

**Expression of *Arabidopsis* MYB transcription factor, *AtMYB111*, in tobacco requires light to modulate flavonol content**

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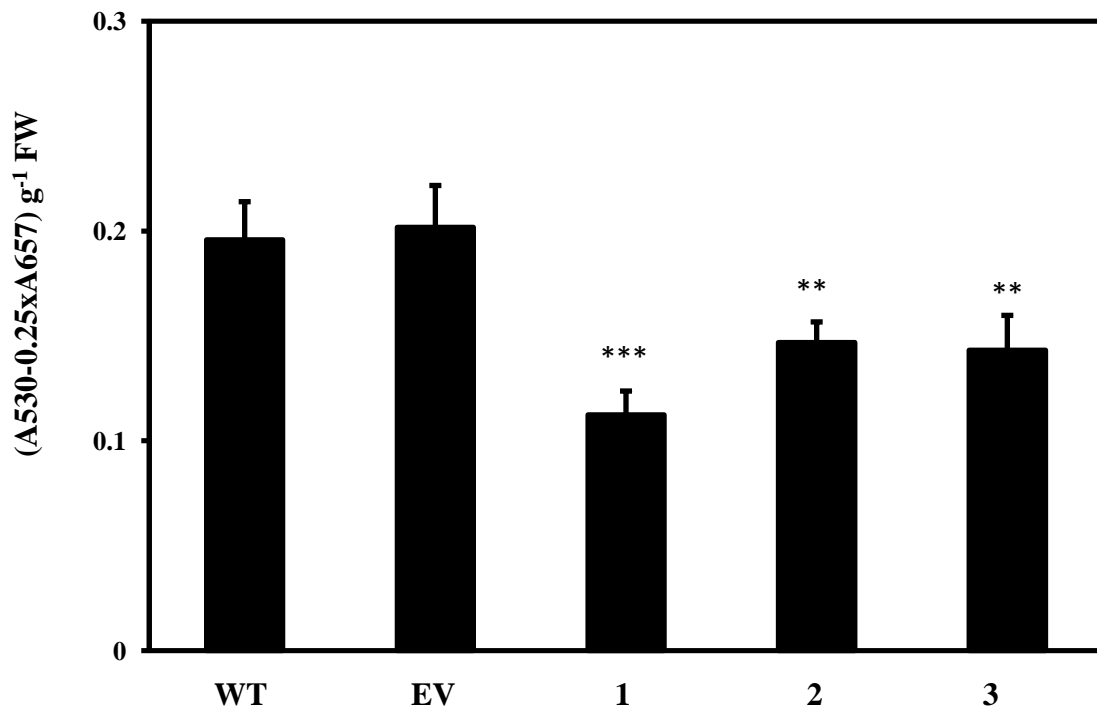
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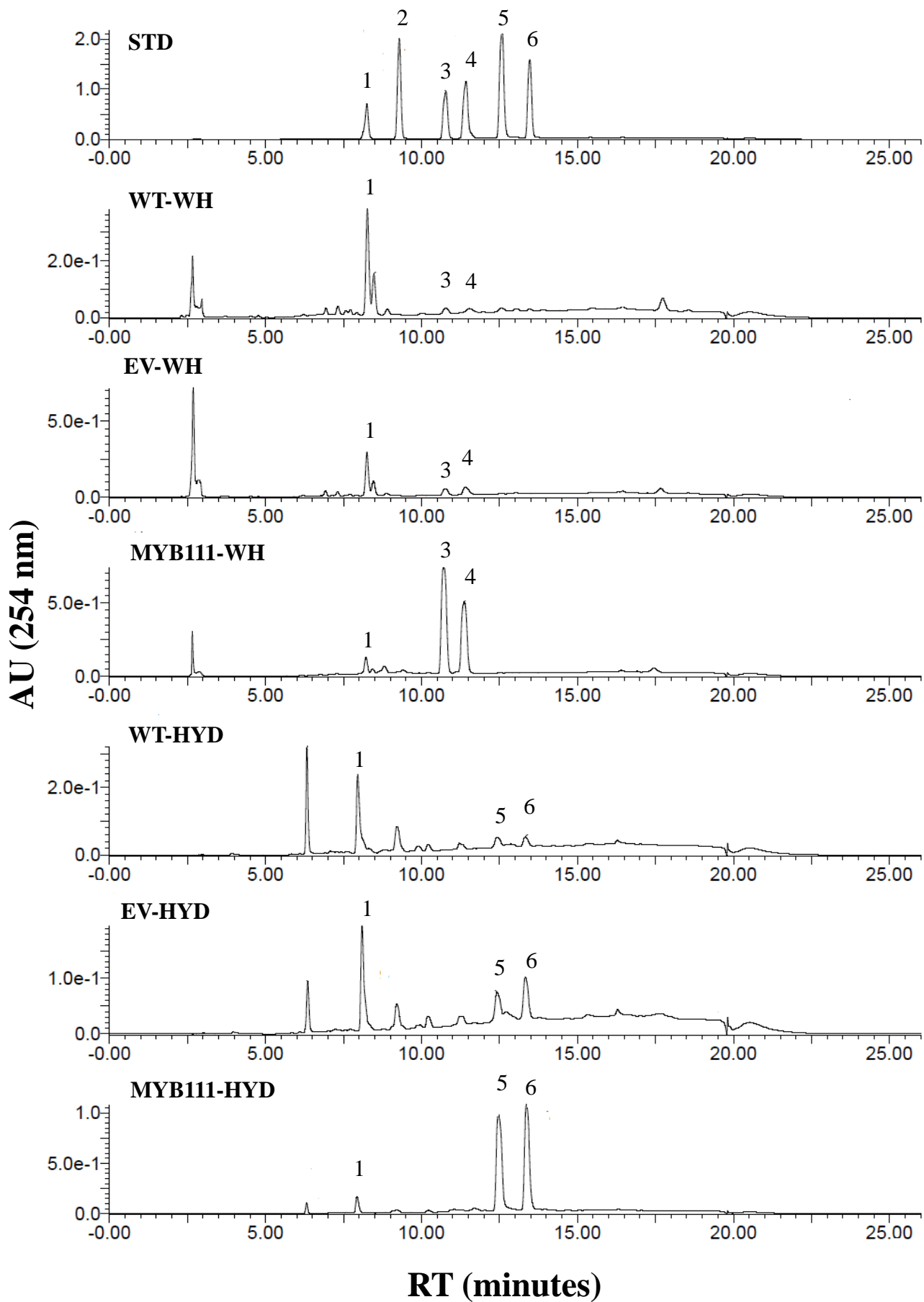
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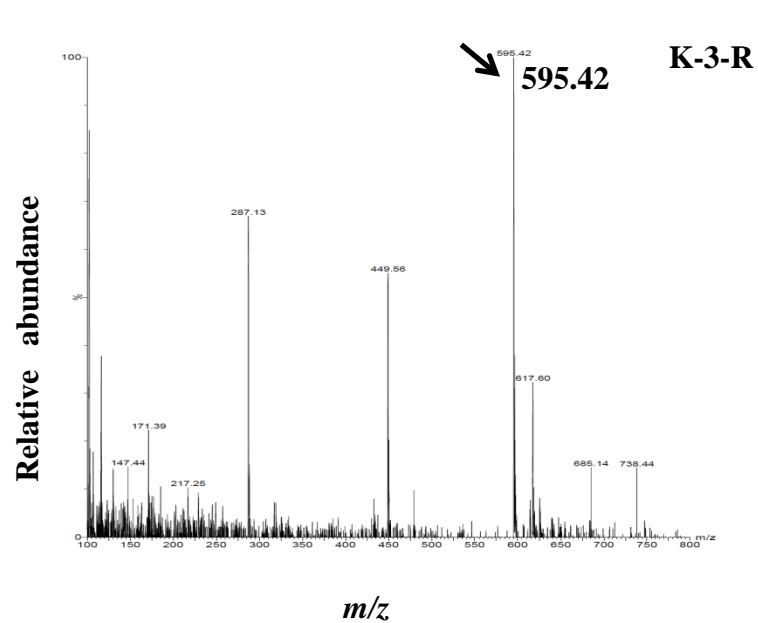
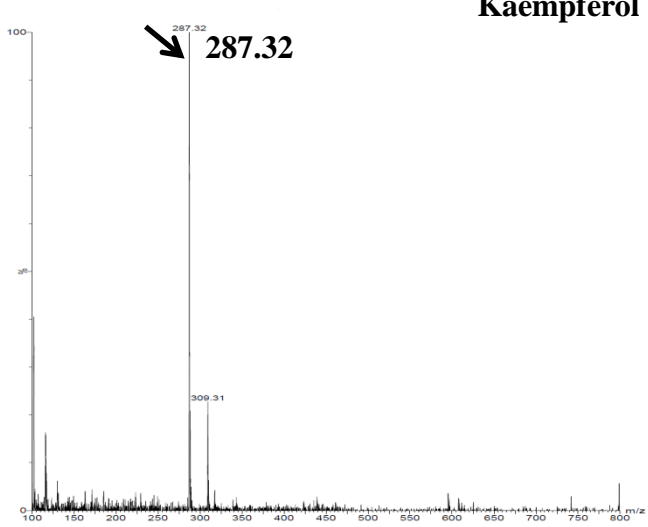
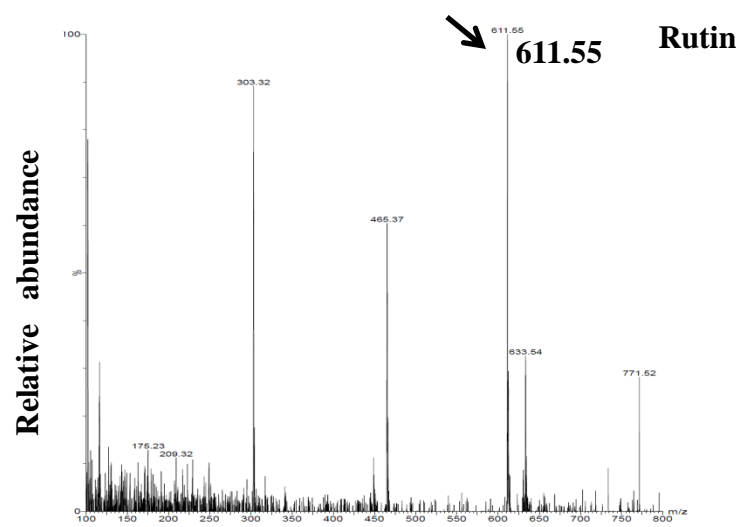
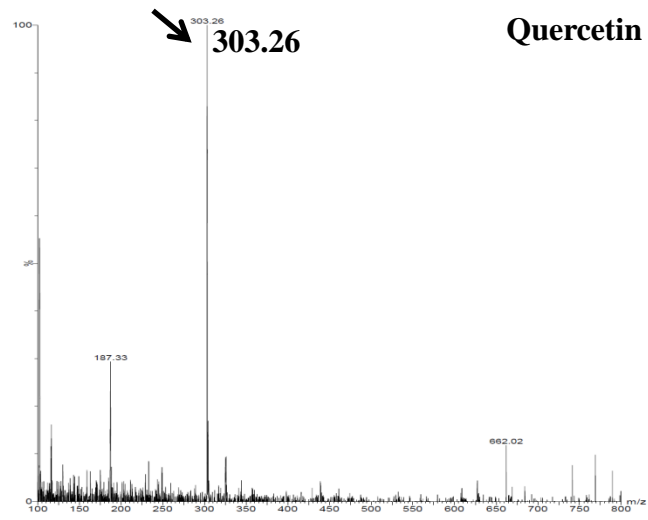
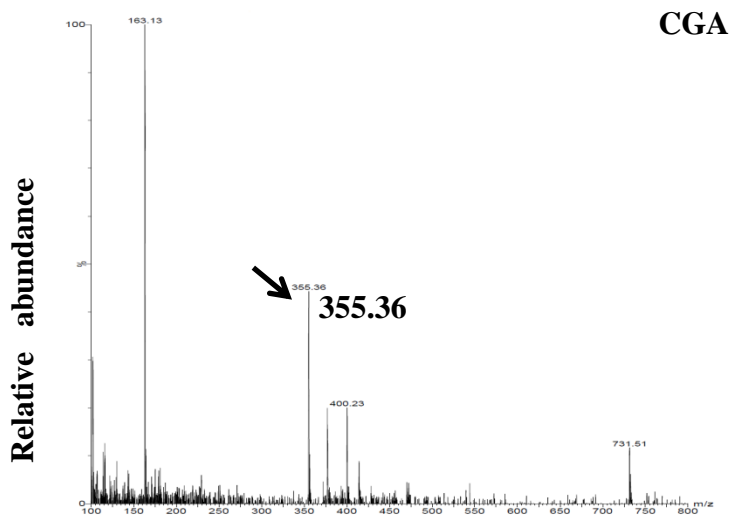
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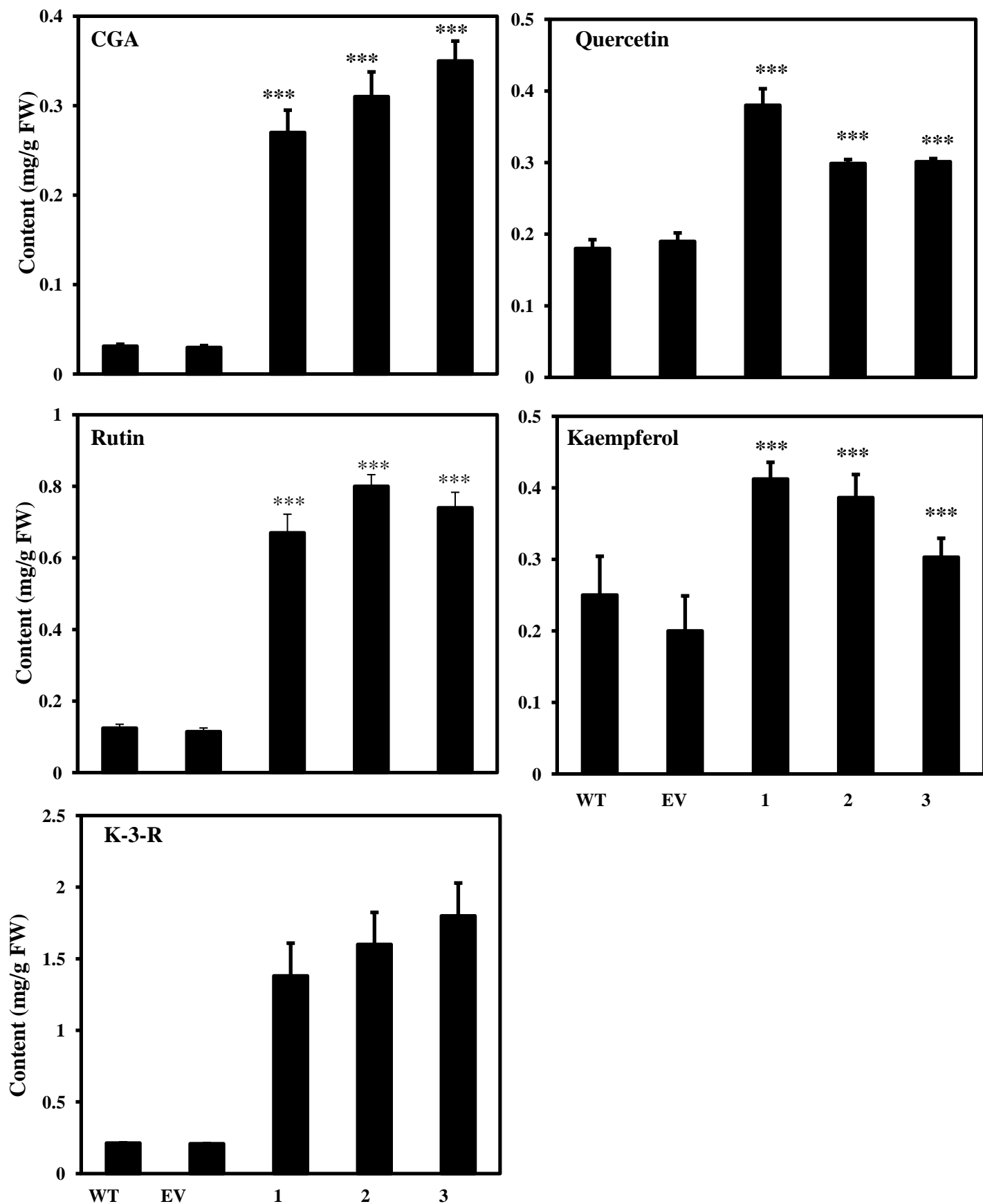




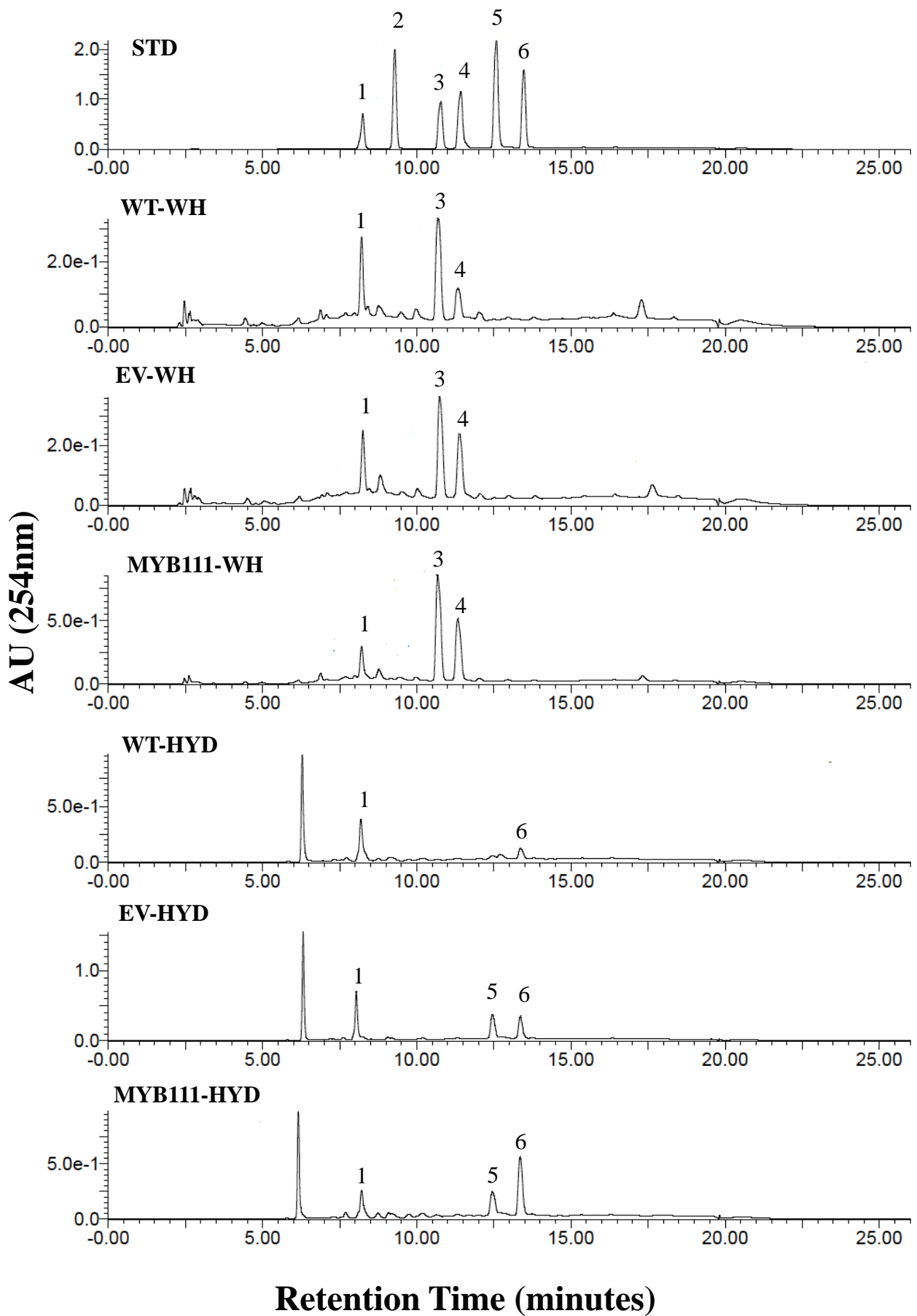
Supplementary Figure S2



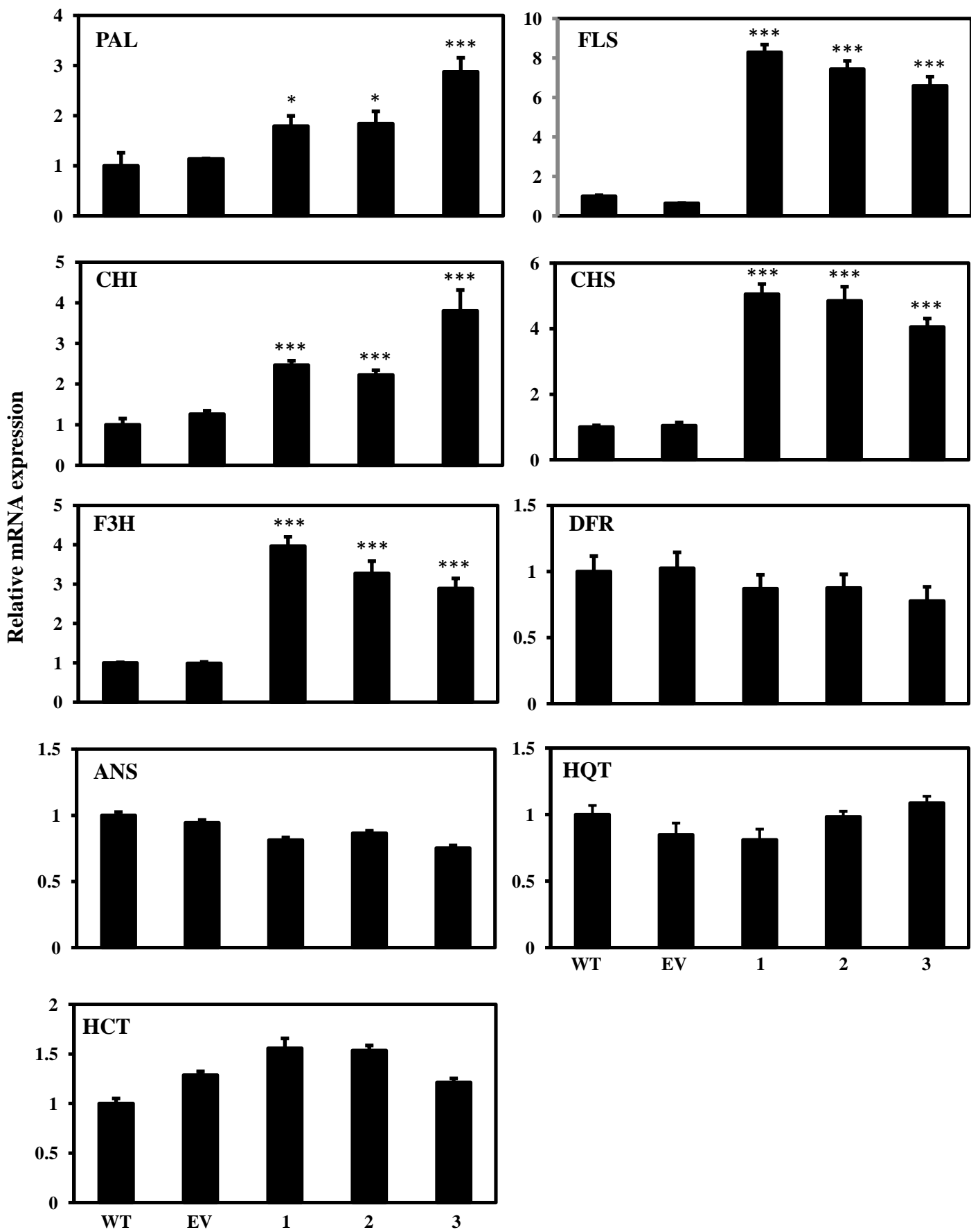
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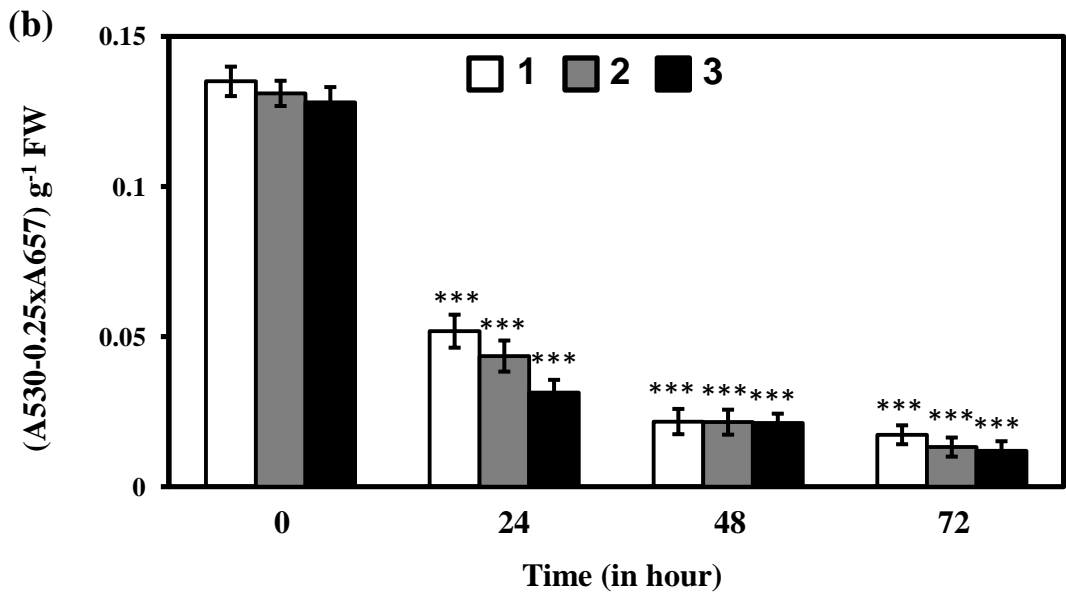
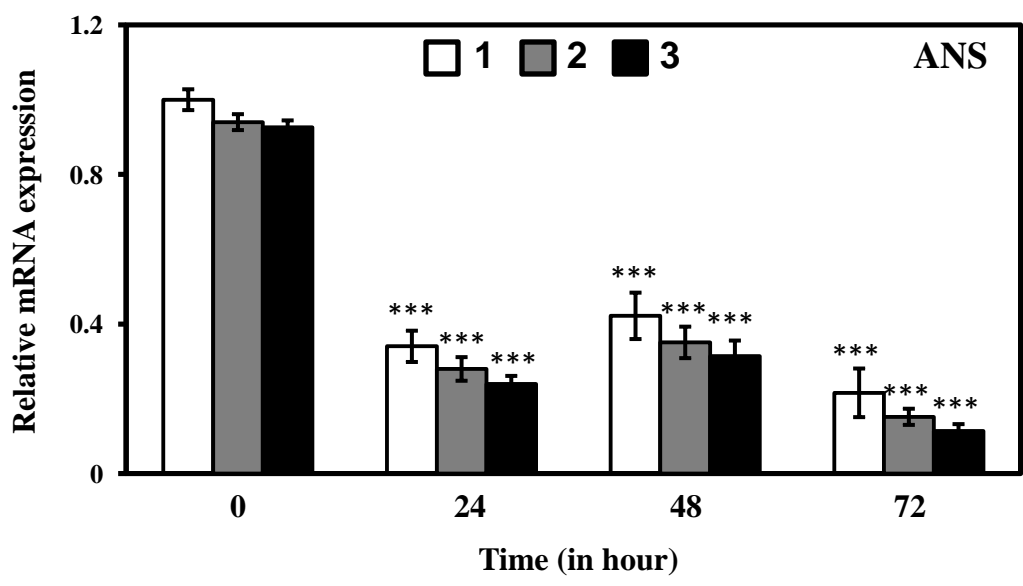
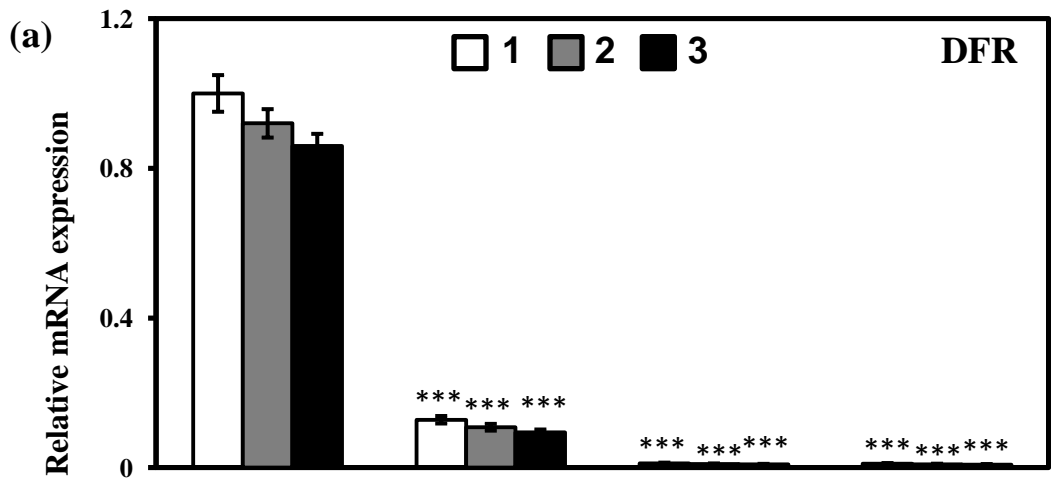
Supplementary Figure S4



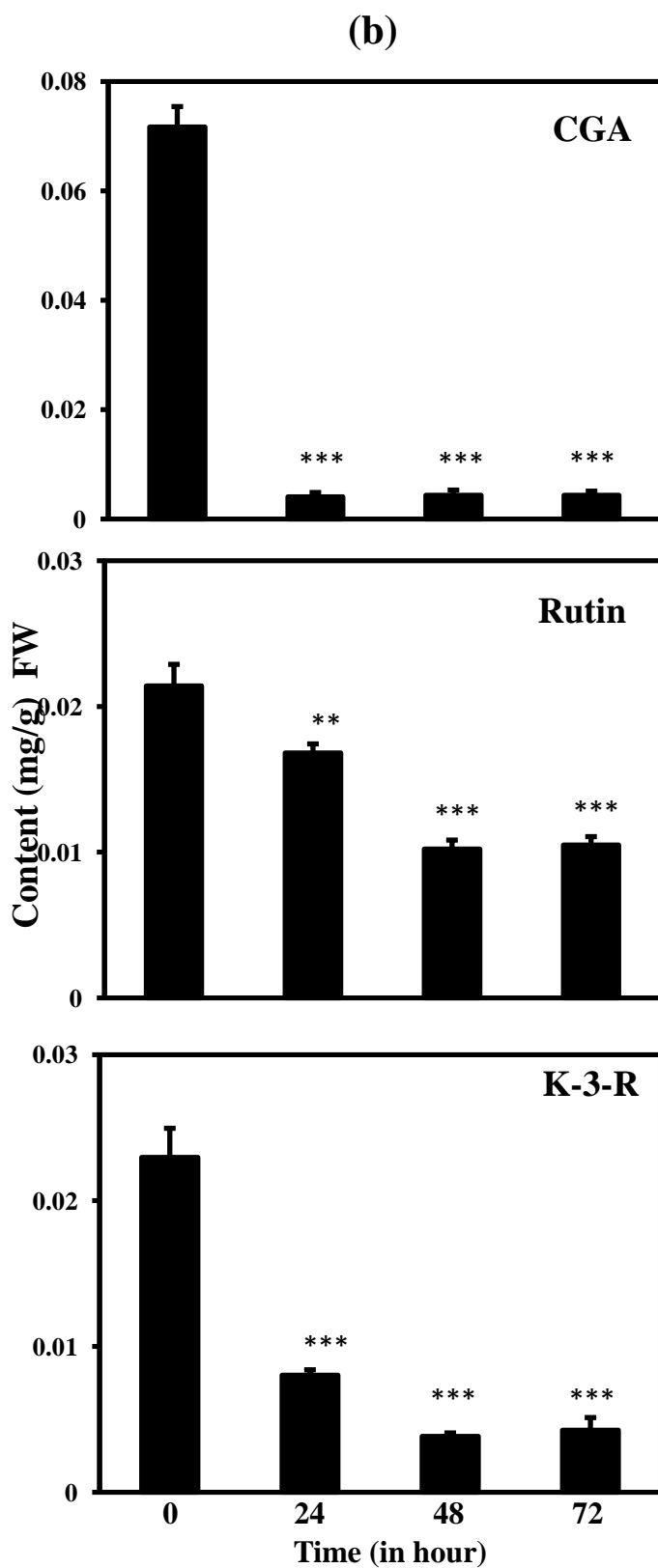
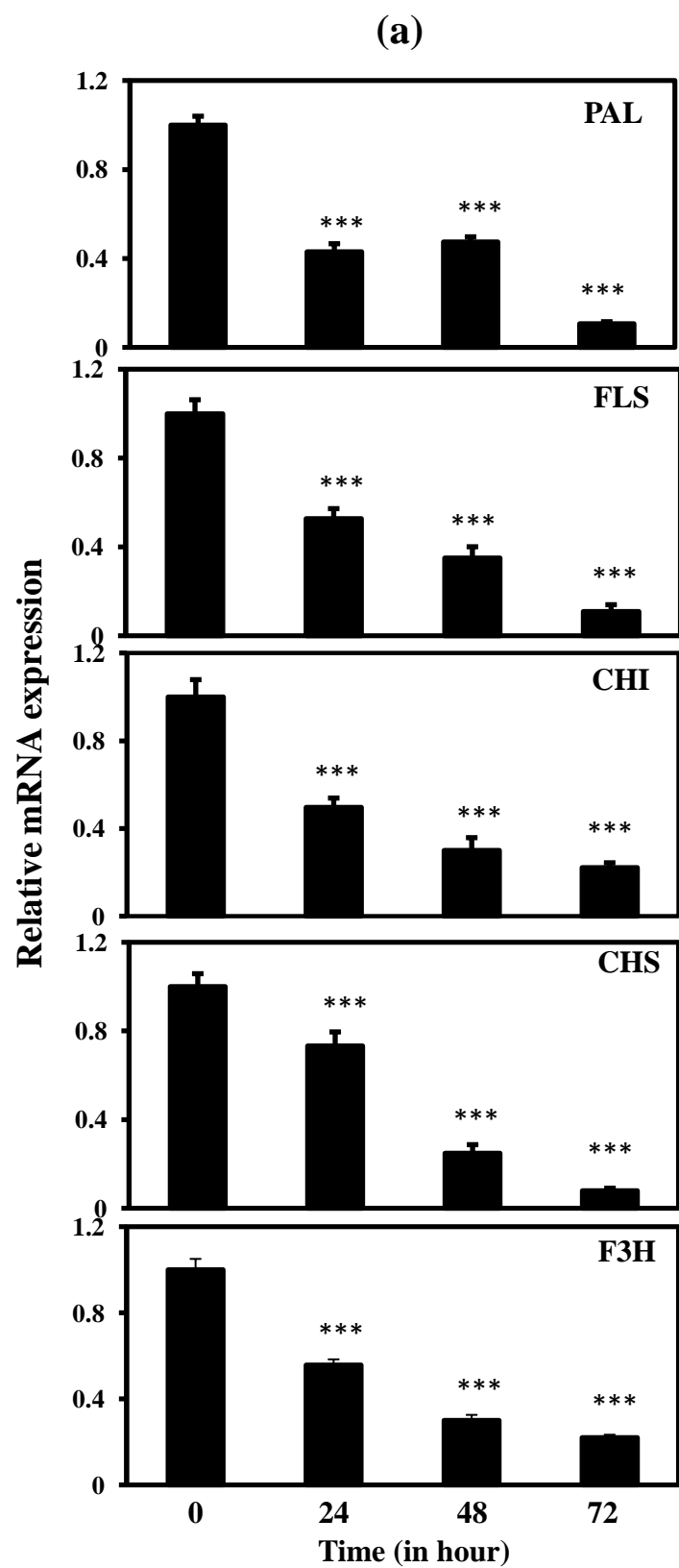
Supplementary Figure S5



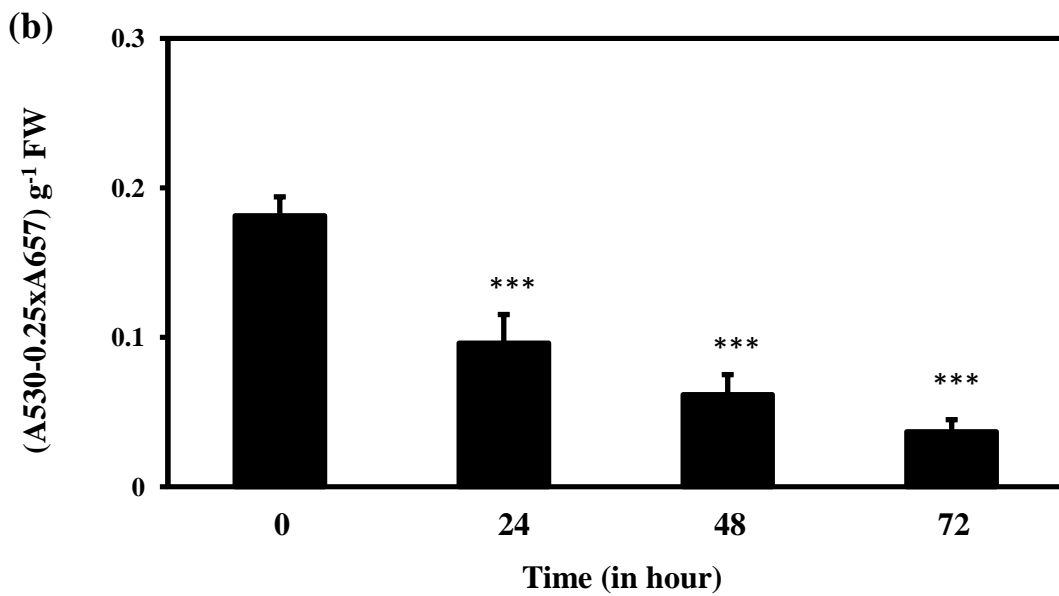
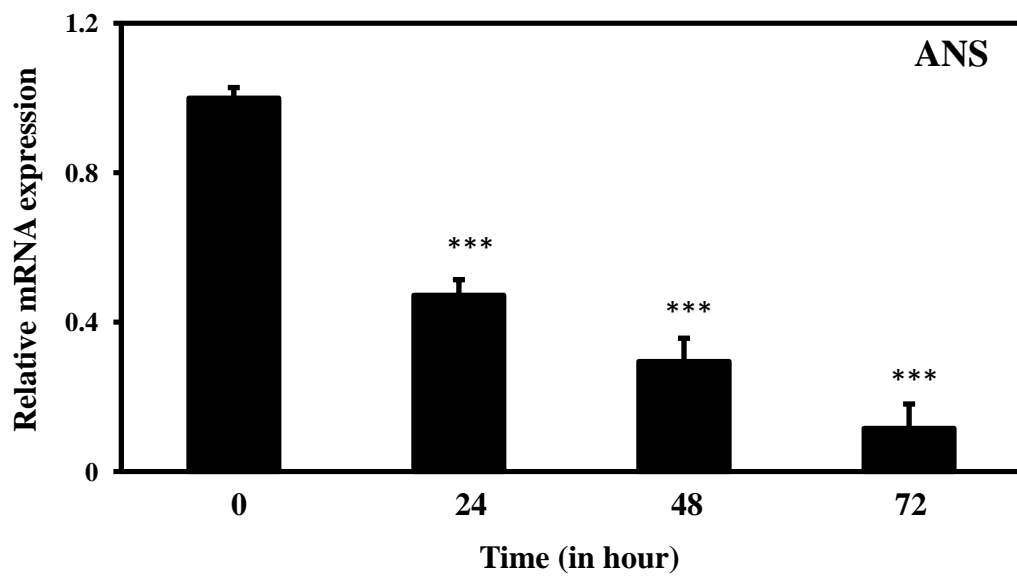
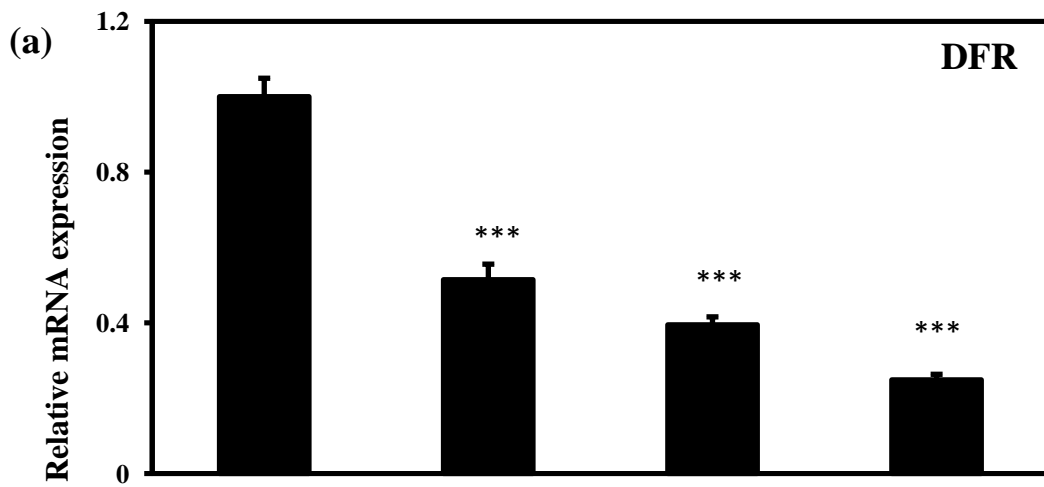
Supplementary Figure S6







Supplementary Figure S8



**Table S1: List of oligonucleotides and their use in the study**

Gene name	Forward (5' to 3')	Reverse (5' to 3')	Purpose
AtMYB111	AACTCACTCTCTCACTCAAGGGTC	TTCTACGCGCGCCTTCTAATGTAC	<i>AtMYB111</i> cDNA isolation
AtMYB111	TGACAGTCTAGACCGAGAAGCAATG	TCCACGAGCTCGGTAGTATCACTCC	Construct preparation
AtMYB111	TGCAACACATCTACCAGGAAG	TTGTGTCCATCTCCGAAAC	Semi quantitative PCR
NtUbi	TCCAGGACAAGGAGGGTAT	GAGACCTCAGTAGACAAAGC	Semi quantitative PCR
NtPAL	GAAGAAAGGTGCATGGACTG	TCTGCAGCTCTTCTGCATC	Real-time PCR
NtCHS	TTGTTCGAGCTTGCTCTGC	AGCCCAGGAACATCTTTGAG	Real-time PCR
NtCHI	GTCAGGCCATTGAAAAGCTC	CTAATCGTCAATGCCCAAC	Real-time PCR
NtF3H	CAAGGCATGTGTGGATATGG	TGTGTCGTTTCAGTCCAAGG	Real-time PCR
NtFLS	TTTGCCACTTGGTGTGTGG	ACTTGACATCATAACCAATGGC	Real-time PCR
NtHQT	ATCAACACATGGTCCGATATAG	ACGTGCTCGAAAGACGATGTTG	Real-time PCR
NtHCT	TAACTACAGCTCCTACGAGATG	GAACGTCCATCTGTTGCTATG	Real-time PCR
NtANS	TGGCGTTGAAGCTCATACTG	GGAATTAGGCACACACTTTGC	Real-time PCR
NtDFR	AACCAACAGTCAGGGGAATG	TTGGACATCGACAGTCCAG	Real-time PCR
NtUbi	GAGGAATGCAGATCTTCGTG	TCCTTGTCCTGGATCTTAGC	Control for Real-time PCR

**Supplementary Figure S1.** Total anthocyanin content in leaves of WT, EV and different transgenic lines (1, 2 and 3).

**Supplementary Figure S2. HPLC profiles illustrating the accumulation of flavonoids in WT, EV and *AtMYB111*-expressing transgenic tobacco plants.** (STD) chlorogenic acid (1), caffeic acid (2), rutin (3), kaempferol-3-*O*-rutinoside (4), quercetin (5), and kaempferol (6). (WT-WH) WT tobacco leaf methanolic extract. (EV-WH) Empty vector control leaf methanolic extract. (MYB111-WH) *AtMYB111*-expressing tobacco leaf methanolic extract. (WT-HYD) WT tobacco leaf acid-hydrolyzed methanolic extract. (EV-HYD) Empty vector control leaf acid-hydrolyzed methanolic extract. (MYB111-HYD) *AtMYB111*-expressing tobacco leaf acid-hydrolyzed methanolic extract. Methanolic as well as acid-hydrolyzed methanolic extract of leaf was separated with HPLC coupled with UV and PDA detector at 254nm.

**Supplementary Figure S3.** MS spectrum of chlorogenic acid (CGA), rutin, kaempferol-3-*O*-rutinoside (K-3-R), quercetin and kaempferol in positive mode.

**Supplementary Figure S4. Phytochemical analysis of petals in WT, EV and different transgenic lines.** Compounds were quantified by separating methanolic as well as acid hydrolysed extracts from the petals WT, EV and transgenic lines using HPLC. The graph shows values  $\pm$  SD of three independent transgenic lines. 1, 2 and 3 represent line 1, line 2 and line 3 respectively.

**Supplementary Figure S5. HPLC profiles for quantification of flavonoids in petal tissue of transgenic, WT and EV control tobacco plants.** (STD) Chlorogenic acid (1), caffeic acid (2), rutin (3), kaempferol-3-*O*-rutinoside (4), quercetin (5), and kaempferol (6). (WT-WH) WT tobacco petals methanolic extract. (EV-WH) Empty vector control petals methanolic extract. (MYB111-WH) *AtMYB111*-expressing tobacco petals methanolic extract. (WT-HYD) WT tobacco petals acid-hydrolyzed methanolic extract. (EV-HYD) Empty vector control petals acid-hydrolyzed methanolic extract. (MYB111-HYD) *AtMYB111*-expressing tobacco petals acid-hydrolyzed methanolic extract. Methanolic as well as acid-hydrolyzed methanolic extract of petals was separated with HPLC coupled with UV and PDA detector at 254nm.

**Supplementary Figure S6.** Quantitative real time expression analysis of the genes involved in phenylpropanoid pathway in petals of WT, EV and different transgenic lines. Expression of structural genes of phenylpropanoid pathway/flavonoid pathway was analyzed using RNA from the petals of different transgenic lines. The graph shows values  $\pm$  SD of three leaves from each of the independent transgenic line. 1, 2 and 3 represent line 1, line 2 and line 3 respectively.

**Supplementary Figure S7. Effect of light on anthocyanin biosynthesis in transgenic tobacco.** (a) Effect of light over the expression of *DFR* and *ANS* genes involved in flavonoid pathway in different transgenic lines. Expression of genes was analyzed by real time PCR using RNA from the leaves of different transgenic lines. (b) Effect of light on total anthocyanin content in different transgenic lines. The graph shows values  $\pm$  SD of three leaves from each of the independent transgenic line. 1, 2 and 3 represent line 1, line 2 and line 3 respectively.

**Supplementary Figure S8. Modulation of flavonoid biosynthesis by light in control (WT) tobacco plant.** (a) Effect of light over the expression of structural genes involved in flavonoid pathway in the WT plants. Expression of structural genes of phenylpropanoid pathway/flavonoid pathway was analyzed by real time PCR using RNA from the leaves of WT plants. (b) Effect of light over flavonoid biosynthesis in WT plants. Phytochemical analysis of methanolic extracts of leaves in WT plants. Compounds were quantified by separating methanolic extracts from the young leaves of WT plants using HPLC.

**Supplementary Figure S9. Effect of light on anthocyanin biosynthesis in control (WT) plant.** (a) Effect of light over the expression of *DFR* and *ANS* genes involved in flavonoid pathway in the WT plants. Expression of genes was analyzed by real time PCR using RNA from the leaves of the WT plants. (b) Effect of light on total anthocyanin content in the WT plants.