



Supplemental Figure S2. Vector construction, targeted insertion and molecular characterization of the *Physcomitrella patens* *dek1ΔlinkerG3/cre#123* mutant line. **A**, Schematic representation of gene targeting using the *pArrow-ΔLinkerG3* vector transformed into the *P. patens* *dek1ΔlinkerG1/cre* background, and elimination of the resistance cassette by Cre-mediated excision. Blue, green and red highlights represent DEK1 MEM, Linker and calpain sequences, respectively (for the DEK1 protein domains, see Figure 1A). Black boxes represent the *loxP* sequence. The numbers in the grey boxes correspond to the exons of the *P. patens* *DEK1* gene. The 5' and 3' targeting sequences (TGS) are boxed. The green triangle with number shows the position of the deleted *DEK1* nucleotides. Annealing sites for primers used for PCR genotyping are shown with arrows (primer sequences can be found in Supplemental Table S3). The hybridization sites of the Southern blotting probes are shown above the schematics. Restriction enzymes used for Southern blotting and their restriction sites are indicated, and the corresponding expected band sizes are also given. **B**, Southern blotting analysis. Southern blotting was performed to confirm insertion of the *npII* resistant cassette in the *dek1ΔlinkerG3#2* mutant and removal of this cassette in the *dek1ΔlinkerG3/cre#123* line after Cre-mediated excision. For comparison the schematics for the WT and *dek1ΔlinkerG1#20* mutant, both of which was included in the Southern blotting analysis, is also provided. Restriction fragments were generated using *Bgl*III and the blot hybridized with a mixture of 5' and 3' TGS probes (A) displayed the expected hybridization signals. Note that locus *PpDEK1ΔLinkerG3/cre#123* harbors one less *Bgl*III site than the *PpDEK1ΔLinkerG3#2* locus due to Cre/*lox* mediated removal of the *npII* resistant cassette. M = marker; WT = wild type, Positive control: WT. **C**, *DEK1* cDNA sequencing. RT-PCR and DNA sequencing was used to analyze the *dek1ΔlinkerG3/cre#123* mutant *DEK1* cDNA showing in-frame fusion of exon 17 and 24 (red box). The RT-PCR product was amplified using primers *Ex7-F* and *Ex30-R* and fully sequenced; for simplicity, only the sequencing result from exon 16 to exon 24 are provided.