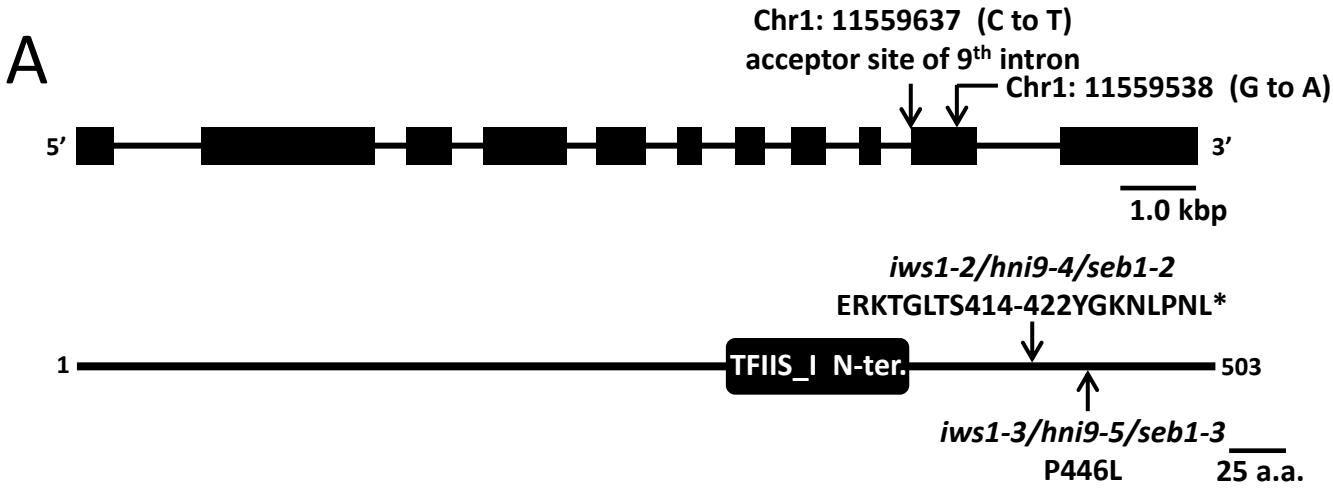
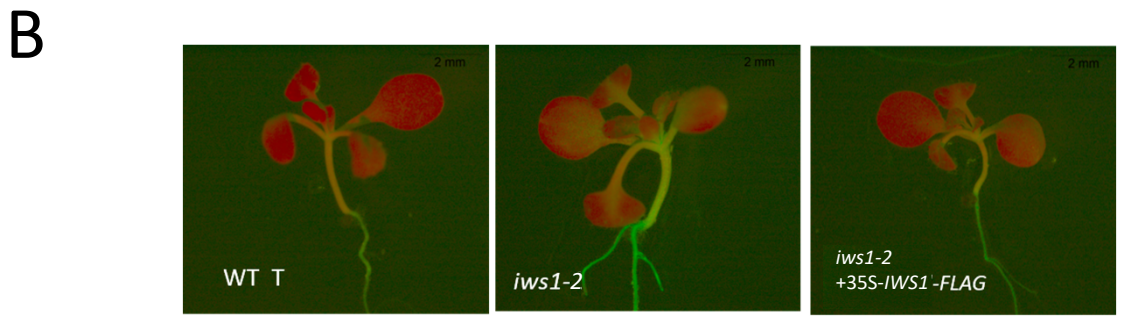
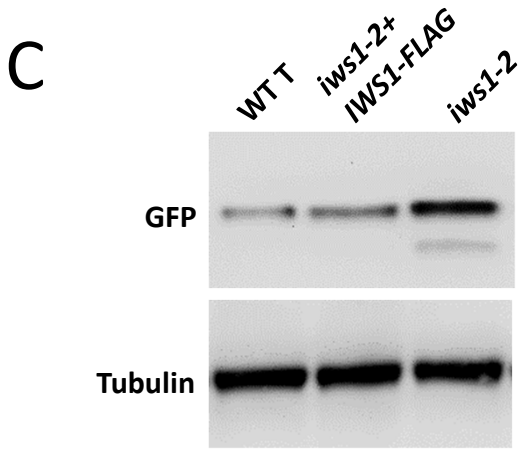
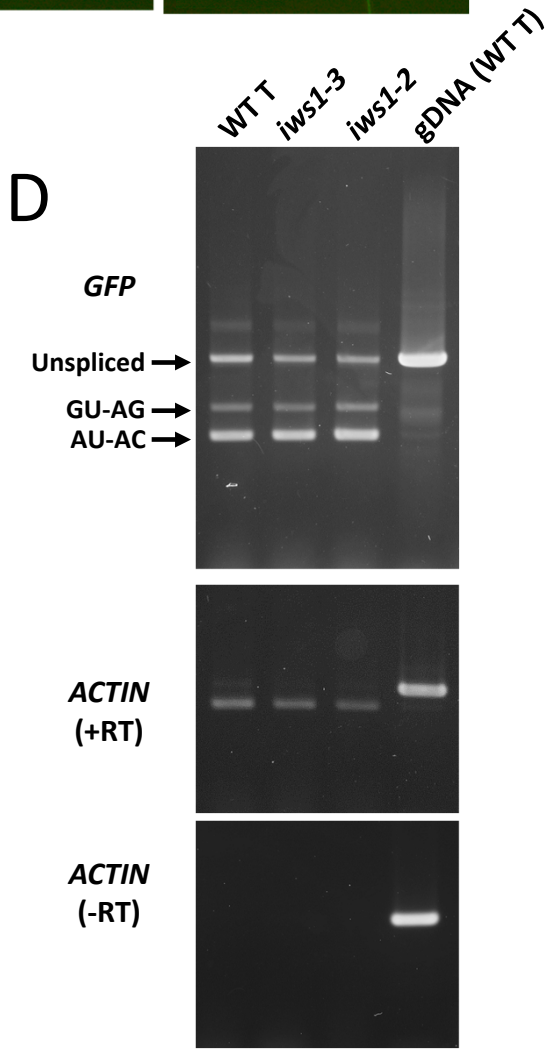
IWS1 –At1g32130









Kanno et al., Figure S4

Figure S4: IWS1

A. Top: Intron-exon structure of the *IWS1* gene (At1g32130) and positions of nucleotide changes in the *iws1-2 and iws1-3* mutants. The *iws1-2* splice site mutation was identified by next generation sequencing. The *iws1-3* mutation (P446L) was identified by Sanger sequencing of candidate genes in an uncategorized hyper-GFP mutant. Bottom: Domain structure of the IWS1 protein (503 amino acids) showing position of the TFIIS_I (N-terminal domain of transcription elongation factor SII) superfamily domain and the consequences of the splice site mutation in *iws1-2* and amino acid substitution in *iws1-3*.
B. GFP fluorescence in seedlings with the indicated genotypes. The *iws1-2* mutant exemplifies the hyper-GFP relative to the WT T line. A complemented *iws1-2* mutant, labeled with '+35S-*IWS1-FLAG*' (35S denotes the 35S promoter of cauliflower mosaic virus), shows a return to more WT T levels of GFP.
C. Western blot showing increased levels of GFP protein, consistent with a hyper-GFP phenotype, in the *iws1-2* mutant relative to the WT T line. The GFP levels in complemented plants (*iws1-2+IWS1-FLAG),* return to approximately the wild-type level. Antibodies to tubulin were used to probe the blot to control for loading levels.
D. Semi-quantitative RT-PCR indicating essentially unchanged levels of the three *GFP* splice variants in the *iws1-2* and *iws1-3* mutants. These results suggest that the hyper-GFP phenotype in the two *iws1* mutants may result from a defect other than a splicing deficiency. Actin, with and without reverse transcriptase (RT), was used as a constitutively expressed control. gDNA, genomic DNA control