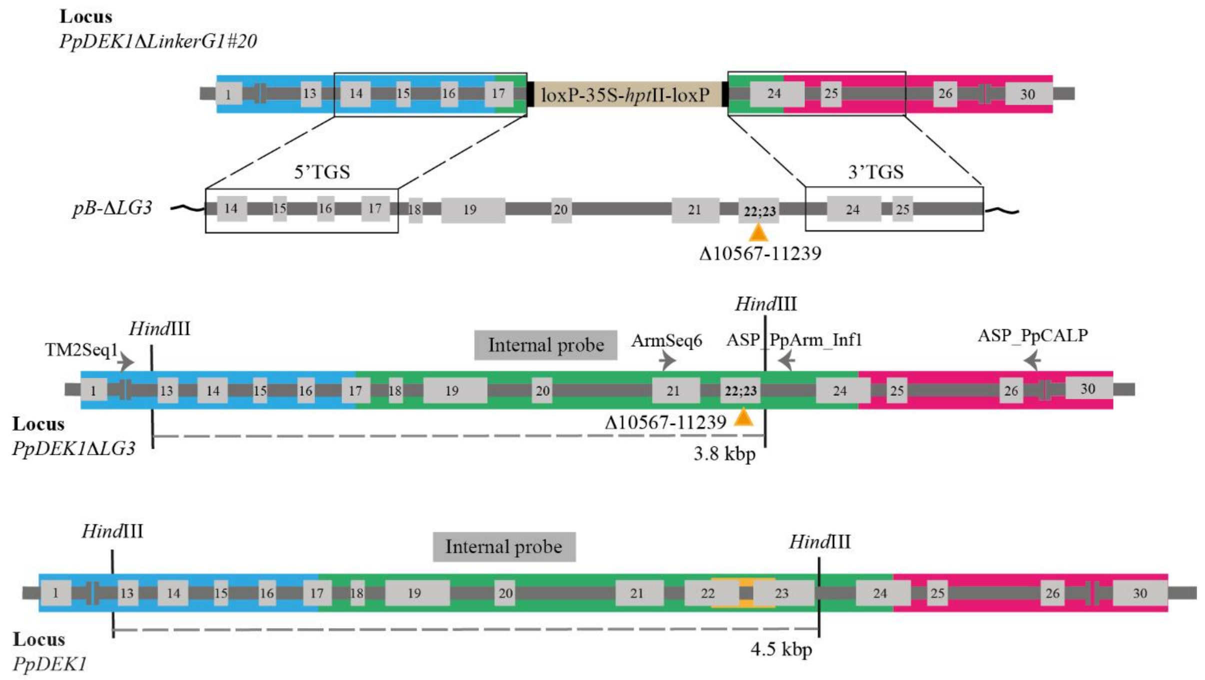
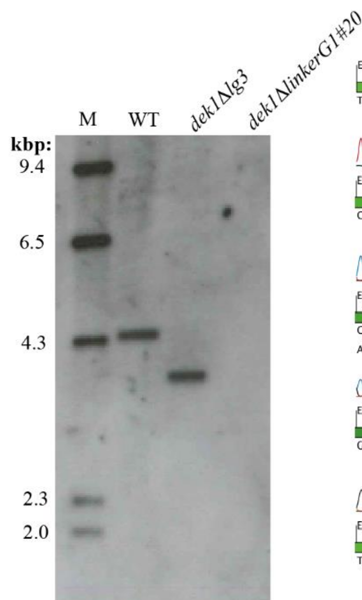
Supplemental Figure S4

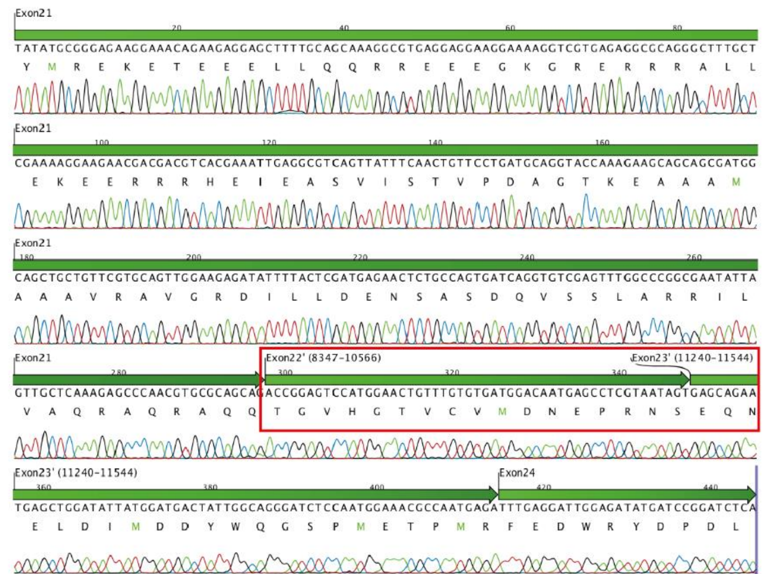
A



B



C



Supplemental Figure S4. Vector construction, targeted insertion of *LinkerΔLG3* and molecular characterization of the *Physcomitrella patens dek1Δlg3* mutant line. A, Schematic representation of gene targeting using the *pB-ΔLG3* vector. Blue, green, yellow and red highlights represent DEK1 MEM, Linker, LG3 and calpain sequences, respectively (for the DEK1 protein domains, see Figure 1A). 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 yellow triangle with numbers 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 site of the Southern Blotting probe is shown above the schematics. The restriction enzyme used for Southern blotting and its restriction sites are indicated, and the corresponding expected band sizes are also shown. B, Southern blotting analysis. Southern blotting was performed to confirm insertion of the *LinkerΔLG3* sequence into the *dek1ΔlinkerG1*#20 mutant. Restriction fragments were generated using *Hind*III and the blot hybridized with the internal probe (A) displayed the expected hybridization signals. M = marker; WT = wild type, Positive control: WT. C, *DEK1* cDNA sequencing. RT-PCR and DNA sequencing was used to analyze the *dek1Δlg3* mutant *DEK1* cDNA showing in-frame fusion of exon 22 and 23 (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 21 to exon 24 are provided.