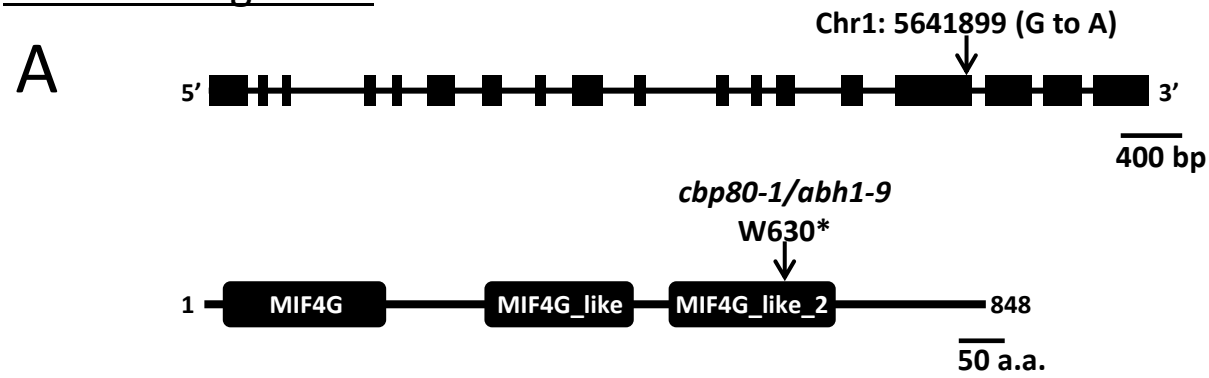
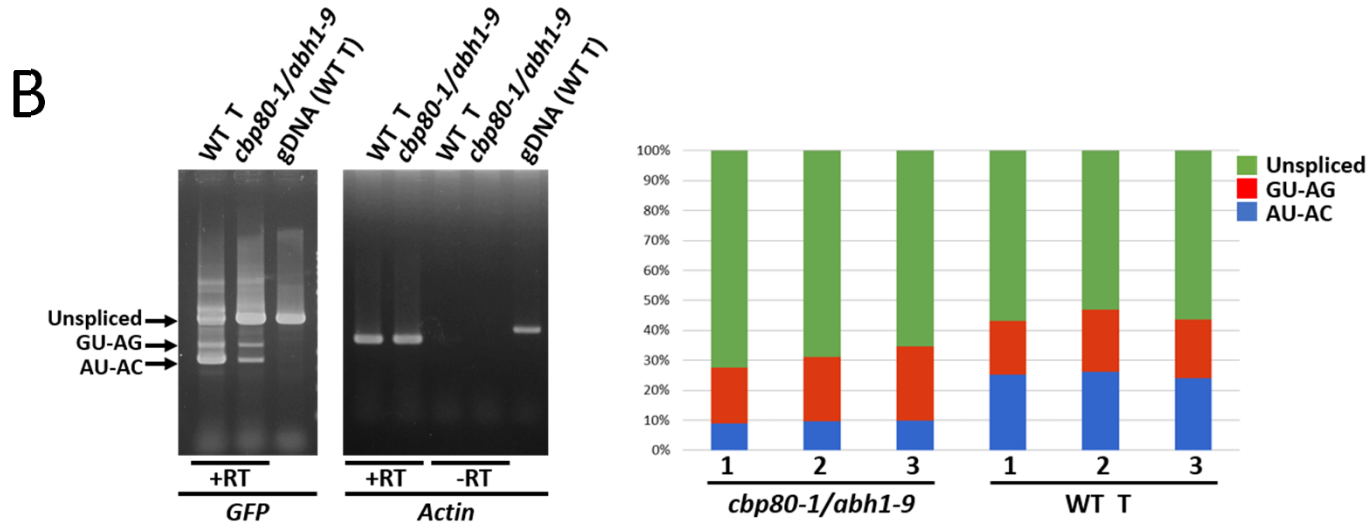
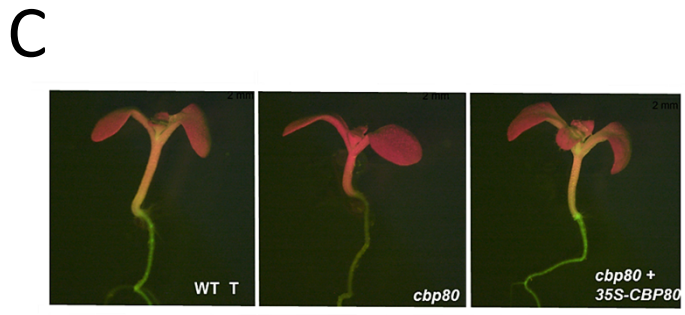
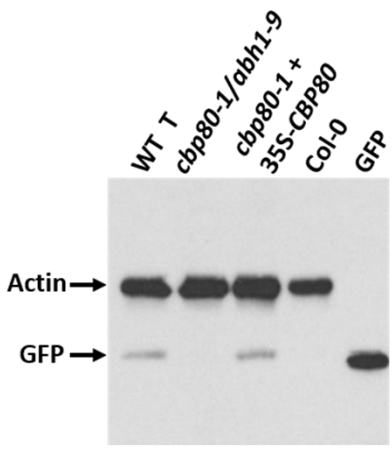
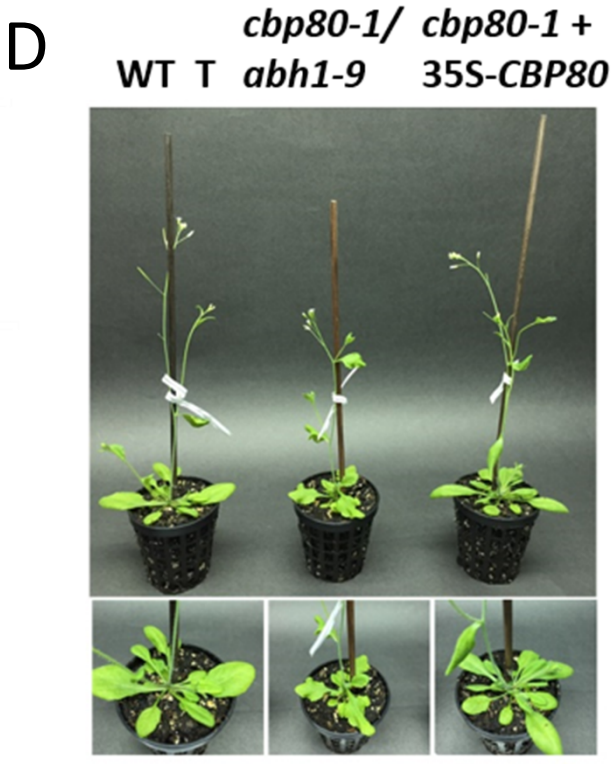
CBP80 –At2g13540











Kanno et al., Suppl Figure S5

Figure S5: CBP80

A. Top: Intron-exon structure of the *CBP80* gene (At2g13540) and position of the nucleotide change in the *cbp80-1* mutant. Bottom: The premature termination codon (asterisk) resulting from the *cbp80-1* mutation is present in one of the three conserved MIF4g-like (Middle domain of eIF4G) domains in the CBP80 protein (848 amino acids)
B. Left: Semi-quantitative RT-PCR indicating elevated levels of unspliced *GFP* pre-mRNA in the *cbp80-1* mutant, consistent with reduced splicing efficiency at both canonical and non-canonical splice sites. Actin, with and without reverse transcriptase (RT), was used as a constitutively expressed control. Right: Quantification of the three *GFP* splice variants in triplicate RNA-seq data from the *cbp80-1* mutant and WT T plants. These results are in accord with the RT-PCR data: the percentage of the translatable AU-AC transcript decreases from ~20% in WT T plants to <10% in the *cbp80-1* mutant. gDNA, genomic DNA control.
C. Top: GFP fluorescence in seedlings with the indicated genotypes. The *cbp80-1* mutant has a GFP-weak phenotype relative to the WT T line. A complemented *cp80-1* mutant, additionally labeled with '+35S-*CDKG2*' (35S denotes the 35S promoter of cauliflower mosaic virus), shows a return to more WT T levels of GFP. Bottom: Western blot showing decreased levels of GFP protein, consistent with a GFP-weak phenotype, in the *cbp80-1* mutant relative to the WT T line. The GFP protein level in complemented plants (*cbp80-1*+35S-*CBP80)*, returned to approximately the level observed in WT T plants. Antibodies to actin were used to probe the blot to control for loading levels. Col-0, non-transgenic Arabidopsis plants of the Col-0 ecotype.
D. Phenotypes of plants with the indicated genotypes. Relative to WT T plants, the *cbp80-1* mutants have serrated rosette leaves, decreased rosette diameter, short stature and reduced seed set. These aberrant phenotypes can be at least partially rescued by introducing a wild-type copy of the *CBP80* gene into the *cbp80-1* mutant (top image: whole plants; bottom image: enlargements of rosettes; Table SX).