Supporting Information

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SI Materials and Methods

Plant Materials and Growth Conditions. *Petunia hybrida* cv. Mitchell (W115) and transgenic *Petunia* seeds were first sown on soil suitable for seedling growth (Sunshine 3 Mix; Sun Gro Horticulture) and, after 1–2 wk, the seedlings were transferred to a soil containing a higher amount of nutrients (Sunshine 4 Mix; Sun Gro Horticulture). *Petunia* plants were kept in a growth room with 12-h light/12-h dark cycling conditions at 25 °C. Occasionally, plants were kept in plant growth incubators (Conviron), which have similar growth conditions. At least 1 wk before experiments, all plants were grown in the growth room and entrained to the same 12-h light/12-h dark conditions. Full-spectrum white fluorescent lamps (Octron F032/950/48; Osram-Sylvania) were set to deliver an approximate fluence rate of 80 μmol/m^2/s. Transgenic *Arabidopsis* plants were grown under 12-h light/12-h dark conditions at 22 °C for the gene expression and LUC imaging assays, under continuous light conditions at 22 °C for the hypocotyl length analysis, and under long-day conditions (16 h of light/8 h of dark) at 22 °C for flowering time experiments.

To clone *PhLHY* cDNA, *Petunia* total RNA was isolated from young leaves approximately 2 wk old harvested in the morning by using TRIzol (Life Technologies) (1), and cDNA was synthesized as previously described (2). The *PhLHY* cDNA was amplified by using the primer set 5′-CACCTTGATGGACCCTTACTC-3′ and 5′-GTTCCCTCGTAGAATTGCACA-3′, which was designed based on the sequence information of EST clones FN031564 and FN003047 and showed high sequence homologies to *Arabidopsis CCA1* and *LHY* cDNAs. The amplified cDNA was cloned into the pENTR/D-TOPO vector (Life Technologies) and the complete cDNA sequences were determined. These sequences matched to EST clone sequences, and the deduced amino acid sequences of PhLHY showed high homology to LHY orthologs (Fig. 2*A* and Fig. S2). The determined *PhLHY* cDNA sequences were deposited into GenBank under the accession no. KP017483. *PhLHY* cDNA was transferred to the pK7WG2 (3) plasmid by a Gateway LR recombination reaction (Life Technologies) to make a *35S*:*PhLHY* binary vector (pK7WG2-PhLHY). Generation of *Petunia* stable *35S*:*PhLHY* transformants was achieved by transformation of excised leaf tissue with an *Agrobacterium tumefaciens* GV3101 strain containing pK7WG2-PhLHY. Segments (1 cm × 1.5 cm) were excised from 2–4-wk-old leaves from plants that were between 5 and 10 wk old, and inoculated with *Agrobacterium* with an OD_{600} of 0.4–0.6. The leaf tissue excisions and *Agrobacterium* were coincubated for 1 d on plates containing coincubation media [1× Linsmaier and Skoog (LS) salts, pH 5.7 (Caisson Laboratories), 3% (wt/vol) sucrose, 1 mg/L 1-naphthaleneacetic acid (NAA), 0.5 mg/L 6-benzylaminopurine (BAP), 100 mg/L acetosyringone]. The explants were then transferred to regeneration/selection plates [1× LS salts, pH 5.7, 3% (wt/vol) sucrose, 1 mg/L NAA, 0.5 mg/L BAP, 100 mg/L ticarcillin, and 100 mg/L kanamycin] for regeneration and selection. After 2–3 wk of selection, regenerated shoots were cut and placed in rooting media [1× LS salts, pH 5.7, 3% (wt/vol) sucrose, 1 mg/L NAA, 100 mg/L ticarcillin, and 100 mg/L kanamycin]. T_1 and T_2 generations were selected by their expression patterns of *PhLHY*, and T_3 and T_4 generations were used for analyses. To generate *Arabidopsis 35S*:*PhLHY* transgenic lines, *Arabidopsis* WT plants (Col-0) that possess a *CCA1*:*LUC* reporter gene (4) were transformed with pK7WG2-PhLHY using the floral dip method (2). Transformants were selected on kanamycin-containing plates as described previously (2). Homozygote T_3 and T_4 plants were used for all analyses.

Collection and GC/MS Analysis of Volatile Compounds. For all FVBP concentration analysis, 2–3 d-old flowers were grown under 12-h light/12-h dark, continuous light, or continuous dark conditions. For analysis of emitted volatiles, flowers were inserted into a three-necked flask collection chamber (inside volume, 250 mL; Fig. S7). Traps were constructed by using Pasteur pipettes with tips cut to a final length of 4.5 inches, loaded with 100 mg of Poropak type Q 80–100 polymer (Waters), held in place by 15 mg of glass wool. Inflow to the collection chamber was purified by Pasteur pipettes loaded with activated charcoal. Flow through the traps was set to 2 L/min, with each time point consisting of 4 h of collection. A detailed diagram of the scent collection system is shown in Fig. S7. The fresh weight of the sampled flowers was taken immediately after scent collection to control for mass effects. The volatiles captured in the traps were eluted with 500 μL of hexane. For endogenous analysis, flowers were harvested at each time point and flash-frozen in liquid nitrogen. Samples were then ground in a Retsch Mixer Mill 400 for 4 × 1 min at 25 frequencies per second, then vortexed with 1 mL hexane for 1 h. Samples were spun down for 10 min at 9,400 × *g*, and 100 μL was then pipetted from the top for analysis. For emitted and endogenous analysis, 1 μL of the hexane elution was injected into a GC/MS device (model 7890A GC system coupled to 5975C inert XL MSD; Agilent Technologies) for quantification of the floral volatiles at each time point (5). Briefly, samples were injected into the inlet and held at 220 °C, and helium was used as the carrier gas at a constant flow of 1 cm^3/min. The initial oven temperature was 45 °C for 4 min, followed by a heating gradient of 10 °C/min to 240 °C, which was held isothermally for 10 min. Chromatogram peaks were identified tentatively with the aid of the NIST mass spectral library (approximately 120,000 spectra) and verified by chromatography with authentic standards. Peak areas for each compound were integrated by using ChemStation software (Agilent Technologies) and are presented in terms of micrograms per gram of fresh floral tissue per hour.

RNA Preparation and Gene Expression Analysis (Quantitative PCR). *Petunia* plants were grown under 12-h light/12-h dark, continuous light, or continuous dark conditions for tissue collection. Young leaf (<2 wk old) and flower tissue (2–3 d postopening corollas, pistil and stamens removed) was collected at the designated time points, then immediately immersed in liquid N_2 for storage at −80 °C. After collection of all samples, total RNA was extracted by TRIzol-based method as described (1). cDNA synthesis and qPCR analysis were performed as previously described (2) with the following differences: 4 μg of total RNA was used to create cDNA, and the following protocol was used for 40–45 cycles: 95 °C for 3 min, and then cycling at 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 20 s. *UBQ* was used as an internal control for normalization (6). Samples were run at least in triplicate, and gene expression values were normalized by the average expression values of hours 0–12. The qPCR primers sequences used in this study are listed in Table S1. RNA isolation, cDNA synthesis, and qPCR of *Arabidopsis* seedlings were performed as previously described (2). Values represent means ± SEM from at least three biological replicates for all gene expression analyses in *Petunia* and *Arabidopsis*. To test for differences in expression patterns over time between transgenic and WT lines, a two-way ANOVA was conducted by using R (www.r-project.org). The categories compared were relative patterns of expression of a gene of interest (*PhPRR5*, *PAL*, *EOBI*, *EOBII*, *CM1*, *ADT*, *CFAT*, *BPBT*, *BSMT1*, *BSMT2*, *EGS*, *EPSPS*, *IGS*, *KAT1*, *ODO1*, *PAAS*, *PhGI*, and

PhLHY) in WT *Petunia* against the pattern of expression in a paired transgenic line (line 37, 46, or 47).

Phylogenetic Analysis. Amino acid sequences for LHY, CCA1, GI, and PRR5 homologs were aligned by using ClustalW (7) on the Cyberinfrastructure or Phylogenetic Research (CIPRES) Science Gateway (www.phylo.org). A phylogenetic tree was generated through Bayesian analysis using MrBayes (8, 9), applying the Jones–Taylor–Thornton (JTT) model (10) of amino acid substitutions. The analysis was run over 5,000,000 Markov chain Monte Carlo generations, sampling every 1,000 with a "burn-in" proportion of 0.25. The final consensus tree was the product of 50% majority rule (11, 12). Maximum likelihood (ML) bootstrap values presented on the phylogenetic tree in Fig. S2*A* were calculated by generating a second phylogenetic tree with a topology constrained to be identical to the first via the ML method by using randomized axelerated maximum likelihood (RAxML) (13), applying the JTT model of amino acid substitution. The bootstrap values were calculated over 1,000 iterations. The final analysis and presentation were accomplished by using Mesquite (mesquiteproject.org) and FigTree (tree.bio.ed.ac.uk/software/figtree) software, respectively. The DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank accession numbers of genes (for deduced amino acid sequences) used in the phylogenetic tree are as follows: *PhLHY*, KP017483; *AtCCA1*, AY519511; *AtLHY*, AK316829; *BrCCA1*, HQ615939; *BrLHYa*, Bra030496; *BrLHYb*, Bra033291; *SlLHYI*, Solyc.10g005080; *PtLHY1*, Potri.002g180800; *PtLHY2*, Potri.014g106800; *PnLHY1*, BAH09384; *PnLHY2*, BAH09385; Cs*LHY*, AY611029; *NaLHY*, JQ424913; *OsCCA1*, NM_001067567; *PpCCA1*a, AB458831; *PpCCA1*b, AB458832; *PvLHY*, AJ420902; *SbCCA1*, TA31430_4558 TA26762_4558; *AtGI*, AT1G22770; *BrGI*, NP_001288824; *PtGI*, XP_002307516; *OsGI*, BAF04134; *AtPRR5*, AT5G24470, *PtAPRR5*, NP_001288827; and *CsPRR5*, ABV53464.

Fluorescent Imaging (Confocal Microscopy). To analyze the intracellular localization of PhLHY-GFP in the flower and the leaf, petals and young leaves *of P. hybrida* cv. Mitchell W115 were transiently transformed with *Agrobacterium* (GV3101) containing pK7WGF2 PhLHY (3) and RFP-H2B (14) Cultures of *Agrobacterium* containing these plasmids were grown to an OD_{600} of 0.5 and resuspended in MES buffer [50 mM MES, pH 5.7, 2 mM NaH_2PO_4, 0.5% (wt/vol) glucose, 100 mM acetosyringone]. After 1 h of incubation in the MES buffer, the *Agrobacterium* solution was injected into flower and leaf tissues via needleless syringe. Two days posttransfection, GFP and RFP images of the tissues were analyzed by using a confocal microscope (TCS SP5; Leica Microsystems).

Flowering Time and Hypocotyl Length Assays. To analyze the effects of *PhLHY* overexpression on flowering time regulation, the seeds of *Arabidopsis* WT (Col-0) and *35S*:*PhLHY* transgenic plants were sown on Sunshine Mix 4 (Sun Gro Horticulture) and stratified in darkness at 4 °C for 2 d to synchronize germination time. Plants were grown under long day (16 h light/8 h dark) conditions at 22 °C. Light was provided by full-spectrum white fluorescent bulbs with a fluence rate of 80 μmol/m^2/s. Flowering time was measured by counting the numbers of rosette and cauline leaves when plants bolted as previously described (2).

For hypocotyl length analysis in *Arabidopsis*, WT (Col-0) and *35S*:*PhLHY* transformants were sown on 1× LS media containing 3% (wt/vol) sucrose, then stratified at 4 °C for 2 d. Plants were grown under continuous light conditions at 22 °C. When the plants were 7 d old, they were scanned and hypocotyl length was measured by using ImageJ software (15). An independent-samples *t* test was performed by using R (www.r-project.org) to compare the lengths of WT and transgenic hypocotyls. For hypocotyl length analysis in *Petunia*, WT (W115) and our *35S*:*PhLHY* lines (nos. 37, 46, and 47) were sown on 1× LS media with 3% (wt/vol) sucrose and grown under continuous light conditions at 25 °C. When plants were 10 d old, they were scanned and analyzed as we did with *Arabidopsis*.

LUC Imaging. *Arabidopsis* WT seedlings (Col-0) and *PhLHY* overexpressors (*35S*:*PhLHY*) harboring a *CCA1*:*LUC* reporter gene (4) were grown on 1× LS media containing 3% (wt/vol) sucrose for 7 d under 12-h light/12-h dark conditions before beginning LUC imaging. At 24 h before imaging, the plants were sprayed with a 5-mM D-luciferin, 0.01% Triton-X solution. The plants were imaged for 5 d under continuous light or 12-h light/12-h dark conditions. Bioluminescence images were captured from plants every 2 h for 15-min exposures by using a high-sensitivity CCD camera (NightOWL; Berthold Technologies), and analyzed by using IndiGO software (Berthold Technologies). Luminescence data were gathered from at least 16 plants per line for *Arabidopsis* and 4 plants per line from *Petunia* flowers. Similar results were obtained from the repeated experiments. White light (~50 μmol/m^2/s) was obtained from halogen lamps (EKE 21V150W; USHIO) filtered with a heat cut filter.

For the transient LUC reporter assay in *P. hybrida* flowers (Fig. 5*D*), we generated *ODO1* promoter-driven firefly LUC reporters (*pODO1*:*LUC*) and PhLHY effecter plasmids. To generate the *pODO1*:*LUC* construct, the 1.2-kB fragment (−1207/−1) of the promoter was cloned from floral genomic DNA using 5′-CAGTTCTTTCAATGTAATTCCGCAG-3′ and 5′-CACTACTGACTCTCAGCTACCACC-3′ primers, and then inserted into the binary firefly LUC vector pFLASH (16). The *pODO1*:*LUC* mEE+mCBS was generated as by mutating the EE and CBS sites as described in *Materials and Methods* for the EMSA assay, before insertion into pFLASH. As the PhLHY effecter, we used pK7WG2-PhLHY plasmid. For a negative control, we used pK7WG2 plasmid containing *GFP* cDNA. The *GFP* cDNA was amplified by using 5′-CACCATGGTGAGCAAGGGCGAGGAG-3′ and 5′-CTACTTGTACAGCTCGTCCAT-3′ primers, and cloned into pENTR/D-TOPO plasmid (Invitrogen). To normalize for transformation efficiency, we used a binary vector containing *Renilla reniformis* LUC (Rluc) expression cassette. To generate the binary vector, we excised *35S* promoter controlled *Rluc* gene from pRTL2-Rluc (17) with HindIII, and ligated into the binary vector pPZP221 (18). Two- to three-day-old flowers were coinfiltrated with *Agrobacterium* transformants containing a reporter (*pODO1*:*LUC* or *pODO1*:*LUC* mEE+mCBS), an effector [*35S*:*LHY* (pK7WG2 LHY), *35S*:*GFP* (pK7WG2 GFP), or nothing], and *35S*:*Rluc*. In addition, the *Agrobacterium* transformant that possesses tomato stunt bushy virus silencing-suppressor p19 plasmid (*35S*:*p19*) was added to all coinfiltrations (19). To prepare for the transfection, after growing each *Agrobacterium* transformant overnight, the appropriate combinations of the transformant cultures (adjusted OD_{600} to 1.0) were mixed, spun down, and resuspended in MES buffer [50 mM MES, pH 5.7, 2 mM NaH_2PO_4, 0.5% (wt/vol) glucose, 100 mM acetosyringone]. After 1 h of incubation in the MES buffer, 0.1 mL of the *Agrobacterium* solution was injected into the corollas of flowers via needleless syringe at zeitgeber time 12 (ZT 12). All plants were entrained to 12-h light/12-h dark cycles and, after 24 h of incubation, flowers were harvested and flash-frozen at ZT 12. Samples were prepared and analyzed based on the protocol of the Dual-Luciferase Assay System (Promega). Soluble proteins were extracted with Passive Lysis Buffer (Promega) supplemented by Complete Protease Inhibitor Mixture tablets (Roche). The activities of firefly and *Renilla* LUCs in the plant extracts were analyzed by using a Victor^3 V plate reader (Perkin–Elmer).

For the time-course LUC activity analysis in *Petunia* flowers (Fig. S6), *P. hybrida* cv. Mitchell (W115) flowers were coinfiltrated with *Agrobacterium* transformants harboring combinations of *pODO1*:*LUC*, *35S*:*PhLHY*, and *35S*:*GFP*. All flowers also received the *Agrobacterium* transformant containing *35S*:*p19*

plasmid. After preparing the *Agrobacterium* solution as described earlier, the *Agrobacterium* solution was injected into the corollas of cut flowers (previously entrained to 12-h light/12-h dark) at ZT 12. Flowers were then immediately sprayed with a 5-mM D-luciferin, 0.01% Triton-X solution, and placed upright in a container filled with 5% (wt/vol) sucrose solution. Beginning at ZT 0 the next day, luminescence was recorded using the NightOWL imaging system (Berthold Industries) as described earlier. Plants were imaged for 48 h while within 12-h light/12-h dark conditions (with lights off for all image collection).

EMSA. For EMSA, we used GST-fused PhLHY protein. To produce the recombinant GST-PhLHY protein, the full length of *PhLHY* cDNA was amplified by using 5′-TATCAGAATTCG-ACCCTTACTCCTCTGGGGAGGAAC-3′ and 5′-ATCAT-AGCGGCCGCTTAAGTAGAAGCTTCTCCTTCCAAGC-3′ primers (the underlined sequences are restriction enzyme recognition sites of EcoRI and NotI, respectively).

The amplified PCR fragment was digested by EcoRI and NotI, and cloned into the EcoRI-NotI sites of the pGEX 4T-1 plasmid (GE Healthcare Life Sciences). The sequences of *PhLHY* cDNA in the pGEX-PhLHY plasmid were verified. The pGEX-PhLHY and pGEX 4T-1 plasmids were transformed into the BL21-CodonPlus (DE3; Stratagene) *Escherichia coli* strain to produce GST-PhLHY and GST proteins, respectively. Production of these proteins and preparation of cell extracts were performed as described previously (20). To induce the expression of GST-PhLHY and GST proteins, 0.1 mM of isopropyl β-D-1-thiogalactopyranoside was added into each bacterial culture (OD_{600}, 0.1). After an additional 4-h incubation at 37 °C, the cell culture was collected by centrifugation and resuspended in the following buffer: 20 mM Hepes·KOH, pH 7.2, 80 mM KCl, 10% (vol/vol) glycerol, 0.1 mM EDTA, 0.1 mM PMSF, 2.5 mM DTT, and Pierce Phospatase Inhibitor Mini Tablets (Thermo Scientific). After sonication and centrifugation, supernatants were collected and used for EMSA. EMSA was performed as previously described (21). A total of 1 μg of cell extracts containing GST-PhLHY or GST proteins were incubated with 100 nM of Cy5-labeled probe in a binding buffer [20 mM Hepes·KOH, pH 7.2, 80 mM KCl, 0.1 mM EDTA, 5% (vol/vol) glycerol, 2.5 mM DTT, 0.2 $\mu g \cdot \mu L^{-1}$ BSA, 500 ng poly dI-dC] and appropriate amounts of unlabeled competitor DNA (5-, 25- and 50-fold molar excess with respect to the labeled probe). The probe sequence of the EE (indicated by underline)-containing region of *ODO1* promoter (22) (*pODO1* EE1) is 5′-[Cy5]ATAAACCTAATAAAAAATATCTT-GATAAAAAATTAA-3′, and the competitor sequences are 5′-ATAAACCTAATAAAAAAT**CGAG**TGATAAAAAATTAA-3′ (mutated nucleotides are shown in bold) for the *pODO1* mutated EE1, 5′-ATAAACCTAATAAAAAATATCTCCATACATAA-TAC-3′ for the *pODO1* EE2, 5′-AAGAAAAGTTGGTAGAT-TTTTTTATATATTTAGG-3′ for the *pODO1* CBS, 5′-ACT-TAATTGTATTAGATATTTCTTGCACCTAAAA-3′ for the *pEPSPS* (accession no. CS050416) EE, and 5′-AAGAGAGA-GAGAGAGATATTTTAACCCAAAAAAAA-3′ for the *pIGS* (GU983699) EE. After incubation for 30 min at room temperature, samples were separated by electrophoresis on 7% (wt/vol) acrylamide gels in 0.25× TBE. Fluorescent gel images were obtained by using a Typhoon FLA 9000 Biomolecular Imager (GE Healthcare Life Sciences).

1. Spitzer B, et al. (2007) Reverse genetics of floral scent: Application of tobacco rattle virus-based gene silencing in Petunia. *Plant Physiol* 145(4):1241–1250.
2. Ito S, et al. (2012) FLOWERING BHLH transcriptional activators control expression of the photoperiodic flowering regulator *CONSTANS* in *Arabidopsis*. *Proc Natl Acad Sci USA* 109(9):3582–3587.
3. Karimi M, Inzé D, Depicker A (2002) GATEWAY vectors for *Agrobacterium*-mediated plant transformation. *Trends Plant Sci* 7(5):193–195.
4. Pruneda-Paz JL, Breton G, Para A, Kay SA (2009) A functional genomics approach reveals CHE as a component of the *Arabidopsis* circadian clock. *Science* 323(5920): 1481–1485.
5. Riffell JA, Lei H, Abrell L, Hildebrand JG (2013) Neural basis of a pollinator's buffet: Olfactory specialization and learning in *Manduca sexta*. *Science* 339(6116): 200–204.
6. Mallona I, Lischewski S, Weiss J, Hause B, Egea-Cortines M (2010) Validation of reference genes for quantitative real-time PCR during leaf and flower development in *Petunia hybrida*. *BMC Plant Biol* 10:4.
7. Larkin MA, et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23(21): 2947–2948.
8. Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8):754–755.
9. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12):1572–1574.
10. Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. *Comput Appl Biosci* 8(3):275–282.
11. Adams EN (1972) Consensus techniques and the comparison of taxonomic trees. *Syst Zool* 21(4):390–397.
12. Nelson G (1979) Cladistic analysis and synthesis: principles and definitions, with a historical note on Adanson's "Familles des plants" (1763-1764). *Syst Zool* 28(1):1–21.
13. Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9):1312–1313.
14. Chakrabarty R, et al. (2007) PSITE vectors for stable integration or transient expression of autofluorescent protein fusions in plants: Probing *Nicotiana benthamiana*-virus interactions. *Mol Plant Microbe Interact* 20(7):740–750.
15. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9(7):671–675.
16. Baudry A, et al. (2010) F-box proteins FKF1 and LKP2 act in concert with ZEITLUPE to control *Arabidopsis* clock progression. *Plant Cell* 22(3):606–622.
17. Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA (2005) FKF1 F-box protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*. *Science* 309(5732):293–297.
18. Hajdukiewicz P, Svab Z, Maliga P (1994) The small, versatile pPZP family of Agrobacterium binary vectors for plant transformation. *Plant Mol Biol* 25(6):989–994.
19. Voinnet O, Rivas S, Mestre P, Baulcombe D (2003) An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *Plant J* 33(5):949–956.
20. Harmer SL, Kay SA (2005) Positive and negative factors confer phase-specific circadian regulation of transcription in Arabidopsis. *Plant Cell* 17(7):1926–1940.
21. Cao S, et al. (2014) A distal CCAAT/NUCLEAR FACTOR Y complex promotes chromatin looping at the *FLOWERING LOCUS T* promoter and regulates the timing of flowering in *Arabidopsis*. *Plant Cell* 26(3):1009–1017.
22. Van Moerkercke A, Haring MA, Schuurink RC (2011) The transcription factor EMISSION OF BENZENOIDS II activates the MYB *ODORANT1* promoter at a MYB binding site specific for fragrant petunias. *Plant J* 67(5):917–928.

Fig. S1. (Continued)

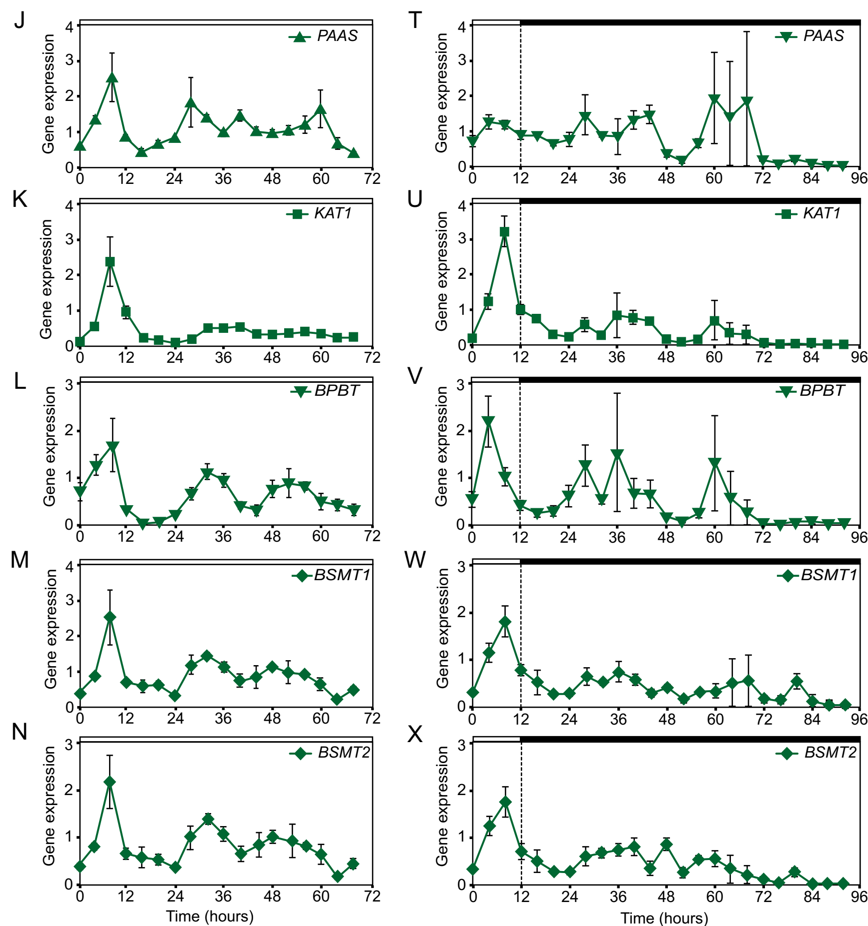

Fig. S1. The floral volatile emission and expression profiles of the genes in the FVBP pathway. (*A*–*D*) Scent expression patterns of methyl benzoate and benzyl benzoate under continuous light (*A* and *B*) and continuous dark (*C* and *D*) conditions. (*Insets*, *C* and *D*) Graphs with enlarged *y*-axes showing the same 32–96 time point results. (*E*–*X*) Expression patterns of the genes in the FVBP pathway under continuous light (*E*–*N*) and continuous dark (*O*–*X*) conditions. Values are relative to *UBQ*, and normalized by the average expression values of hours 0–12. Results represent mean ± SEM from three biological replicates. The line and marker color of the graphs corresponds to its placement within the greater FVBP pathway. White and black bars at the top indicate periods of light and dark, respectively.

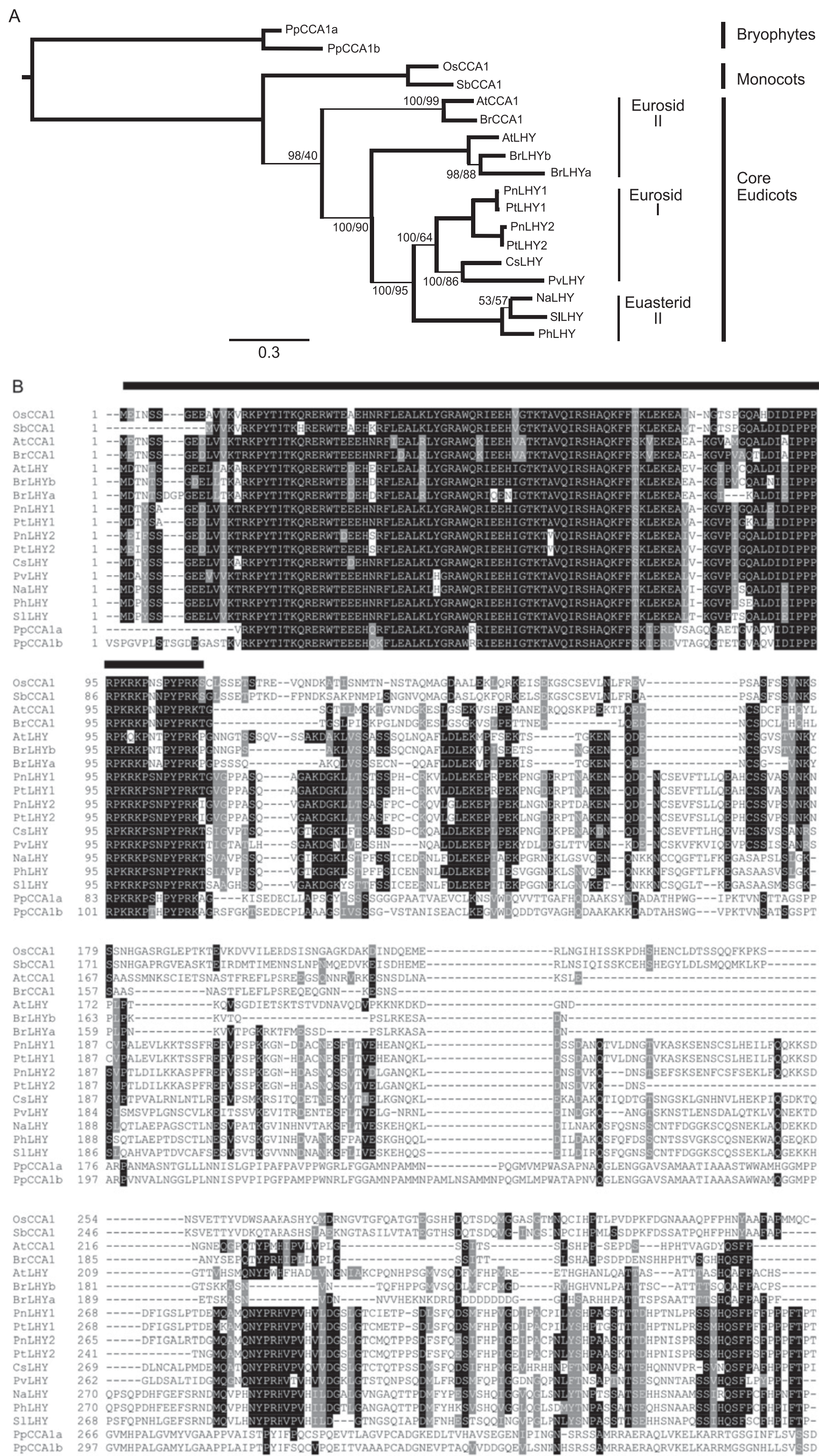

Fig. S2. (Continued)

Fig. S2. (Continued)

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OsCCA1         --------------------------------------------------
SbCCA1         --------------------------------------------------
AtCCA1         --------------------------------------------------
BrCCA1         --------------------------------------------------
AtLHY          --------------------------------------------------
BrLHYb         --------------------------------------------------
BrLHYa         --------------------------------------------------
PnLHY1         --------------------------------------------------
PtLHY1         --------------------------------------------------
PnLHY2         --------------------------------------------------
PtLHY2         --------------------------------------------------
CsLHY          --------------------------------------------------
PvLHY          --------------------------------------------------
NaLHY          --------------------------------------------------
PhLHY          --------------------------------------------------
SlLHY          --------------------------------------------------
PpCCA1a   847  SRTLSLSIGAGGLKNADSSVSARTEKYSGVGFVPYQRVSIHVEATVAKL
PpCCA1b   883  SQTLFLSVGTGGLKNAVSNVPTRTVKYSGVGFVPYQRASTRTEPLVDKL
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C

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AtGI    1 MASSSSERWIDGLQFSSLLWPPPRDPQQHKDQVVAYVEYFGQFTS--EQFPDDIAELVRHQYPSTEKRLLDDVLAMFVLHHPEHGHAVILPIISCLIDG
BrGI    1 MTSPTSSERWTDGLQFSSLLWSPPRDPQQHKDQVVAYVEYFGQFTS--EQFPDDIAELVRNQYPSTEKRLLDDVLAMFVLHHPEHGHAVILPIISCLIDG
PtGI    1 -MASSSERWIDGLQFSSLFWPPPQDAQQRKAQITAYVDYFGQCTS--EHFPDDISELIRNRYPSKDKRLFDDVLATFVLHHPEHGHAVVLPIISCIIDG
OsGI    1 --MSASNEKWIDGLQFSSLFWPPPQDSQQKQAQILAYVEYFGQFTADSEQFPEDIAQLIQSCYPSKEKRLVDEVLATFVLHHPEHGHAVVHPILSRIIDG
PhGI    1 ----------------------------------------------------------------------------------------------------

AtGI   99 SLVYSKEAHPFASFISLVCPSSENDYSEQWALACGEILRILTHYNRPIYKTEQQNGDTERNCLSKATTSGSPT-SEPKAGSPTQHERKPLRPLSPWISDI
BrGI   99 TLVYSKEAHPFASFISLVSPNSENDYSEQWALACGEILRILTHYNRPIYKTEQQNGETES----KASTSGSPTLSEAKAVSPGQHERKPLRPLSPWISDI
PtGI   98 TLVYDRSSPPFASFISLVCPGSENEYSEQWALACGEILRILTHYNRPIYKREQQNNETDRSSSDSHATSSESAEGKSTSMPLVQQERKPFRPLSPWITDI
OsGI   99 TLSYDRNGFPFMSFISLFSHTSEKEYSEQWALACGEILRVLTHYNRPIFKVDHQHSEAECSSTSDQASSCESMEKRANGSPRNEPDRKPLRPLSPWITDI
PhGI    1 ----------------------------------------------------------------------------------------------------

AtGI  198 LLAAPLGIRSDYFRWCSGVMGKYAAG-ELKPPTI------------ASRGSGKHPQLMPSTPRWAVANGAGVILSVCDDEVARYETATLTAVAVPALLLP
BrGI  195 LLAAPLGIRSDYFRWCSGVMGKYAAG-ELKPPTIGECPSPLATTMGSTRGSGKHPQYMPSTPRWAVANGAGVILSVCDDEVARYETATLTAVAVPALLLP
PtGI  198 LLAAPLGIRSDYFRWCSGVMGKYAAG-ELKPPTT-----------TSSRGSGKHPQLIPSTPRWAVANGAGVILSVCDEEVARYETATLTAAAVPALLLP
OsGI  199 LLAAPLGIRSDYFRWCGGVMGKYAAGGELKPPTT-----------AYSRGSGKHPQLMPSTPRWAVANGAGVILSVCDEEVARYETANLTAAAVPALLLP
PhGI    1 ----------------------------------------------------------------------------------------------------

AtGI  285 PPTTSLDEHLVAGLPALEPYARLFHRYYAIATPSATQRLLLGLLEAPPSWAPDALDAAVQLVELLRAAEDYASGVRLPRNWMHLHFLRAIGIAMSMRAGV
BrGI  294 PPTTSLDEHLVAGLPALEPYARLFHRYYAIATPSATQRLLLGLLEAPPSWAPDALDAAVQLVELLRAAEDYASGVRLPRNWMHLHFLRAIGIAMSMRAGV
PtGI  286 PPTTALDEHLVAGLPALEPYARLFHRYYAIATPSATQRLLLGLLEAPPSWAPDALDAAVQLVELLRAAEDYASGIRLPRNWMHLHFLRAIGTAMSMRAGI
OsGI  288 PPTTPLDEHLVAGLPPLEPYARLFHRYYAIATPSATQRLLFGLLEAPPSWAPDALDAAVQLVELLRAAEDYDSGMRLPKNWMHLHFLRAIGTAMSMRAGI
PhGI    1 ----------------------------------------------------------------------------------------------------

AtGI  385 AADAAAALLFRILSQPALLFPPLSQVEGVEIQHAPIGGYSSNYRKQIEVPAAEATIEATAQGIASMLCAHGPEVEWRICTIWEAAYGLIPLNSSAVDLPE
BrGI  394 AADAAAALLFRILSQPALLFPPLSQAEGVEIKHAPIGGYGSNYRKQIEVPAAEATIEATAQGIASMLCAHGPEVEWRICTIWEAAYGLIPLNSSAVDLPE
PtGI  386 AADAAAALLFRILSQPALLFPPLRQVEGVEVQHEPLGGYISCYRKQIEVPAAEATIEATAQGIASMLCAHGPEVEWRICTIWEAAYGLIPLSSSAVDLPE
OsGI  388 AADTSAALLFRILSQPTLLFPPLRHAEGVELHHEPLGGYVSSYKRQLEVPASEATIDATAQGIASMLCAHGPDVEWRICTIWEAAYGLLPLSSSAVDLPE
PhGI    1 ----------------------------------------------------------------------------------------------------

AtGI  485 IIVATPLQPPILSWNLYIPLLKVLEYLPRGSPSEACLMKIFVATVETILSRTFPPESSRELTRKARSSFTTR-SATKNLAMSELRAMVHALFLESCAGVE
BrGI  494 IIVATPLQPPILSWNLYIPLLKVLEYLPRGSPSEACLMKIFVATVETILSRTFPPETS---IRKARASLATRSSATKNLAMAELRAMVHALFLESCAGVE
PtGI  486 IIVATPLQPPLLSWNLYIPLLKVLEYLPRGSPSEACLMKIFVATVEAILQRTFPPEASREQTRRTRY-FSSLGPASKNLAVAELRTMVHSLFLESCASVE
OsGI  488 IVVAAPLQPPTLSWSLYLPLLKVFEYLPRGSPSEACLMRIFVATVEAILRRTFPSETS-EQSRKPR-------SQSKNLAVAELRTMIHSLFVESCASMD
PhGI    1 ----------------------------------------------------------------------------------------------------

AtGI  584 LASRLLFVVLTVCVSHEAQSSGSKRPRSEYASTTENIEANQPVSNNQTANRKSRNVKGQGPVAAFDSYVLAAVCALACEVQLYPMISGGGNFSNSAVAGT
BrGI  591 IASRLLFVVLTVCVSHEAQSSGSKRRRSEEDATAE---ENQ---DNQTSNRKSRNVKGQGPVAAFDSYVLAAVCALACEVQLYPMISGGGNFSNSAVAAT
PtGI  585 LASRLLFVVLTVCVSHEAHSRGSKRPRGEENDLPEDGTEDSQSTSEMRRNMKSRRMKKQGPVAAFDSYVLAAVCALACELQIFPFVSRGSNHSTSKHSET
OsGI  580 LASRLLFVVLTVCVSHQALPGGSKRPTGSDNHSSEEVTNDSRLT-----NGRNRCKKRQGPVATFDSYVLAAVCALSCELQLFPFISKNGNHSNLKDSIK
PhGI    1 ----------------------------------------------------------------------AAVCALSRELQLFTLISRGTNHSDPKIIMD

AtGI  684 ITKPVKINGSSKEYGAGIDSAISHTRRILAILEALFSLKPSSVGTPWSYSSSEIVAAAMVAAHISELFRRSKALTHALSGLMRCKWDKEIHKRASSLYNL
BrGI  685 ITKSVKINGSSNEYGAGIDSAIKHTRRILAILEALFSLKPSSVGTPWSYSSSEIVAAAMVAAHISELFRRSKALTHALSGLMRCKWDKEIHKRASSLYNL
PtGI  685 VAKPAKLNGAVSEFQTSLNSAIHHTHRILSILEALFSLKPSTIGTSWSYSSNEIVAAAMVAAHVSELFRRSKACMHALSVLMRCKWDNEIYTRASSLYNL
OsGI  675 IVIPGKTTGISNELHNSISSAILHTRRILGILEALFSLKPSSVGTSWSYSSNEIVAAAMVAAHVSELFRRSRPCLNALSALKQCKWDAEISTRASSLYHL
PhGI   31 EAKP--ANDSSIELRNGIHSAVRHTRRILAILEALFSLKPSSVGTSWSYSSNEIVAAAMVAAHISDLFRRSKACMQGLAILIRCKWDNEIHSRASSLYNL

AtGI  784 IDVHSKVVASIVDKAEPLEAYLKNTPVQKDSVTCLNWKQENTCASTTCFDT-AVTSASRTEMNPRG-NHKYARHSDEGSGR--PSEKGIKDFLLDASDLA
BrGI  785 IDVHSKVVASIVDKAEPLEAYLKNAPVQKDSLACVNWKQQNNTSSAAGFGTAAVTSTSRNEMAPRGGNHKYARHSDEGSGSRSSSDKGIKDLLLDASDLA
PtGI  785 IDVHSKAVASIVNKAEPLGAHLH-APVWKDSIVCSDGNKQNRSASTGCFNSGQSSALQSTELVHSETKLKCGRASHSEEGSGSTSGKGIAGLPLDASDLA
OsGI  775 IDLHGKTVTSIVNKAEPLEAHLTLTPVKKDEPPIEE---KNINSSDGGALEKKDASRSHRKNGFARPLLKCAEDVILNGDVASTSGKAIASLQVEASDLA
PhGI  129 IDIHSKTVASIVNKAEPLEAYLIHAPILKERPXCLNGKKHYKYTNRNCLTAEEPSVSQCKD---------------------------------------

AtGI  880 NFLTADRLAGFYCGTQKLLRSVLAEKPELSFSVVSLLWHKLIAAPEIQPTAESTSAQQGWRQVVDALCNVVSATPAKAAAAVVLQAERELQPWIAKDDEE
BrGI  885 NFLTADRLAGFYRGTQVLLRSILAEKPELSFSVVSLLWHKLIASPEIQPTAESTSAQQGWRQVVDALCNVVSATPAKAAAAVVLQAERELQPWIAKDDEE
PtGI  884 NFLTMHRHIGFNCSAQVLLRSVLPEKQELCFSVVSLLWQKLIASPETQPSAESTSAQQGWRQVVDALCNVVSASPTIAATAVVLQAERELQPWIAKDDDS
OsGI  872 NFLTMDRNGGYRG-SQTLLRSVLSEKQELCFSVVSLLWQKLIASPEMQMSAESTSAHQGWRKVVDALCDIVSASPTKASAAIVLQAEKDLQPWIARDDEQ
PhGI      ----------------------------------------------------------------------------------------------------

AtGI  980 GQKMWKINQRIVKVLVELMRNHDRPESLVILASASDLLLRATDGMLVDGEACTLPQLELLEATARAIQPVLAWGPSGLAVVDGLSNLLKCRLPATIRCLS
BrGI  985 GQKMWKINQRIVKVMVELMRNHDRPESLVILASASDLLLRATDGMLVDGEACTLPQLELLEATARAIQPVLAWGPSGLAVVDGLSNLLKCRLPATIRCLS
PtGI  984 GQIMWRINQRIVKLIVELMRNHDTPESLVILASASDLLLRATDGMLVDGEACTLPQLELLEATARAVQPVLQWGESGFAVADGLSNLLKCRLPATIRCLS
OsGI  971 GQKMWRVNQRIVKLIAELMRNHDSPEALVILASASDLLLRATDGMLVDGEACTLPQLELLEVTARAVHLIVEWGDSGVSVADGLSNLLKCRLSTTIRCLS
PhGI      ----------------------------------------------------------------------------------------------------

AtGI 1080 HPSAHVRALSTSVLRDIMNQSSIP-IKVTPKLP---TTEKNGMNSPSYRFFNAASIDWKADIQNCLNWEAHSLLSTTMPTQFLDTAARELGCTISLSQ-
BrGI 1085 HPSAHVRALSTSVLRDIMNQSGITTTKATPKPPPTITTEKNGTDSPSYRFFNAAAIDWKADIQKCLNWEAHSLLSTTMPTQFLDTAARELGCTISMSSQ
PtGI 1084 HPSAHVRALSTSVLRDIQHTGSIK---PASKLT-----HRNGIHGPSYQYLRSDVINWQADIEKCLTWEAHSRLATGMPVHHLDTAAKELGCTISI---
OsGI 1071 HPSAHVRALSMSVLRDILNSGQIN---SSKLIQG---EHRNGIQSPTYQCLAASIINWQADVERCIEWEAHSRRATGLTLAFLTAAAKELGCPLTC---
PhGI      ---------------------------------------------------------------------------------------------------
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Fig. S2. (Continued)

PNAS PNAS PNAS PNAS

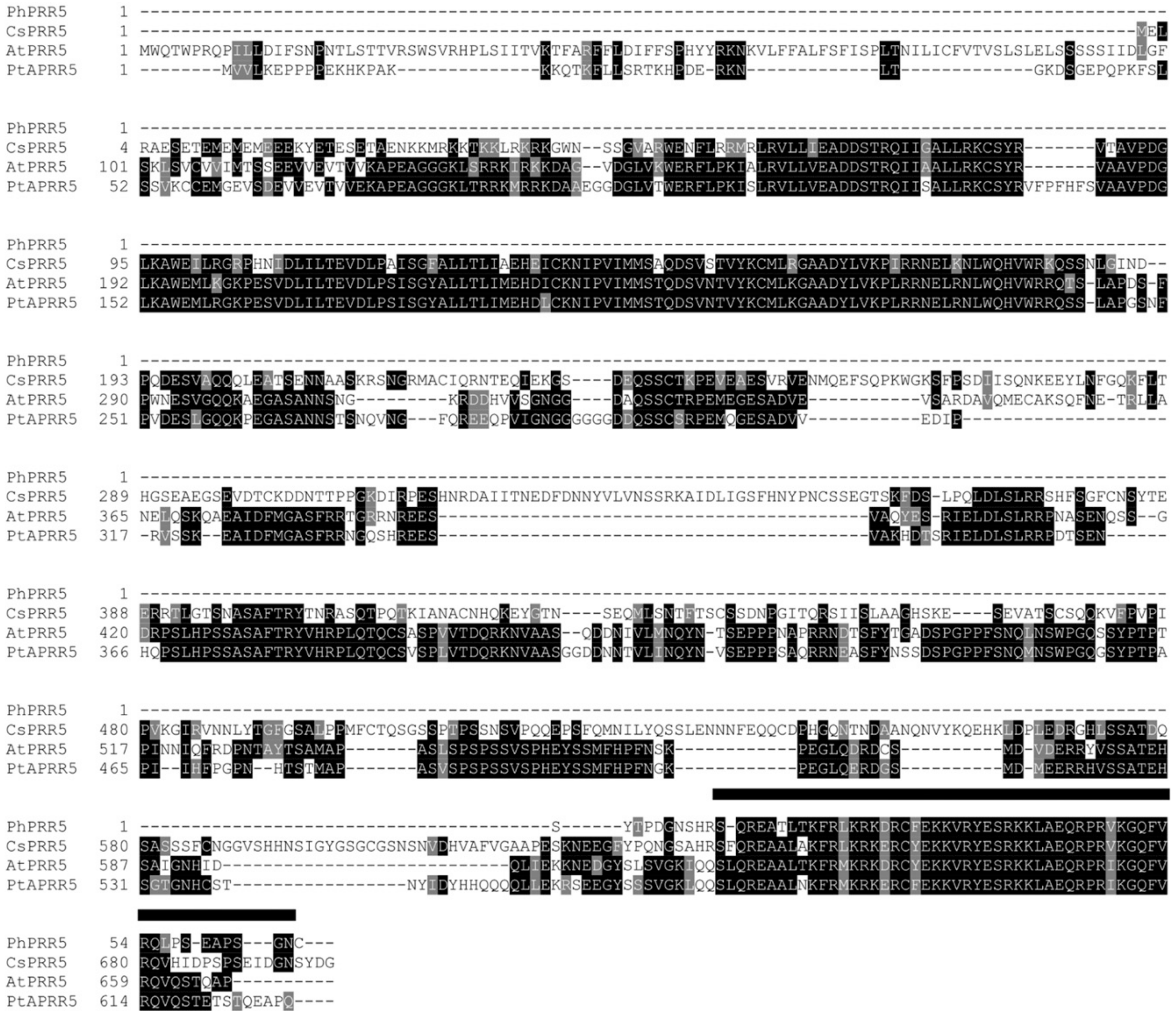

Fig. S2. (*A*) *PhLHY* is a homolog of the circadian clock gene LHY. Composite phylogenetic tree displaying the relationship of *PhLHY* with *LHY* and *CCA1* homologs. *LHY* and *CCA1* homologs used are from *Phaseolus vulgaris* (PvLHY), *Castanea sativa* (CsLHY), *Populus trichocarpa* (PtLHY1, PtLHY2), *Populus nigra* (PnLHY1, PnLHY2), *Nicotiana attenuata* (NaLHY), *Solanum lycopersicum* (SlLHY), *Arabidopsis thaliana* (AtLHY, AtCCA1), *Brassica rapa* (BrCCA1, BrLHYa, BrLHYb), *Sorghum bicolor* (SbCCA1), and *Oryza sativa* (OsCCA1), with designated outgroup *Physcomitrella patens* (PpCCA1a, PpCCA1b). Support values preceding branching are from Bayesian posterior probability analysis and maximum-likelihood analysis in format: Bayesian value/maximum likelihood value. Support values below 100/100 are shown, corresponding to adjacent thinned branches. (*B*) Amino acid alignment of LHY and CCA1 protein orthologs found in flowering and nonflowering plants. Proteins aligned are as follows: *P. vulgaris* (PvLHY), *C. sativa* (CsLHY), *P. trichocarpa* (PtLHY1, PtLHY2), *P. nigra* (PnLHY1, PnLHY2), *N. attenuata* (NaLHY), *S. lycopersicum* (SlLHY), *A. thaliana* (AtLHY, AtCCA1), *B. rapa* (BrCCA1, BrLHYa, BrLHYb), *S. bicolor* (SbCCA1), *O. sativa* (OsCCA1), and *P. patens* (PpCCA1a, PpCCA1b). Areas shaded in black represent portions of the proteins that display a high degree (>0.5) of agreement throughout all analyzed orthologs. (*C*) Amino acid alignment of GI homologs found in plants and *P. hybrida*. To identify putative *GI* homolog in *P. hybrida*, we screened *P. hybrida* EST database and found that the EST clone FN036363 contained a DNA fragment that showed a strong homology to *GI* cDNA. Proteins aligned as follows: *A. thaliana* GI (AtGI), *B. rapa GI* (BrGI), *P. trichocarpa* (PtGI), *O. sativa* (OsGI), and *P. hybrida* GI, (PhGI). PhGI amino acid sequences are deduced from the DNA sequences found in the EST clone, identified by a black bar running over the sequence. The same DNA sequences were used for designing qPCR primers. (*D*) Amino acid alignment of PRR5 homologs found in plants and the *P. hybrida*. To identify putative *PRR5* homolog in *P. hybrida*, we screened *P. hybrida* EST database and found that the EST clone FN035819 contained a DNA fragment that showed a strong homology to *PRR5* cDNA. Proteins aligned as follows: *C. sativa* PRR5 (CsPRR5), *A. thaliana* PRR5 (AtPRR5), *P. trichocarpa* APRR5, (PtAPRR5), and *P. hybrida* (PRR5). PhPRR5 amino acid sequences are deduced from the DNA sequences found in the EST clone, identified by a black bar running over the sequence. The same DNA sequences were used for designing qPCR primers.

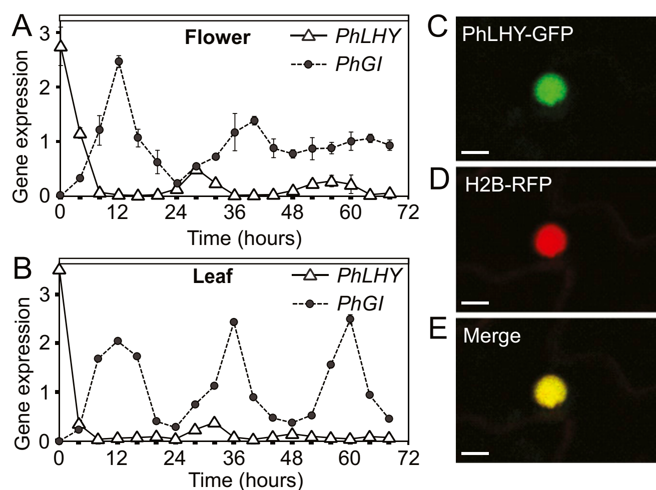

Fig. S3. Putative *Petunia* clock gene homologs show rhythmic gene expression patterns in *Petunia* leaf and flower tissue under continuous light conditions, and PhLHY is localized in the nucleus in leaf cells. (*A* and *B*) Under continuous light conditions, *PhLHY* and *PhGI* oscillations both dampen in flower (*A*), but only *PhLHY* dampens in leaf (*B*). Results represent mean ± SEM from three biological replicates. White bar at the top indicates period of light. (*C–E*) Confocal microscope images of the *Petunia* leaf epidermal cell. GFP fluorescence of PhLHY-GFP protein (*C*), RFP fluorescence of H2B-RFP protein used as a reference for nuclear localization (*D*), and a merged image of these (*E*) are shown. (Scale bar: 10 mm.)

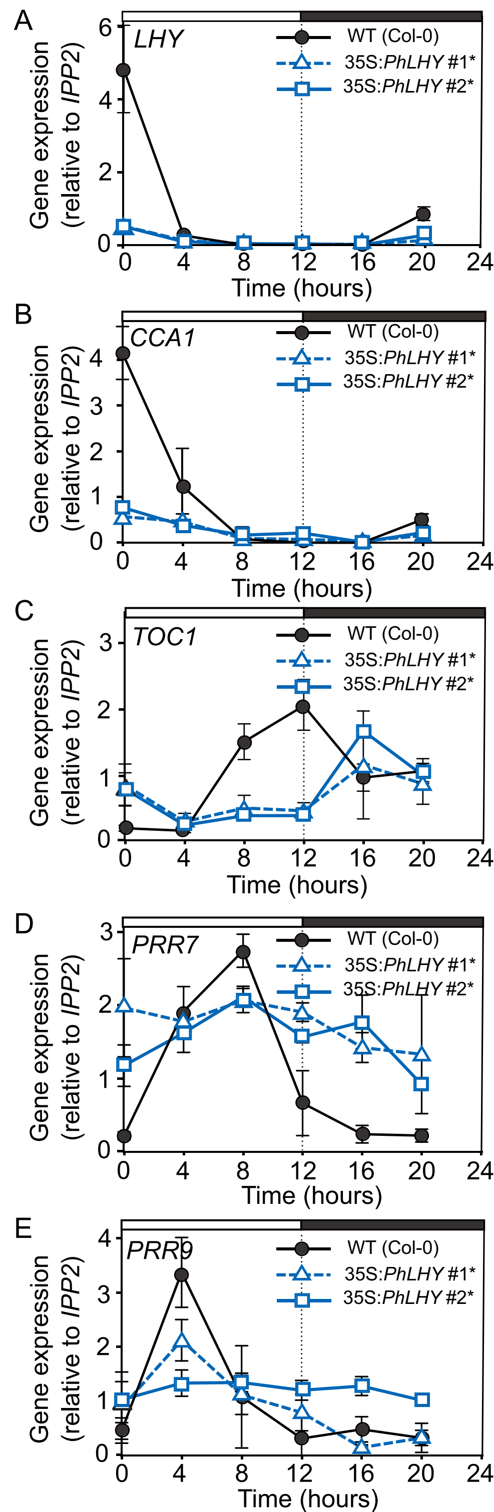

Fig. S4. Comparison of gene expression profiles of five core clock genes in *35S:PhLHY Arabidopsis* transgenic lines and WT Col-0 under 12-h light/12-h dark conditions over 24 h. The genes analyzed were *LHY* (*A*), *CCA1* (*B*), *TOC1* (*C*), *PRR7* (*D*), *and PRR9* (*E*). Results shown represent means ± SEM from three biological replicates. Black and white bars at the top indicate periods of light and dark, respectively. (**P* < 0.05, expression pattern differs from the one in WT plants; two-way ANOVA.)

Fig. S5. (Continued)

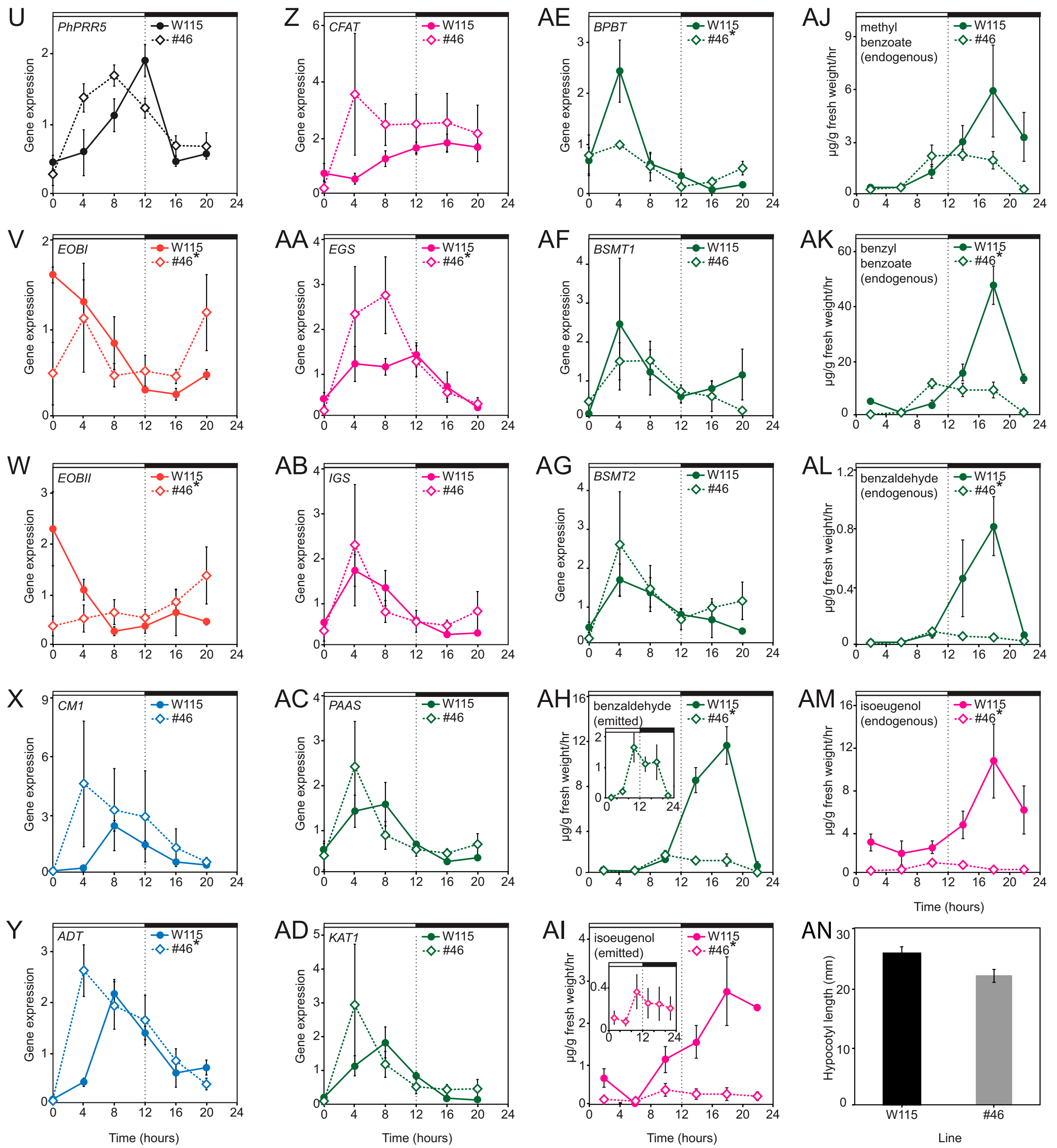

Fig. S5. (Continued)

Fig. S5. *PhLHY* influences gene expression patterns and emission of floral volatiles in the FVBP pathway. (*A–M*, *U–AG*, and *AO–BA*) Daily expression patterns of transcription factor genes and enzyme genes related to the FVBP pathway in a transgenic line with constitutive (line 37) and reduced (lines 46 and 47) *PhLHY* expression (line 37) under 12-h light/12-h dark conditions. Values are relative to *UBQ*, and normalized by the average expression values of hours 0–12. (*N*, *O*, *AH–AI*, *BB*, and *BC*) Daily scent expression patterns of methyl benzoate and benzyl benzoate in lines 37, 46, and 47 and (*Insets*) graphs with enlarged *y*-axes showing the same 0–24 time point results. (*P–S*, *AJ–AM*, and *BD–BG*) Daily endogenous volatile compounds in lines 37, 46, and 47. Results in Fig. S5 represent mean ± SEM from three biological replicates. The line and marker color of the graphs corresponds to its placement within the greater FVBP pathway. White and black bars at the top indicate periods of light and dark, respectively (*$P < 0.05$, expression profiles, scent emission patterns, and daily endogenous volatile compounds of transgenic lines differ significantly from WT *Petunia*; two-way ANOVA). (*T*, *AN*, and *BH*) Developing hypocotyl length in millimeters in a comparison between transgenic lines 37, 46, and 47 and WT *Petunia* W115. (*$P < 0.05$, developing hypocotyl lengths that differ significantly from W115 *Petunia*; Student *t* test.)

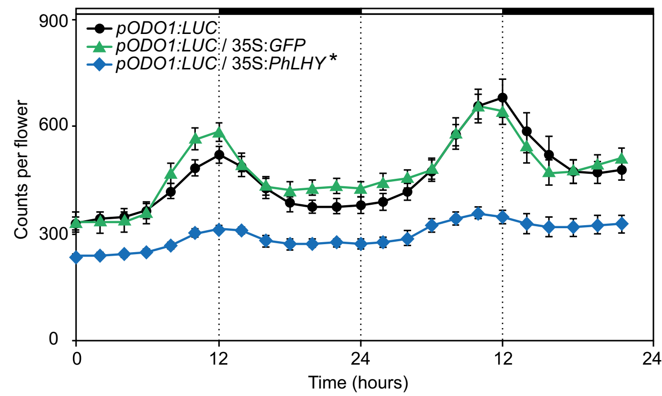

Fig. S6. Constitutive expression of PhLHY through transient transformation of flowers suppresses *pODO1:LUC* in vivo. *ODO1* promoter-driven LUC (*pODO1:LUC*) is used as a reporter (Fig. 5C). *pODO1:LUC* was coinfiltrated with *35S:PhLHY* or *35S:GFP*. White and black bars at the top indicate periods of light and dark, respectively. Results represent means ± SEM ($n = 8$). (*$P < 0.05$, *pODO1:LUC/35S:PhLHY* is the only expression profile differing significantly from the one in *pODO1:LUC*; two-way ANOVA.)

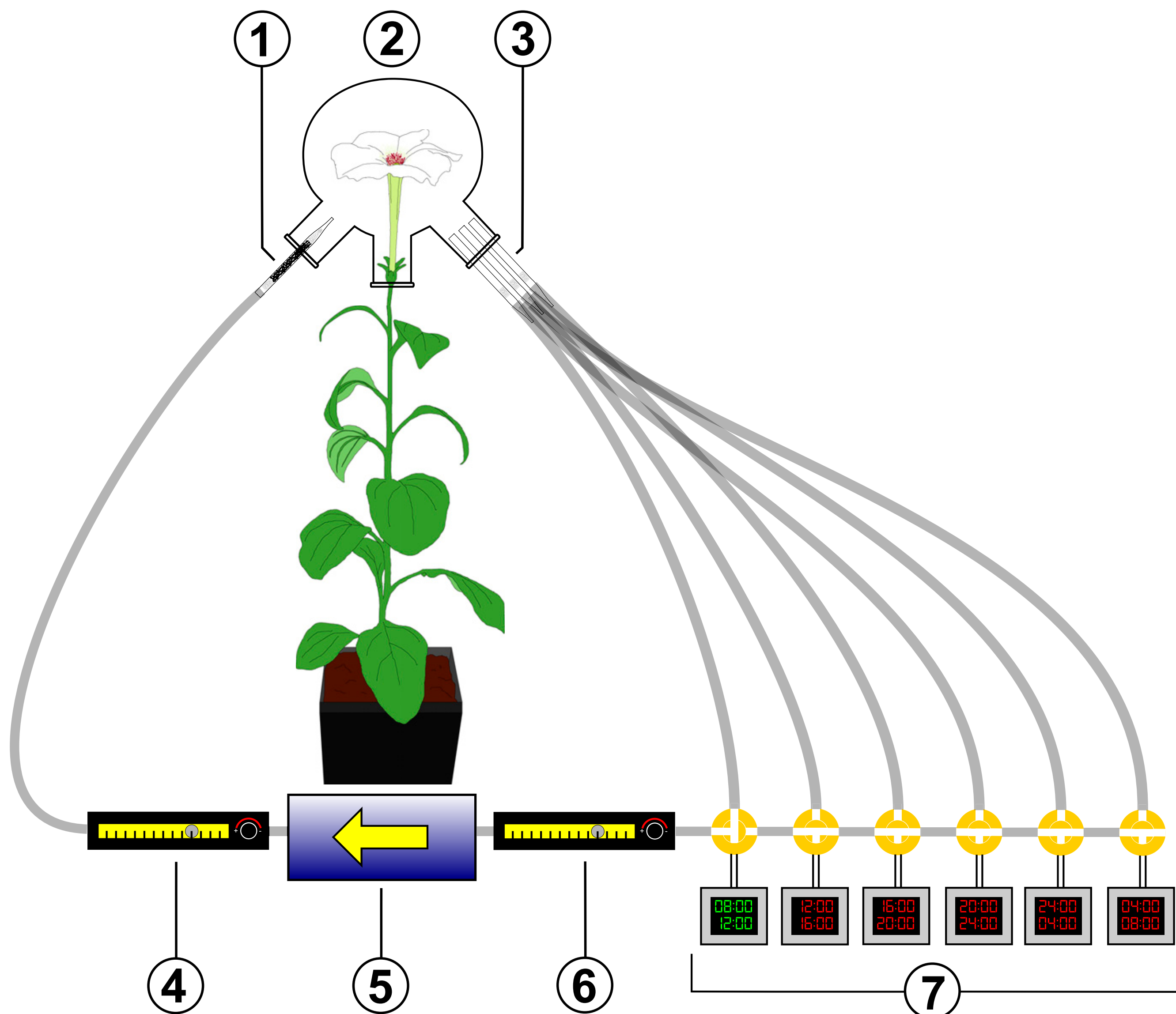

Fig. S7. Diagram of volatile collection apparatus. 1, Charcoal filter for introduced air; 2, Floral chamber (three-necked flask); 3, Volatile collection traps (Poropak); 4, Flow control for introduction of filtered air; 5, Unidirectional air pump (electric motor); 6, Flow control for suction to volatile collection traps; and 7, Timer-regulated solenoid switches (one trap open per time point).

Table S1. Quantitative PCR primer sequences used in this study

Gene name	Forward primer (5′–3′)	Reverse primer (5′–3′)	Accession no.	Species
ADT	GCGTGAAGCAGTAGACGACA	GACGTTGCTTGAATCGTCCT	FJ790412	*P. hybrida*
BPBT	TGGTGGACCAGCTAAAGGAG	CAATTGAGCATCACCCTTCA	AY611496	*P. hybrida*
BSMT1	GTGGTCGAAAAACCCGAATA	CACGACCACCTATGAAGTGC	AY233465	*P. hybrida*
BSMT2	TCATAGGTGGTCGAGGTGCT	GACATGGGATAATTCCCTGTGG	AY233466	*P. hybrida*
CFAT	CCAATGCCTAGCCCTAACAA	GGACGCTTCTTCACATCACA	DQ767969	*P. hybrida*
CM1	CCCTGCTGTTGAAGAGGCTA	GCATGATCCAGTCCCCATAC	CO805161	*P. hybrida*
EGS	TCTGACCCTGCTAAGGGAAA	TTTGATCAGCCAATTGCATC	EF467241	*P. hybrida*
EOBI	CCTTAGCTCGATCTGCTGGT	CACCTGTTTCCCCACTTAGC	KC182627	*P. hybrida*
EOBII	CAAGCAGCTTCTTCAGAGCAAA	AATTAGGGCCTGCTTGGAAAGT	EU360893	*P. hybrida*
EPSPS	TGGCTCAAGGGATACAAACC	GCTGTAGCCACTGATGCTGA	M21084	*P. hybrida*
IGS	CCACGTCAAAAGAGTGAGCA	CCAGTGGTTTTCTCCCAAGA	DQ372813	*P. hybrida*
KAT1	GCTACAGGTGCACGTTGTGT	AAAGATCGTCCACAGCATCC	FJ657663	*P. hybrida*
ODO1	CATGCACCACTGATGAATCC	ATGGCGAATCGATAAGAGGA	AY705977	*P. hybrida*
PAAS	TGTCGATGAAACCCAAGTGA	ACCACATTCCAGGCCATATC	DQ243784	*P. hybrida*
PAL	GGGTCCTTCAAGGCATGATA	GTTGCCAAAGATTCCAGCAT	AY705976	*P. hybrida*
PhCAB	CTTGCCAAGTCGTGTTGATG	TTCACCTTGAGCTCAGCAAA	K00972	*P. hybrida*
PhGI	TCTGCCGTCCGTCATACTCG	ATGCAAGCCTTGGAGCGTCT	FN036363	*P. hybrida*
PhPRR5	TTCGTTTGAAGCGGAAAGAT	TACCCGATGGAGCCTCACTA	FN035819	*P. hybrida*
PhLHY	ACCGACAATGGAACTTGGAG	TTCTCCTTCCAAGCGAAGTC	KP017483	*P. hybrida*
UBQ	TGGAGGATGGAAGGACTTTGG	CAGGACGACAACAAGCAACAG	SGN-U207515	*P. hybrida*
CCA1	CCAGATAAGAAGTCACGCTCAGAA	GTCTAGCGCTTGACCCATAGCT	AT2G46830	*A. thaliana*
LHY	GACTCAAACACTGCCCAGAAGA	CGTCACTCCCTGAAGGTGTATTT	AT1G01060	*A. thaliana*
IPP2	GTATGAGTTGCTTCTCCAGCAAAG	GAGGATGGCTGCAACAAGTGT	AT3G02780	*A. thaliana*
PRR7	CTGCACTCGTTATATCGTTACTG	GGCATGATCACCTCTGTTAG	AT5G02810	*A. thaliana*
PRR9	CCAATGAGGAAAAACGAG	GCACCACTTCCTTGATCTG	AT2G46790	*A. thaliana*
TOC1	CTCTCCTTTCAGAGTGTTCTTATC	CACAGGGATTCTGCGAAG	AT5G61380	*A. thaliana*