

## **New *Phytologist* Supporting Information:**

**Article title:** DEK1 displays a strong subcellular polarity during *Physcomitrella patens* three-dimensional growth

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**Article acceptance date:** 24 December 2019

The following Supporting Information is available for this article:

**Figure S1:** Cre recombinase treatment evaluation.

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**Figure S3:** Bud count analysis of *dek1-tomato<sup>int</sup>* and *dek1-tomato<sup>int</sup>/dek1<sup>o</sup>*.

**Figure S4:** Gametangia development is morphologically similar between WT and *dek1-tomato<sup>int</sup>*.

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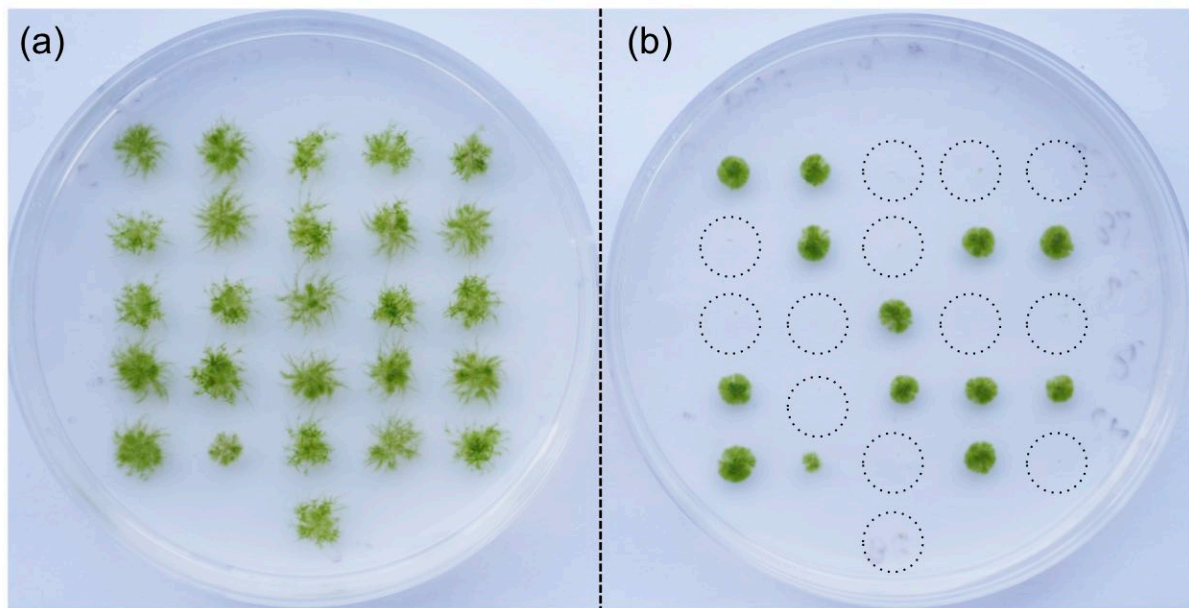
**Figure S9:** DEK1-TOMATO<sup>INT</sup> during antheridia development.

**Figure S10:** Schematic representation of the *dek<sup>o</sup>* generation in *dek1-tomato<sup>int</sup>* background and transformant PCR genotyping.

**Figure S11:** *dek1-tomato<sup>int</sup>/dek1<sup>o</sup>* transcript analysis.

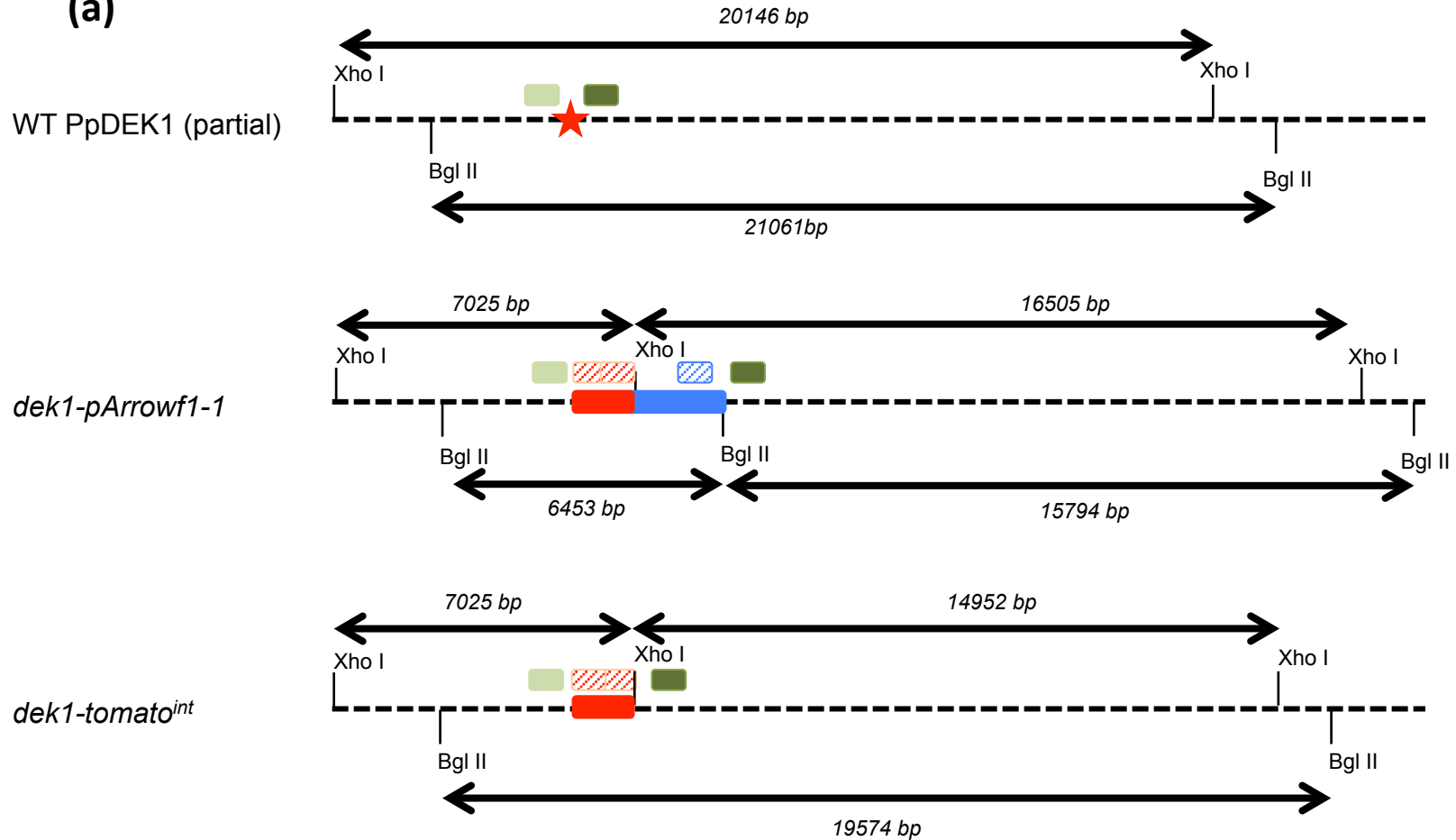
**Figure S12:** *dek1-tomato<sup>int</sup>/dek1<sup>o</sup>* transcript sequence analysis.

**Table S1:** List of the primers used in this study



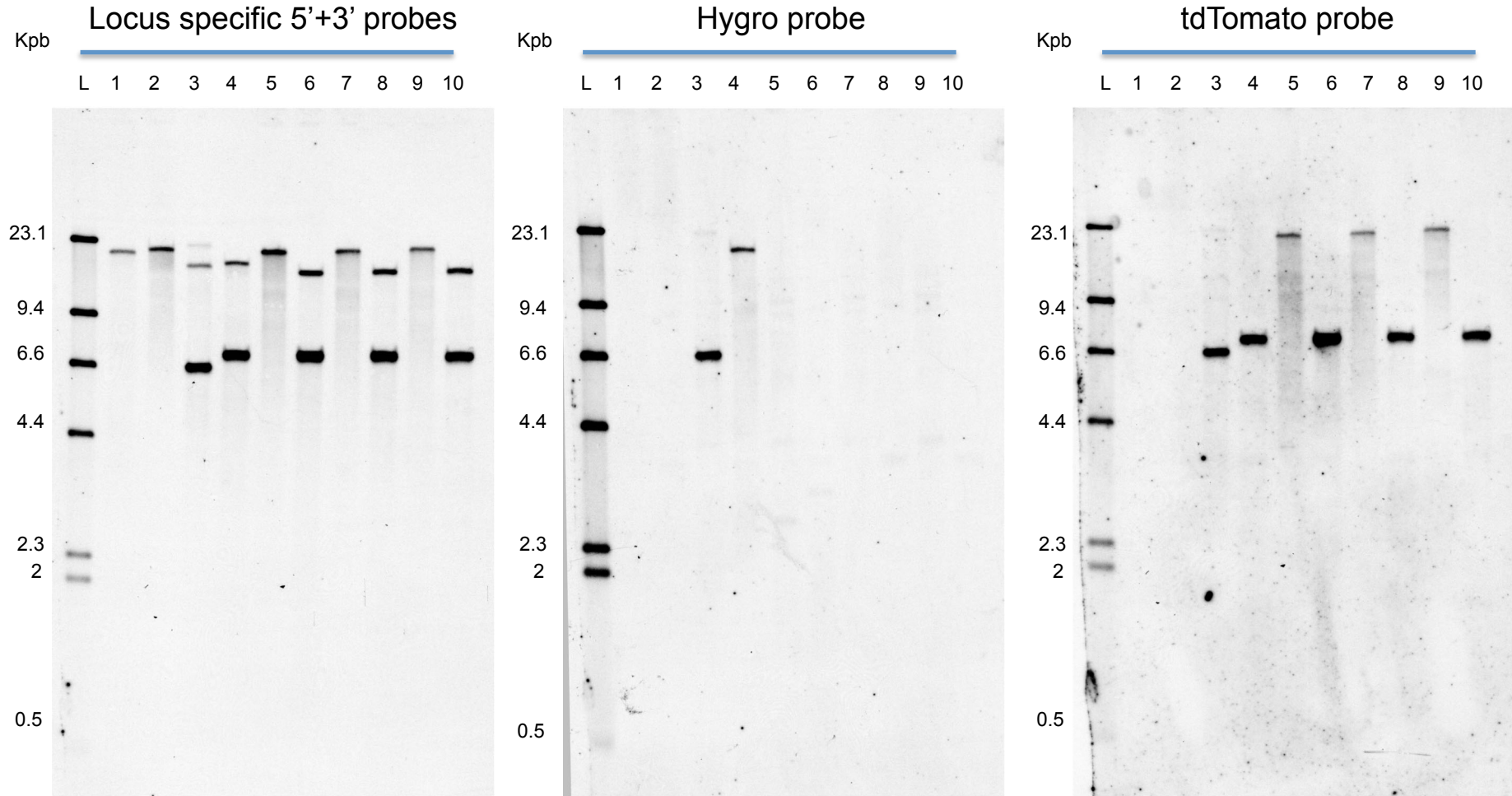
**Figure S1:** Cre recombinase treatment evaluation. Regenerated *dek1-pArrowf1-1* protoplasts transiently transformed with the Cre recombinase were duplicated and inoculated unto (a) BCDA and (b) BCDA+ 25 µg/ml hygromycin B. Pictures were taken after 15 days of growth in standard conditions. Dotted circles in (b) point to inocula that died on selective medium without displaying any growth, indicating the successful resistance cassette excision.

(a)

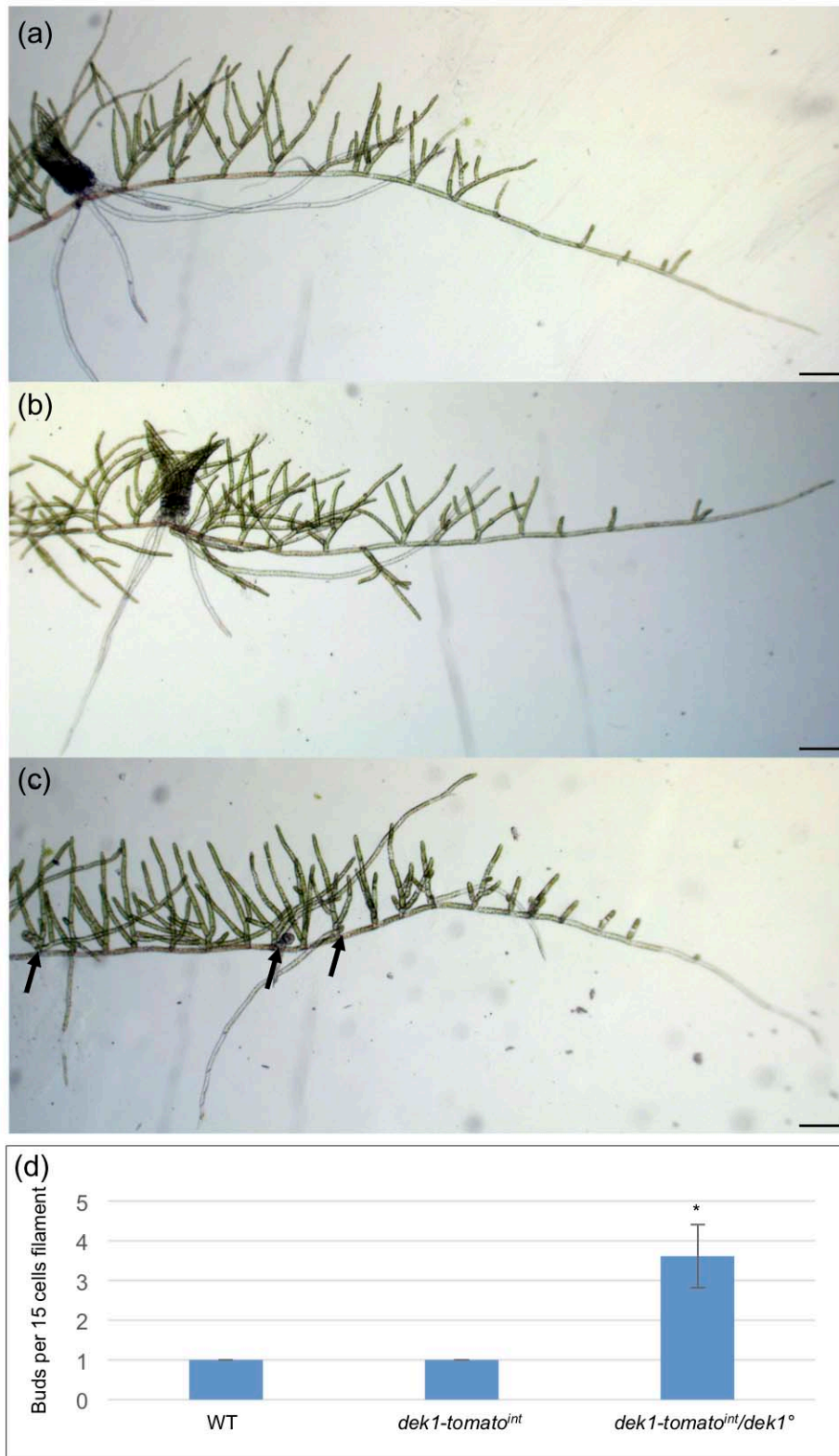


**Figure S2:** Southern blot analysis of *dek1-pArrowf1-1* and *dek1-tomato<sup>int</sup>* strains. (a) Illustration of the restriction cut pattern predictions for WT, *dek1-pArrowf1-1* and *dek1-tomato<sup>int</sup>* strains at *tdTomato* insertion locus. Dashed line: *Physcomitrella patens* genome. Red star: insertion site of *tdTomato* sequence in WT. Bold line with arrow: DNA fragment detected by the different probes with predicted size in base pairs. Red box: *tdTomato* sequence. Blue box: hygromycin resistance cassette. Light green box: 5' probe. Dark green box: 3' probe. Dashed red box: *tdTomato* probe. Dashed blue box: hygromycin probe.

**(b)**

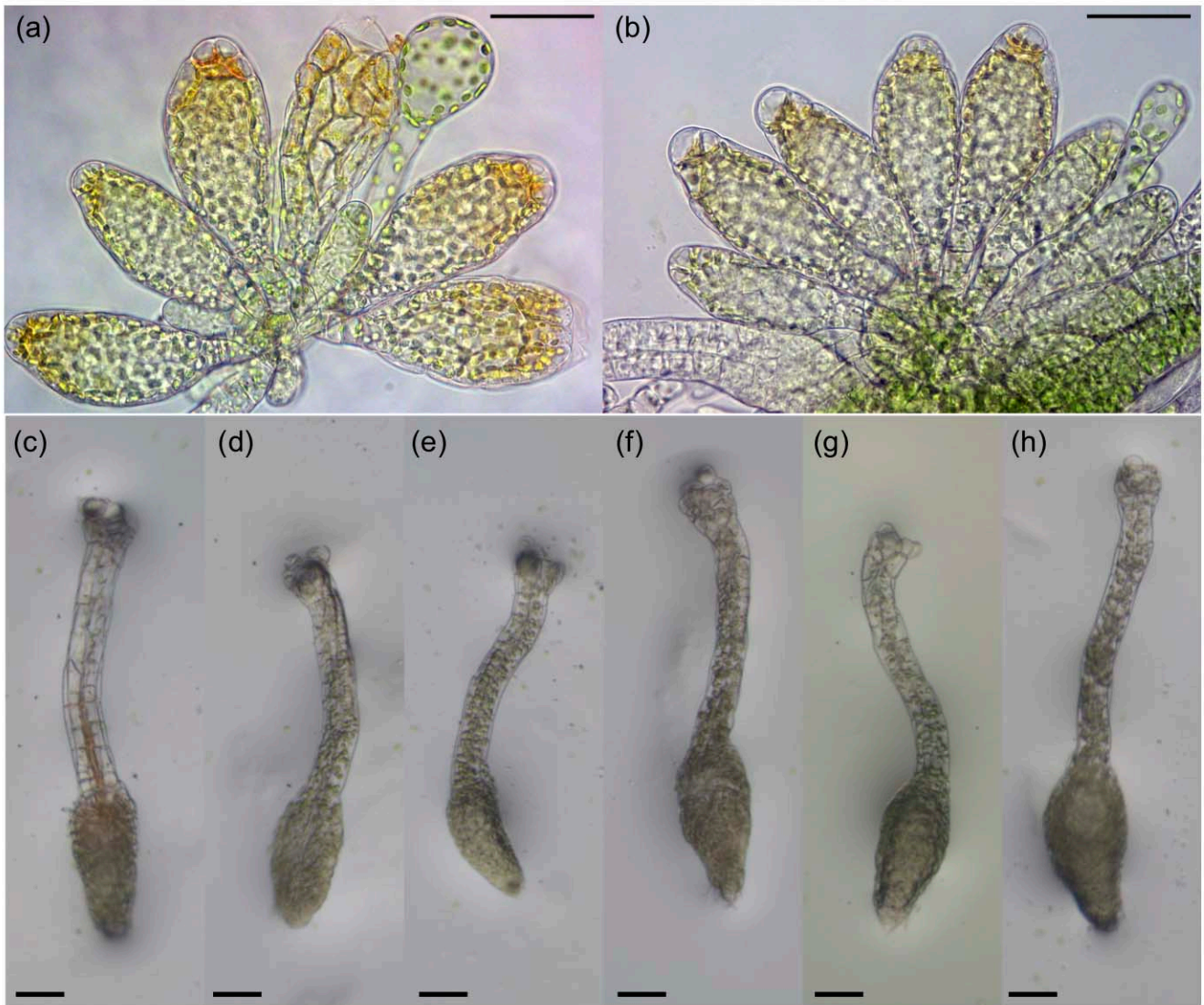


**Figure S2:** Southern blot analysis of *dek1-pArrowf1-1* and *dek1-tomato<sup>int</sup>* strains. (b) L: Lambda phage cut with Hind III. Genomic DNAs: lanes 1,2 WT, lanes 3,4: *dek1-pArrowf1-1*; 5,6: *dek1-tomato<sup>int</sup>-1*, lanes 7,8: *dek1-tomato<sup>int</sup>-2*, lanes 9,10: *dek1-tomato<sup>int</sup>-3*. Restriction digests: lanes 1, 3, 5, 7 and 9: Bgl II (not a cutter of WT and *dek1-tomato<sup>int</sup>*, cutter of *dek1-pArrowf1-1*); lanes 2, 4, 6, 8 and 10: Xho I (not a cutter of WT cutter of both transgenic types).

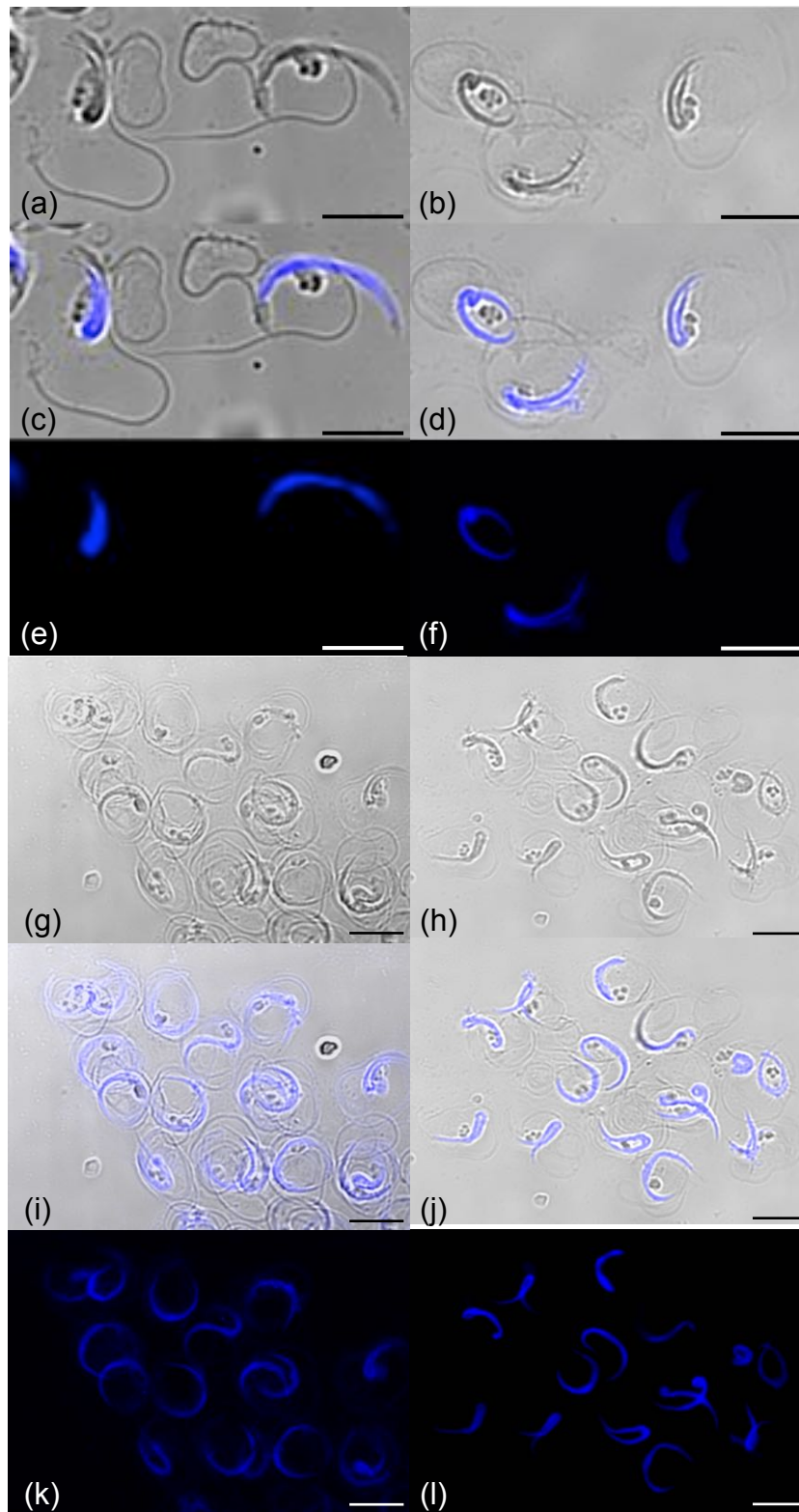


**Figure S3:** Bud count analysis of *dek1-tomato<sup>int</sup>* and *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>*. (a) Two week-old WT filament showing a single developing gametophore. (b) Two week-old *dek1-tomato<sup>int</sup>* filament showing a single developing gametophore. (c) Two week-old *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>* filament showing multiple small buds (black arrows). Bar : 200  $\mu$ m. (d) Buds were counted on two week-old cultured filaments with more than 15 cells. Data were acquired on three independent cultures for each strain,  $n = 105$  for each strain, error bar shows the standard deviation, \* denotes a significant difference in bud number between *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>* and both WT and *dek1-tomato<sup>int</sup>* using T-test,  $p$  value  $< 0.01$ .



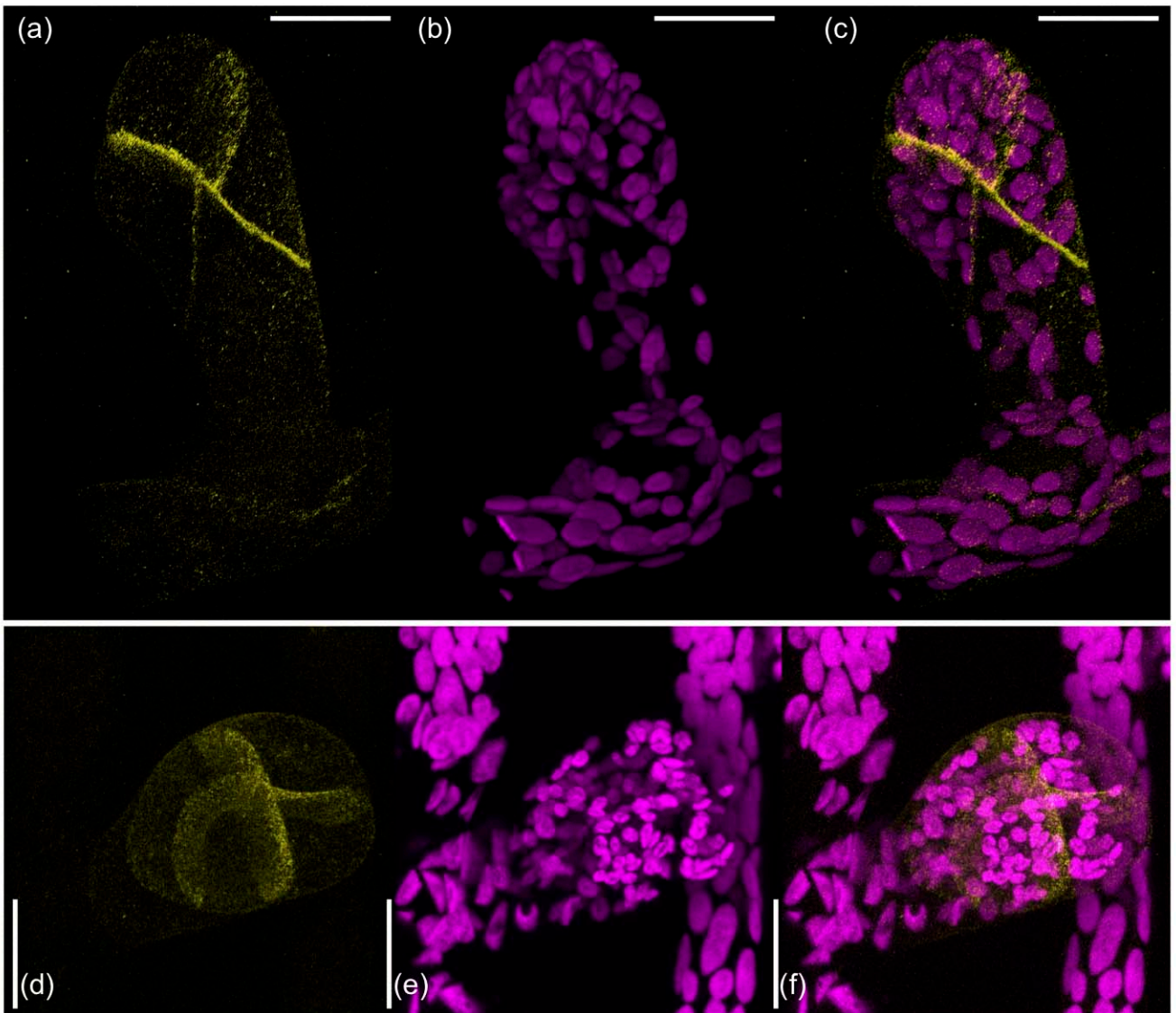


**Figure S4:** Gametangia are morphologically similar between WT and *dek1-tomato<sup>int</sup>*. (a) Antheridia bundle from WT isolated 21 days after culture transfer to 15°C. (b) Antheridia bundle from *dek1-tomato<sup>int</sup>* isolated 21 days after culture transfer to 15°C. Bar in (a) and (b): 10  $\mu$ m. (c-e) Mature archegonia with fertilization canal open from WT isolated between 21 and 30 days after transfer to 15°C. (f-h) Mature archegonia with fertilization canal open *dek1-tomato<sup>int</sup>* isolated between 21 and 30 days after transfer to 15°C. Bar in (c-h): 50  $\mu$ m. Albeit gametangia from both strains appear similar, *dek1-tomato<sup>int</sup>* is self-sterile.



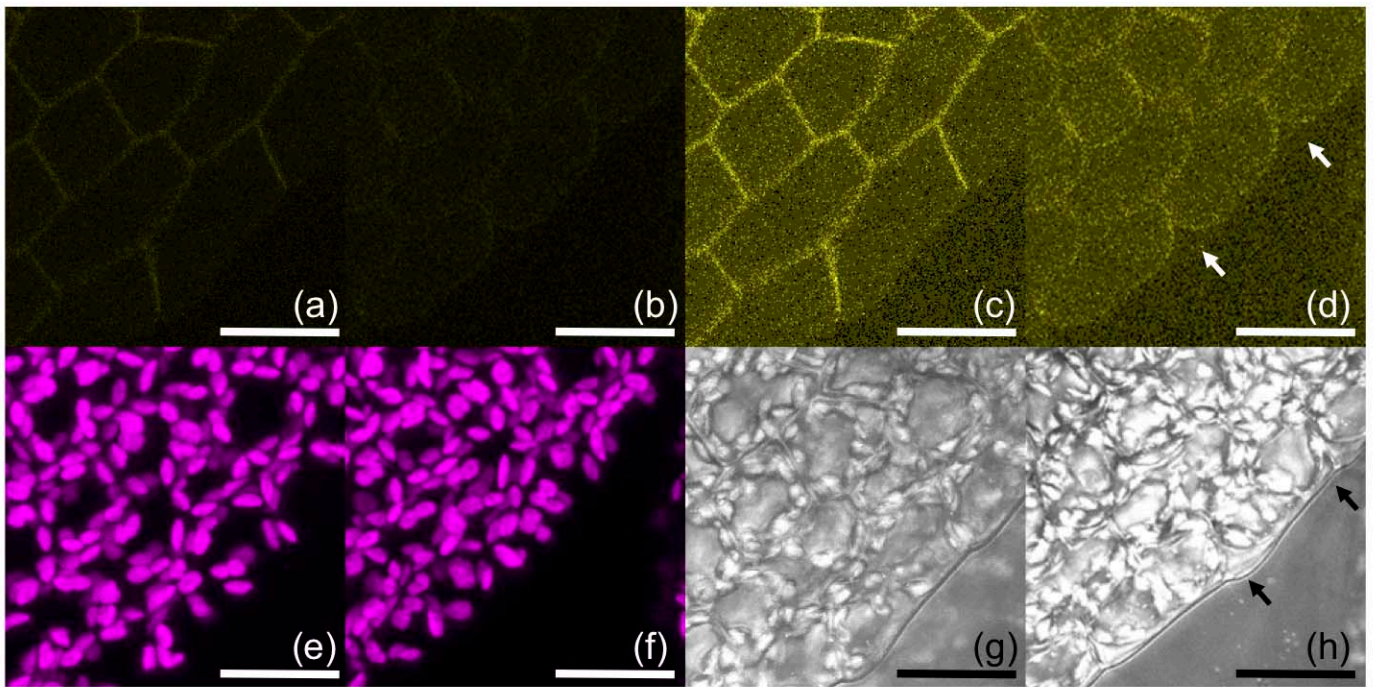
**Figure S5:** Spermatozooids are morphologically similar in WT and *dek1-tomato<sup>int</sup>*. Mature spermatozooids from both strains display the moss biflagellate motile apparatus, the fasciculate nucleus (DAPI-stained) and the plastid and mitochondrial bodies. Spermatozooids from WT (a, c, e, g, i, k) and *dek1-tomato<sup>int</sup>* (b, d, f, h, j, l) isolated 21 days after culture transfer to 15°C. (a, b, g, h): white light image; (c, d, i, j): merged white light and DAPI staining fluorescence images. (e, f, k, l): DAPI staining fluorescence images. Bar in all panels : 10  $\mu$ m.



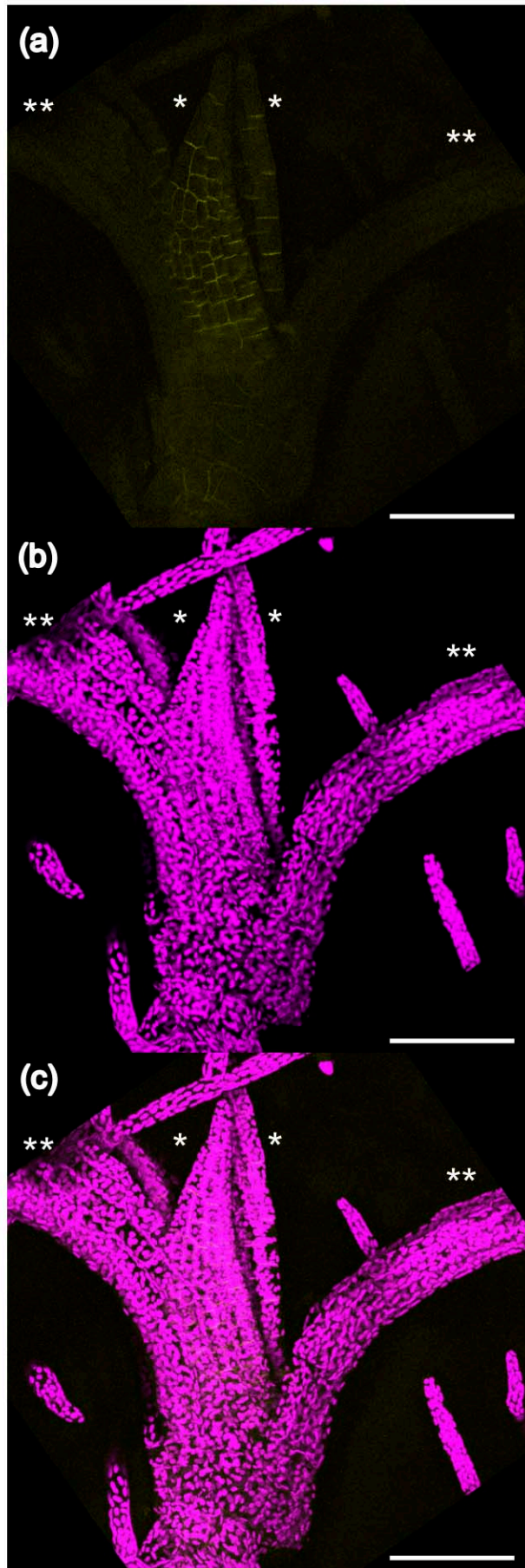


**Figure S6:** DEK1-TOMATO<sup>INT</sup> fluorescent signal during bud formation. DEK1-TOMATO<sup>INT</sup> signal at four (a-c) and five (d-f) celled bud stage. During the early stage the signal remains weak and is restricted to the contiguous cell face of bud cell. (a, d): DEK1-TOMATO<sup>INT</sup> specific fluorescent signal. (b, e): Chlorophyll auto-fluorescence specific signal. (c, f) merged picture with both fluorescent signals. Bar in all images: 20  $\mu$ m.



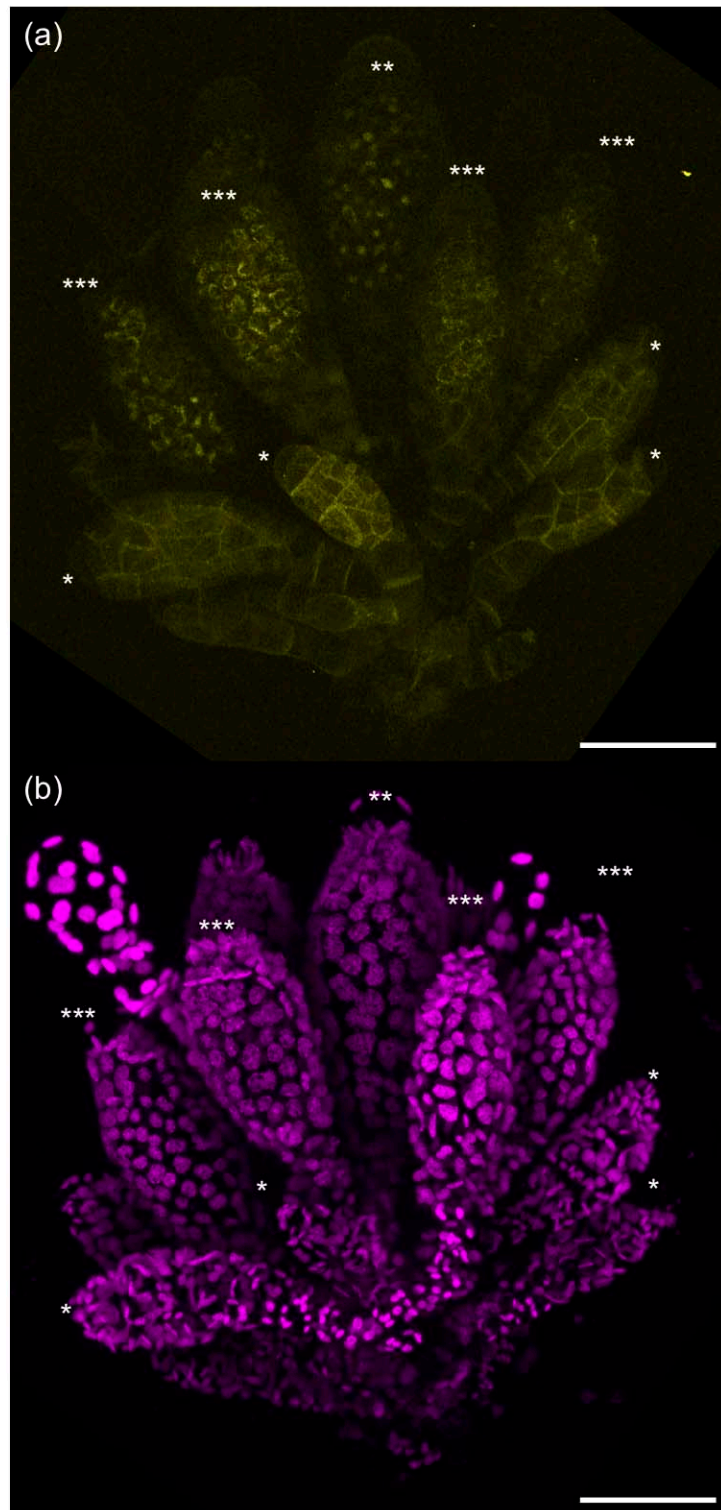


**Figure S7:** DEK1-TOMATO<sup>INT</sup> localizes at the plasma membrane. Lateral detail of a *dek1-tomato<sup>int</sup>* phyllid in water (a, c, e and g) and after 30 sec in mannitol 8,5% (b, d, f and h). (a, b) DEK1-TOMATO<sup>INT</sup> specific fluorescent signal in non saturated condition. (c, d) same image as (a,b), but DEK1-TOMATO<sup>INT</sup> specific fluorescent overexposed signal. (e, f): Chlorophyll auto-fluorescence specific signal, (g, h): False color projection. Arrows point to the plasma membrane detachment from the cell wall under plasmolysis condition. Note that the signal decreases rapidly upon treatment. (a-f) : images are maximal projection of the confocal stack spanning the depth of the phyllid. (g, h): images are single slice acquisition median to the the stack used in (a-f). Bar: 20  $\mu$ m.

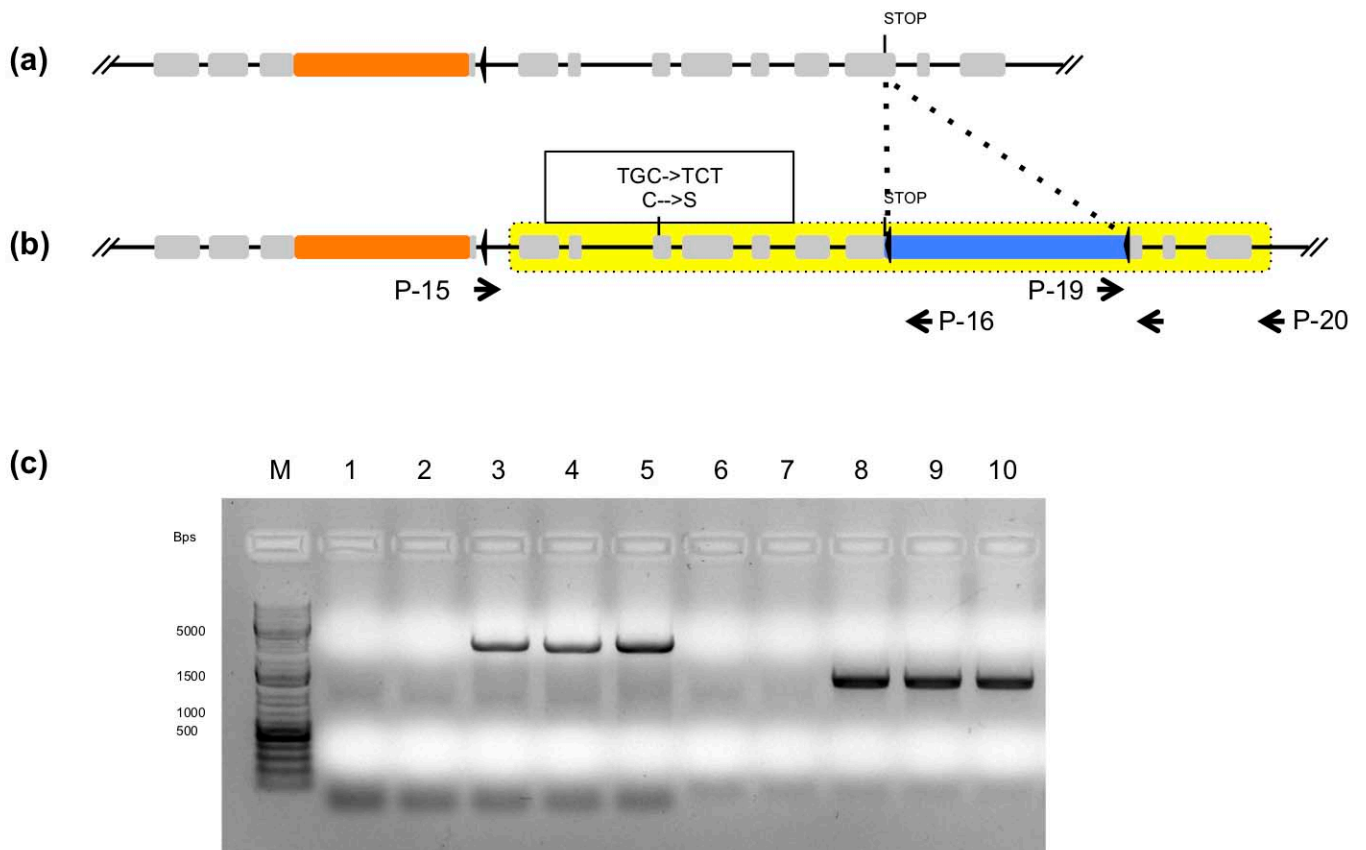


**Figure S8:** DEK1-TOMATO<sup>INT</sup> is present in developing phyllid but absent in mature phyllid. Bottom of a *dek1-tomato<sup>int</sup>* gametophore with two inner developing phyllids (\*) that displays polarized DEK1-TOMATO<sup>INT</sup> signal and two outer mature phyllids (\*\*) without fluorescent signal. (a) DEK1-TOMATO<sup>INT</sup> specific fluorescent signal. (b) Chlorophyll auto-fluorescence specific signal. (c) merged picture with both fluorescent signals. Bar in all images: 100  $\mu$ m.



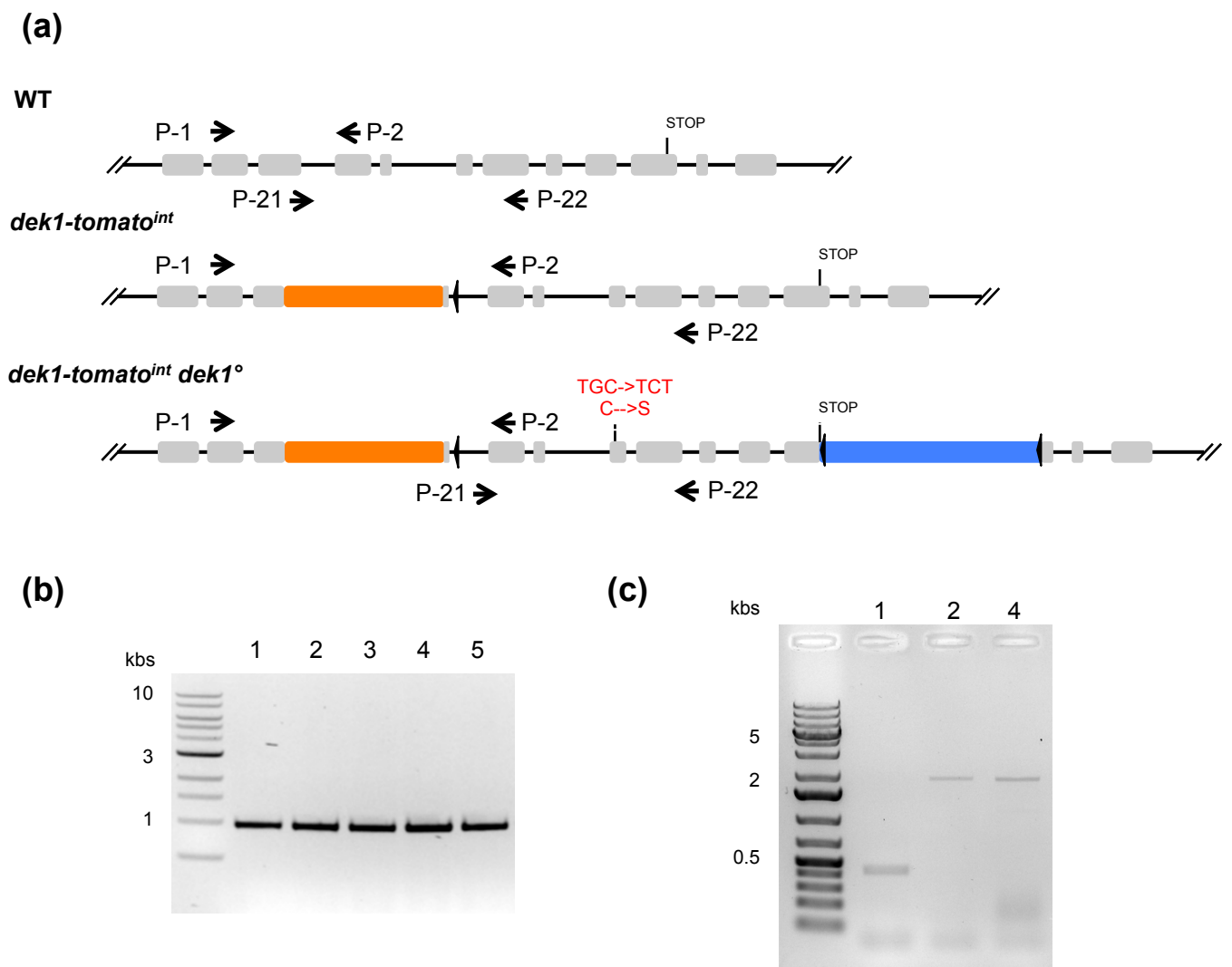


**Figure S9:** DEK1-TOMATO<sup>INT</sup> during antheridia development. Antheridia bundle with antheridia at different developmental stages displaying distinct DEK1-TOMATO<sup>INT</sup> signal. \*: Growing antheridia prior spermatogenesis is displaying DEK1-TOMATO<sup>INT</sup> signal at the interface of divided cells. \*\*: Antheridia during early spermatogenesis is showing a dotted signal in future spermatozoid. \*\*\*: Antheridia during late spermatogenesis is showing the characteristic fasciculate shaped body of *P.patens* spermatozoid. Note that both \*\* and \*\*\* the signal in the antheridial jacket is almost absent. (a) DEK1-TOMATO<sup>INT</sup> specific fluorescent signal. (b): Chlorophyll auto-fluorescence specific signal. Bar: 40  $\mu$ m.

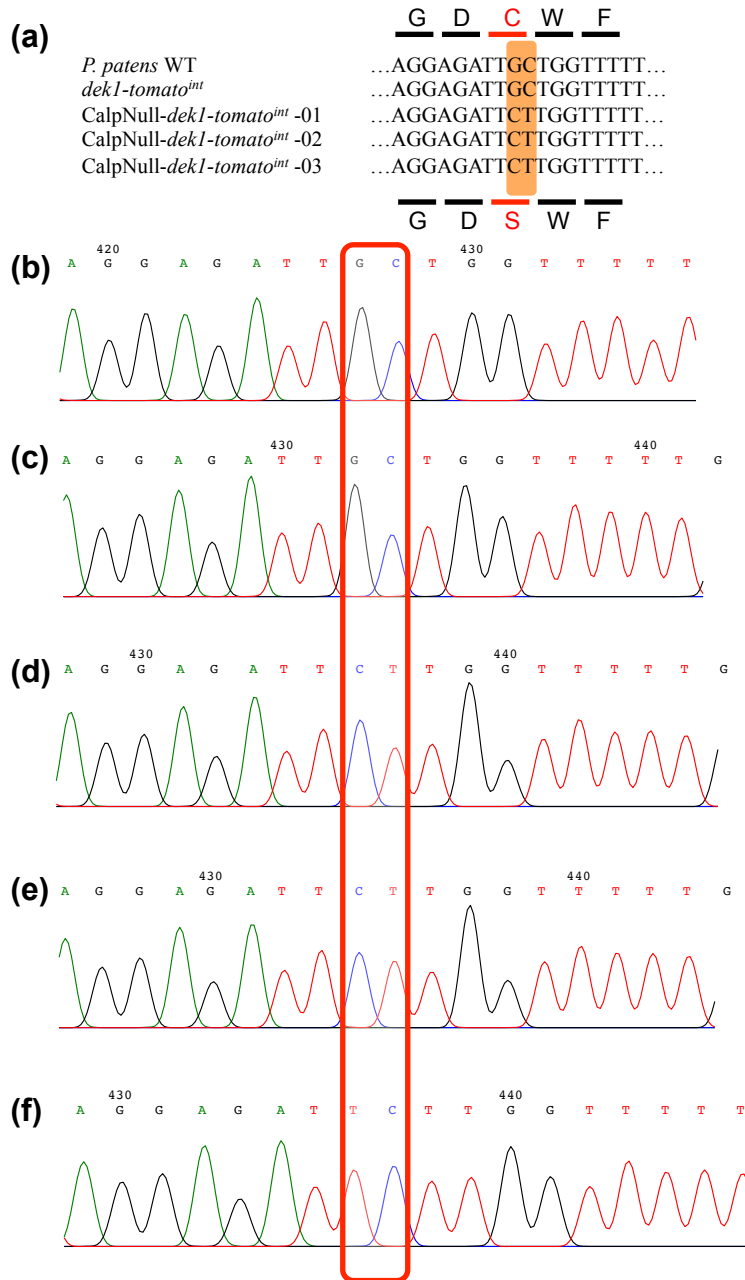


**Figure S10:** Schematic representation of the *dek<sup>0</sup>* generation in *dek1-tomato<sup>int</sup>* background and transformant PCR genotyping. (a): Schematic representation of *dek1-tomato<sup>int</sup>* locus around point mutation site. (b): Schematic representation of *dek1-tomato<sup>int</sup>/dek<sup>0</sup>* locus after transformation around the point mutation site. Black bar shows the genomic sequence. The grey boxes are exon sequences. The orange box is the dTomato sequence. The blue box corresponds to the 35S:NptIII-CamVter resistance cassette sequence. Black arrowhead: pLox sites. Yellow box corresponds to the transformation vector pArrowf1 sequence. (c): PCR genotyping confirming the transformants proper insertion pattern. M: Molecular ladder. Lanes 1, 6: WT. Lanes 2, 7: *dek1-tomato<sup>int</sup>*. Lanes 3-5 and 8-10: *dek1-tomato<sup>int</sup>/dek<sup>0</sup>*#1-3. Lanes 1-5, PCR genotyping from the 5' insertion of the vector using the primer pair P-15/P-16 (no band in WT and *dek1-tomato<sup>int</sup>*, a band 3266 bps in *dek1-tomato<sup>int</sup>/dek<sup>0</sup>*). Lanes 6-10, PCR genotyping confirmation the 3' targeting of the vector using the primer pair P-19/P-20 (no band in WT and *dek1-tomato<sup>int</sup>*, one band of 1440 bps in *dek1-tomato<sup>int</sup>/dek<sup>0</sup>*).





**Figure S11:** *Dek1-tomato<sup>int</sup>/dek1<sup>°</sup>* transcript analysis. (a) Schematic representation of the analyzed locus in WT, *dek1-tomato<sup>int</sup>* and *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>* strains. Black lane: genomic sequence. Grey box: exon. Orange box: tdTomato sequence. Blue box: G418 resistance cassette. Arrow: primer position with their respective names. (b) RT-PCR fragment of the partial DEK1 transcript covering the mutated Cys->Ser mutation amplified with the primer pair P-21 and P-22. Lane 1: WT, lane 2: *dek1-tomato<sup>int</sup>*, lane 3: *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>-01*, lane 4: *dek1-tomato<sup>int</sup>dek1<sup>°</sup>-02*, lane 5: *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>-03*. (c) RT-PCR fragment of the partial DEK1 transcript including the tdTomato sequence amplified with the primers P-1 and P-2. Lane 1: WT, lane 2: *dek1-tomato<sup>int</sup>*, lane 3: *dek1-tomato<sup>int</sup>-dek1<sup>°</sup>-02*.



**Figure S12:** *Dek1-tomato<sup>int</sup>/dek1<sup>°</sup>* transcript sequence analysis. (a) Local alignment of the predicted sequence of the modified locus in the analyzed strains. In red: modified amino acids. Orange shading: modified nucleotides. (b-f). Chromatogram sequencing trace of the mutated site obtained by sequencing the fragment showed in Fig. S11 (b). (b): WT, (c): *dek1-tomato<sup>int</sup>*, (d): *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>-01*, (e): *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>-02*, (f): *dek1-tomato<sup>int</sup>/dek1<sup>°</sup>-03*. The red box points to the WT and mutated nucleotides.

| Name | Sequence                           | Descriptive name  |
|------|------------------------------------|---|
| P-1  | ACA CAC CTG TGT AGC TGA TG         | cDEK1 tomato-fw   |
| P-2  | ATC TCG CTC GTA GCT GAG ATC        | cDEK1 tomato-rew  |
| P-3  | TCA ACT GTT CCT GAT GCA GGT AC     | gDEK1 insertion-fw  |
| P-4  | AGA TTC TCT GTG TCT GCG ATT AGA C  | gDEK1 insertion-rev   |
| P-5  | ACAGATAGCTGGGCAATGGA               | 35Sm-rev  |
| P-6  | TTTCGCTCATGTGTTGAGCAT              | CamVter-fw  |
| P-7  | TGG CAG CTG CTG TTC GTG C          | Insertion DEK1 DIG-probe 5'-fw                                  |
| P-8  | TCA TCT TCG GTT AAA CAG CGA C      | Insertion DEK1 DIG-probe 5'-rev                                 |
| P-9  | TGT AAG CTT GGA GTC TGC ATG TTC    | Insertion DEK1 DIG-probe 3'-fw                                  |
| P-10 | AAA GCT GTT AGC TTG ATA TGA CCG    | Insertion DEK1 DIG-probe 3'-rev                                 |
| P-11 | TGA ACT CAC CGC GAC GTC TGT C      | DIG-probe HptII-fw  |
| P-12 | ATC GGC GAG TAC TTC TAC ACA G      | DIG-probe HptII-rev   |
| P-13 | CTA CTT GTA CAG CTC GTC CAT G      | DIG-probe mcherry-rev   |
| P-14 | CAC CAT GGT GAG CAA GGG CGA GGA G  | DIG-probe mcherry-fw  |
| P-15 | TTC ATA GAC GGA GGT TTC GAT GGA    | genotyping <i>dek1-tomato<sup>mt</sup>/dek<sup>0</sup></i> fw1  |
| P-16 | GGA GCC ACC TTC CTT TTC CA         | genotyping <i>dek1-tomato<sup>mt</sup>/dek<sup>0</sup></i> rev2 |
| P-17 | CAC TCG CGT GTT GCC TTA AGC A      | genotyping <i>dek1-tomato<sup>mt</sup>/dek<sup>0</sup></i> fw3  |
| P-18 | GTC AGG CCT CCT TCAC GTT TCG       | genotyping <i>dek1-tomato<sup>mt</sup>/dek<sup>0</sup></i> rev4 |
| P-19 | AGG GTT CTT ATA GGG TTT CGC TCA TG | genotyping <i>dek1-tomato<sup>mt</sup>/dek<sup>0</sup></i> fw5  |
| P-20 | TGG CAC AAA TTT GAC CAA CAA ATC T  | genotyping <i>dek1-tomato<sup>mt</sup>/dek<sup>0</sup></i> rev6 |
| P-21 | TGGAAACGCCAATGAGATTTGAGG           | RT DEK1 mutation site-rev                                       |
| P-22 | GTCGACCTCTCGTACCTGTAAAAGAG         | RT DEK1 mutation site-rev                                       |

**Table S1:** Primers used in the present study.