Supplementary Figure 1. Flowering time distribution of sr45-1 versus sr45-1+FWA
Supplementary Figure 2. Confirmation of sr45-1 de novo phenotype. (A) Flowering time of randomly selected T₂ sr45-1+FWA transformants. FWA construct has a Basta® resistance gene as a selection marker that allows testing for the presence of FWA construct. Red-dotted line depicts the flowering time of untransformed sr45-1 mutants grown under the same conditions. (B) RT-PCR showing FWA expression of a selection of the above-mentioned lines. UBQ10 expression is showed as a loading control. (C) Bisulfite cutting assay, showing FWA methylation status in the above-mentioned lines. Genomic DNA is digested with BglII to destroy the endogenous FWA gene before bisulfite treatment. DNA methylation of transgenic FWA was assayed by PCR from bisulfite-treated DNA followed by Clal digestion. CG methylation protects the Clal site from bisulfite conversion. Black arrow indicates the unmethylated size. (D) Flowering time of homozygous T₃ sr45-1+SR45 complemented lines after FWA transformation. (E) RT-PCR and bisulfite cutting assay showing SR45 expression and partial restoration of methylation at FWA. UBQ10 expression is showed as a loading control.
Supplementary Figure 3. *FLC* de-repression enhancement. (A) Flowering time of Columbia, *sr45-1*, *dcl3-1* and *sr45-1, dcl3-1* double mutant. (B) RT-PCR showing the expression of *FLC* in the above-mentioned lines. *UBQ10* expression is showed as a loading control.