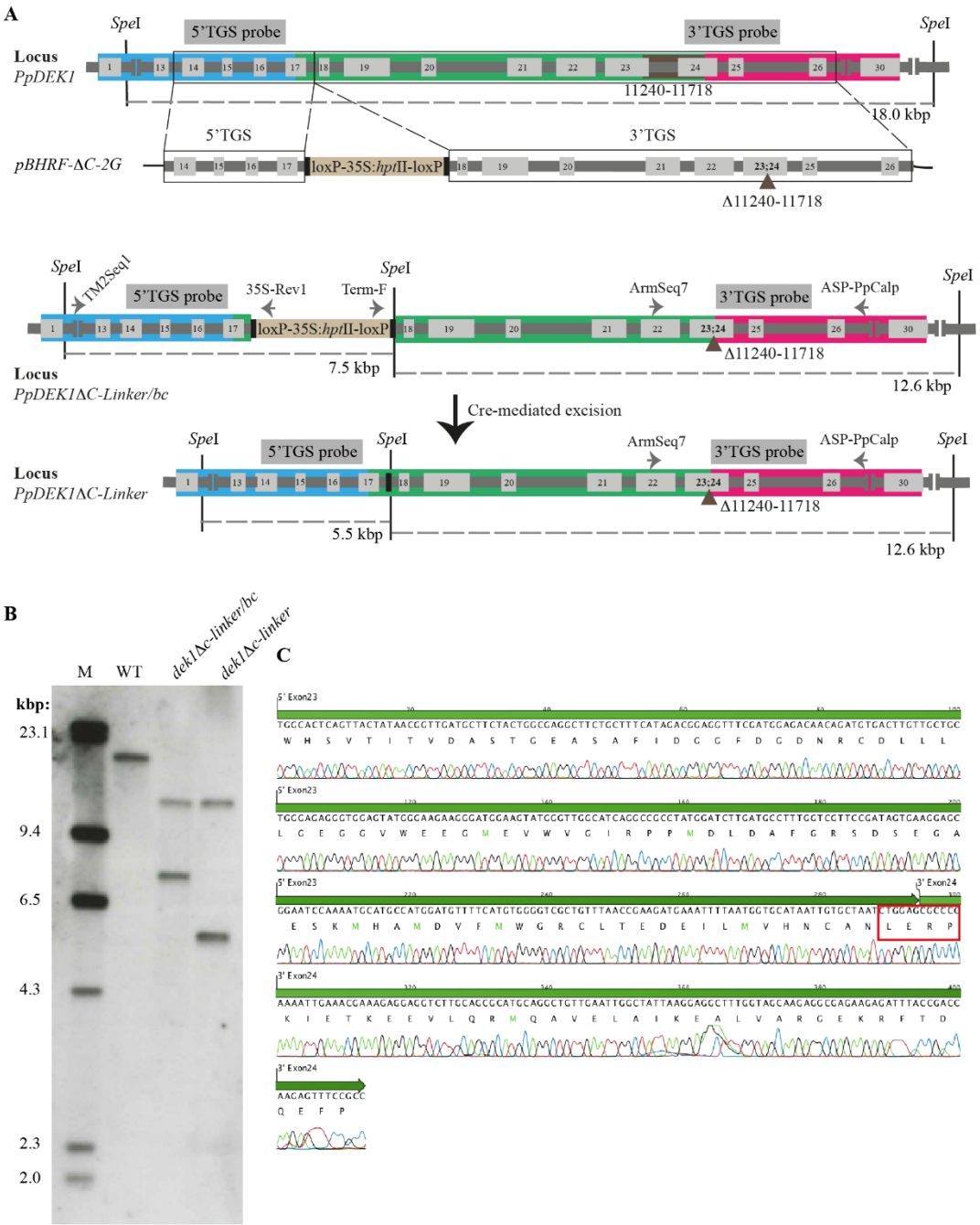
Supplemental Figure S6



Supplemental Figure S6. Vector construction, targeted insertion and molecular characterization of the *Physcomitrella patens dek1Δc-linker* mutant line. A, Schematic representation of gene targeting using the *pBHRF-ΔC-2G* vector. Blue, green, brown and red highlights represent DEK1 MEM, Linker, C-segment 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 brown 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 sites of the Southern blotting probes are 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 *hptII* resistance cassette into the WT. Restriction fragments were generated using *Spe*I and the blot hybridized with a mixture of 5' and 3' TGS probes (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 *dek1Δc-linker* mutant *DEK1* cDNA showing in-frame fusion of exon 23 and 24 (red box). The RT-PCR product was amplified using primers *Ex7-F* and *Ex30-R* and fully sequenced; only the sequencing result from exon 23 to exon 24 are provided.