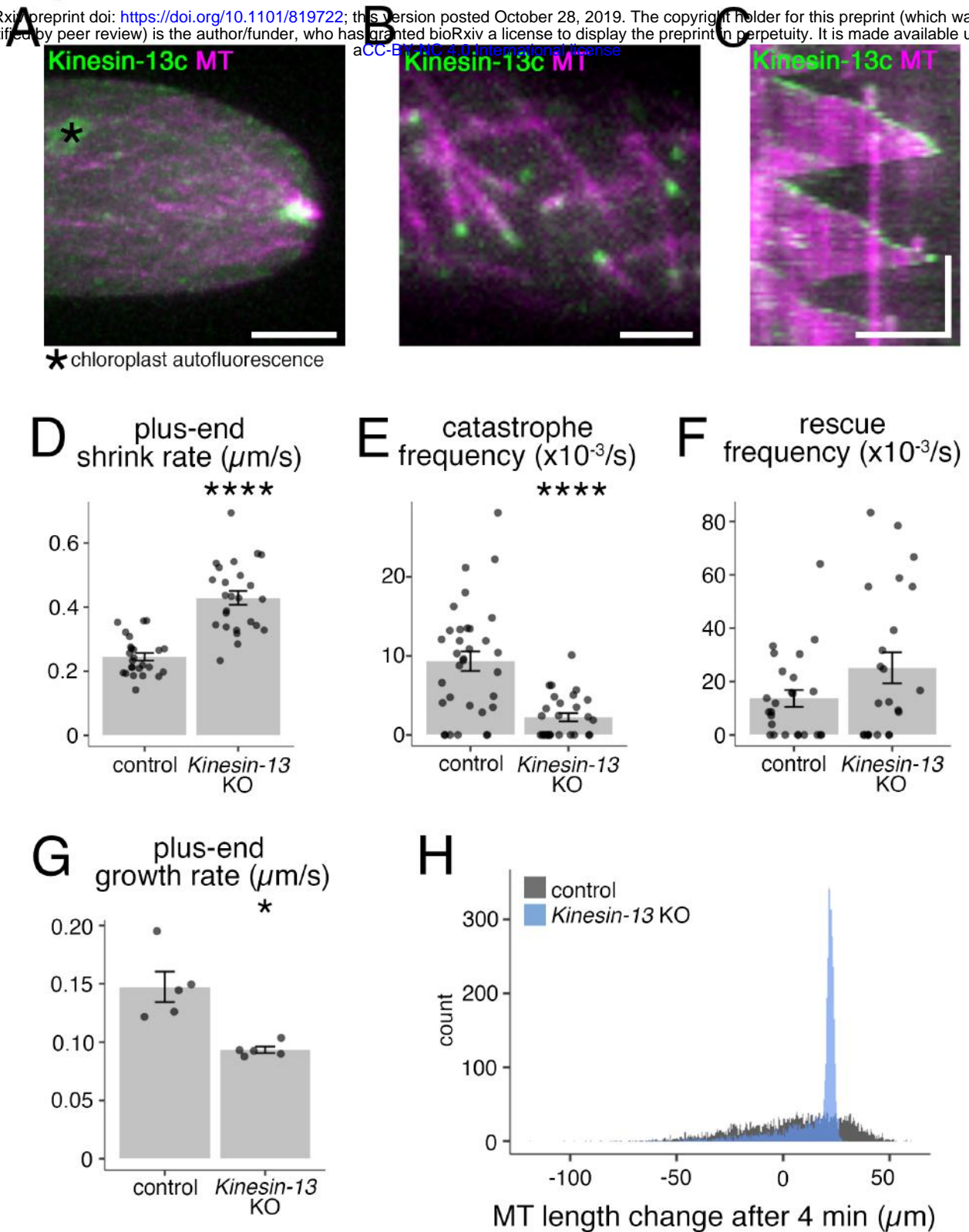


Figure 5: *Kinesin-13* and *-8* KO moss have wavy protonema filaments correlated with unstable MT foci positioning.

(A) Protonema filaments of control (*GFP-tubulin/histoneH2B-mRFP*), *Kinesin-13* KO (GPH0438#30), *Kinesin-8* KO (GPH0433#9) moss. Bar, 50  $\mu\text{m}$ . (B) Waviness of protonema filaments measured as frequency of wavy bend ( $>18^\circ$ ) of protonema filaments over measured lengths. Control (*GFP-tubulin/histoneH2B-mRFP*),  $0.006 \pm 0.001 \mu\text{m}^{-1}$  (mean  $\pm$  SEM; N = 28 filaments); *Kinesin-13* KO (GPH0438#30),  $0.024 \pm 0.002 \mu\text{m}^{-1}$  (mean  $\pm$  SEM; N = 26 filaments; p-value  $< 0.0001$ ); *Kinesin-8* KO (GPH0433#7),  $0.022 \pm 0.005 \mu\text{m}^{-1}$  (mean  $\pm$  SEM; N = 8 filaments; p-value  $< 0.01$ ); *Cerulean/Kinesin-13* KO (GPH0903#1),  $0.022 \pm 0.004 \mu\text{m}^{-1}$  (mean  $\pm$  SEM; N = 12); *Kinesin-13b(full-length)-Cerulean/Kinesin-13* KO (GPH0899#10),  $0.004 \pm 0.001 \mu\text{m}^{-1}$  (mean  $\pm$  SEM; N = 8; p-value  $< 0.0001$ ). Points represent individual protonema filaments, results shown are from one experiment of at least four independent experiments. (C) MT foci at tip of caulonema cell of control (*GFP-tubulin/histoneH2B-mRFP*), *Kinesin-13* KO (GPH0438#30), *Kinesin-8* KO (GPH0433#9) moss. Images were acquired with z-sections at 0.3  $\mu\text{m}$  intervals for 20  $\mu\text{m}$  range, and maximum z-projections are displayed. Bottom panels show overlaid time series. Bar, 10  $\mu\text{m}$ ; colours in time series indicate different time points as labelled in top panels. (D) MT foci positions were tracked using 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 colours) 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

bioRxiv preprint doi: <https://doi.org/10.1101/819722>; this version posted October 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.



**Figure 6: *Kinesin-13* localises to the interphase MT network and depletion of *Kinesin-13* results in increased shrink rate, reduced catastrophe frequency, increased rescue frequency, and reduced growth rate.**

**(A)** MT foci of *Kinesin-13c-Citrine/mCherry-tubulin* (GPH0100#15) moss. Image was acquired at 0.3  $\mu\text{m}$  intervals for 10  $\mu\text{m}$  range; shown is maximum z-projection. Bar, 5  $\mu\text{m}$ . **(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  $\mu\text{m}$ . **(C)** Kymograph of MT growth taken from imaging as in (B), taken every 3 s. Vertical bar, 2 min; horizontal bar, 5  $\mu\text{m}$ . **(D)** Interphase MT plus-end shrink rate of control (*GFP-tubulin/histoneH2B-mRFP*,  $0.245 \pm 0.012 \mu\text{m/s}$  (mean  $\pm$  SEM; N = 25 cells)) and *Kinesin-13* KO (GPH0438#30,  $0.429 \pm 0.021 \mu\text{m/s}$  (mean  $\pm$  SEM; N = 25 cells; p-value < 0.0001)) 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*,  $9.3 \pm 1.2 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N = 33 cells)) and *Kinesin-13* KO (GPH0438#30,  $2.2 \pm 0.5 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N = 28 cells; p-value < 0.0001)). Points represent individual cells; results shown are from two independent experiments. **(F)** Interphase MT rescue frequency of control (*GFP-tubulin/histoneH2B-mRFP*,  $14 \pm 3 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N = 25 cells)) and *Kinesin-13* KO (GPH0438#30,  $25 \pm 6 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; 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,  $0.147 \pm 0.013 \mu\text{m/s}$  (mean  $\pm$  SEM; N = 5 cells, 50 MTs)) and *Kinesin-13* KO moss (GPH0577#11,  $0.093 \pm 0.003 \mu\text{m/s}$  (mean  $\pm$  SEM; N = 5 cells, 50 MTs; p-value < 0.05)). Points represent individual cells. **(H)** Simulation of MT growth of 4,000 MTs in 4 min based on a probability model established using MT dynamics parameters from *in vivo* interphase MT dynamics analyses (D–G) (refer to Materials & Methods, and Table S4). 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  $\mu\text{m}$  in length. For MTs under *Kinesin-13* KO conditions, the distribution of MT length was narrower, with 50% of all MTs between 11.5 to 22.6  $\mu\text{m}$  lengths, whereas the longest 25% of MTs ranged from 22.6 to 29.4  $\mu\text{m}$  in length. Histogram bin width = 0.5  $\mu\text{m}$ .

# Figure 7

bioRxiv preprint doi: <https://doi.org/10.1101/819722>; this version posted October 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.

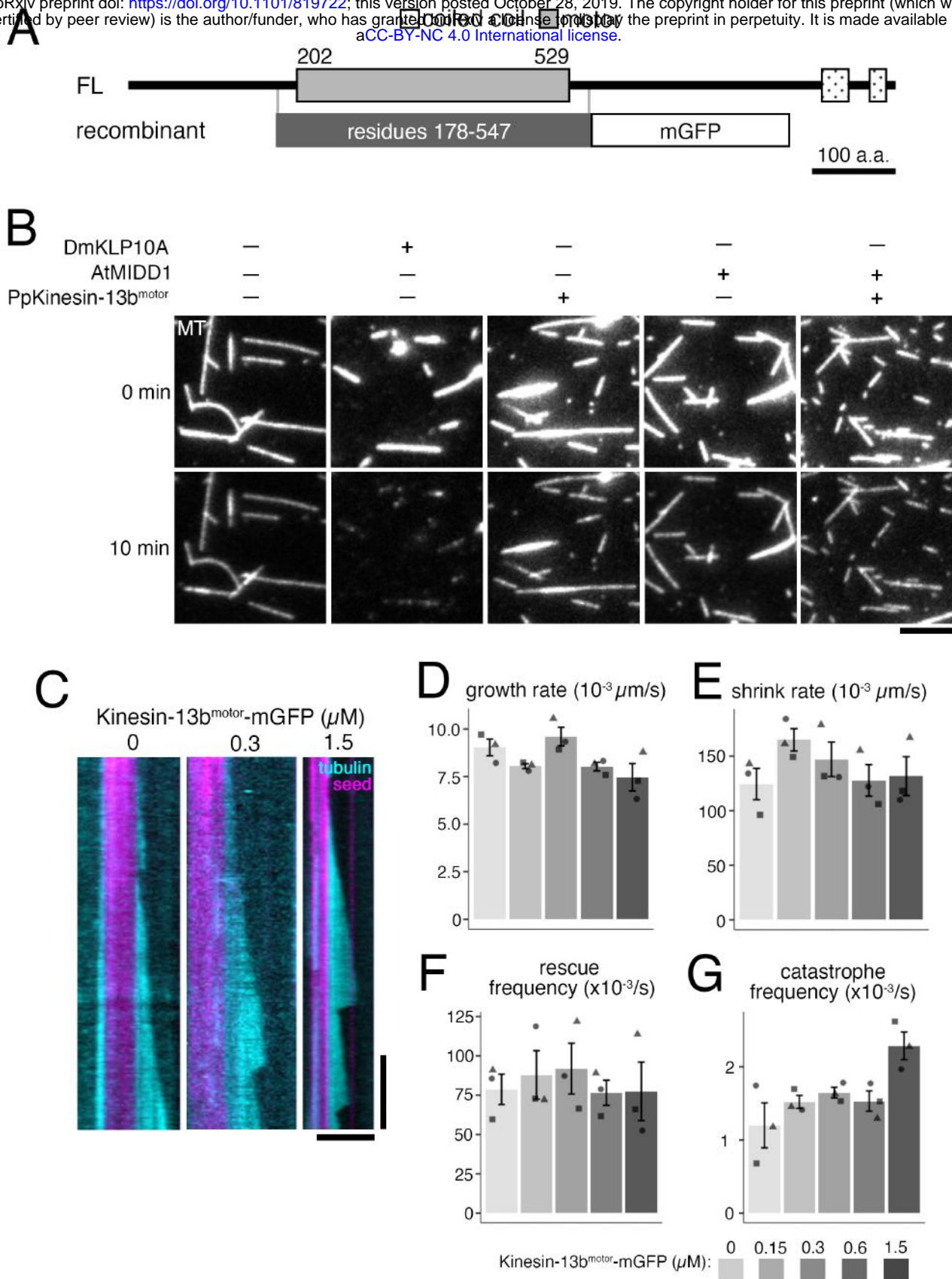
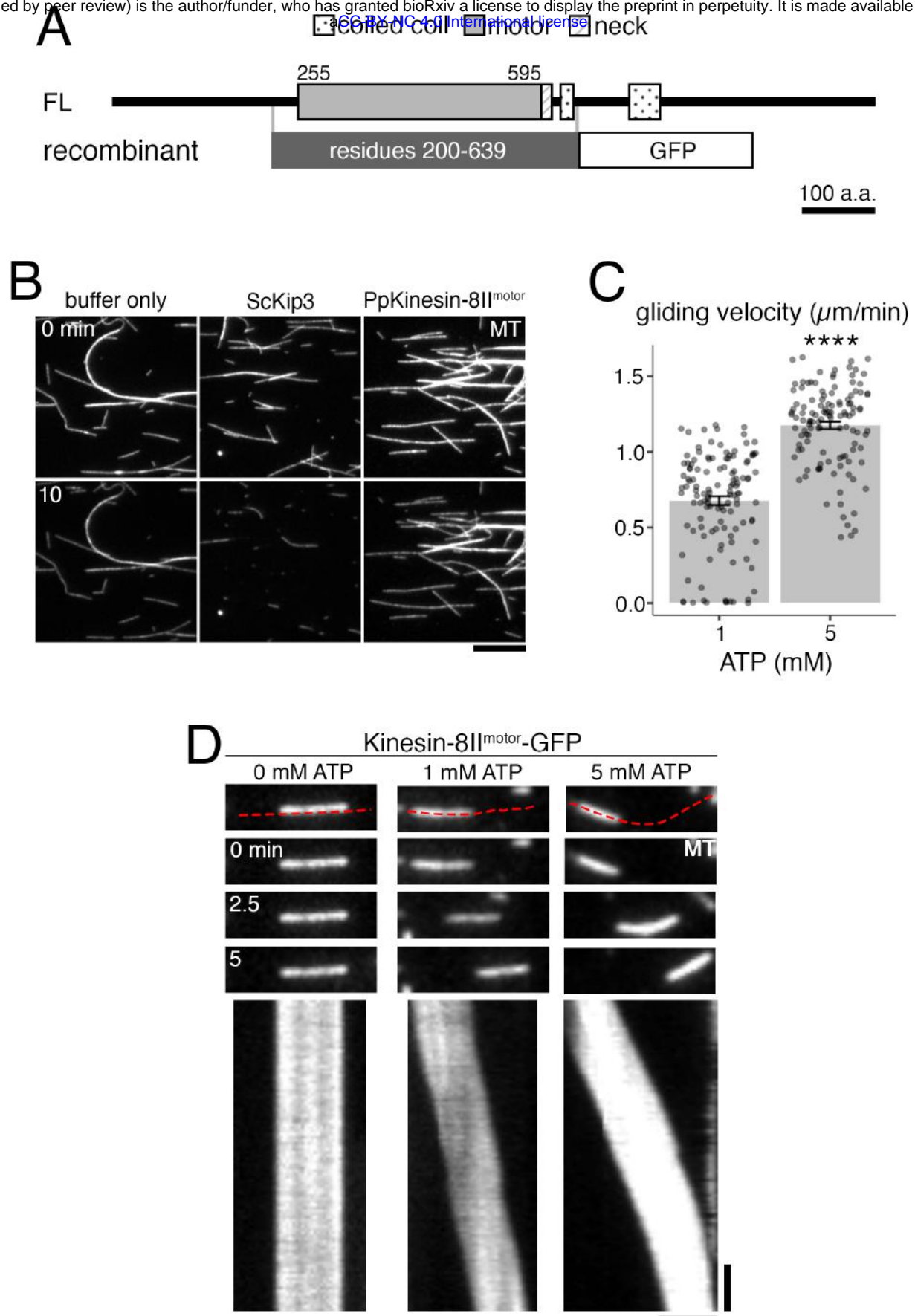


Figure 7: Recombinant Kinesin-13 does not depolymerise stabilised GMPCPP-MT seeds but shows MT catastrophe inducing activity.

**(A)** Protein domains of Kinesin-13b and recombinant Kinesin-13b<sup>motor</sup>-mGFP construct. Protein domains were determined using InterPro. His-tag for affinity purification was attached to C-terminus of the recombinant protein. **(B)** *In vitro* MT depolymerisation assay using GMPCPP-stabilised MT seeds was performed using purified DmKLP10A, recombinant Kinesin-13b<sup>motor</sup>-mGFP construct, AtMIDD1, AtMIDD1 and Kinesin-13b<sup>motor</sup>-mGFP construct, and also under buffer only conditions. Only DmKLP10A successfully depolymerised MT seeds. The slight reduction in intensity in the bottom panels is due to photobleaching during imaging. All proteins were used at 200 nM except for AtMIDD1 which was at 100 nM. Bar, 5  $\mu\text{m}$ . **(C)** Representative kymographs of *in vitro* MT dynamics polymerisation assays with Kinesin-13b<sup>motor</sup>-mGFP construct at 0, 0.3, and 1.5  $\mu\text{M}$ . Time-lapse imaging was performed with TIRF microscopy taken every 3 s. Brightness and contrast was manually adjusted. Vertical bar, 2 min; horizontal bar, 5  $\mu\text{m}$ . **(D-G)** *In vitro* MT dynamics parameters were analysed from time-lapse imaging of *in vitro* MT dynamics polymerisation assays with Kinesin-13b<sup>motor</sup>-mGFP construct at 0, 0.15, 0.3, 0.6, and 1.5  $\mu\text{M}$  taken using TIRF microscopy at every 3 s. In particular, growth rate was observed to reduce slightly, from  $9.0 \pm 0.4 \times 10^{-3} \mu\text{m/s}$  (mean  $\pm$  SEM; N = 3) in buffer only conditions, to  $7.5 \pm 0.7 \times 10^{-3} \mu\text{m/s}$  (mean  $\pm$  SEM; N = 3) in 1.5  $\mu\text{M}$  protein. Catastrophe frequency was observed to reproducibly increase with high concentrations of Kinesin-13b<sup>motor</sup>-mGFP, having a catastrophe frequency of  $2.3 \pm 0.2 \times 10^{-3}/\text{s}$  (mean  $\pm$  SEM; N = 3) at 1.5  $\mu\text{M}$  protein, compared to  $1.2 \pm 0.3 \times 10^{-3}/\text{s}$  in buffer only conditions. Points represent mean values from independent experiments.

# Figure 8

bioRxiv preprint doi: <https://doi.org/10.1101/819722>; this version posted October 28, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC 4.0 International license.



**Figure 8: Recombinant Kinesin-8 motor does not depolymerise MTs but shows MT gliding activity.**

**(A)** Protein domains of Kinesin-8II and recombinant Kinesin-8II<sup>motor</sup>-GFP construct. Protein domains were identified using InterPro. His-tag for affinity purification was attached to C-terminus of the recombinant protein. **(B)** *In vitro* MT depolymerisation assay using GMPCPP-stabilised MT seeds was performed using purified ScKip3, recombinant Kinesin-8II<sup>motor</sup>-GFP, and also under buffer only conditions. Only ScKip3 showed MT depolymerisation activity. The slight reduction in intensity in 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-8II<sup>motor</sup>-GFP. 1 mM ATP,  $0.68 \pm 0.03$  μm/min (mean ± SEM; N = 124 MTs); 5 mM ATP,  $1.18 \pm 0.02$  μm/min (mean ± SEM; N = 121 MTs, p-value < 0.0001). **(D)** *In vitro* MT gliding assay using GMPCPP-stabilised MTs on Kinesin-8II<sup>motor</sup>-GFP which was immobilised on glass, at 0, 1, and 5 mM ATP. Red dotted line in top panel indicates segmented line used to draw kymographs (bottom panels). Gliding activity of Kinesin-8II<sup>motor</sup>-GFP was verified in 3 independent experiments. Vertical bar, 45 s; horizontal bar, 2 μm.

## Parsed Citations

- Collonnier, C., Epert, A., Mara, K., Maclot, F., Guyon-Debast, A., Charlot, F., White, C., Schaefer, D.G., and Nogue, F. (2017). CRISPR-Cas9-mediated efficient directed mutagenesis and RAD51-dependent and RAD51-independent gene targeting in the moss *Physcomitrella patens*. *Plant Biotechnol J* 15, 122-131.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Cove, D. (2005). The moss *Physcomitrella patens*. *Annu Rev Genet* 39, 339-358.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Deng, Z.Y., Liu, L.T., Li, T., Yan, S., Kuang, B.J., Huang, S.J., Yan, C.J., and Wang, T. (2015). OsKinesin-13A is an active microtubule depolymerase involved in glume length regulation via affecting cell elongation. *Sci Rep* 5, 9457.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Desai, A., Verma, S., Mitchison, T.J., and Walczak, C.E. (1999). Kin I kinesins are microtubule-destabilizing enzymes. *Cell* 96, 69-78.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Doonan, J.H., Cove, D.J., and Lloyd, C.W. (1985). Immunofluorescence microscopy of microtubules in intact cell lineages of the moss, *Physcomitrella patens*. I. Normal and CIPC-treated tip cells. *J Cell Sci* 75, 131-147.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Doonan, J.H., Cove, D.J., and Lloyd, C.W. (1988). Microtubules and microfilaments in tip growth: evidence that microtubules impose polarity on protonemal growth in *Physcomitrella patens*. *J Cell Sci* 89, 533-540.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Edzuka, T., and Goshima, G. (2019). *Drosophila* kinesin-8 stabilizes the kinetochore-microtubule interaction. *J Cell Biol* 218, 474-488.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Eng, R.C., and Wasteneys, G.O. (2014). The microtubule plus-end tracking protein ARMADILLO-REPEAT KINESIN1 promotes microtubule catastrophe in *Arabidopsis*. *Plant Cell* 26, 3372-3386.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Fujikura, U., Elsaesser, L., Breuninger, H., Sanchez-Rodriguez, C., Ivakov, A., Laux, T., Findlay, K., Persson, S., and Lenhard, M. (2014). Atkinesin-13A modulates cell-wall synthesis and cell expansion in *Arabidopsis thaliana* via the THESEUS1 pathway. *PLoS Genet* 10, e1004627.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Ganem, N.J., Upton, K., and Compton, D.A. (2005). Efficient mitosis in human cells lacking poleward microtubule flux. *Curr Biol* 15, 1827-1832.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gatt, M.K., Savoian, M.S., Riparbelli, M.G., Massarelli, C., Callaini, G., and Glover, D.M. (2005). Klp67A destabilises pre-anaphase microtubules but subsequently is required to stabilise the central spindle. *J Cell Sci* 118, 2671-2682.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gell, C., Bormuth, V., Brouhard, G.J., Cohen, D.N., Diez, S., Friel, C.T., Helenius, J., Nitzsche, B., Petzold, H., Ribbe, J., Schaffer, E., Stear, J.H., Trushko, A., Varga, V., Widlund, P.O., Zanic, M., and Howard, J. (2010). Microtubule dynamics reconstituted in vitro and imaged by single-molecule fluorescence microscopy. *Methods Cell Biol* 95, 221-245.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gicking, A.M., Swentowsky, K.W., Dawe, R.K., and Qiu, W. (2018). Functional diversification of the kinesin-14 family in land plants. *FEBS Lett* 592, 1918-1928.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Goshima, G., and Vale, R.D. (2003). The roles of microtubule-based motor proteins in mitosis: comprehensive RNAi analysis in the *Drosophila* S2 cell line. *J Cell Biol* 162, 1003-1016.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Goshima, G., and Scholey, J.M. (2010). Control of mitotic spindle length. *Annu Rev Cell Dev Biol* 26, 21-57.  
Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Gupta, M.L., Jr., Carvalho, P., Roof, D.M., and Pellman, D. (2006). Plus end-specific depolymerase activity of Kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle. Nat Cell Biol 8, 913-923.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Hertzer, K.M., Ems-McClung, S.C., Kline-Smith, S.L., Lipkin, T.G., Gilbert, S.P., and Walczak, C.E. (2006). Full-length dimeric MCAK is a more efficient microtubule depolymerase than minimal domain monomeric MCAK. Mol Biol Cell 17, 700-710.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Hildebrandt, E.R., and Hoyt, M.A. (2000). Mitotic motors in *Saccharomyces cerevisiae*. Biochim Biophys Acta 1496, 99-116.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Hirokawa, N., Noda, Y., Tanaka, Y., and Niwa, S. (2009). Kinesin superfamily motor proteins and intracellular transport. Nat Rev Mol Cell Biol 10, 682-696.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Hiwatashi, Y., Sato, Y., and Doonan, J.H. (2014). Kinesins have a dual function in organizing microtubules during both tip growth and cytokinesis in *Physcomitrella patens*. Plant Cell 26, 1256-1266.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Homma, N., Takei, Y., Tanaka, Y., Nakata, T., Terada, S., Kikkawa, M., Noda, Y., and Hirokawa, N. (2003). Kinesin superfamily protein 2A (KIF2A) functions in suppression of collateral branch extension. Cell 114, 229-239.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Howard, J., and Hyman, A.A. (2007). Microtubule polymerases and depolymerases. Curr Opin Cell Biol 19, 31-35.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Jonsson, E., Yamada, M., Vale, R.D., and Goshima, G. (2015). Clustering of a kinesin-14 motor enables processive retrograde microtubule-based transport in plants. Nat Plants 1.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Kline-Smith, S.L., Khodjakov, A., Hergert, P., and Walczak, C.E. (2004). Depletion of centromeric MCAK leads to chromosome congression and segregation defects due to improper kinetochore attachments. Mol Biol Cell 15, 1146-1159.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Kobayashi, T., Tsang, W.Y., Li, J., Lane, W., and Dynlacht, B.D. (2011). Centriolar kinesin Kif24 interacts with CP110 to remodel microtubules and regulate ciliogenesis. Cell 145, 914-925.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Kofuji, R., and Hasebe, M. (2014). Eight types of stem cells in the life cycle of the moss *Physcomitrella patens*. Curr Opin Plant Biol 17, 13-21.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Kosetsu, K., de Keijzer, J., Janson, M.E., and Goshima, G. (2013). MICROTUBULE-ASSOCIATED PROTEIN65 is essential for maintenance of phragmoplast bipolarity and formation of the cell plate in *Physcomitrella patens*. Plant Cell 25, 4479-4492.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Kozgunova, E., and Goshima, G. (2019). A versatile microfluidic device for highly inclined thin illumination microscopy in the moss *Physcomitrella patens*. Sci Rep in press.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Lee, T., Langford, K.J., Askham, J.M., Bruning-Richardson, A., and Morrison, E.E. (2008). MCAK associates with EB1. Oncogene 27, 2494-2500.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Leong, S.Y., Yamada, M., Yanagisawa, N., and Goshima, G. (2018). SPIRAL2 Stabilises Endoplasmic Microtubule Minus Ends in the Moss *Physcomitrella patens*. Cell Struct Funct 43, 53-60.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Li, W., Moriwaki, T., Tani, T., Watanabe, T., Kaibuchi, K., and Goshima, G. (2012). Reconstitution of dynamic microtubules with *Drosophila* XMAP215, EB1, and Sentin. *J Cell Biol* 199, 849-862.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Lopez-Obando, M., Hoffmann, B., Gery, C., Guyon-Debast, A., Teoule, E., Rameau, C., Bonhomme, S., and Nogue, F. (2016). Simple and Efficient Targeting of Multiple Genes Through CRISPR-Cas9 in *Physcomitrella patens*. *G3 (Bethesda)* 6, 3647-3653.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Lu, L., Lee, Y.R., Pan, R., Maloof, J.N., and Liu, B. (2005). An internal motor kinesin is associated with the Golgi apparatus and plays a role in trichome morphogenesis in *Arabidopsis*. *Mol Biol Cell* 16, 811-823.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Maney, T., Wagenbach, M., and Wordeman, L. (2001). Molecular dissection of the microtubule depolymerizing activity of mitotic centromere-associated kinesin. *J Biol Chem* 276, 34753-34758.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Mayr, M.I., Hummer, S., Bormann, J., Gruner, T., Adio, S., Woehlke, G., and Mayer, T.U. (2007). The human kinesin Kif18A is a motile microtubule depolymerase essential for chromosome congression. *Curr Biol* 17, 488-498.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Menand, B., Calder, G., and Dolan, L. (2007). Both chloronemal and caulonemal cells expand by tip growth in the moss *Physcomitrella patens*. *J Exp Bot* 58, 1843-1849.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Mennella, V., Rogers, G.C., Rogers, S.L., Buster, D.W., Vale, R.D., and Sharp, D.J. (2005). Functionally distinct kinesin-13 family members cooperate to regulate microtubule dynamics during interphase. *Nat Cell Biol* 7, 235-245.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Menon, S., and Gupton, S.L. (2016). Building Blocks of Functioning Brain: Cytoskeletal Dynamics in Neuronal Development. *Int Rev Cell Mol Biol* 322, 183-245.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Miki, H., Okada, Y., and Hirokawa, N. (2005). Analysis of the kinesin superfamily: insights into structure and function. *Trends Cell Biol* 15, 467-476.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Miki, T., Nishina, M., and Goshima, G. (2015). RNAi screening identifies the armadillo repeat-containing kinesins responsible for microtubule-dependent nuclear positioning in *Physcomitrella patens*. *Plant Cell Physiol* 56, 737-749.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Miki, T., Nakaoka, Y., and Goshima, G. (2016). Live cell microscopy-based RNAi screening in the moss *Physcomitrella patens*. *Methods Mol Biol* 1470, 225-246.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Miki, T., Naito, H., Nishina, M., and Goshima, G. (2014). Endogenous localizome identifies 43 mitotic kinesins in a plant cell. *Proc Natl Acad Sci U S A* 111, E1053-1061.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Moores, C.A., Yu, M., Guo, J., Beraud, C., Sakowicz, R., and Milligan, R.A. (2002). A mechanism for microtubule depolymerization by Kln1 kinesins. *Mol Cell* 9, 903-909.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Moriwaki, T., and Goshima, G. (2016). Five factors can reconstitute all three phases of microtubule polymerization dynamics. *J Cell Biol* 215, 357-368.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

Nakaoka, Y., Kimura, A., Tani, T., and Goshima, G. (2015). Cytoplasmic nucleation and atypical branching nucleation generate endoplasmic microtubules in *Physcomitrella patens*. *Plant Cell* 27, 228-242.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Nakaoka, Y., Miki, T., Fujioka, R., Uehara, R., Tomioka, A., Obuse, C., Kubo, M., Hiwatashi, Y., and Goshima, G. (2012). An inducible RNA interference system in *Physcomitrella patens* reveals a dominant role of augmin in phragmoplast microtubule generation. *Plant Cell* 24, 1478-1493.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Nebenfuhr, A., and Dixit, R. (2018). Kinesins and Myosins: Molecular Motors that Coordinate Cellular Functions in Plants. *Annu Rev Plant Biol* 69, 329-361.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Niwa, S., Nakajima, K., Miki, H., Minato, Y., Wang, D., and Hirokawa, N. (2012). KIF19A is a microtubule-depolymerizing kinesin for ciliary length control. *Dev Cell* 23, 1167-1175.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Oda, Y., and Fukuda, H. (2013). Rho of plant GTPase signaling regulates the behavior of Arabidopsis kinesin-13A to establish secondary cell wall patterns. *Plant Cell* 25, 4439-4450.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Ogawa, T., Saijo, S., Shimizu, N., Jiang, X., and Hirokawa, N. (2017). Mechanism of Catalytic Microtubule Depolymerization via KIF2-Tubulin Transitional Conformation. *Cell Rep* 20, 2626-2638.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Ovechkina, Y., Wagenbach, M., and Wordeman, L. (2002). K-loop insertion restores microtubule depolymerizing activity of a "neckless" MCAK mutant. *J Cell Biol* 159, 557-562.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Reddy, A.S., and Day, I.S. (2001). Kinesins in the Arabidopsis genome: a comparative analysis among eukaryotes. *BMC Genomics* 2, 2.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Rogers, G.C., Rogers, S.L., and Sharp, D.J. (2005). Spindle microtubules in flux. *J Cell Sci* 118, 1105-1116.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Rogers, G.C., Rogers, S.L., Schwimmer, T.A., Ems-McClung, S.C., Walczak, C.E., Vale, R.D., Scholey, J.M., and Sharp, D.J. (2004). Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase. *Nature* 427, 364-370.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Rounds, C.M., and Bezanilla, M. (2013). Growth mechanisms in tip-growing plant cells. *Annu Rev Plant Biol* 64, 243-265.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Sabry, J.H., O'Connor, T.P., Evans, L., Toroian-Raymond, A., Kirschner, M., and Bentley, D. (1991). Microtubule behavior during guidance of pioneer neuron growth cones in situ. *J Cell Biol* 115, 381-395.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Sbalzarini, I.F., and Koumoutsakos, P. (2005). Feature point tracking and trajectory analysis for video imaging in cell biology. *J Struct Biol* 151, 182-195.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Schmidt, S., and Smertenko, A. (2019). Identification and characterization of the land-plant-specific microtubule nucleation factor MACET4. *J Cell Sci* 132.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Shen, Z., Collatos, A.R., Bibeau, J.P., Furt, F., and Vidali, L. (2012). Phylogenetic analysis of the Kinesin superfamily from *Physcomitrella*. *Front Plant Sci* 3, 230.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Shipley, K., Hekmat-Nejad, M., Turner, J., Moores, C., Anderson, R., Milligan, R., Sakowicz, R., and Fletterick, R. (2004). Structure of a kinesin microtubule depolymerization machine. *EMBO J* 23, 1422-1432.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Soppina, V., and Verhey, K.J. (2014). The family-specific K-loop influences the microtubule on-rate but not the superprocessivity of kinesin-3 motors. *Mol Biol Cell* 25, 2161-2170.**



Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Stumpff, J., von Dassow, G., Wagenbach, M., Asbury, C., and Wordeman, L. (2008).** The kinesin-8 motor Kif18A suppresses kinetochore movements to control mitotic chromosome alignment. *Dev Cell* 14, 252-262.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Stumpff, J., Wagenbach, M., Franck, A., Asbury, C.L., and Wordeman, L. (2012).** Kif18A and chromokinesins confine centromere movements via microtubule growth suppression and spatial control of kinetochore tension. *Dev Cell* 22, 1017-1029.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Tanaka, E., Ho, T., and Kirschner, M.W. (1995).** The role of microtubule dynamics in growth cone motility and axonal growth. *J Cell Biol* 128, 139-155.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Uehara, R., Tsukada, Y., Kamasaki, T., Poser, I., Yoda, K., Gerlich, D.W., and Goshima, G. (2013).** Aurora B and Kif2A control microtubule length for assembly of a functional central spindle during anaphase. *J Cell Biol* 202, 623-636.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Unsworth, A., Masuda, H., Dhut, S., and Toda, T. (2008).** Fission yeast kinesin-8 Klp5 and Klp6 are interdependent for mitotic nuclear retention and required for proper microtubule dynamics. *Mol Biol Cell* 19, 5104-5115.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Vidali, L., Augustine, R.C., Kleinman, K.P., and Bezanilla, M. (2007).** Profilin is essential for tip growth in the moss *Physcomitrella patens*. *Plant Cell* 19, 3705-3722.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Walczak, C.E., and Heald, R. (2008).** Mechanisms of Mitotic Spindle Assembly and Function 265, 111-158.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Walczak, C.E., Gayek, S., and Ohi, R. (2013).** Microtubule-depolymerizing kinesins. *Annu Rev Cell Dev Biol* 29, 417-441.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**West, R.R., Malmstrom, T., Troxell, C.L., and McIntosh, J.R. (2001).** Two related kinesins, klp5+ and klp6+, foster microtubule disassembly and are required for meiosis in fission yeast. *Mol Biol Cell* 12, 3919-3932.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Wu, S.Z., and Bezanilla, M. (2018).** Actin and microtubule cross talk mediates persistent polarized growth. *J Cell Biol* 217, 3531-3544.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yamada, M., and Goshima, G. (2018).** The KCH Kinesin Drives Nuclear Transport and Cytoskeletal Coalescence to Promote Tip Cell Growth in *Physcomitrella patens*. *Plant Cell* 30, 1496-1510.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yamada, M., Miki, T., and Goshima, G. (2016).** Imaging Mitosis in the Moss *Physcomitrella patens*. *Methods Mol Biol* 1413, 263-282.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yoshida, M.W., Yamada, M., and Goshima, G. (2019).** Moss Kinesin-14 KCBP Accelerates Chromatid Motility in Anaphase. *Cell Struct Funct* 44, 95-104.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

## Movie legends

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

GFP-tubulin and histoneH2B-mRFP were imaged with spinning disc confocal microscopy. NEBD, 0 min; Playback at 10 fps at 1 min intervals; left, basal side; right, apical side.

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

GFP-tubulin on the mitotic spindle at metaphase was photobleached in a strip (0 min), and the migration of the photobleached strip towards the poles can be observed. Playback at 20 fps at 3 s intervals.

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

**GFP-tubulin was imaged with epifluorescence microscopy. Playback at 30 fps at 3 min intervals.**

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

**The MT foci was imaged with spinning disc confocal microscopy. Movies are maximum z-projections of z-stacks taken every 0.3  $\mu\text{m}$  for a 20  $\mu\text{m}$  range. Playback at 15 fps at 3 min intervals.**

**Movie 5. Localisation of Kinesin-13 during interphase**

**Kinesin-13c is shown representatively for the other two paralogues, which show similar localisation. Localisation at the MT foci (top panel) and the interphase endoplasmic MT array (bottom panel) were imaged with spinning disc confocal microscopy and oblique illumination fluorescence microscopy respectively. Playback at 15 fps at 3 s intervals.**