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Supplementary Material

Expression and functional analysis of *PhEOL1* and *PhEOL2* during flower senescence in petunia

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Table S1. Primer sequences of *PhEOL1*, *PhEOL2*, *PhACS2* and *PhACS3* used in quantitative real-time PCR

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
PhEOL1	AGTGCCCTGGAGATAAAG	CACAATACTCTGACCGTTT
PhEOL2	ATAGGTGATGTTATGGGTGCC	TCCCCTCCTCCTATTGTCCTT
PhACS2	TTGTTGATTCCTCGGCTAC	CTATCGTTCTCGGTCGTTG
PhACS3	CTGGTTTCGTGTATGTTTTG	ACTTTGCTGATGATTCTGCT
PhActin	TGCTGATCGAATGAGCAAGGAA	GGAGCAACAACCTTAATCTTC
PhCYP	AGGCTCATCATTCCACCGTGT	TCATCTGCGAACTTAGCACCG

Table S2. Primer sequences of *PhEOL1* and *PhEOL2* used in VIGS

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
PhEOL1	AGGCTCAGAACAAGGCATCA	CGGAATTCGCTCACCACCATCACATCC
PhEOL2	GCGGATCCCTGCTTATGACGAGATGACCA	CGGAATTCACGACTATGAAGTTCCAGCA
PhCHS	GATCTCGAGTGGAGGCATTCCAACCATTG	CCAGAGCTCATTCAAGACCTTCACCAG

Table S3. Primer sequences of *PhEOL1*, *PhACS2* and *PhACS3* used in yeast 2-hybrid

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
PhEOL1	CTGAATTCATGTCTACATCTAGAGACAACG	GCGTCGACTCATGTATTTTGTCGTGCTTGA
PhACS2	CCGGAATTCAAACAATGAAGCTTTTATCAGA	AGTCATCGATGACTATCGTTCTCGGTCGTTGA
PhACS3	CTGAATTCATGAAGATGTTGTCAGAGAAAG	CGGGATCCCTATCGTTCTCTTTGACGATCA

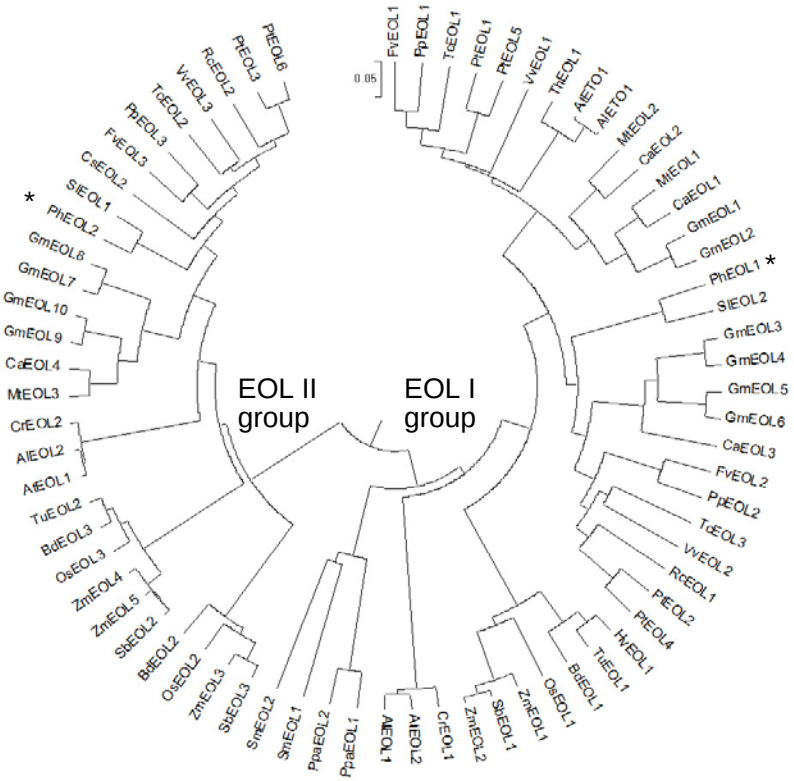

Fig. S1. Phylogenetic tree of EOLs. Two petunia PhEOLs (asterisk) were aligned with *Arabidopsis thaliana* AtETO1 (NP_001030839), AtEOL1 (NP_192177), AtEOL2 (NP_200663), *Solanum lycopersicum* SlEOL1 (NP_001234175), SlEOL2 (XP_004247013), *Arabidopsis lyrata* AlETO1 (XP_002877822), AlEOL1 (XP_002866273), AlEOL2 (XP_002872824), *Capsella rubella* CrEOL1(EOA12867), CrEOL2 (EOA23457), *Thellungiella halophila* ThEOL1 (BAJ34198), *Theobroma cacao* TcEOL1 (EOY07113), TcEOL2 (EOX94657), TcEOL3 (EOY34563), *Fragaria vesca* FvEOL1 (XP_004302535), FvEOL2 (XP_XP_004294790), FvEOL3 (XP_004290632), *Prunus persica* PpEOL1 (EMJ09575), PpEOL2 (EMJ09914), PpEOL3 (EMJ02952), *Glycine max* GmEOL1 (XP_003516976), GmEOL2 (XP_003520346), GmEOL3 (XP_003544898), GmEOL4 (XP_003519262), GmEOL5 (XP_003551231), GmEOL6 (XP_003539361), GmEOL7 (XP_003521261), GmEOL8 (XP_003554270), GmEOL9 (XP_003518903), GmEOL10 (XP_003536706), *Cicer arietinum* CaEOL1 (XP_004506795), CaEOL2 (XP_004492482), CaEOL3 (XP_004500295), CaEOL4 (XP_004495171), *Vitis vinifera* VvEOL1 (XP_002269998), VvEOL2 (XP_002278414), VvEOL3 (XP_002280519), *Medicago truncatula* MtEOL1 (XP_003604576), MtEOL2 (XP_003623335), MtEOL3 (XP_003590582), *Populus trichocarpa* PtEOL1 (XP_002309154), PtEOL2 (XP_002313975), PtEOL3 (XP_002306795), PtEOL4 (XP_002298492), PtEOL5 (XP_002323609), PtEOL6 (XP_002302093), *Ricinus communis* RcEOL1 (XP_002521192), RcEOL2 (XP_002520939), SbEOL1 (XP_002468027), *Sorghum bicolor* SbEOL2 (XP_002449762), SbEOL3 (XP_002459418), *Zea mays* ZmEOL1 (DAA44871), ZmEOL2 (AFW88685), ZmEOL3 (NP_001147844), ZmEOL4 (DAA42183), *Brachypodium distachyon* BdEOL1 (XP_003558142),

BdEOL2 (XP_003557494), BdEOL3 (XP_003577420), *Hordeum vulgare* HvEOL1 (BAJ99623), *Oryza sativa* OsEOL1 (EEE58870), OsEOL2 (NP_001059027), OsEOL3 (ABA94447), *Physcomitrella patens* PpaEOL1 (XP_001754017), PpaEOL2 (XP_001766795), *Selaginella moellendorffii* SmEOL1 (XP_002973474), SmEOL2 (XP_002982871), *Triticum urartu* TuEOL1 (EMS47068), TuEOL2 (EMS59877), *Cucumis sativus* CsEOL2 (XP_004145366). The amino acid sequences of Arabidopsis EOLs were obtained from The Arabidopsis Information Resource or the National Center for Biotechnology Information database. The amino acid sequences were analyzed with Vector NTI (version 9.0.0; Invitrogen), and the phylogenetic tree was constructed with MEGA (version 3.1) using a bootstrap test of phylogeny with minimum evolution test and default parameters.

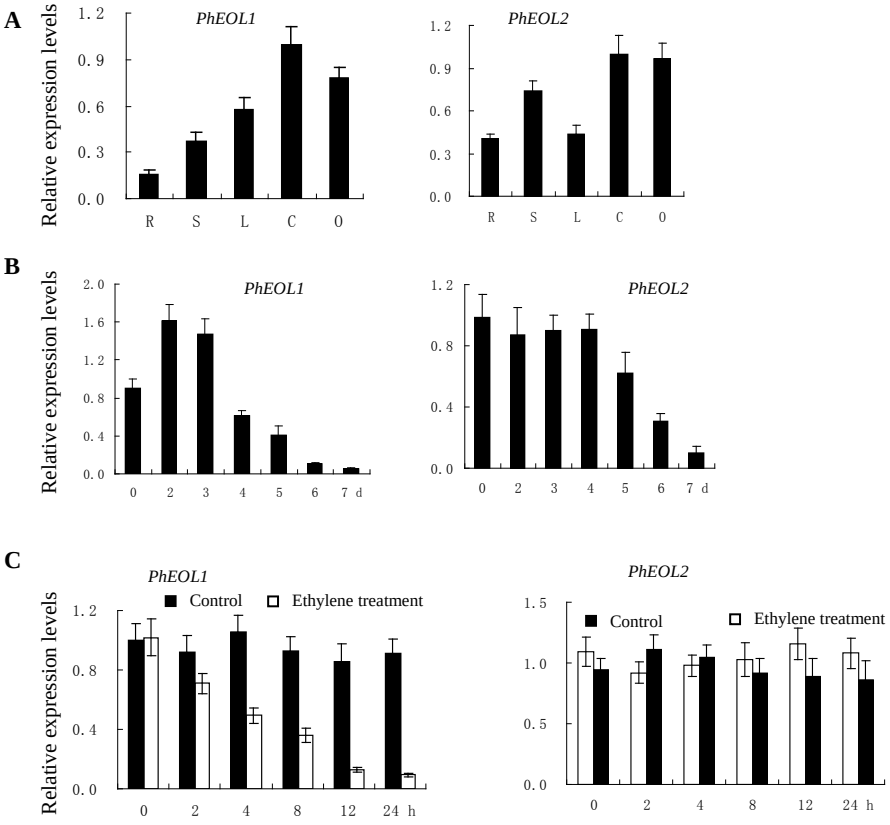

Fig. S2. Expression of *PhEOL1* and *PhEOL2* determined by quantitative real-time PCR with the internal reference gene *PhCYP* in different organs (A), in corollas during natural flower senescence (B), and in corollas in response to 2 µl l^{-1} exogenous ethylene (C). R, roots; L, leaves; S, stems; C, corollas; O, ovaries. Relative expression levels are shown as fold change values. Data are presented as the mean ± SD (n = 3). Different letters mean significant difference at P=0.05 level.

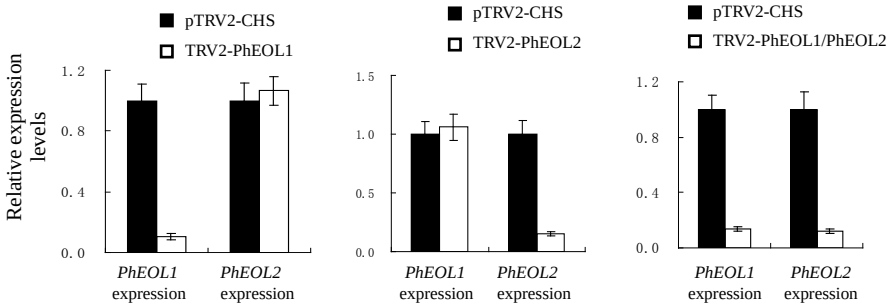

Fig. S3. Effects of TRV2-CHS/PhEOL1 (left), TRV2-CHS/PhEOL2 (middle) and TRV2-CHS/ PhEOL1/PhEOL2 (right) treatment on the expression of *PhEOL1* and *PhEOL2* in white flowers on day 4 after opening as determined by quantitative real-time PCR with the internal reference gene *PhCYP*, respectively. Relative expression levels are shown as fold change values. Data are presented as the mean ± SD (n = 3). Different letters mean significant difference at P=0.05 level.

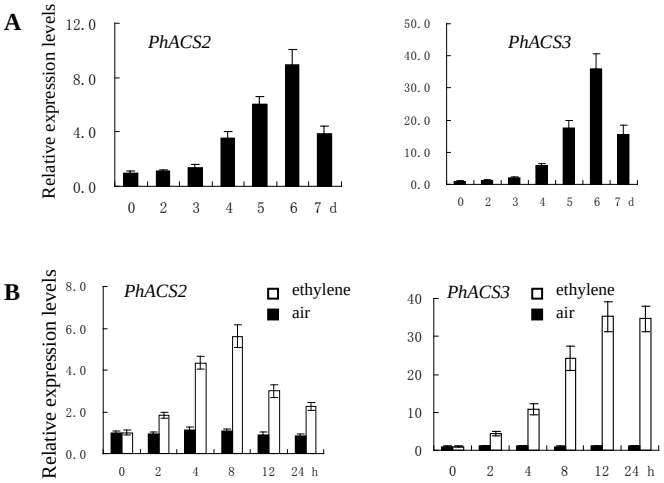

Fig. S4. Expression of *PhACS2* and *PhACS3* in the corollas determined by quantitative real-time PCR with the internal reference gene *PhCYP* during natural flower senescence (A), and in response to 2 μl l^{-1} exogenous ethylene (B). Relative expression levels are shown as fold change values. Data are presented as the mean ± SD (n = 3). Different letters mean significant difference at P=0.05 level.