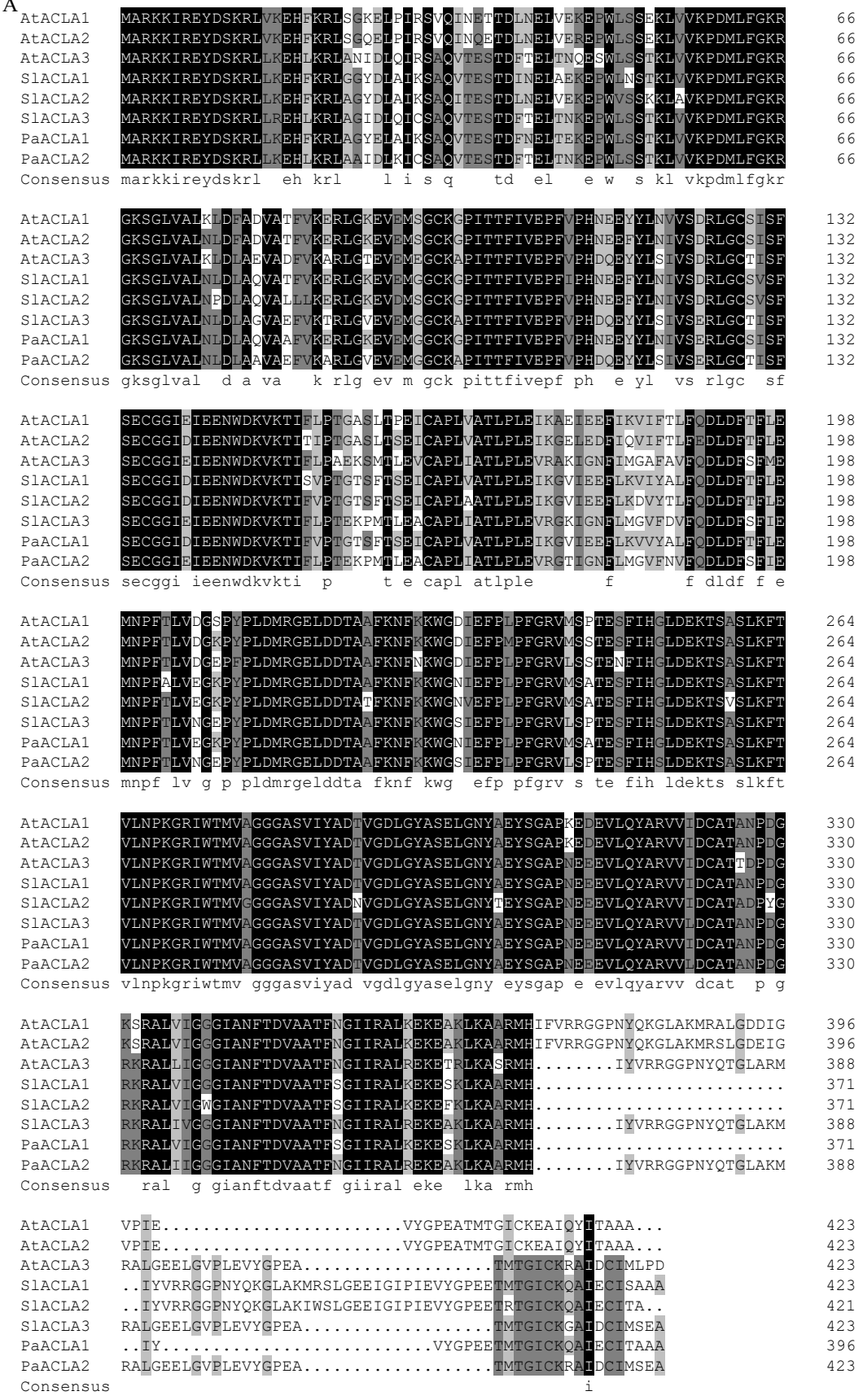
A
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AtACLA3 MARKKIREYDSKRLLKEHLKRLANIDLQIRSAQVTESTDFTELTNQESWLSSTKLVVKPDMLFGKR 66
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SlACLA2 MARKKIREYDSKRLLKEHFKRLAGYDLAIKSAQITESTDLNELVEKEPWVSSKKLAVKPDMLFGKR 66
SlACLA3 MARKKIREYDSKRLLREHLKRLAGIDLQICSAQVTESTDFTELTNKEPWLSSTKLVVKPDMLFGKR 66
PaACLA1 MARKKIREYDSKRLLKEHFKRLAGYELAIKSAQVTESTDFNELTEKEPWLSSTKLVVKPDMLFGKR 66
PaACLA2 MARKKIREYDSKRLLKEHLKRLAAIDLKICSAQVTESTDFTELTNKEPWLSSTKLVVKPDMLFGKR 66
Consensus markkireydskrl eh krl l i s q td el e w s kl vkpdmlfgkr
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AtACLA2 GKSGLVALNLDFADVATFVKERLGKEVEMSGCKGPITTFIVEPFVPHNEEFYLNIVSDRLGCSISF 132
AtACLA3 GKSGLVALKLDLAEVADFVKARLGTEVEMEGCKAPITTFIVEPFVPHDQEYYLSIVSDRLGCTISF 132
SlACLA1 GKSGLVALNLDLAQVATFVKERLGKEVEMGGCKGPITTFIVEPFIPHNEEFYLNIVSDRLGCSVSF 132
SlACLA2 GKSGLVALNPDLAQVALLLKERLGKEVDMSGCKGPITTFIVEPFVPHNEEFYLNIVSDRLGCSVSF 132
SlACLA3 GKSGLVALNLDLAGVAEFVKTRLGVEVEMGGCKAPITTFIVEPFVPHDQEYYLSIVSERLGCTISF 132
PaACLA1 GKSGLVALNLDLAQVAAFVKERLGKEVEMGGCKGPITTFIVEPFVPHNEEYYLNIVSERLGCSISF 132
PaACLA2 GKSGLVALNLDLAAVAEFVKARLGVEVEMGGCKAPITTFIVEPFVPHDQEYYLSIVSERLGCTISF 132
Consensus gksglval d a va k rlg ev m gck pittfivepf ph e yl vs rlgc sf
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AtACLA3 SECGGIEIEENWDKVKTIFLPAEKSMTLEVCAPLIATLPLEVRAKIGNFIMGAFAVFQDLDFSFME 198
SlACLA1 SECGGIDIEENWDKVKTISVPTGTSFTSEICAPLVATLPLEIKGVIEEFLKVIYALFQDLDFTFLE 198
SlACLA2 SECGGIDIEENWDKVKTIFVPTGTSFTSEICAPLAATLPLEIKGVIEEFLKDVYTLFQDLDFTFLE 198
SlACLA3 SECGGIEIEENWDKVKTIFLPTEKPMTLEACAPLIATLPLEVRGKIGNFLMGVFDVFQDLDFSFLE 198
PaACLA1 SECGGIDIEENWDKVKTIFVPTGTSFTSEICAPLVATLPLEIKGVIEEFLKVVYALFQDLDFTFLE 198
PaACLA2 SECGGIEIEENWDKVKTIFLPTEKPMTLEACAPLIATLPLEVRGTIGNFLMGVFNVFQDLDFSFLE 198
Consensus secggi ieenwdkvkti p t e capl atlple f f dldf f e
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SlACLA2 MNPFTLVEGKPYPLDMRGELDDTATFKNFKKWGNVEFPLPFGRVMSATESFIHGLDEKTSVSLKFT 264
SlACLA3 MNPFTLVNGEPYPLDMRGELDDTAAFKNFKKWGSIEFPLPFGRVLSPTESFIHSLDEKTSASLKFT 264
PaACLA1 MNPFTLVEGKPYPLDMRGELDDTAAFKNFKKWGNIEFPLPFGRVMSATESFIHGLDEKTSASLKFT 264
PaACLA2 MNPFTLVNGEPYPLDMRGELDDTAAFKNFKKWGSIEFPLPFGRVLSPTESFIHSLDEKTSASLKFT 264
Consensus mnpf lv g p pldmrgelddta fknf kwg efp pfgrv s te fih ldekts slkft
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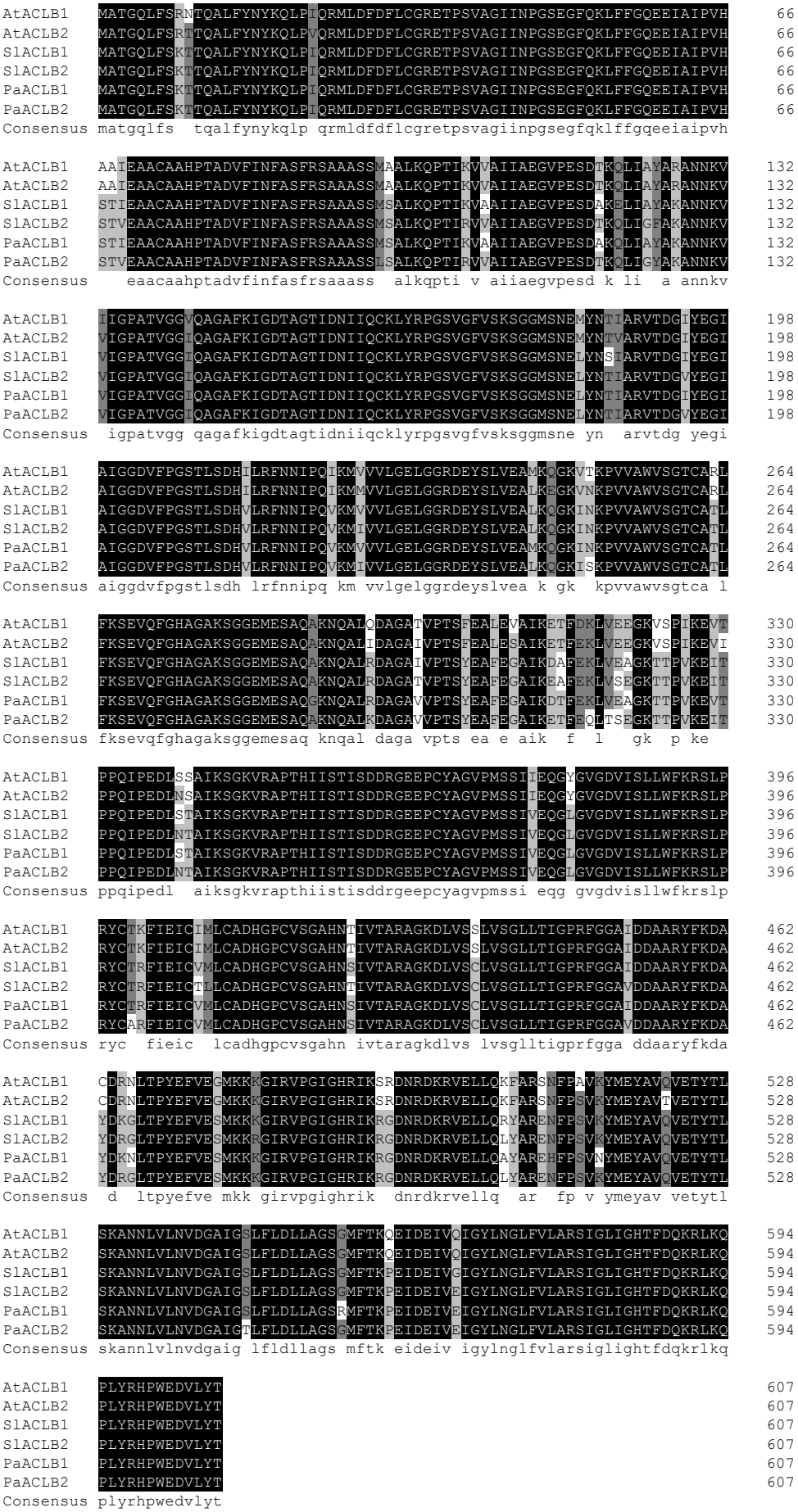
AtACLA3 VLNPKGRIWTMVAGGGASVIYADTVGDLGYASELGNYAEYSGAPNEDEVLQYARVVIDCATTDPDG 330
SlACLA1 VLNPKGRIWTMVAGGGASVIYADTVGDLGYASELGNYAEYSGAPNEDEVLQYARVVIDCATANPDG 330
SlACLA2 VLNPKGRIWTMVGGGGASVIYADNVGDLGYASELGNYTEYSGAPNEDEVLQYARVVIDCATADPYG 330
SlACLA3 VLNPKGRIWTMVAGGGASVIYADTVGDLGYASELGNYAEYSGAPNEDEVLQYARVVLDCATANPDG 330
PaACLA1 VLNPKGRIWTMVAGGGASVIYADTVGDLGYASELGNYAEYSGAPNEDEVLQYARVVIDCATANPDG 330
PaACLA2 VLNPKGRIWTMVAGGGASVIYADTVGDLGYASELGNYAEYSGAPNEDEVLQYARVVLDCATANPDG 330
Consensus vlnpkgriwtmv gggasviyad vgdlgyaselgny eysgap e evlqyarvv dcat p g
AtACLA1 KSRALVIGGGIANFTDVAATFNGIIRALKEKEAKLKAARMHIFVRRGGPNYQKGLAKMRALGDDIG 396
AtACLA2 KSRALVIGGGIANFTDVAATFNGIIRALKEKEAKLKAARMHIFVRRGGPNYQKGLAKMRSLGDEIG 396
AtACLA3 RKRALLIGGGIANFTDVAATFNGIIRALREKETRLKASRMH.....IYVRRGGPNYQTGLARM 388
SlACLA1 RKRALVIGGGIANFTDVAATFSGIIRALKEKESKLKAARMH..... 371
SlACLA2 RKRALVIGWGIANFTDVAATFSGIIRALKEKEFKLKAARMH..... 371
SlACLA3 RKRALIVGGGIANFTDVAATFNGIIRALREKEAKLKAARMH.....IYVRRGGPNYQTGLAKM 388
PaACLA1 RKRALVIGGGIANFTDVAATFSGIIRALKEKESKLKAARMH..... 371
PaACLA2 RKRALIIGGGIANFTDVAATFNGIIRALREKEAKLKAARMH.....IYVRRGGPNYQTGLAKM 388
Consensus ral g gianftdvaatf giiral eke lka rmh
AtACLA1 VPIE.....VYGPEATMTGICKEAIQYITAAA... 423
AtACLA2 VPIE.....VYGPEATMTGICKEAIQYITAAA... 423
AtACLA3 RALGEELGVPLEVYGPEA.....TMTGICKRAIDCIMLPD 423
SlACLA1 ..IYVRRGGPNYQKGLAKMRSLGEEIGIPIEVYGPEETMTGICKQAIECISAAA 423
SlACLA2 ..IYVRRGGPNYQKGLAKIWSLGEEIGIPIEVYGPEETRTGICKQAIECITA.. 421
SlACLA3 RALGEELGVPLEVYGPEA.....TMTGICKGAIDCIMSEA 423
PaACLA1 ..IY.....VYGPEETMTGICKQAIECITAAA 396
PaACLA2 RALGEELGVPLEVYGPEA.....TMTGICKRAIDCIMSEA 423
Consensus i

B


AtACLB1
AtACLB2
SlACLB1
SlACLB2
PaACLB1
PaACLB2
Consensus

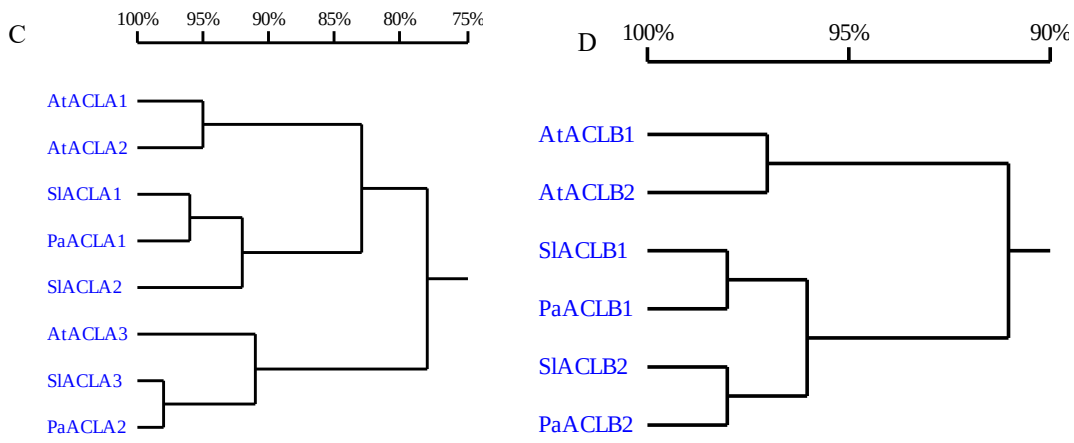



**Figure S1** Predicted amino acid sequence alignments and neighbour-joining trees of ACLAs and ACLBs. (A) Predicted amino acid sequence alignments of petunia PaACLAs with *Arabidopsis thaliana* AtACLA1 (AT1G10670), AtACLA2 (AT1G60810), AtACLA3 (AT1G09430), *Solanum lycopersicum* SlACLA1 (Solyc05g005160.3), SlACLA2 (Solyc04g039670.3) and SlACLA3 (Solyc01g101040.3). Conserved residues are shaded in black. Grey shading indicates similar residues in six out of eight of the sequences. Slight grey shading indicates similar residues in five out of eight of the sequences. (B) Predicted amino acid sequence alignments of petunia PaACLBs with AtACLB1 (AT3G06650), AtACLB2 (AT5G49460), SlACLB1 (Solyc01g059880.3) and SlACLB2 (Solyc12g099260.1). Conserved residues are shaded in black. Grey shading indicates similar residues in five out of six of the sequences. Light grey shading indicates similar residues in four out of six of the sequences. (C) and (D) Neighbour-joining trees among proteins encoded by the *ACLA*- and *ACLB*-like genes using DNAMAN.

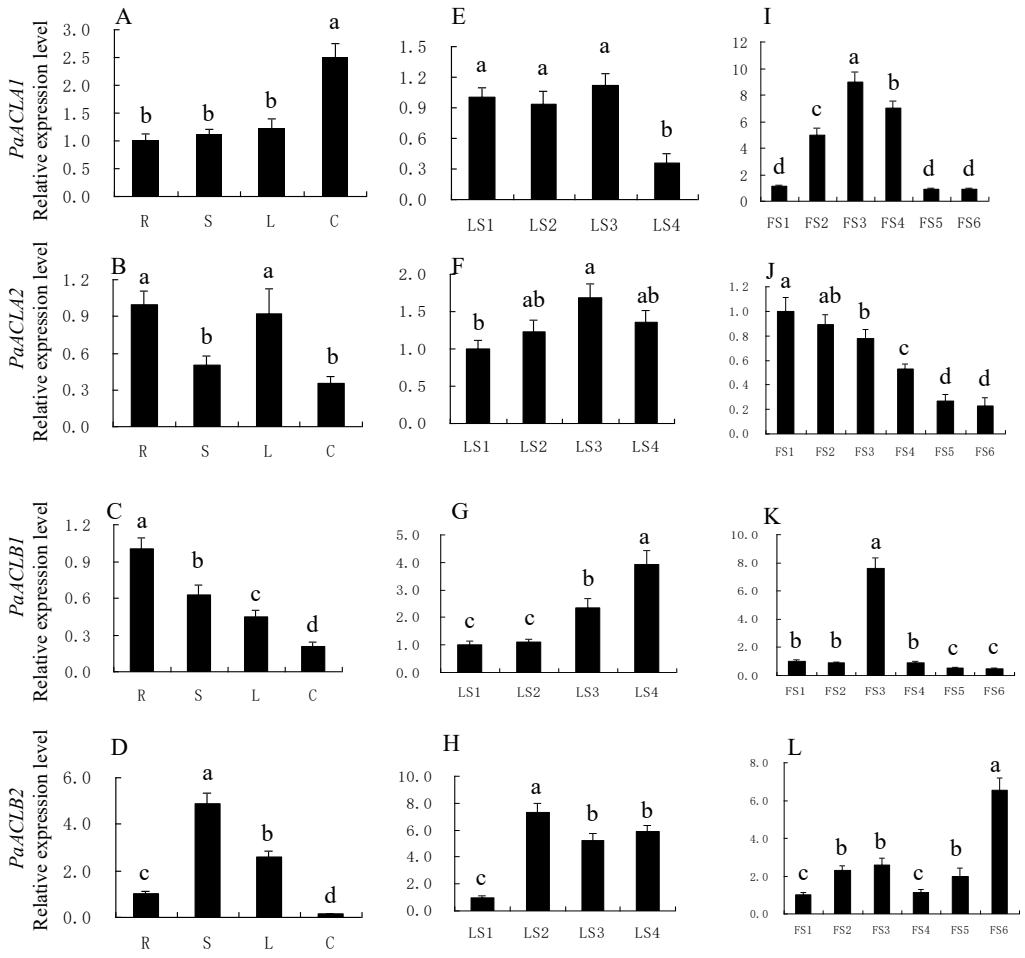



**Figure S2** Expression patterns of *PaACLAs* and *PaACLBs* determined by quantitative real-time PCR using *Actin* as the internal reference gene. (A-D) Expression of *PaACLA1* (A), *PaACLA2* (B), *PaACLB1* (C) and *PaACLB2* (D) in different organs. R, roots; S, stems; L, leaves; C, corollas. (E-H) Expression of *PaACLA1* (E), *PaACLA2* (F), *PaACLB1* (G) and *PaACLB2* (H) during leaf development. LS1, young leaves, 1 cm; LS2, growth leaves, 3 cm; LS3, mature leaves, 4 cm; LS4, old leaves. (I-L) Expression of *PaACLA1* (I), *PaACLA2* (J), *PaACLB1* (K) and *PaACLB2* (L) during flower development. S1-S6, six flower development stages. FS1 (0.5 cm in length), FS2 (1.0 cm), FS3 (2.0 cm), FS4 (3.0 cm), FS5 (4.0 cm) and FS6 (anthesis). *Actin* (accession no. FN014209) was used as the internal reference gene to quantify the cDNA abundance. Data are presented as the means ± SD (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) with 3 biological replicates.

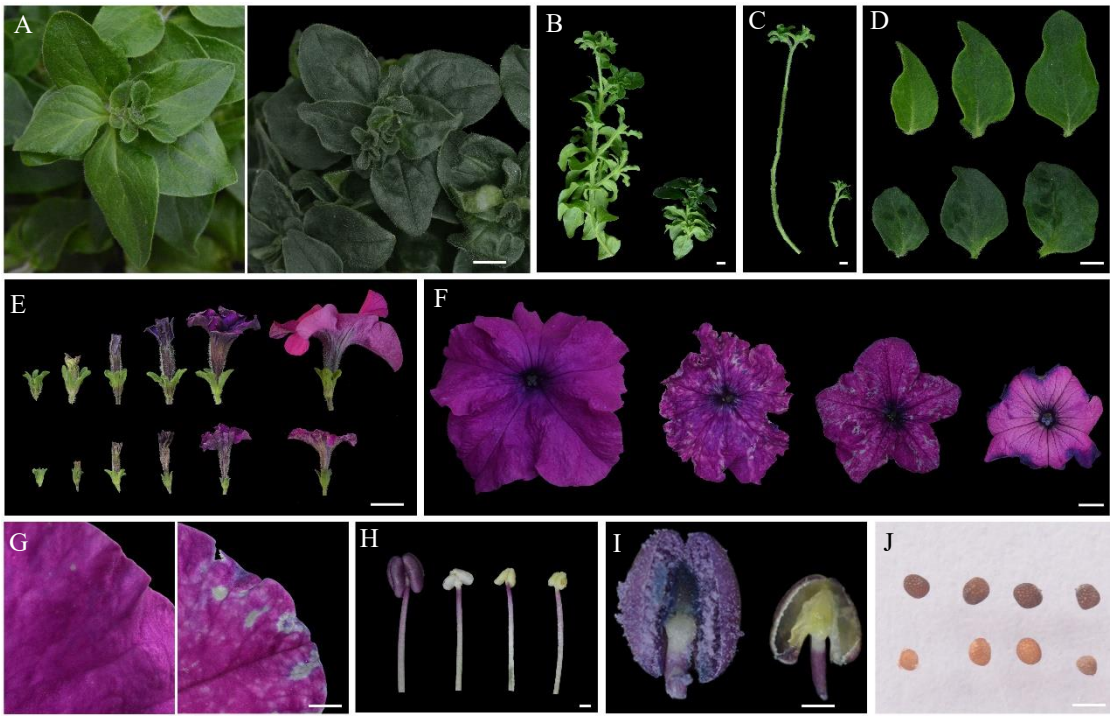



**Figure S3** Phenotypical alteration of VIGS-mediated silencing of *PaACLB1* and *PaACLB2* in plants. (A-C) pTRV2- (left) and pTRV2-PaACLB1-B2-treated plant (right) branches from five-week-old plants. (D) Abnormal leaves of pTRV2-PaACLB1-B2-treated plants (right) and controls (left). (E) The six developmental stages of petunia buds of pTRV2-PaACLB1-B2-treated plants (bottom) and control (top). (F) Abnormal flowers of pTRV2-PaACLB1-B2-treated plants (right three) and controls (left). (G) Petals with transparent spots of pTRV2-PaACLB1-B2-treated plants (right) and control (left). (H) Abnormal anthers of pTRV2-PaACLB1-B2-treated plants (right three) and controls (left). (I) The anthers without pollen of pTRV2-PaACLB1-B2-treated plants (right) and control (left). (J) The seeds from pTRV2-PaACLB1-B2-treated plants (bottom) and control (top). Bars=1 cm in (A-C) and (F); bars=0.5 cm in (D); bars=2 cm in (E); bars=0.3 cm in (G); bars= 0.1 mm in (H); bars= 0.05 mm in (I) and (J).

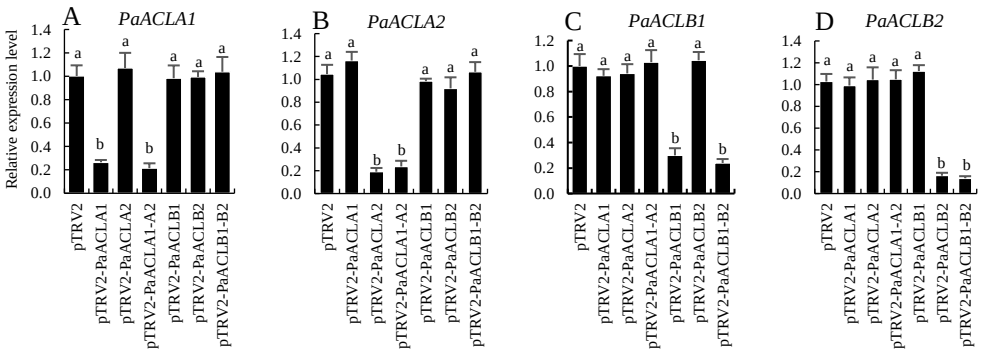



**Figure S4** Expression of *PaACLAs* and *PaACLBs* determined using quantitative real-time PCR. (A-D) Expression of *PaACLA1* (A), *PaACLA2* (B), *PaACLB1* (C) and *PaACLB2*(D) in corollas of the control plants and *PaACLAs*- and *PaACLBs*-silenced plants. *Cyclophilin* (accession no. EST883944) was used as the internal reference gene to quantify the cDNA abundance. Data are presented as the means ± SD (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) with 3 biological replicates. P-values ≤0.05 were considered significant.

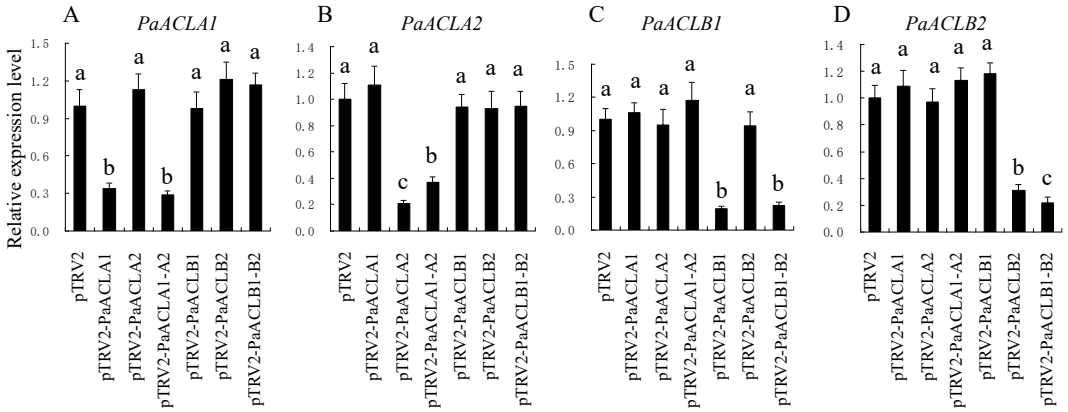



**Figure S5** Expression of *PaACLAs* and *PaACLBs* determined by quantitative real-time PCR using *Actin* as the internal reference gene. (A-D) Expression of *PaACLA1* (A), *PaACLA2* (B), *PaACLB1* (C) and *PaACLB2*(D) in corollas of the control plants and *PaACLAs*- and *PaACLBs*-silenced plants. *Actin* (accession no. FN014209) was used as the internal reference gene to quantify the cDNA abundance. Data are presented as the means ± SD (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) with 3 biological replicates. P-values ≤0.05 were considered significant.

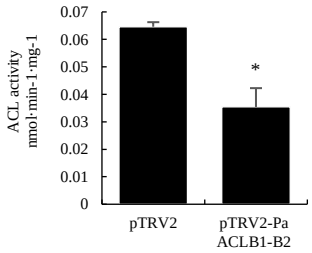
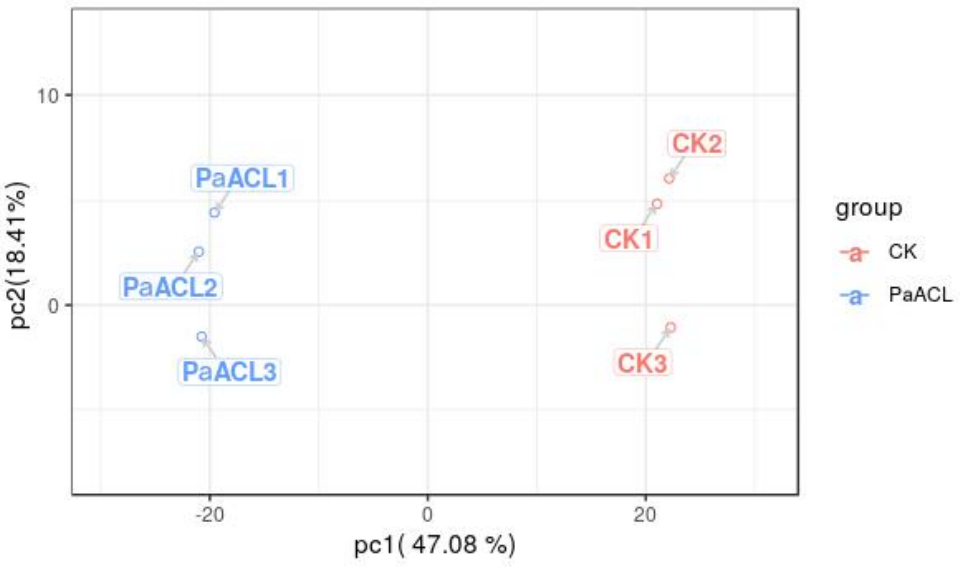



**Figure S6** ACL activities of *PaACLB1-B2* silencing flowers and control. The data represents the mean and SD of three separate extractions.

A




B

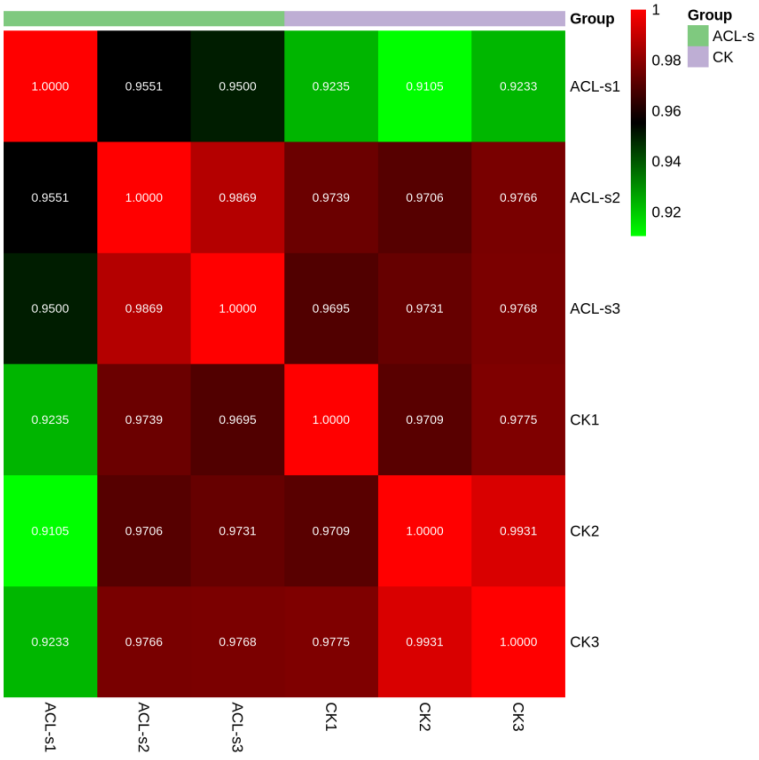



**Figure S7** Repeatability analysis of metabolome and transcriptome. A, PCA score of mass spectrum data of samples and quality control samples in the metabolome. B, Repeatability test between samples in the transcriptome.

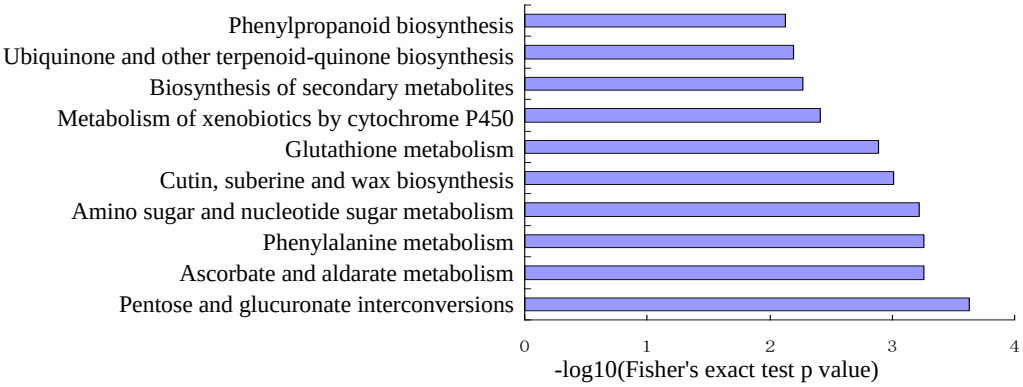



**Figure S8** KEGG pathway enrichment analysis of differentially expressed genes in petunia corollas of *PaACLB1-B2*-silenced plants and control. The significance level was set at P <0.05 (Fischer's exact test). The data come from Supplemental Data Table 6.

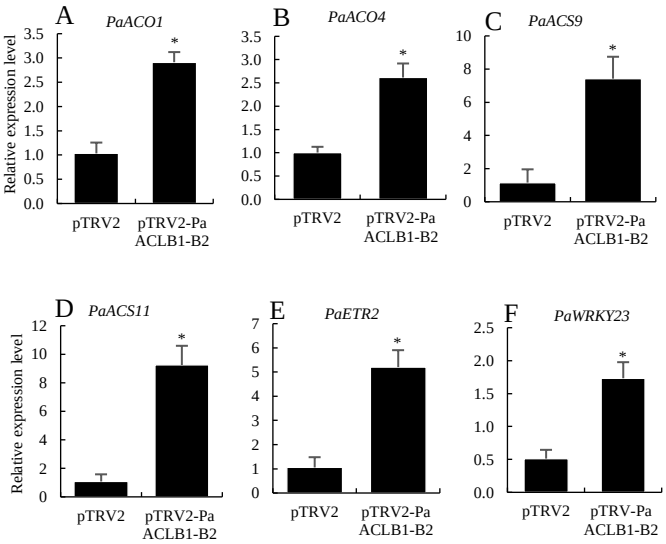



**Figure S9** Confirmation of 6 senescence-related gene expression data by qPCR. Relative expression levels are shown as fold change values. *Cyclophilin* (accession no. EST883944) was used as the internal reference gene to quantify the cDNA abundance. Data are presented as the means ± SD (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) with 3 biological replicates. P-values ≤0.05 were considered significant.

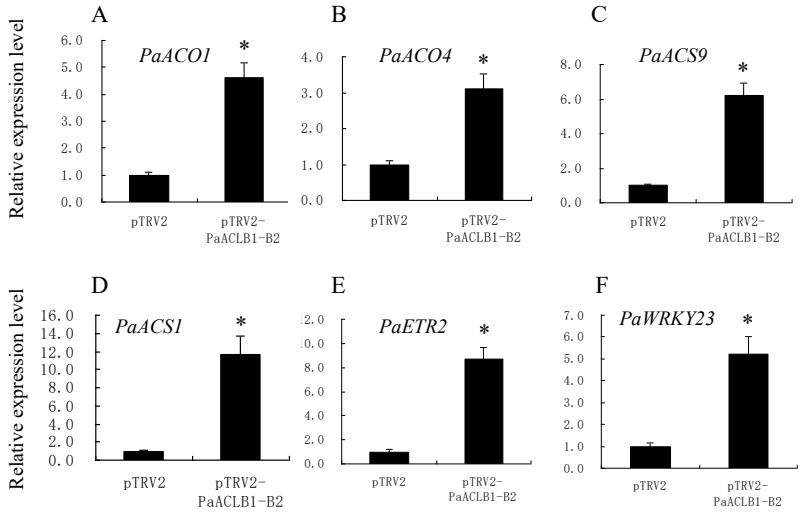



**Figure S10** Confirmation of 6 senescence-related gene expression data by quantitative real-time PCR using *Actin* as the internal reference gene. Relative expression levels are shown as fold change values. *Actin* (accession no. FN014209) was used as the internal reference gene to quantify the cDNA abundance. Data are presented as the means ± SD (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) with 3 biological replicates. P-values ≤0.05 were considered significant.

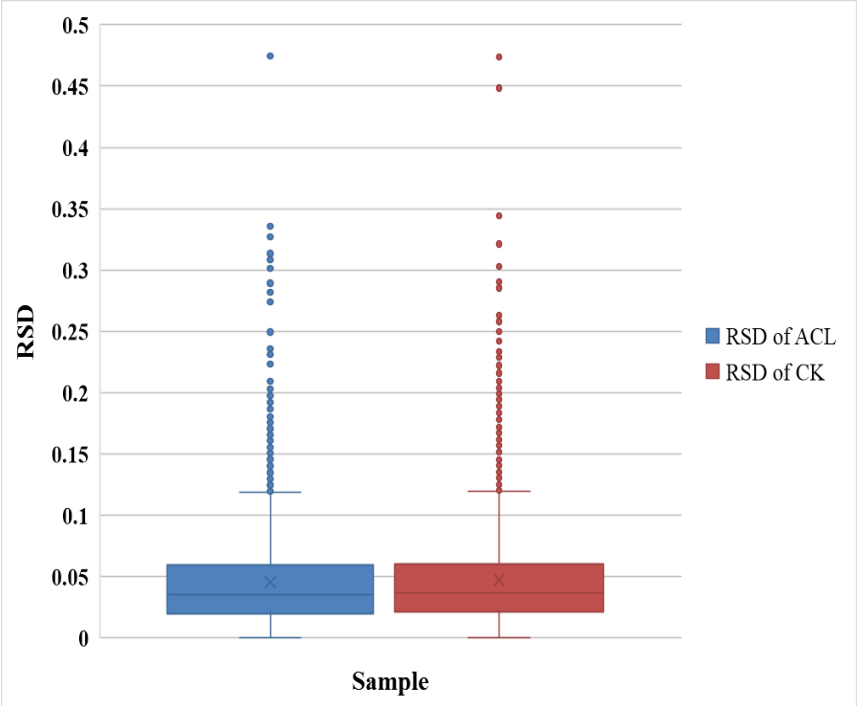



**Figure S11** Repeatability test between samples in the proteome of petunia corollas of *PaACLB1-B2*-silenced plants and control.

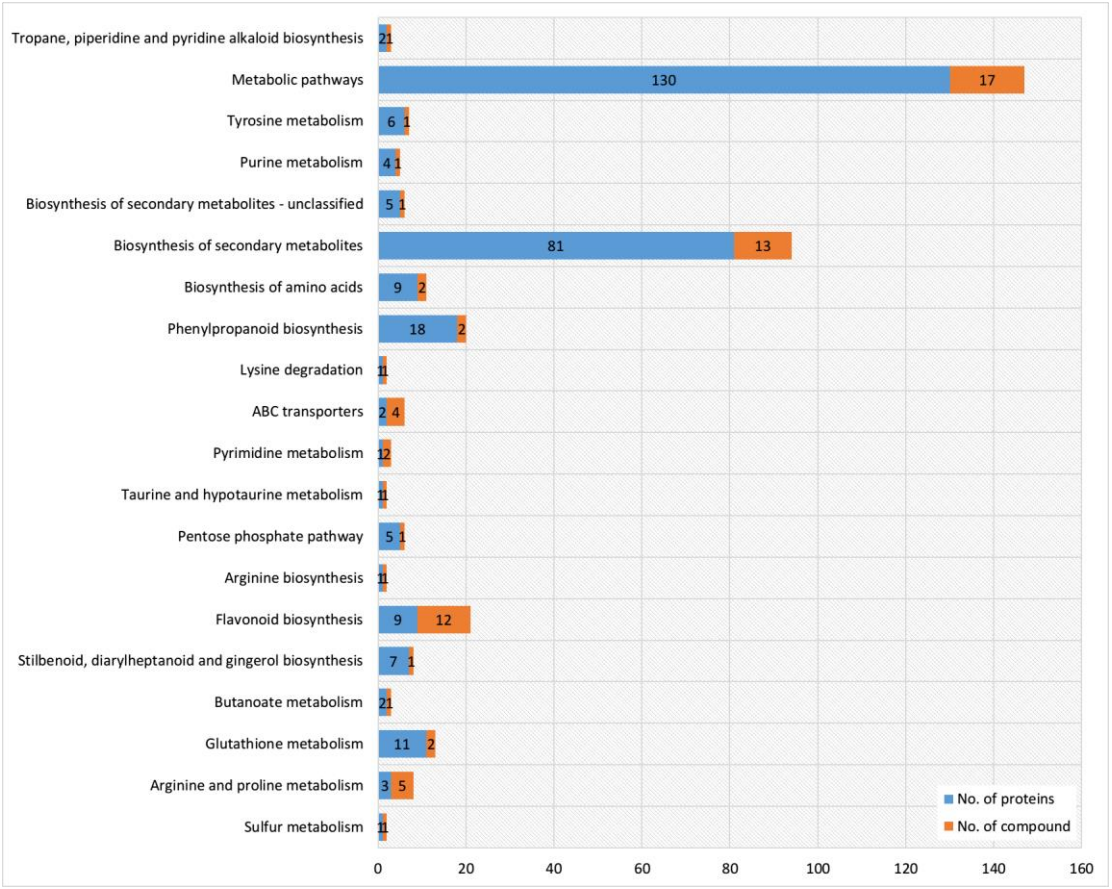



**Figure S12** Distribution of KEGG pathways of differential proteins and differential metabolites.

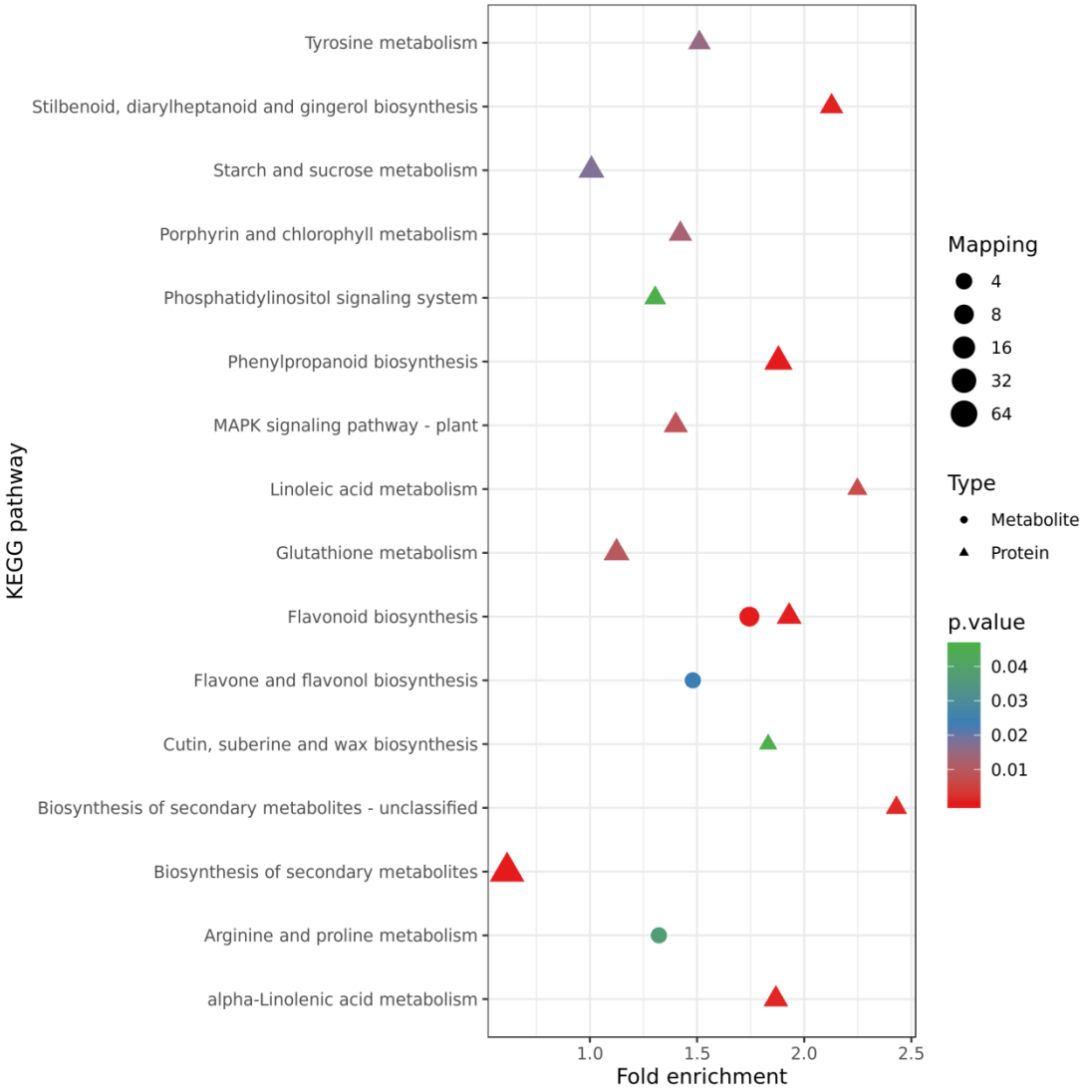
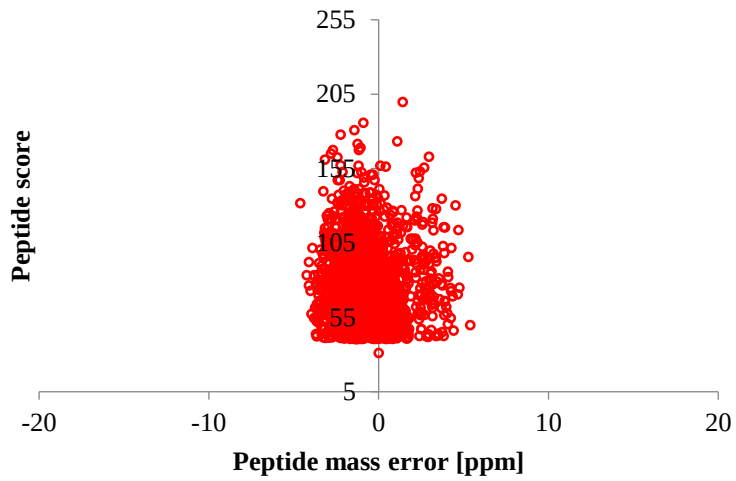



**Figure S13** The bubble chart of KEGG enrichment analysis. The abscissa is the ratio of differential metabolites/proteins in the pathway to the background metabolite/protein identified in the pathway, and the ordinate is the corresponding entry in the KEGG. The triangle represents the metabolite, and the circle represents the protein. Shape size indicates the number of proteins or metabolites in the pathway. The shape colour indicates the significance of channel enrichment.

A




B

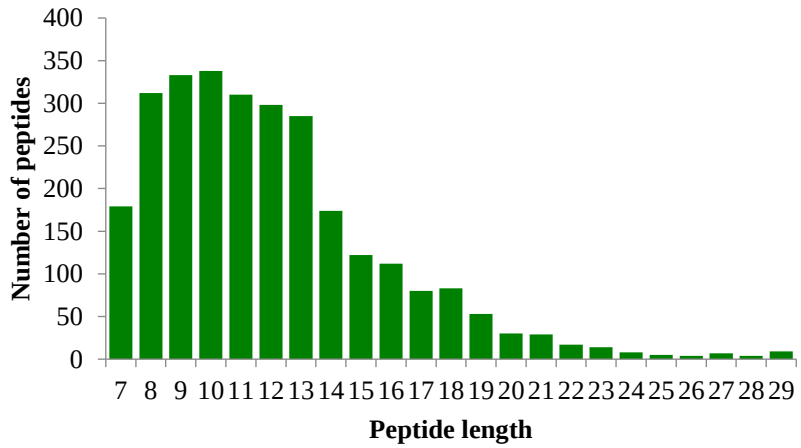



**Figure S14** Quality control of mass spectrometry data. (A) The mass shift distribution of the identified peptide segment; (B) the length distribution of the identified peptide segment.

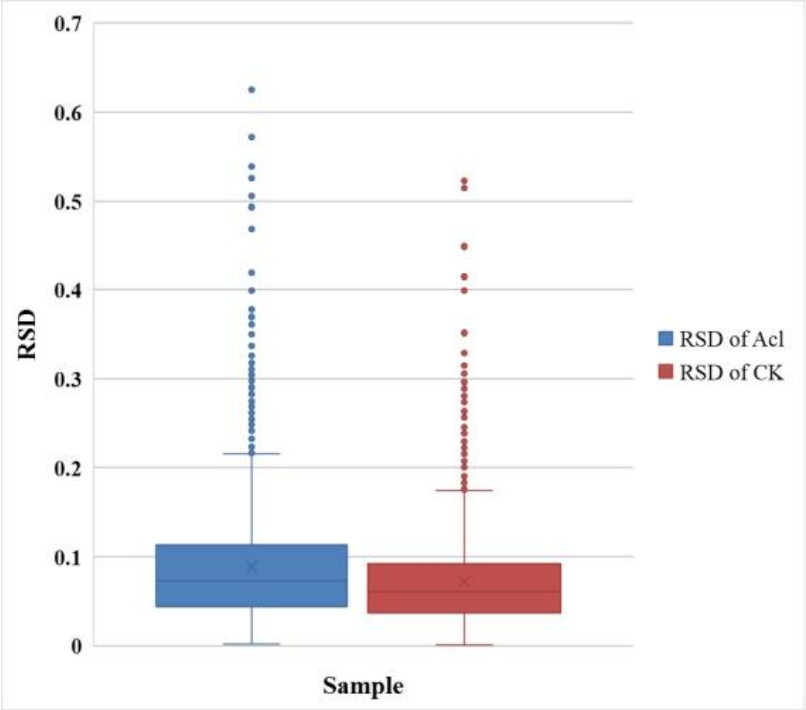
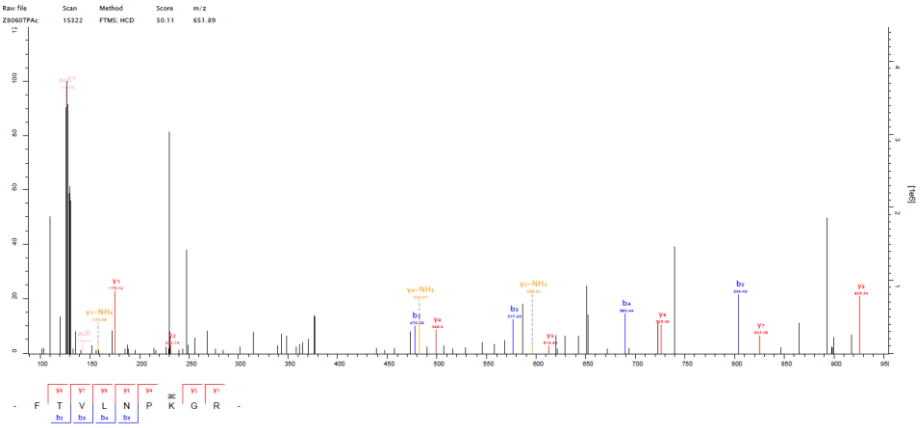
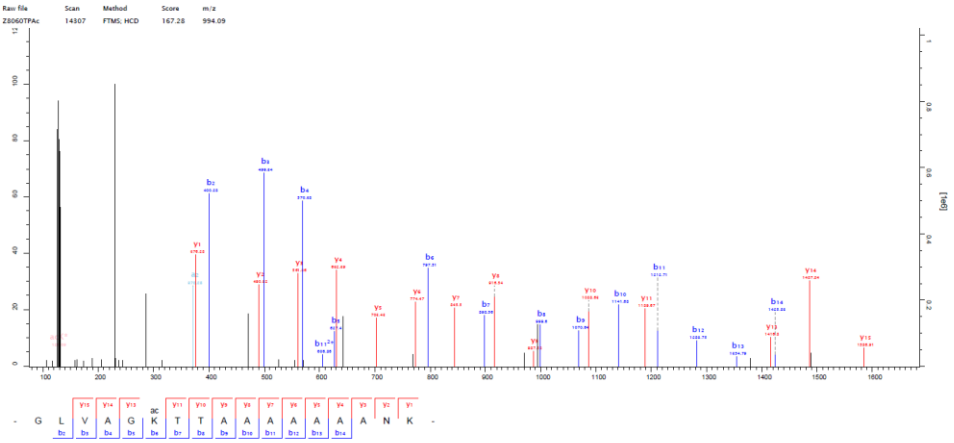



**Figure S15** Repeatability test between samples in protein lysine acetylome.

A



B



C

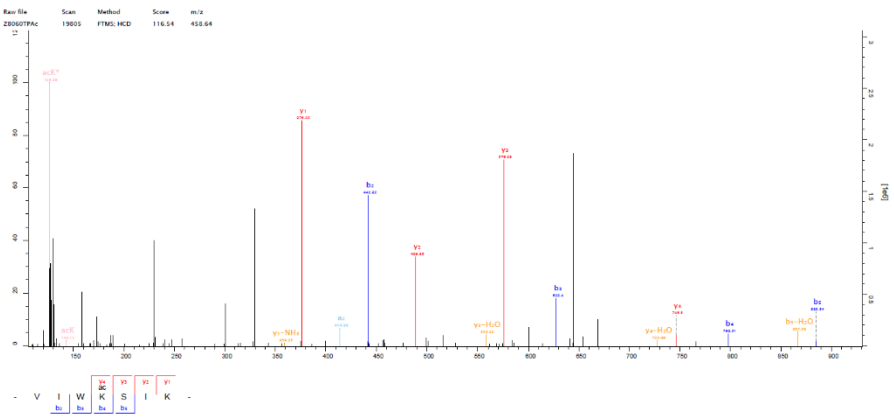
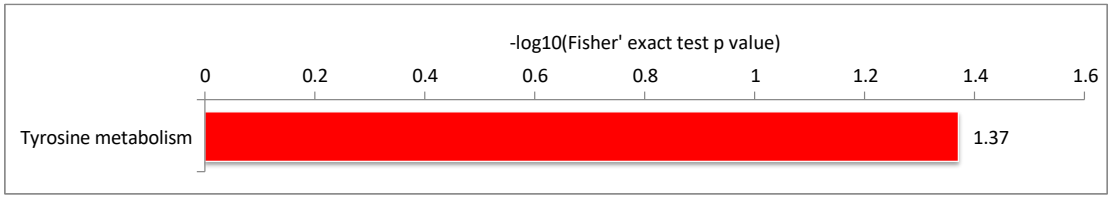


**Figure S16** MS/MS spectra of lysine acetylation of several proteins. A, ATP-citrate lyase PaACLA1_269Kac (Peaxi162Scf00357g00428); B, isocitrate dehydrogenase IDH26Kac (Peaxi162Scf00705g00316); C, histone H2A.11_13Kac (Peaxi162Scf00608g00918).

A




B

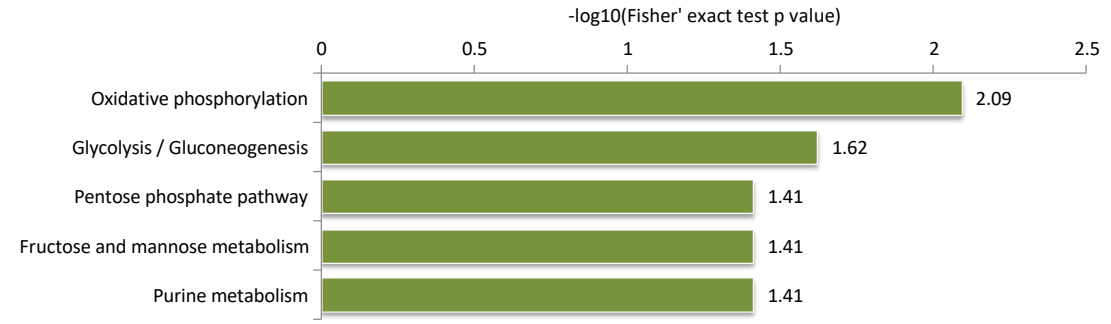



**Figure S17** KEGG pathway enrichment analysis of proteins with upregulated and downregulated Kac sites. KEGG pathway enrichment analysis of proteins with upregulated (A) and downregulated (B) Kac sites. The significance level was set at $P < 0.05$ (Fischer's exact test). The data come from Supplemental Data Table 9.

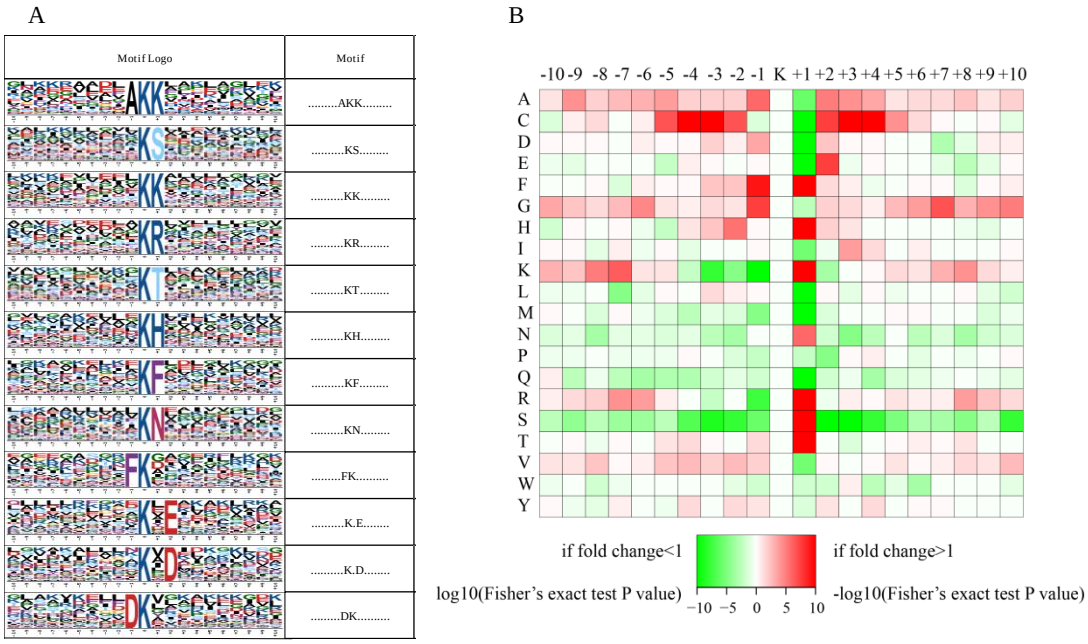



**Figure S18** Motif analysis of all the identified Kac sites in petunia. A, Acetylation motifs and the conservation of Kac sites. The height of each letter corresponds to the frequency of that amino acid residue in that position. The central K refers to the acetylated lysine. B, Amino acid sequence properties of ubiquitylation sites. The heat map shows significant position-specific under- or overrepresentation of amino acids flanking the modification sites.

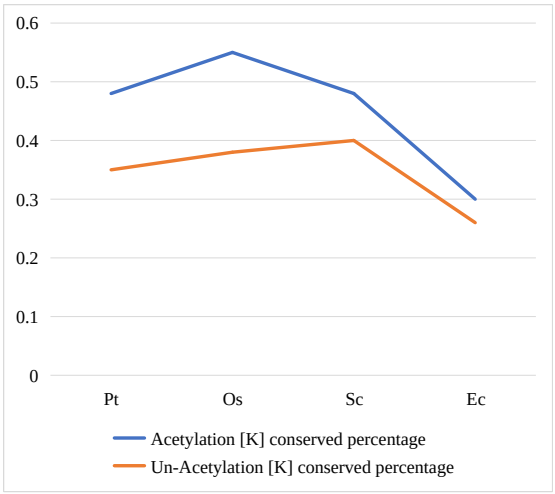



**Figure S19** Evolutionary conservation of acetylated and non-acetylated lysines on protein orthologues in selected species. Abbreviations: Ec, *Escherichia coli*; Os, *Oryza sativa* japonica; Pt, *Phaeodactylum tricornutum*; Sc, *Saccharomyces cerevisiae*.

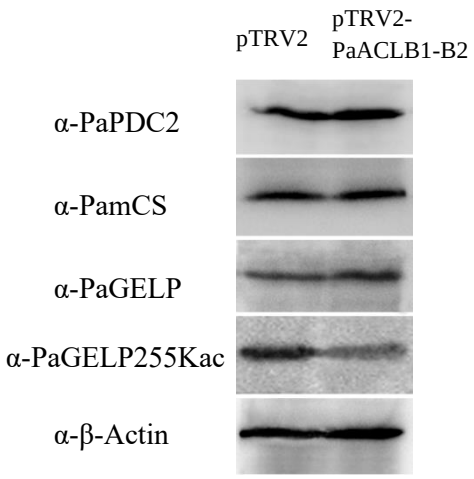
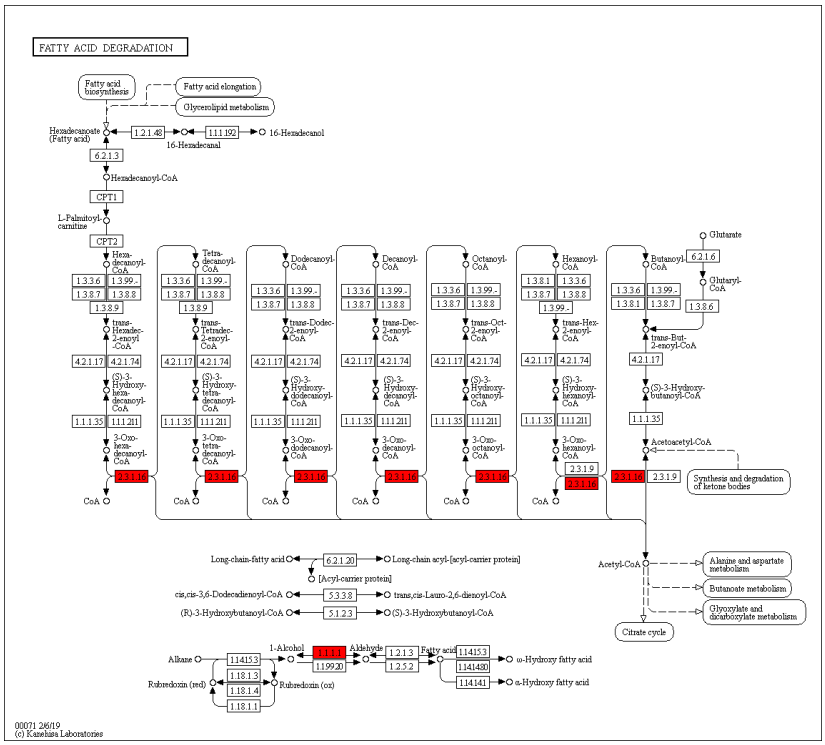



**Figure S20** Confirmation of proteome (the first three) and acetylome data (the fourth one). Western blot analysis of petunia corollas treatment with pTRV2 and pTRV2-PaACLB1-B2. Representative results from three independent experiments are indicated.

A




B

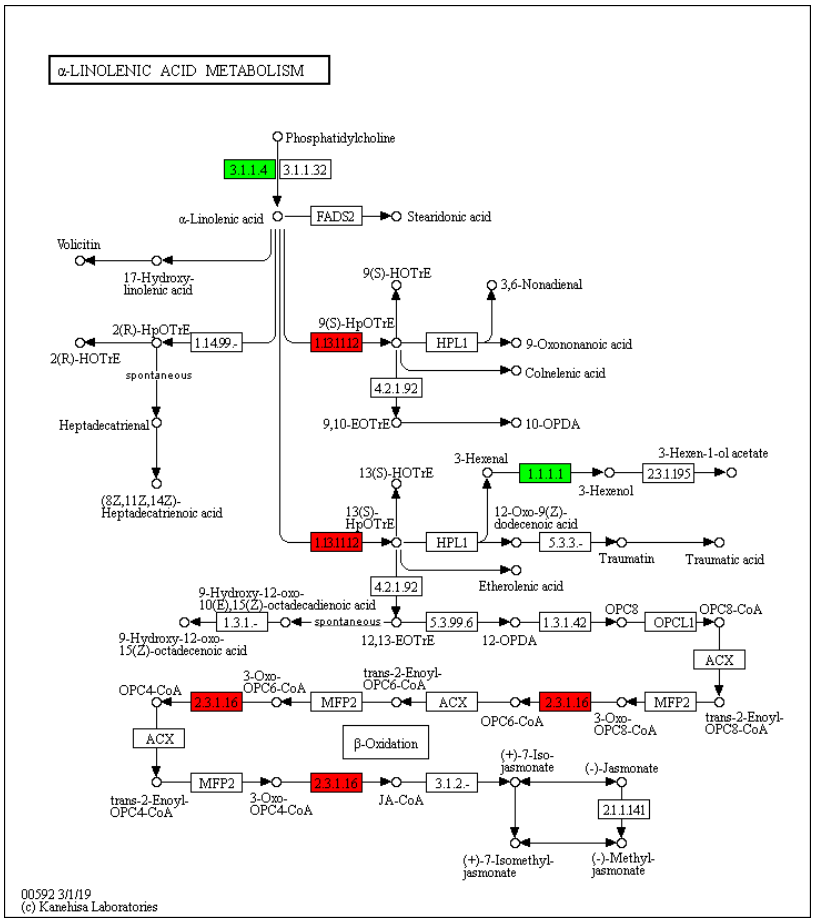



**Figure S21** Differential proteins with acetylation sites in the opposite direction were enriched in the fatty acid degradation (A) and alpha-linolenic acid metabolism (B) KEGG pathways. Red box

indicates up-regulation and green box indicates down-regulation in acetylation level based on the statistical significance in this study.

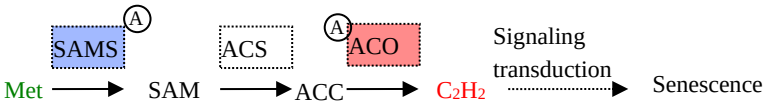



**Figure S22** Effects of *PaACLB1-B2* silencing on the proteins engaged in ethylene biosynthesis in petunia. Differentially expressed proteins based on statistical significance in this study are framed in boxes, and differentially acetylated proteins have round boxes. The red box indicates upregulation; the green box indicates downregulation; and the blue indicates no significant changes upon pTRV2-PaACLB1-B2 treatment. Abbreviations: A, acetylation; ACC, 1-aminocyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; Met, methionine; SAM, S-adenosylmethionine; SAMS, S-AdoMet synthetase.

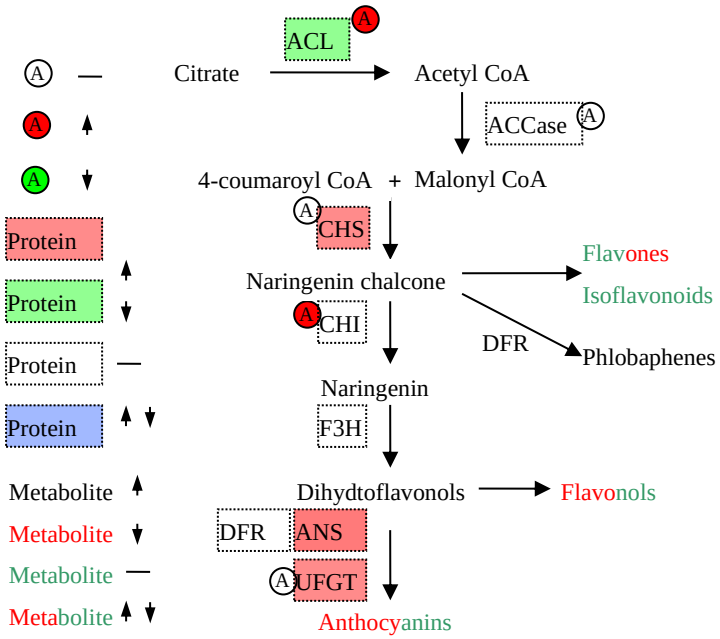



**Figure S23** Effects of *PaACLB1-B2* silencing on anthocyanin and other flavonoid biosynthesis and the proteins engaged in their biosynthesis pathway in petunia. Differentially expressed proteins based on statistical significance in this study are framed in boxes, and differentially acetylated proteins are framed with circles. The red box indicates upregulation; the green box indicates downregulation; and the blue indicates no significant changes upon pTRV2-PaACLB1-B2 treatment. Abbreviations: 3GT, UDP-glucose:flavonoid 3-O-glycosyl transferase; 4CL, 4-coumarate:CoA ligase; A, acetylation;; ACCase, acetyl-CoA carboxylase; ANS, anthocyanin synthase; C4H, cinnamate-4-hydroxylase; CHS, chalcone synthase; CHI, chalcone flavanone isomerase; DFR, dihydroflavonol 4-reductase; F3H, flavanone 3ß-hydroxylase.