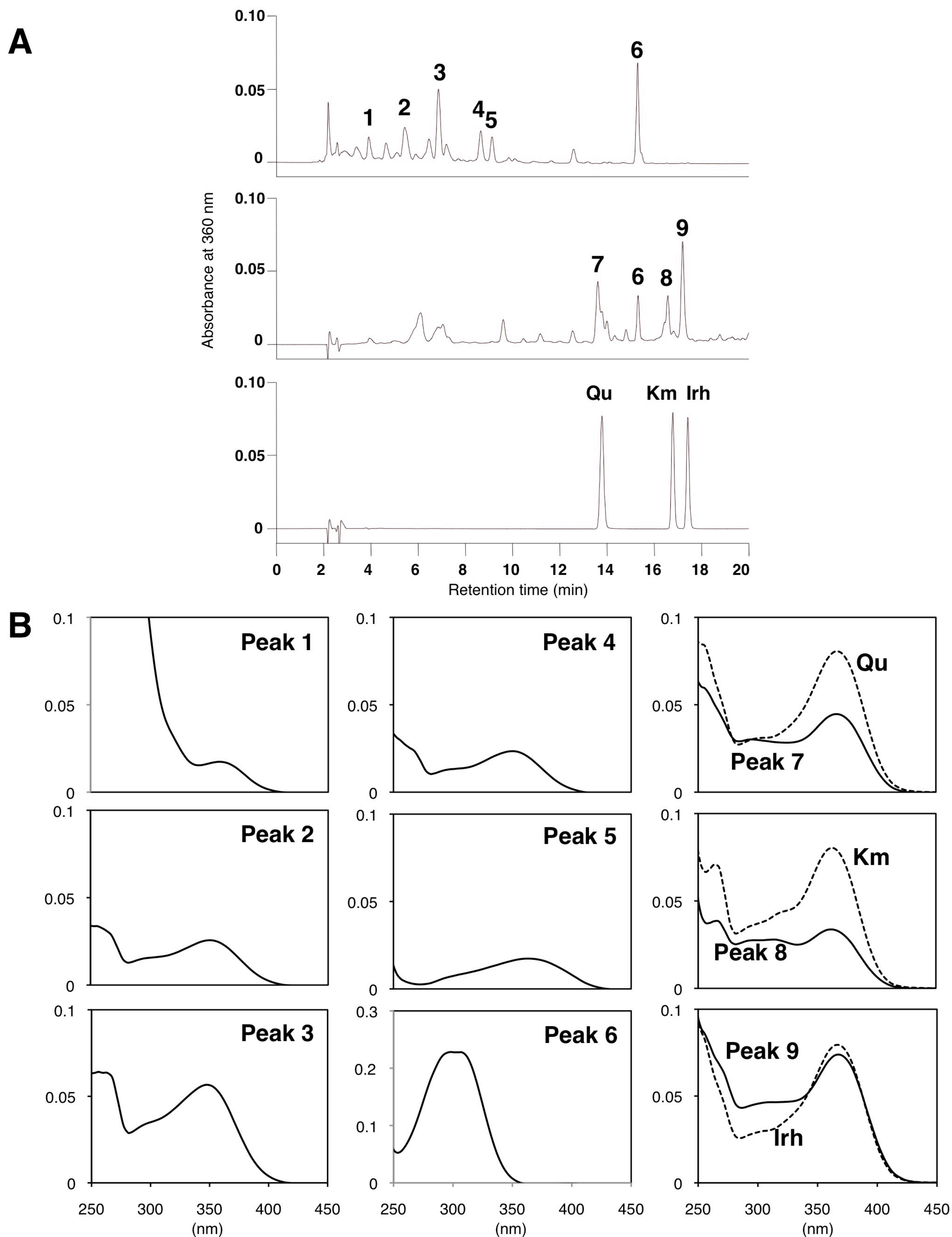
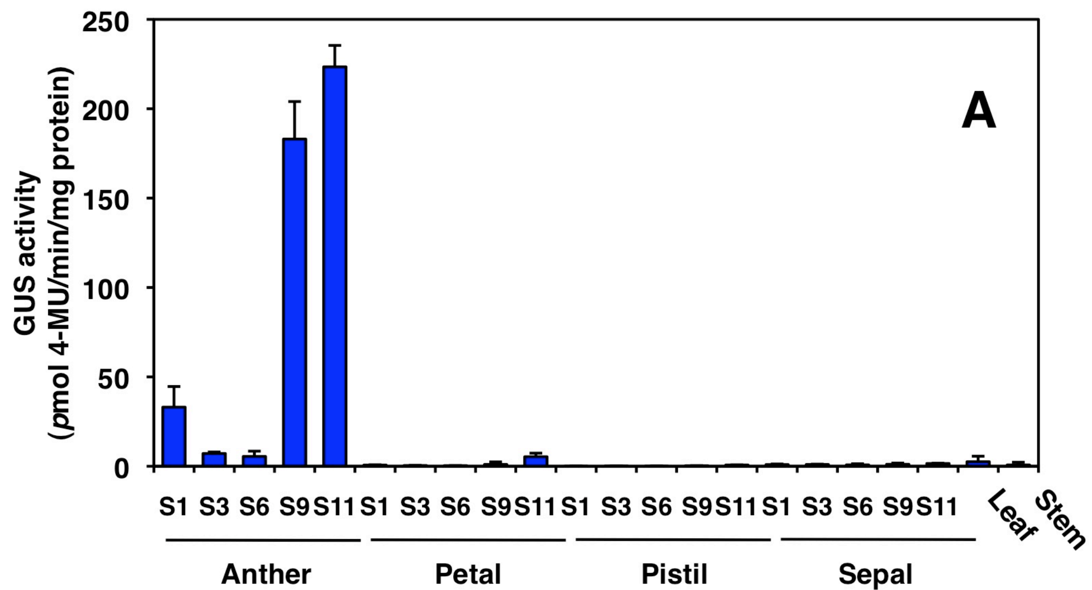
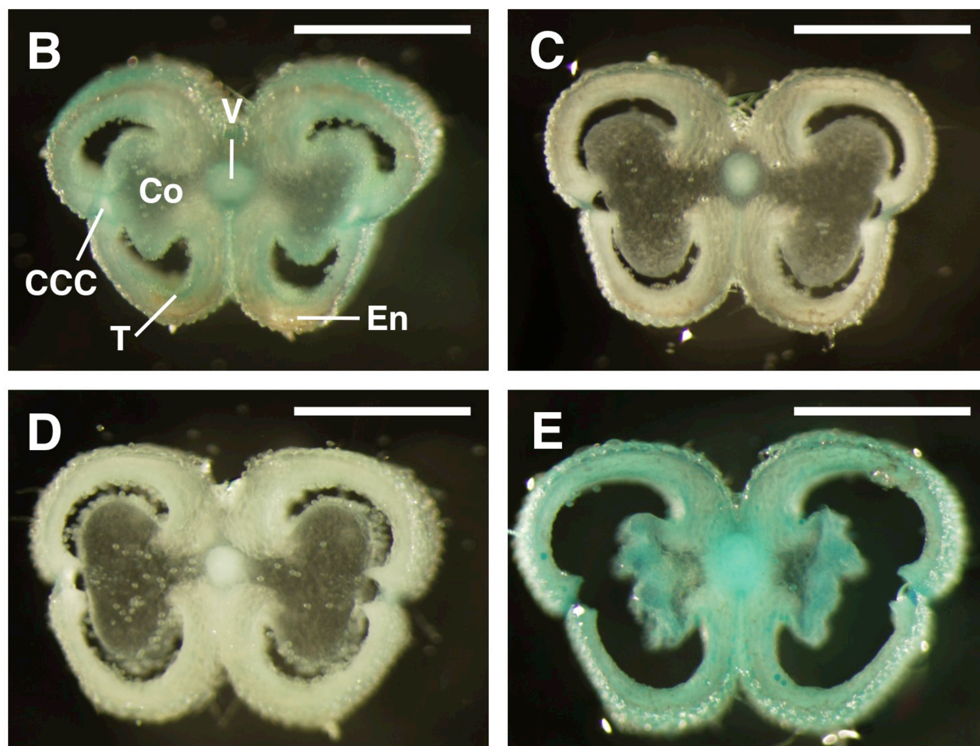


Supplemental Figure S1. HPLC profiles of methanolic extracts from gentian anther.

(A) Methanolic extracts (upper) and hydrolyzed extracts (middle) from gentian anther at developmental stage 3 were separated by HPLC as described in the Materials and methods. Quercetin (Qu), kaempferol (Km) and isorhamnetin (Irh) were also separated as authentic standards (lower).

(B) UV-visible light absorbance spectra of peaks 1 to 9 shown in (A) and authentic standards are indicated.



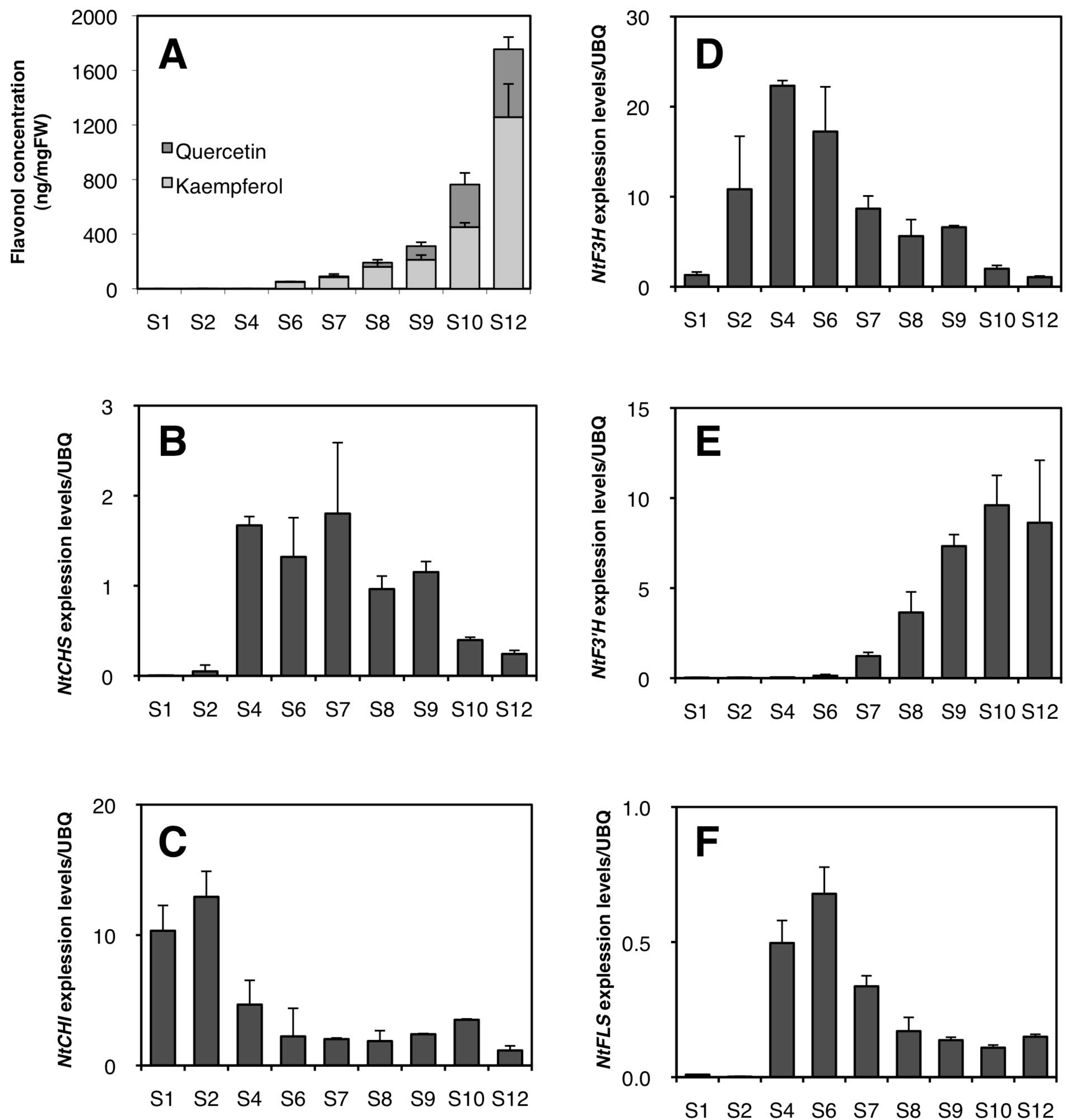


Supplemental Figure S2. Fluorometric and histochemical GUS assays in GtFLSproGUS transgenic tobacco line no. 7.

The GUS assays were performed as described in Figure 5.

(A) Fluorometric GUS assay, (B–E) histochemical GUS assay. Scale bar = 1 mm.

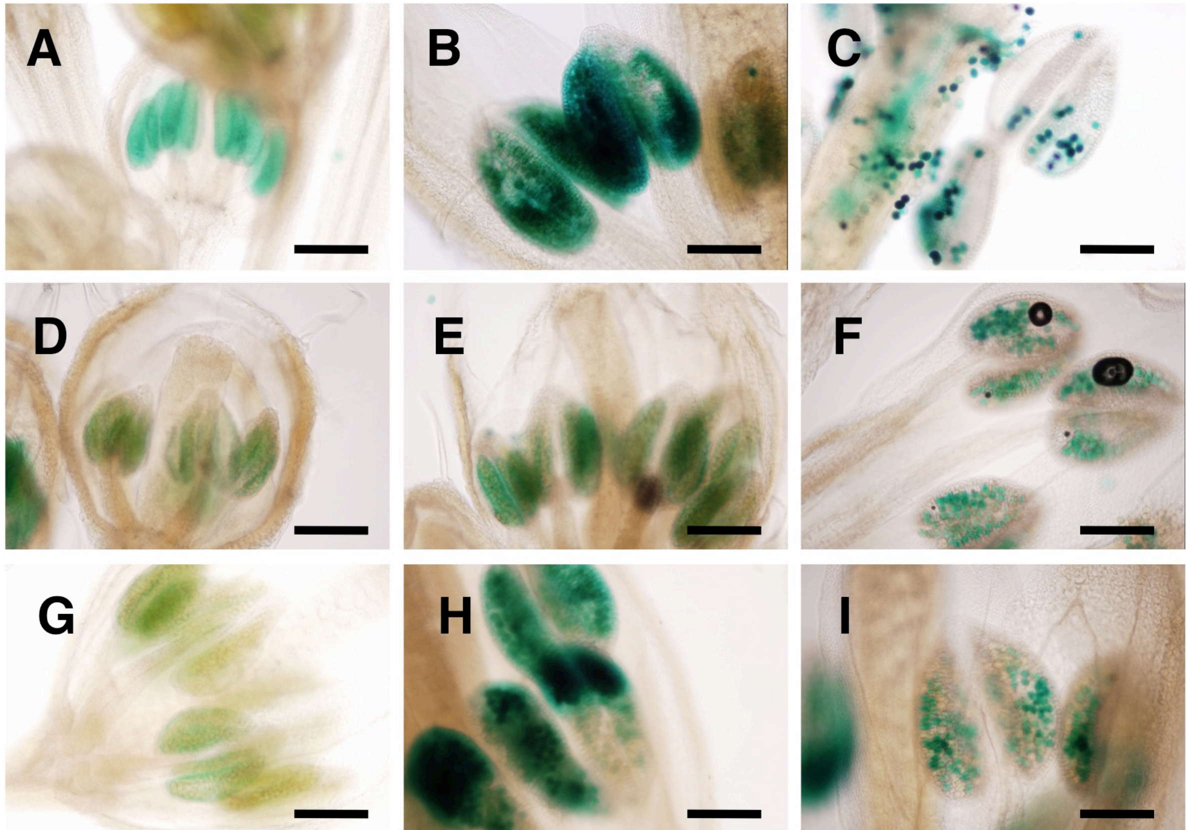
(B) Co, connective; CCC, circular cell cluster; En, endothecium; T, tapetum; V, vascular bundle.



Supplemental Figure S3. Temporal profiles of flavonol accumulation and expression of their biosynthetic genes in tobacco anther.

(A) Flavonol accumulation in *Nicotiana tabacum* cv. SR-1 anthers. Flavonols were extracted from tobacco anthers at different stages as defined by Koltunow et al. (1990). The assay was performed as described in Figure 2.

(B–F) Real-time PCR analysis of flavonoid biosynthetic genes in tobacco anther. Total RNA isolated from anthers at nine different stages were analyzed. Ubiquitin transcriptions were also investigated as an internal expression standard and used to calibrate the expression levels of each gene. The error bars represent standard deviations of three independent experiments. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; FLS, flavonol synthase.



Supplemental Figure S4. Histochemical GUS assay in GtFLSproGUS transgenic *Arabidopsis* line nos. 1 (A–C), 4 (D–E) and 7 (G–I).

The histochemical GUS assays were performed as described in Figure 6. Scale bar = 100 µm.