A


25 a.a.

## B



## C



D

A. Top: Intron-exon structure of the CWC16a gene (At1g25682) and position of the nucleotide changes in the $c w c 16 a-1$ mutant (Kanno et al., 2017a) and in two new mutants, $c w c 16 a-2$ and $c w c 16 a-3$ (identified in this study). The domain structure of the CWC16a protein ( 310 amino acids) and the positions of the premature termination codons (asterisks) resulting from the cwc $16 a-1$ and $c w c 16 a-2$ mutations and the frame shift resulting from the $c w c 16 a-3$ mutation are shown below.
B. Expected positions of T-DNA insertions in five putative $c w c 16 b$ alleles examined for this study. For two lines, SALK-009736C and SALK_132471, the T-DNA was found integrated as expected (https://www.arabidopsis.org/) using the primers shown in Table S1. However, we were unable to demonstrate that CWC16b transcripts were eliminated in these two lines. For three lines, however, SALK_152264, SALK_527_G04 and SALK_053475, the T-DNA could not be detected at the expected insertion siteusing the primers shown in Table S1.
C. Quantification of the three GFP splice variants in triplicate RNA-seq data from the cwc 16a mutants. The percentage of the translatable AU-AC transcript increases from $\sim 40 \%$ in the WT T line to $70-80 \%$ in the three $c w c 16 a$ mutants. This increase, which occurs at the expense of the GU-AG and unspliced transcripts, presumably accounts in large part for the hyper-GFP phenotype of $c w c 16 a$ mutants.
D. Top: GFP fluorescence in seedlings with the indicated genotypes. The $c w c 16 a$ mutants all have a hyper-GFP phenotype relative to the WT T line. Bottom: GFP Western blot confirming elevated levels of GFP protein relative to the WT T line in the three $c w c 16 a$ mutants.

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

