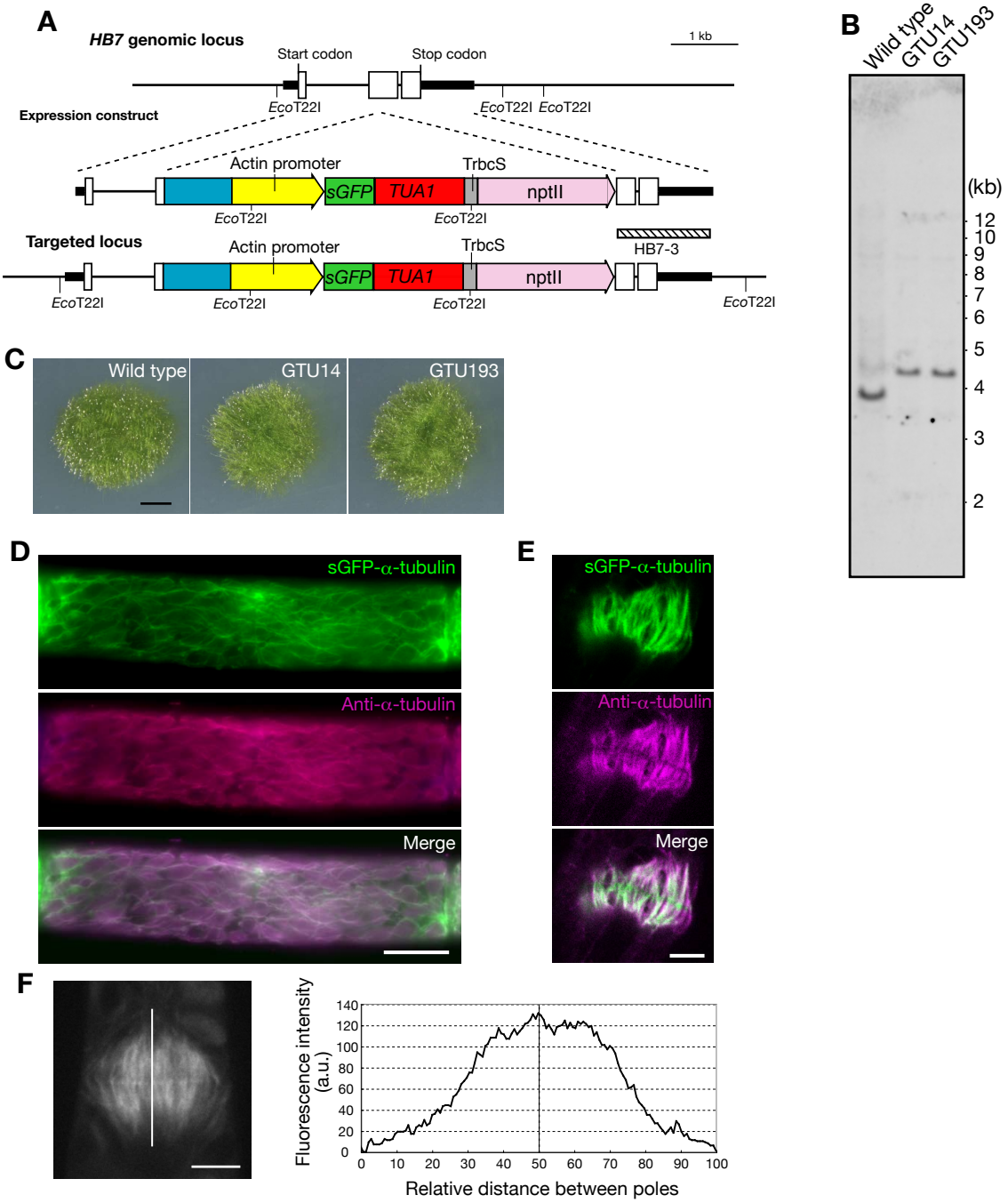
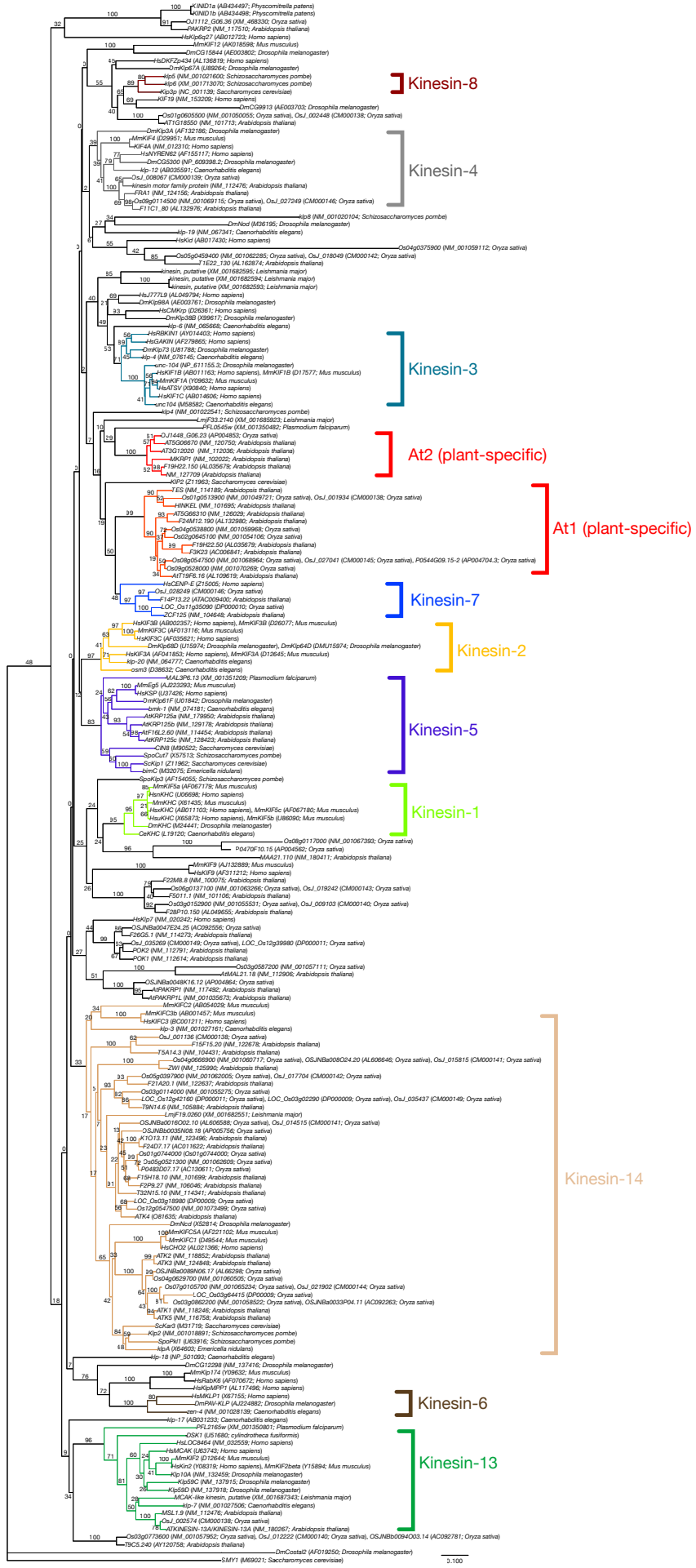
Supplemental Data. Hiwatashi et al. (2008) Kinesins are indispensable for interdigitation of phragmoplast microtubules in the moss *Physcomitrella patens*.



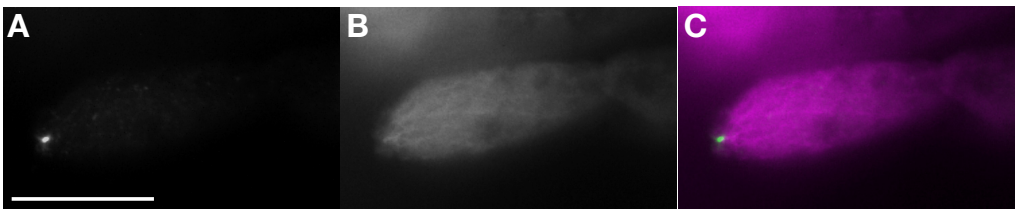
Supplemental Figure 1. Generation of the Lines Expressing the sGFP and α-tubulin Fusion Proteins.

(A) A schematic for the insertion into the *HB7* locus of a DNA fragment containing the rice actin promoter (yellow arrow; McElroy et al., 1990), the *sGFP* gene (green box; Chiu et al., 1996), *Physcomitrella patens* α-tubulin gene *TUA1* (red box; AB096718), a Pea rbcS terminator (TrbcS; gray box), and a neomycin phosphotransferase II expression cassette (nptII; pink arrow; Nishiyama et al., 2000). A blue box represents the vector region of TN90. Open boxes and thick lines represent *HB7* coding and untranslated regions, respectively. The probe used in **(B)** is indicated as a hatched line. **(B)** DNA gel-blot analysis of insertion mutant lines (GTU14 and GTU193). Genomic DNA was digested with *Eco*T22I. **(C)** Protonemal colonies of the GTU14 and GTU193 mutant and wild-type lines. **(D)** and **(E)** Immunodetection of α-tubulin protein in the GTU193 line using anti-α-tubulin. Top, middle, and bottom panels show sGFP-α-tubulin signals, anti-α-tubulin signals, and a merged image of both, respectively. A chloronemal cell at interphase **(D)** and a caulonemal cell at cytokinesis showing a phragmoplast **(E)**. A false color composite image shows sGFP-α-tubulin fusion protein in green and anti-α-tubulin in magenta. **(F)** A phragmoplast in cytokinesis (left) and a profile plot of fluorescence intensity (right) of the GTU193 insertion mutant line expressing sGFP-α-tubulin. A chloronemal apical cell is shown. Changes in fluorescence intensity along a white line connecting the two poles (left) are indicated in the right figure. Bars = 20 μm in **(C)**, 10 μm in **(D),** and 4 μm in **(E)** and **(F)**.

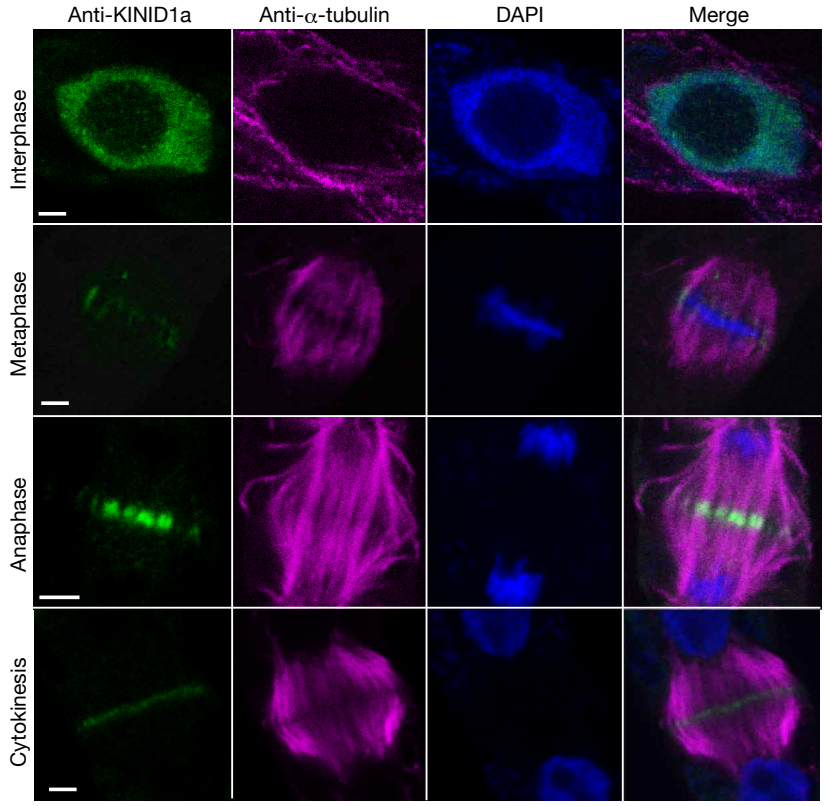
Supplemental Figure 2. GUS Activity in the Gene-Trap Line *Apical1*.
GUS activity in caulonema (left) and young gametophore (right) cells in the gene-trap line *Apical1*. Arrows indicate apical cells. Bars = 20 μm.



Supplemental Figure 3. Neighbor-joining Tree of Kinesin Superfamily Genes Including *KINID1a* and *KINID1b*. This tree is unrooted. Local bootstrap values with 1000 replicates are shown on branches. Horizontal branch length is proportional to the estimated evolutionary distance. Subfamily assignments (Lawrence et al., 2004) are designated on the right. Genes with identical amino acid sequences within the region used for the phylogenetic analysis are combined in the same branch. Accession numbers of genes with assigned names are shown in parentheses before the species name. Genes having no assigned name are represented by their locus tag.

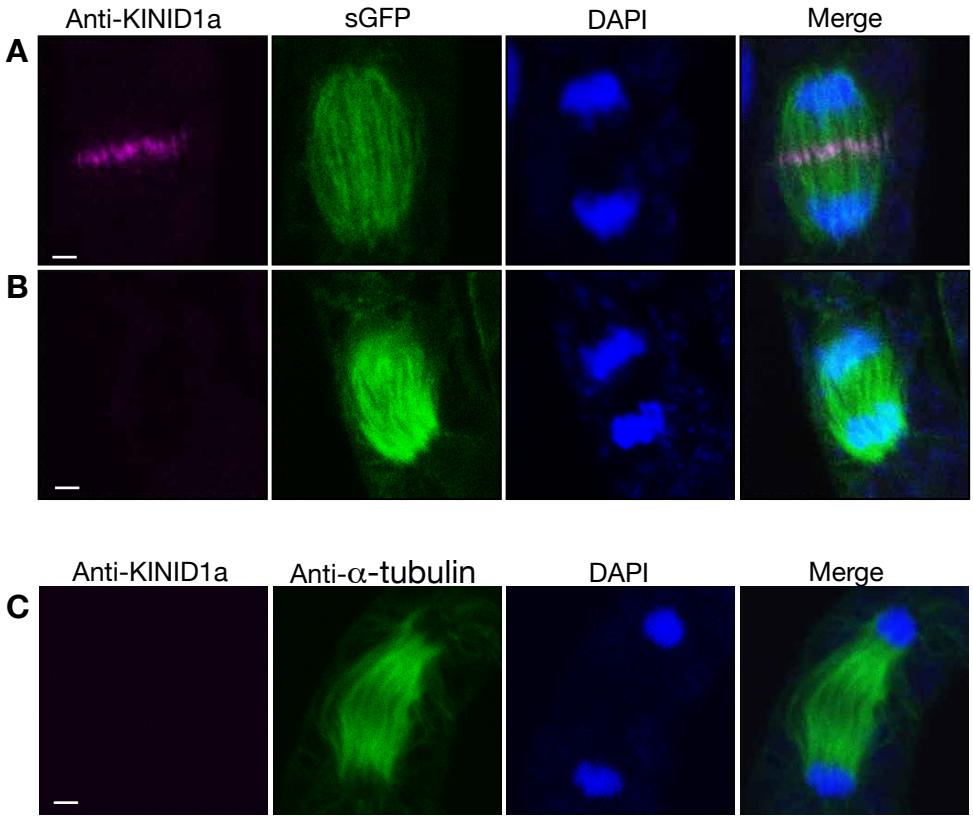


Supplemental Figure 4. Immunodetection of KINID1a Protein with Anti-KINID1a Antibody in a Caulonemal Apical Cell Indicating a Signal in Cytoplasm.
KINID1a **(A)** and microtubules with anti-α-tubulin **(B)** are shown. A false color merged image **(C)** shows KINID1a in green and α-tubulin in magenta. A bar = 2 μm.



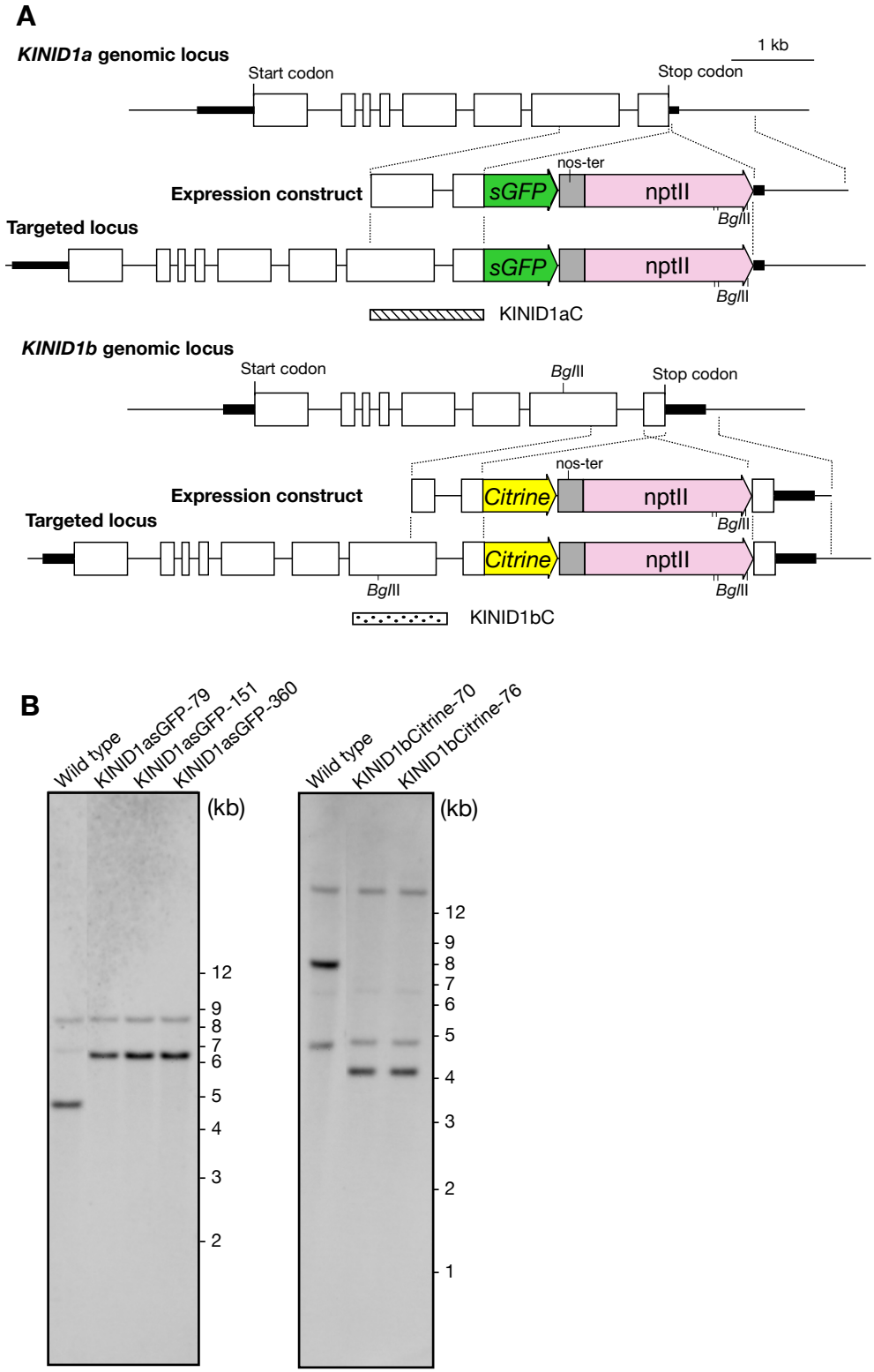
Supplemental Figure 5. Immunodetection of KINID1a Protein with Anti-KINID1a Antibody in Chloronemal Apical Cells.

Triple localization of KINID1a with anti-KINID1a, microtubules with anti-α-tubulin, and DNA with DAPI in chloronemal apical cells at interphase, metaphase, anaphase, and cytokinesis. The false color merged images show KINID1a in green, α-tubulin in magenta, and DNA in blue. Bars = 2 μm.



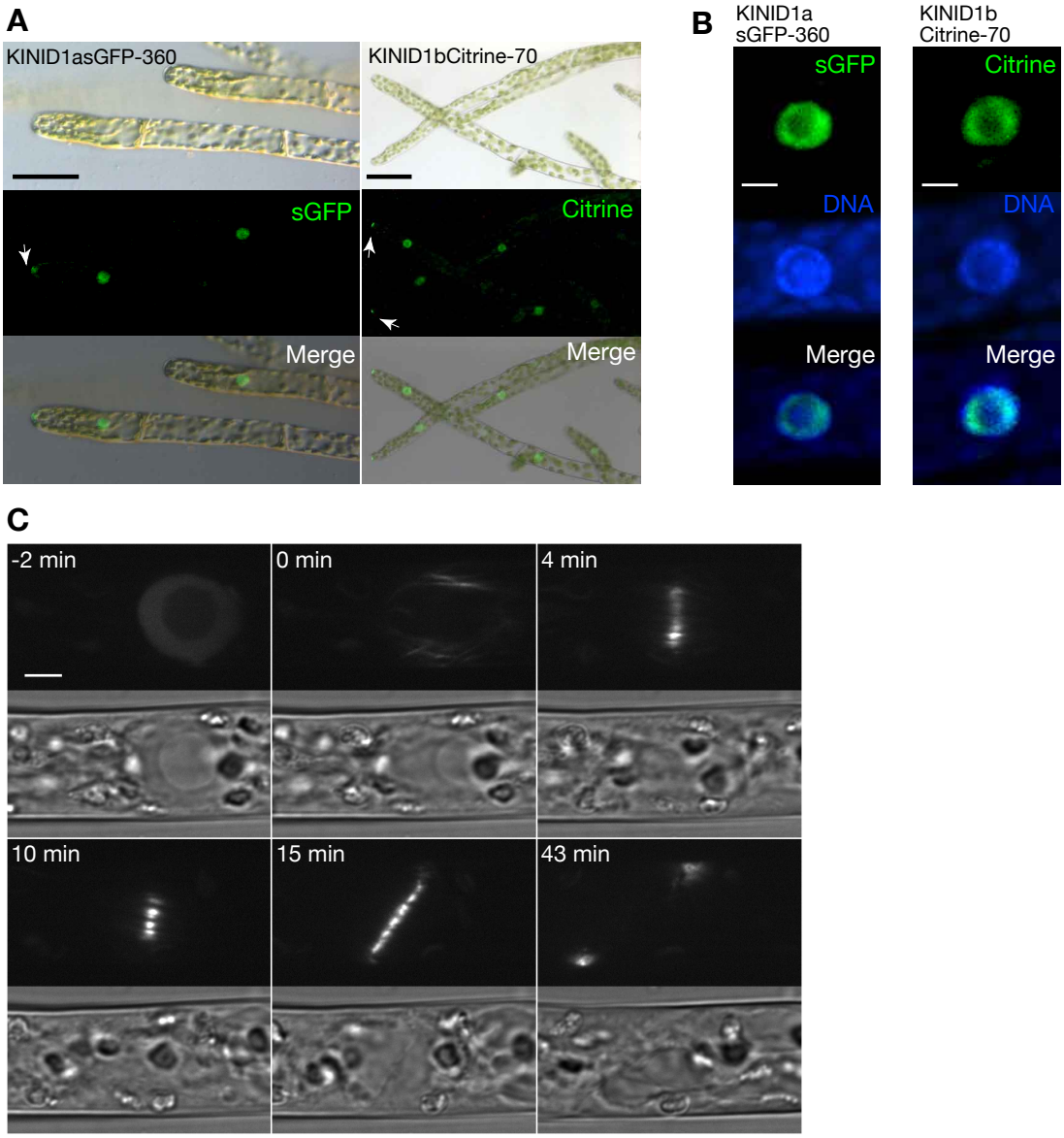
Supplemental Figure 6. Immunodetection of KINID1a Protein with Anti-KINID1a Antibody in Caulonemal Apical Cells of the *KINID1a* Single-deletion Mutant Line and the *KINID1a* and *KINID1b* Double-deletion Mutant Line Expressing sGFP-α-tubulin Fusion Protein.

(A) A caulonemal apical cell of the GTU193 line. **(B)** A caulonemal apical cell of the GTU193 line in which both KINID1a and KINID1b were deleted (the Δkinu1aΔkinu1bGTU193-126 line). Images of a cell stained with anti-KINID1a antibody (Anti-KINID1a), a cell showing GFP fluorescence for α-tubulin (GFP), and a cell stained with DAPI (DAPI). False color merged images (Merge) show KINID1a in green, α-tubulin in magenta, and DNA in blue. **(C)** A caulonemal apical cell of the Δkinu1a-168 line. Images of a cell stained with anti-KINID1a antibody (Anti-KINID1a), a cell with anti-α-tubulin antibody (Anti-α-tubulin), and a cell stained with DAPI (DAPI). False color merged images (Merge) show KINID1a in green, α-tubulin in magenta, and DNA in blue. Bars = 2 μm.



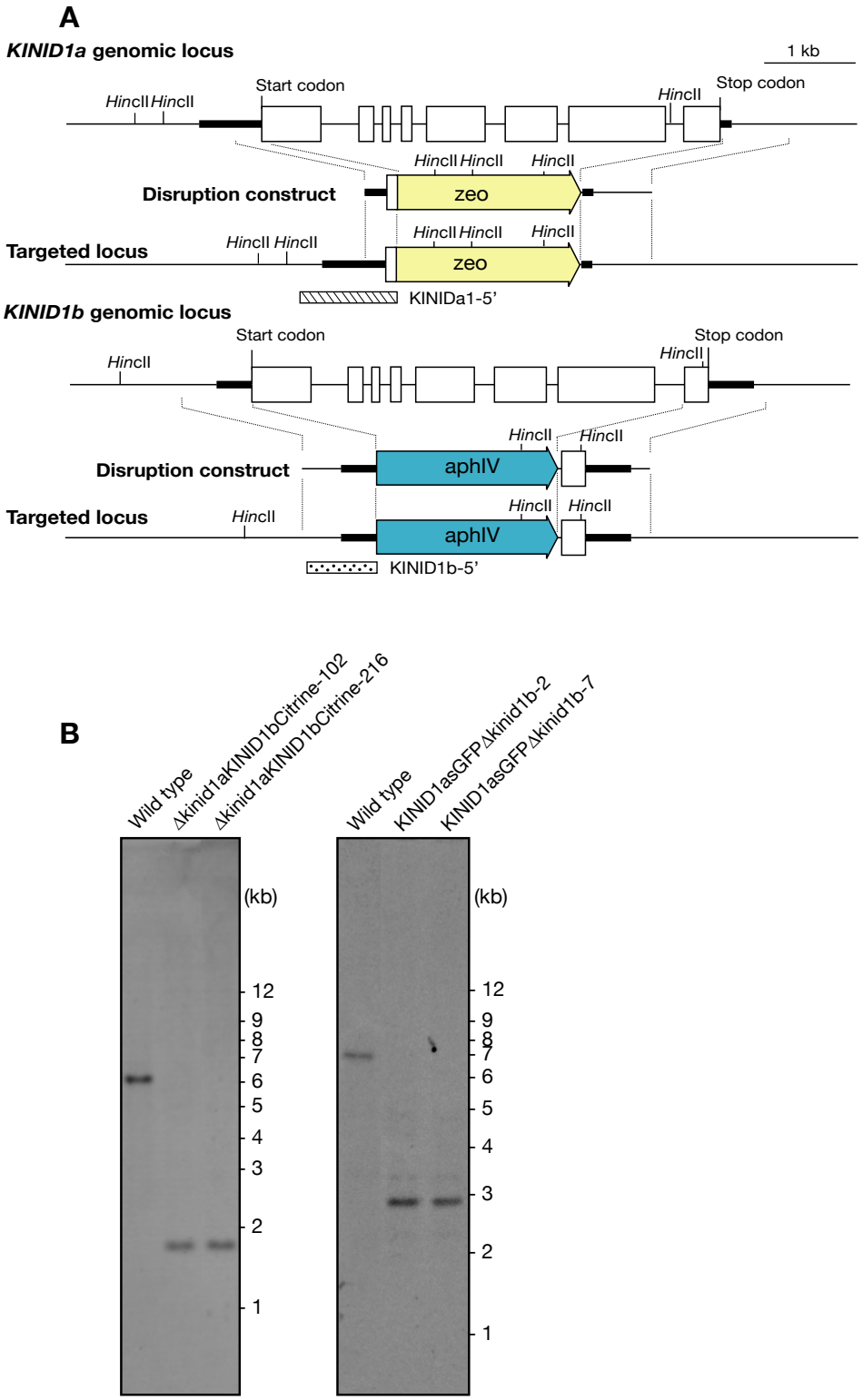
Supplemental Figure 7. Construction of the KINID1a-sGFP and KINID1b-Citrine Lines.

(A) Schematics of targeting the *KINID1a* and *KINID1b* loci. White boxes represent the *KINID1a* and *KINID1b* coding regions. Closed boxes indicate the 5' and 3' untranslated regions. Probes used in **(B)** are indicated by hatched and dotted boxes. Green and yellow arrows denote the *sGFP* (*sGFP*; Chiu et al., 1996) and *Citrine* genes (*Citrine*; Griesbeck et al., 2001), respectively. A gray box and a pink arrow indicate the nos terminator (nos-ter; Nishiyama et al., 2000) and the neomycin phosphotransferase II expression cassette (nptII; Nishiyama et al., 2000), respectively. **(B)** DNA gel-blot analysis of targeted lines. Genomic DNA of wild type, KINID1a-sGFP (right panel), and KINID1b-Citrine (left panel) was digested with *Bgl*II.



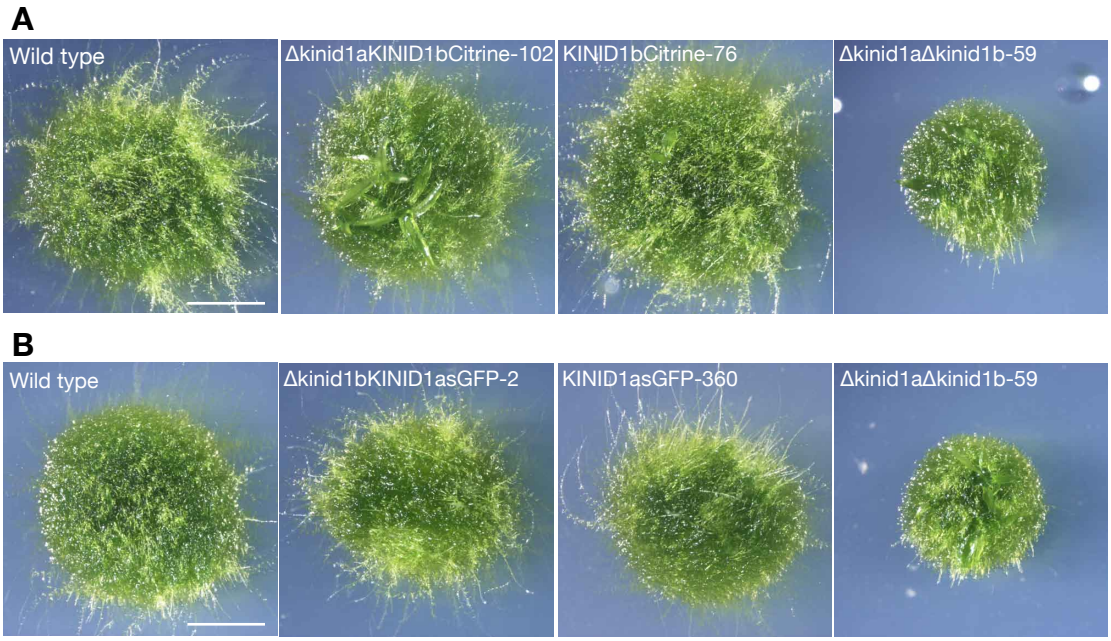
Supplemental Figure 8. Localized Fluorescent Signals of KINID1a-sGFP and KINID1b-Citrine Fusion Proteins in Interphase.

(A) Caulonemal cells of KINID1asGFP-360 (left panels) and KINID1bCitrine-70 (right panels) lines. A bright field image (top), a fluorescence image (middle), and a composite image (bottom) for each line are shown. Arrows indicate signals in the cytoplasm. **(B)** Localization of signals in nuclei of caulonemal apical cells of KINID1asGFP-360 (left panels) and KINID1bCitrine-70 (right panels) lines. Fluorescence image of sGFP- or Citrine-expressing cells (top), fluorescence image of cells stained with Hoechst33342 (middle), and a merged image (bottom). **(C)** Time-lapse observations of the localization dynamics of the KINID1b-Citrine fusion protein. Images were recorded every 1 min. Frames from a time-lapse movie of a caulonemal apical cell in M-phase were selected. An image showing nuclear envelope breakdown is set at 0 min. The time progression before or after the breakdown is shown in the upper left corner of the images. The upper and lower panels show KINID1b-Citrine fluorescence and the bright field image, respectively. Bars = 50 μm in **(A)**, 20 μm in **(B)**, and 10 μm in **(C)**.



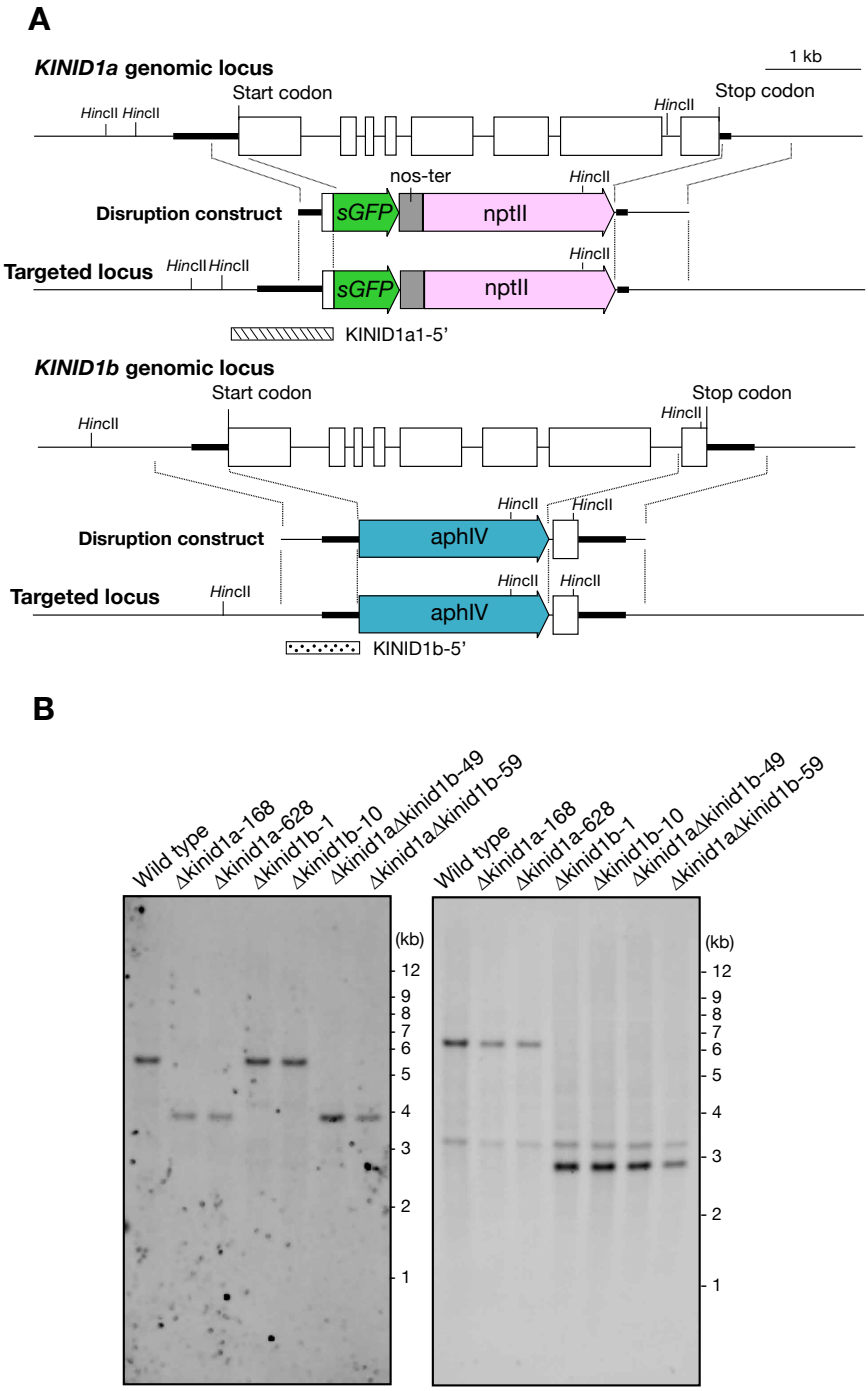
Supplemental Figure 9. Generation of *KINID1a*-Deletion Mutant Lines Expressing the KINID1b-Citrine Fusion Protein and *KINID1b*-Deletion Mutant Lines Expressing the KINID1a-sGFP Fusion Protein.

(A) Schematics of the disruption of the *KINID1a* and *KINID1b* loci. White boxes represent *KINID1a* and *KINID1b* coding regions. Closed boxes indicate 5' and 3' untranslated regions. Probes used for DNA-blot analyses are indicated by hatched and dotted boxes. A yellow arrow shows the bleomycin expression cassette (zeo; p35S-Zeo [EF451822]) and a blue arrow indicates the hygromycin phosphotransferase expression cassette (aphIV; pTN86 [AB267705]). **(B)** DNA gel-blot analyses of the deletion lines. Genomic DNA of wild type and deletion lines was digested with *Hin*cII and hybridized with KINID1a-5' (left panel) and KINID1b-5' (right panel) probes.

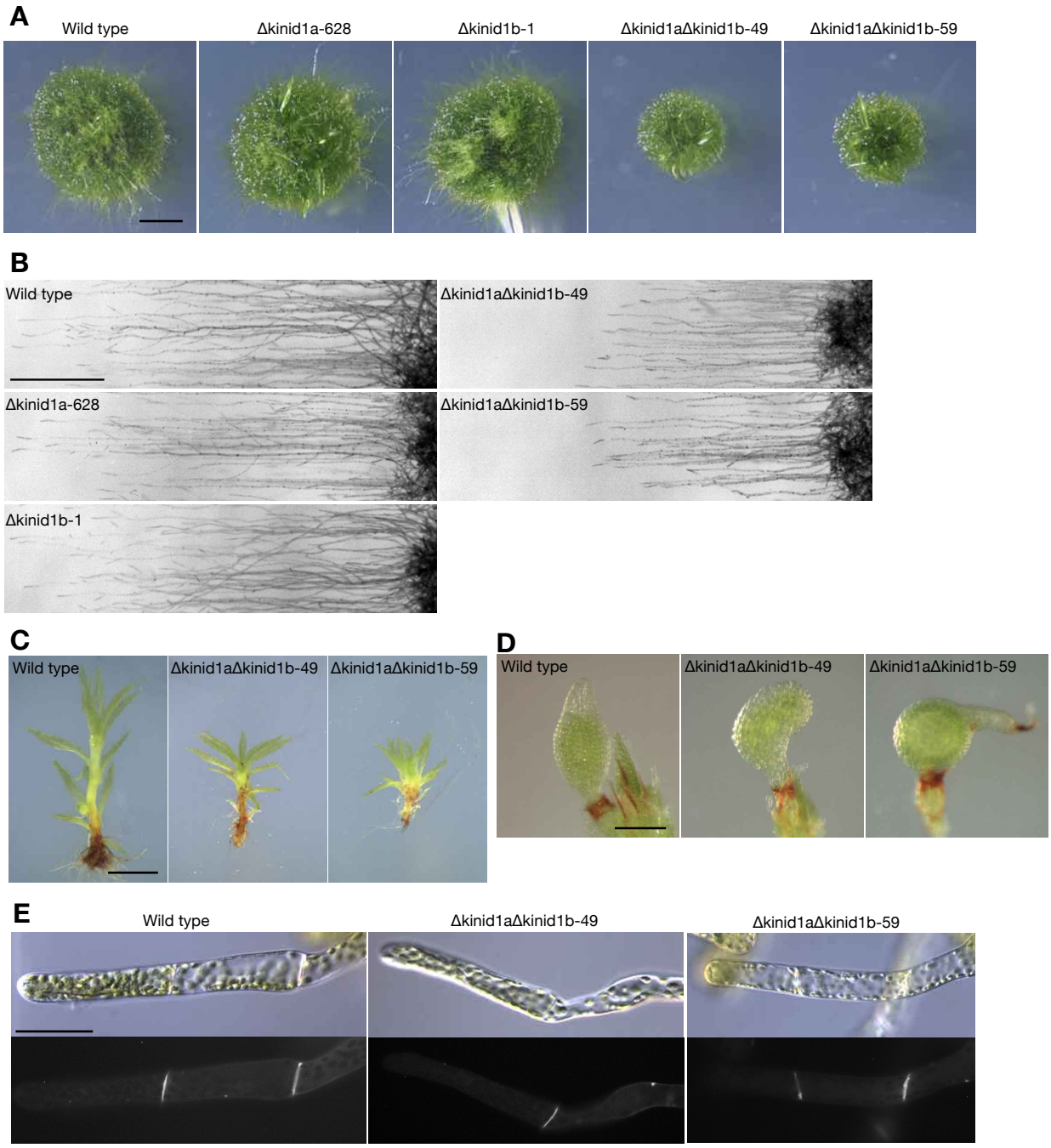


Supplemental Figure 10. Colony of the *KINID1a* Deletion Mutant Line Expressing the KINID1b-Citrine Fusion Protein and the *KINID1b* Deletion Mutant Line Expressing the KINIDa1-sGFP Fusion Protein.

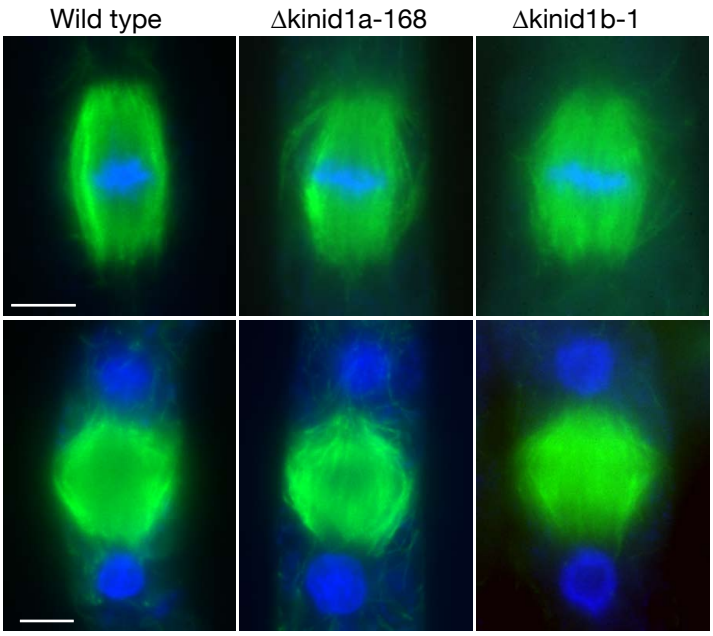
(A) Colony of the wild type, the *KINID1a* single-deletion mutant line expressing the KINID1b-Citrine fusion protein, the line expressing the KINID1b-Citrine fusion protein, and the *KINID1a* and *KINID1b* double-deletion mutant line. **(B)** Colony of the wild type, the *KINID1b* single-deletion mutant line expressing the KINID1a-sGFP fusion protein, the line expressing the KINID1a-sGFP fusion protein, and the *KINID1a* and *KINID1b* double-deletion mutant line. Each colony was incubated for 10 days. Bars = 2 mm.



Supplemental Figure 11. Construction of *KINID1a* and *KINID1b* Deletion Mutant Lines.
(A) Schematic of the construction of the *KINID1a* and *KINID1b* deletion mutants. White boxes represent *KINID1a* and *KINID1b* coding regions. Closed boxes indicate 5' and 3' untranslated regions. Probes used for DNA gel-blot analyses are indicated by hatched and dotted boxes. A green arrow, a gray box, a pink arrow, and a blue arrow indicate *sGFP* gene (*sGFP*; Chiu et al., 1996), nos terminator (nos-ter; Nishiyama et al., 2000), neomycin phosphotransferase II expression cassette (nptII; Nishiyama et al., 2000), and aminoglycoside phosphotransferase IV cassette (aphIV; pTN86 [AB267705]), respectively. **(B)** DNA gel-blot analyses of the deletion lines. Genomic DNA of wild type and deletion lines was digested with *Hinc*II and hybridized with KINID1a-5' (left panel) or KINID1b-5' (right panel) probes.

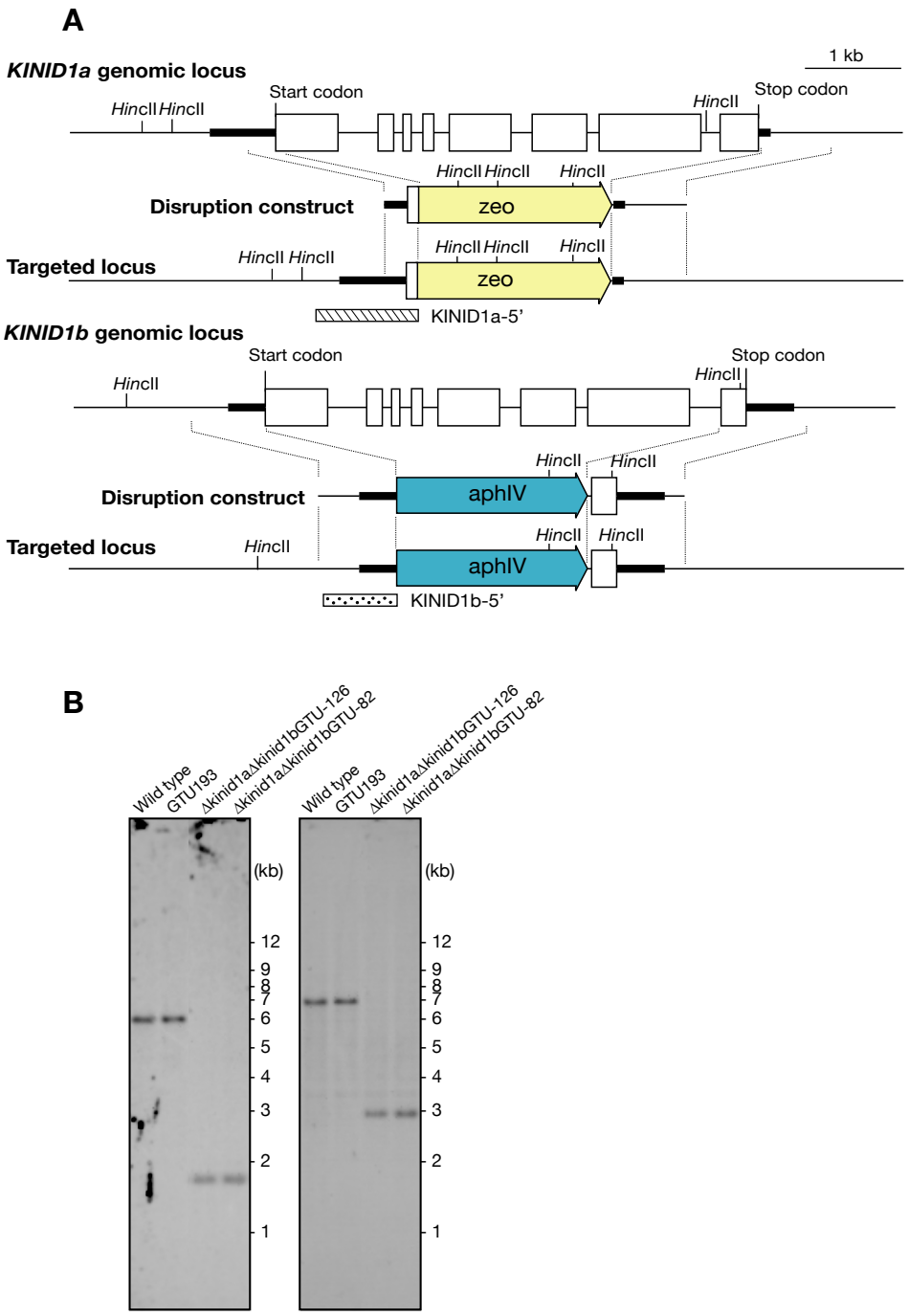


Supplemental Figure 12. Phenotypes of the *KINID1a* and *KINID1b* Deletion Mutants and the Wild type. (A) Protonemal colonies of *KINID1a* and *KINID1b* deletion mutants and wild type. *KINID1a* deletion mutant (Δkinid1a-628), *KINID1b* deletion mutant (Δkinid1b-1), and *KINID1a* and *KINID1b* double-deletion mutants (ΔkinidΔkinid1b-49 and 59) are shown. Each colony was incubated for 10 days after inoculation. **(B)** Red-light-grown protonemata of the *KINID1a* deletion mutant (Δkinid1a-628), *KINID1b* deletion mutant (Δkinid1b-1), *KINID1a* and *KINID1b* double-deletion mutants (ΔkinidΔkinid1b-49 and 59), and wild-type. Protonemal cells were incubated under unilateral red light for 7 days after inoculation. **(C)** Gametophores of the double-deletion mutants (ΔkinidΔkinid1b-49 and 59) and wild type. Gametophores with 11 leaves are shown. **(D)** Sporophytes of the double-deletion mutants (ΔkinidΔkinid1b-49 and 59) and wild type. **(E)** Caulonemal cells stained with aniline blue. The wild type (left), the ΔkinidΔkinid1b-49 line (middle), and ΔkinidΔkinid1b-59 line (right) are shown. Bars = 2 mm in **(A)**, 1 mm in **(B)** and **(C)**, 200 μm in **(D)**, and 50 μm in **(E)**.



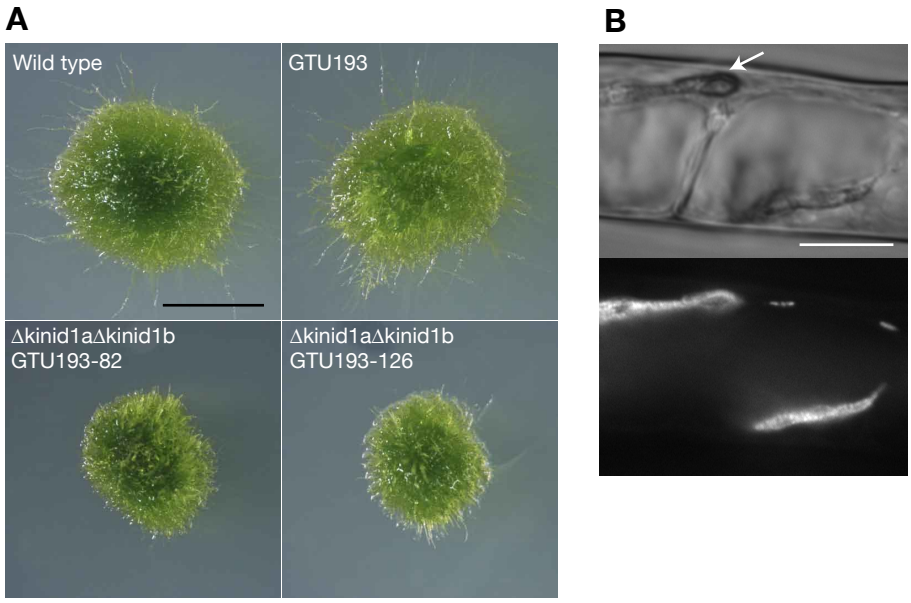
Supplemental Figure 13. A Spindle and Phragmoplast in the *KINID1a* and *KINID1b* Single-Deletion Mutants and the Wild Type.

Top and bottom panels show a spindle and a phragmoplast, respectively. Microtubules and DNA were stained with anti-α-tubulin antibody (green) and with DAPI (blue), respectively. A bar = 5 μm.



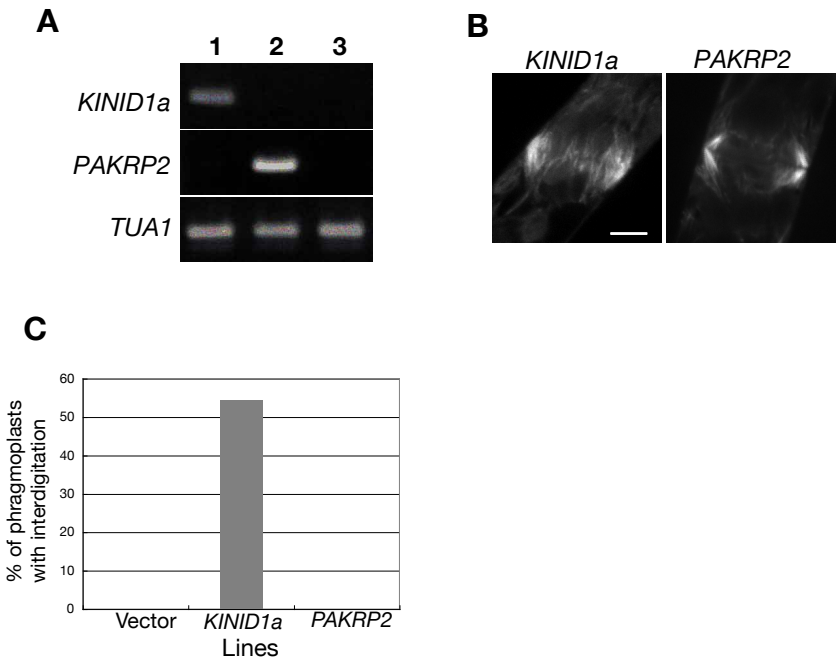
Supplemental Figure 14. Generation of *KINID1a* and *KINID1b* Double-Deletion Mutant Lines Expressing the sGFP-α-tubulin Fusion Protein.

(A) Schematics of the disruption of the *KINID1a* and *KINID1b* loci. White boxes represent *KINID1a* and *KINID1b* coding regions. Closed boxes indicate 5' and 3' untranslated regions. Probes used for DNA-blot analyses are indicated by hatched and dotted boxes. A yellow arrow shows the bleomycin expression cassette (zeo; p35S-Zeo [EF451822]) and a blue arrow indicates the hygromycin phosphotransferase expression cassette (aphIV; pTN86 [AB267705]). **(B)** DNA gel-blot analyses of the deletion lines. Genomic DNA of wild type and deletion lines was digested with *Hinc*II and hybridized with KINID1a-5' (left panel) and KINID1b-5' (right panel) probes.



Supplemental Figure 15. Phenotypes of the *KINID1a* and *KINID1b* Double-deletion Mutant Lines Expressing the sGFP-α-tubulin Fusion Protein.

(A) Protonemal colonies of wild type, the mutant line expressing the fusion protein of sGFP (Chiu et al., 1996) and α-tubulin (GTU193), and *KINID1a* and *KINID1b* double-deletion mutants expressing the sGFP-α-tubulin fusion protein (ΔkinidΔkinid1bGTU193-82 and 126). Each colony was incubated for 10 days after inoculation. **(B)** An incomplete septum of the ΔkinidΔ kinid1bGTU193-82 line. A bright field image (top) and an image of chloroplast autofluorescence (bottom) are shown. An arrow indicates the chloroplast. Bars = 2 mm in **(A)**, and 10 μm in **(B)**.



Supplemental Figure 16. Complemetation test with KINID1a and PAKRP2 in the *KINID1a* and *KINID1b* double-deletion mutant line.

(A) RT-PCR for *KINID1a*, *PAKRP2*, and *TUA1* with cDNA using protonemata of the Δkinid1aΔ kinid1bGTU193-126 line transformed with the expresson vector containing *KINID1a* (1), *PAKRP2* (2), or no insert (3), together with the expression vector containing *DsRed2*. **(B)** Phragmoplasts of caulonemal cells in the line transformed with the expression vector containing *KINID1a* (left panel) or *PAKRP2* (right panel). A bar = 5 μm. **(C)** Percetage of observed phragmoplasts with the interdigitation. Eleven caulonemal cells were examined.

Supplemental Table 1. Primers used in this work.

Primer	Sequence
GUS-R2	5’-ATTTCACGGGTTGGGGTTTCTACAGGACGT-3’
adapter	5’-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT-3’
UAP	5’-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3’
229-2p1	5'-CTGGTGAGGACGAGGATTCC-3’
229-2p2	5'-ATGTCGTCTTGCTTATGCAG-3’
API1MF1	5'-GGTGCNGGNAARAGYCAYACNATG-3’
API1MF2	5'-ATCTAYAAYGARGARATHTAYGA-3’
API1MR1	5'-GARTAYGGNGCNAARGCNAAGTG-3’
API1MR2	5'-CAYARRATCATNARDATYTT-3’
api1LF5	5’-CGGTGTAAGTGATGGTTCGATGAG-3’
api1LR9	5’-TTGGGAAGGCACATTCCTCAG-3’
229-2p3SalI	5’-TGCTGTCGACGAAGCACCTC-3’
api1R400ClaI	5’-CGGGAGTTGGTCGACTTTCACAG-3’
api1F2820SalI	5’-GGACGCACAG TCGACTCACAGC-3’
api1R3940ClaI	5’-AGAAATGTATCGATGCAGCGAG-3’
api1LF12SphI	5’-GATAGCATGCAATCAATCTGCCTGATCTG-3’
api1LR11NotI	5’-TATTGCGGCCGCTTTCACCAATTGTCACTGC-3’
api1LF13XhoI	5’-AAGCTCGAGAACCGAGAATAACAACAATGACA-3’
api1LR390HindIII	5’-CTTAAGCTTGGGAGACATCATCGATGC-3’
M13-21	5’-TGTAAAACGACGGCCAGT-3’

api1LF4780ApaI	5’-TCCGGGGCCCCATACTACACCGATGT-3’
api1LR5760SalI	5’-GGAAGTCGACGATGCAACGAGTTGAGTTGACG-3’
api1F2820SalI	GGACGCACAGTCGACTCACAGC
api1R3940Cla	AGAAATGTATCGATGCAGCGAG
api1LF9	5’-TACCACAAATCCAGCCAATCAAG-3’
api1LR5220	5’-TCTCAAAGTCGCAGGACAAAAC-3’
229-2p4	5’-CAGCCTTCAAATCAGTGTCCC-3’
api1R400ClaI	5’-CGGGAGTTGGTCGACTTTCACAG-3’
api1LF10	5’-CTCGAGGGTGGTGCCATTTCCGA-3’
api1F3010SalI-1	5’-GTCAGGTCGACGGAAGTGATGC-3’
api1R3940STOPEco52I	5’-TATCCGGCCGTTAGATGCAGCGAGTAGAGT-3’
API1F300cacc	5’-CACCATGACAACGATGATGTCCCCGAA-3’
API1R3930	5’-GATGCAGCGAGTAGAGTTGACAATGTG-3’
AtAPI1F130	5’-CACCATGGCACCGACACCATCTTCTTC-3’
AtAPI1R2860NheI	5’-GAGAGCTAGCCAAAGGCTGCTACATC-3’
MS257-f1	5’-TGGAGAGGTTGTCGGTGGACTAC-3’
MS257stop	5’-TCAGTAGTCGTCGTCCTCCGGAC-3’
API1F1100	5’-TGCATGGTCACGCTTGATG-3’
API1R1800	5’-CTCAGCCCGTACCCTCATC-3’
AtAPI1F930	5’-CTGCATTATCATACTTGATGTGCC-3’
AtAPI1R1410	5’-AGCTGCTTTTGTGCCTCATTCC-3’

Supplemental methods

Construction of Plasmids for Gene Targeting

Primer sequences are listed in Supplemental Table 1 online. Construction of plasmids for gene targeting is described in Supplemental Methods online. All mutant lines obtained by gene targeting were verified by DNA gel-blot analysis.

For the sGFP-α-tubulin knock-in line, *sGFP* was cloned from pTH-2 (Chiu et al., 1996), and a DNA fragment covering the coding region of α-tubulin was PCR-amplified using a full-length cDNA clone as a template, digested with *Spe*I and *Xho*I, and cloned into the *Spe*I-*Xho*I site of p35S-sGFP-ad2-talin (generous gift of Dr. T. Kagawa) to make p35S-sGFP-ad2-tubulin. A *Sal*I-*Xho*I fragment excised from p35S-sGFP-ad2-tubulin was blunted and cloned into an *Eco*RV site of pTN90 containing the rice *Actin* promoter (McElroy et al., 1990), an rbcS terminator, a modified nptII cassette from pTN80, and genomic fragments of a homeobox protein from *P. patens* (*HB7*). The plasmid was digested with *Not*I for gene targeting.

To construct KINID1a-sGFP lines, an *KINID1a* DNA fragment spanning the 843rd residue to the C-terminus just prior to the stop codon was PCR-amplified with api1F2820SalI and api1R3940ClaI primers, digested with *Sal*I and *Cla*I, and cloned into the *Sal*I and *Cla*I site of pGFPmutNPT containing the coding region of *sGFP* (Chiu et al., 1996) whose start codon was replaced with the sequence TTG, and an NPTII cassette (Nishiyama et al., 2000), thereby creating an in-frame fusion of the *KINID1a* coding sequence and sGFP to produce pAPI1GFP-5'. A 1.1-kb genomic DNA fragment

downstream of a stop codon of *KINID1a* was amplified by TAIL-PCR (Liu et al., 1995), cloned into pGEM-T (Promega), and named p1-1. The *Not*I-*Sac*II DNA fragment of p1-1 was cloned into the *Not*I and *Sac*II sites of pAPI1GFP-5' to make pAPI1GFP. The pAPI1GFP was digested with *Apa*I and *Not*I for gene targeting.

pCit-npt containing a coding region for Citrine (Griesbeck et al., 2001) with the start codon replaced by the sequence TTG, the nopaline synthase polyadenylation signal (nos-ter), and an NPTII cassette (Nishiyama et al., 2000) flanked by two loxP sites was used to construct the KINID1b-Citrine lines. A *KINID1b* DNA fragment spanning the 974th residue to the C-terminus just prior to a stop codon was amplified with api1LF4780ApaI and api1LR5760SalI primers. The amplified fragment was digested with *Apa*I and *Sal*I and cloned into the *Apa*I and *Sal*I site of pCit-npt, thereby creating an in-frame fusion of the coding sequence of *KINID1b* and *Citrine* resulting in the plasmid pAPI1LCitrine-5'. A 1.1-kb genomic DNA fragment downstream of a stop codon of *KINID1b* was amplified with api1LF12SphI and api1LR11NotI primers and cloned into pGEM-T (Promega) to make pAPI1L-3'. The *Sph*I-*Not*I fragment of pAPI1L-3' was cloned into the *Sph*I-*Not*I sites of pAPI1LCitrine-5' to produce pAPI1LCitrine. The pAPI1LCitrine was digested with *Apa*I and *Not*I for gene targeting.

To construct the mRFP-α-tubulin lines, we cloned a PCR fragment of mRFP (Campbell et al., 2002) into the *Aor*51HI site of pUGW0 (Nakagawa et al., 2007) and digested the resultant plasmid with *Xba*I and *Sac*I to obtain a fragment containing mRFP and a gateway cassette (Invitrogen). We blunted the fragment and cloned it into

the *Hpa*I site of the pCMAK1 plasmid containing the E7113 promoter (Mitsuhara et al., 1996), a nos terminator from pTH-2 (Chiu et al., 1996), a zeocin resistant cassette from p35S-Zeo (EF451822), and a *P. patens* genomic region from the lamda213 clone (Schaefer and Zryd, 1997). The plasmid so formed was named pBS213-7113-mRFP-G. A PCR-fragment covering an α-tubulin coding region amplified using p35S-GFP-ad2-tubulin as a template was cloned into pENTR/D-TOPO (Invitrogen) and inserted into pBS213-7113-mRFP-G using the Gateway LR Clonase enzyme mix (Invitrogen) to make p7113-mRFP-tub. The p7113-mRFP-tub was linearized with *Kpn*I for gene targeting.

To delete the *KINID1a* gene, a genomic DNA fragment of *KINID1a* was amplified using 229-2p3SalI and api1R400ClaI primers. An amplified fragment was digested with *Sal*I and *Cla*I and cloned into the *Kpn*I-*Sal*I site of the pGFPmut-NPTII plasmid and named pAPI1dis-5'. A *Not*I-*Sac*II fragment excised from p1-1 was inserted into the *Not*I-*Sac*II site of pAPI1dis-5' to make pAPI1dis. A fragment covering *sGFP* and an NPTII cassette of the pAPI1dis was replaced with a zeocin resistant cassette from p35S-Zeo (EF451822) with *Cla*I and *Not*I to make pAPI1-zeo. The pAPI1dis and the pAPI1-zeo were linearized with *Sal*I and *Sac*II for gene targeting.

To delete the *KINID1b* gene, a genomic DNA fragment was amplified with api1LF12SphI and api1LR11NotI primers. The amplified fragment was digested with *Sph*I and *Not*I and cloned into the *Sph*I-*Not*I site of pTN86 to make papi1Ldis-3'. A genomic DNA fragment was amplified with api1LF13XhoI and api1LR390HindIII

primers. The amplified fragment was digested with *Xho*I and *Hin*dIII, resulting in two fragments (138 and 766 bp). The 766 bp-fragment was cloned into the *Xho*I and *Hin*dIII sites of pAPI1Ldis-3' to make pAPI1Ldis. The fragment was amplified with M13-21 and api1LR11NotI primers using pAPI1Ldis as a template and digested with *Xho*I. This fragment was used for gene targeting.

DNA Gel-Blot Analysis

Procedures for nucleic acids extraction were described previously (Hiwatashi et al., 2001). Genomic DNA was digested with restriction enzymes, run on agarose gels, and transferred to nylon membranes according to previously described procedures (Hiwatashi et al., 2001). Probe labeling, hybridization, and detection were performed using an AlkPhos Direct Labeling and Detection System (GE Healthcare) according to the supplier's instructions. A PCR fragment amplified using pAPI1GFP as template with the api1F2820SalI and the api1R3940Cla primers was used as the KINID1aC probe. A PCR fragment amplified using genomic DNA of wild-type *P. patens* as template with the api1LF9 and the api1LR5220 primers, and with the 229-2p4 and the api1R400ClaI primers was used as the KINID1bC and KINID1a-5' probe, respectively. A PCR-fragment amplified using pAPI1Ldis as a template with the api1LF10 and the api1LR390HindIII primer was used as the KINID1b-5' probe.

Complementation Test with KINID1a and PAKRP2 in the *KINID1a* and

***KINID1b* Double-Deletion Mutant Line.**

We used pBS213-7113-GHA plasmid containing a E7113 promoter (Mitsuhara et al., 1996), a gateway cassette and a HA tag from pGWB14 (Nakagawa et al., 2007), a nos terminator from pTH-2 (Chiu et al., 1996), a zeocin resistant cassette from p35S-Zeo (EF451822), and a *P. patens* genomic region from the lamda213 clone (Schaefer and Zryd, 1997). A PCR-fragment covering a putative start codon to a stop codon of *KINID1a* and *PAKRP2* was amplified with the API1F300cacc and API1R3930 primers and the AtAPI1F130 and AtAPI1R2860NheI primers, respectively. The amplified fragment was cloned into pENTR/D-TOPO (Invitrogen) and inserted into pBS213-7113-GHA using the Gateway LR Clonase enzyme mix (Invitrogen) to make p7113-KINID1a and p7113-PAKRP2.

The *KINID1a* and *KINID1b* double-deletion mutant line, Δkinid1aΔkinid1bGTU193-126, was transformed with p7113-KINID1 or p7113-PAKRP2, together with a pTKM-DsRED2 containing the rice *Actin* promoter (McElroy et al., 1990), DsRed2 (Clontech Laboratories Inc.), and an rbcS terminator as a visual marker. The transformed protoplasts were incubated on PRM/B (Nishiyama et al., 2000) supplemented with 2% (w/v) sucrose for 3 days and then transferred on BCD plates with 1 mM $CaCl_2$ (Nishiyama et al., 2000). After incubation for 7 days under polarized white light, the regenerated protonemata showing the DsRed2 fluorescence were observed.

For RT-PCR, total RNA was extracted from regenerated protonemata using the RNeasy Micro kit (QIAGEN) and subjected to on-column DNase treatment according to the Manufacture's instructions (QIAGEN). The cDNA was synthesized from 0.1 μg of total RNA using Superscript III First-strand systhesis system for RT-PCR (Invitrogen). PCR was performed with Blend Taq (TOYOBO) using the API1F1100 and API1R1800 primers, the AtAPI1F930 and AtAPI1R1410 primers, and the MS257-f1 and MS257stop primers for *KINID1a*, *PAKRP2*, and *TUA1*, respectively.

Accession Numbers

Sequence data from this article can be found in GenBank/EMBL data libraries under the following accession numbers: *Physcomitrella patens* genes *TUA1* (AB096718), *HB7* (AB028078); pTN80 (AB267704); pTN86 (AB267705); p35S-Zeo (EF451822).

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