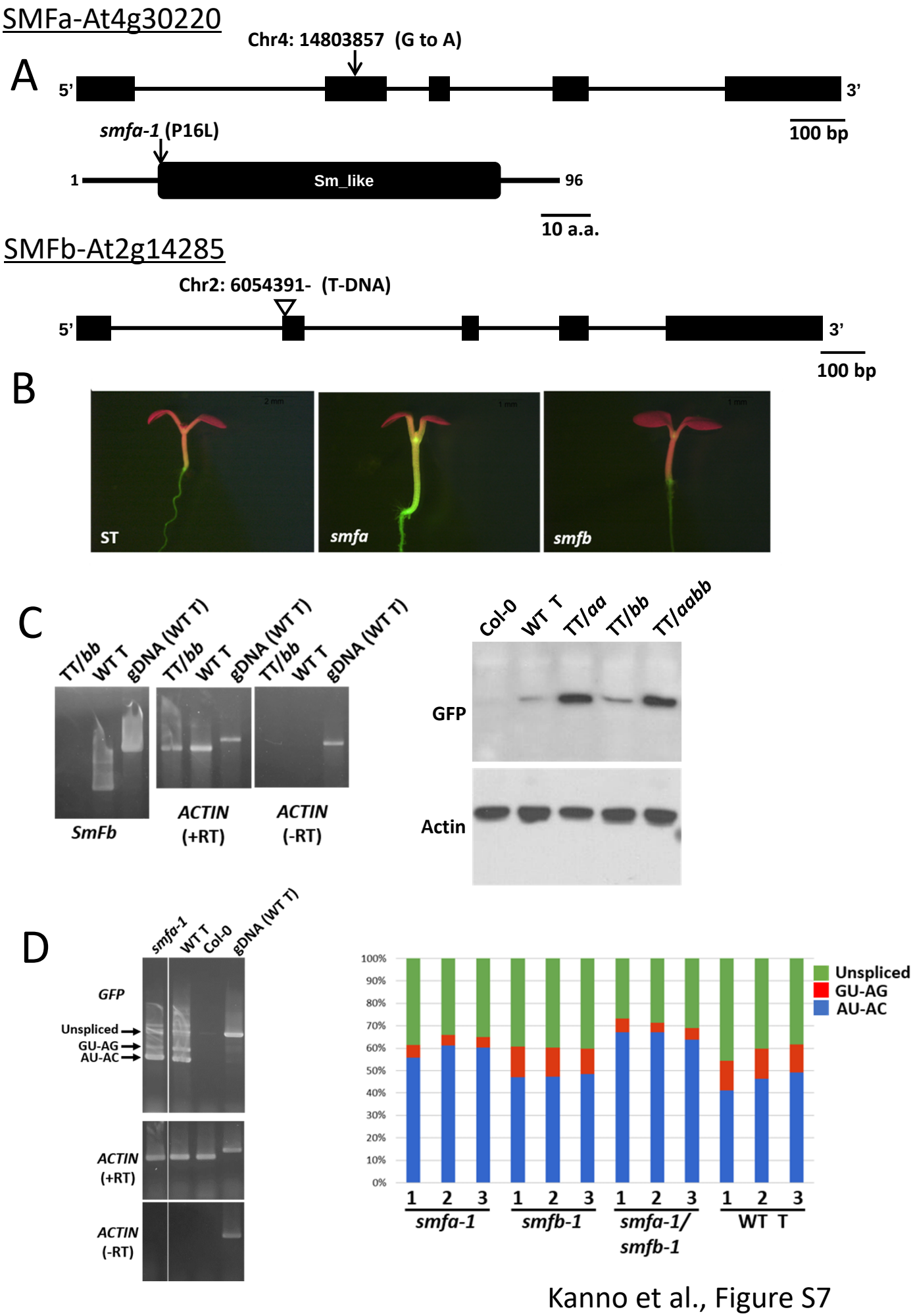

Kanno et al., Figure S7

Figure S7: Redundancy test of *SMFa* and *SMFb*

A. Top: Intron-exon structure of the *SMFa* gene (At4g30220) and position of the nucleotide change and amino acid substitution in the *smfa-1* mutant (Kanno et al., 2017a). Bottom: Intron-exon structure of the *SMFb* gene (At2g14285) and position of the T-DNA insertion in the *smfb-1* mutant (SAIL_608_B05).
B. GFP fluorescence in seedlings with the indicated genotypes. The *smfa-1* mutant displays a hyper-GFP phenotype relative to the WT T line (Kanno et al., 2017a). By contrast, the *smfb-1* mutant, which appears to be a complete knock-out (Part C), has a GFP level similar to the WT-T line.
C. Left: RT-PCR to demonstrate loss of the corresponding wild-type *SMFb* transcript in the *smfb-1* insertion mutant (TT/bb). Actin, with and without reverse transcriptase (RT), was used as a constitutively expressed control. gDNA, genomic DNA used as a control. Right: Western blot indicating increased level of GFP protein in the *smfa-1* mutant (*TT/aa*) and *smfa smfb* double mutant (*TT/aabb*) relative to the WT T line, and no increase above the wild-type level in the *smfb-1 (TT/bb)* mutant. GFP protein was not detected in non-transgenic Col-0 plants. Antibodies to actin were used to probe the blot to control for loading levels.
D. Left: Semi-quantitative RT-PCR indicating levels of the three *GFP* splice variants in the *smfa-1* mutant. Consistent with the hyper-GFP phenotype of the *smfa-1* mutant, an increased level of the translatable AU-AC transcript and decreased levels of the GU-AG and unspliced transcripts were observed. These results were mirrored in the RNA-seq experiments (Right), which revealed an increase of the AU-AC transcript from ~ 35-50% in WT T plants and the *smfb-1* mutant to 55-70% in *smfa* and *smfaabb* double mutants. Corresponding decreases in the GU-AG and unspliced transcripts were also apparent from the RNA-seq results. These results suggest increased splicing efficiency at noncanonical splice sites in the GFP pre-mRNA in the *smfa-1* mutant. gDNA, genomic DNA control

Kanno, T., W. D. Lin, J. L. Fu, A. J. M. Matzke, and M. Matzke, 2017a A genetic screen implicates a CWC16/Yju2/CCDC130 protein and SMU1 in alternative splicing in *Arabidopsis thaliana.* RNA 23(7): 1068–1079. https://doi.org/10.1261/rna.060517.116