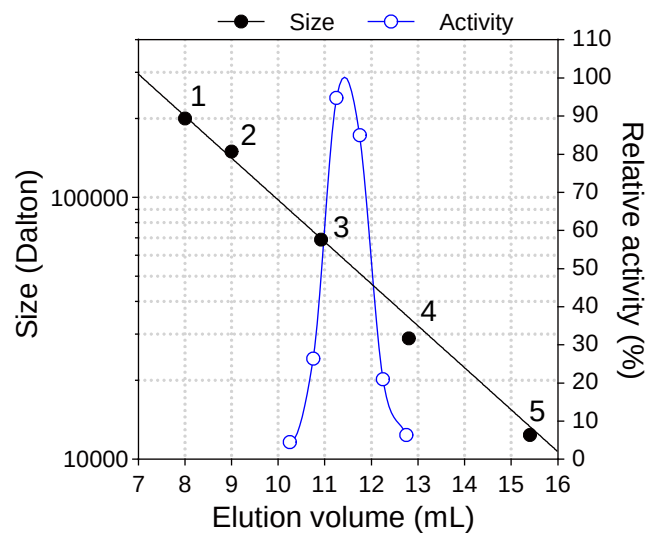
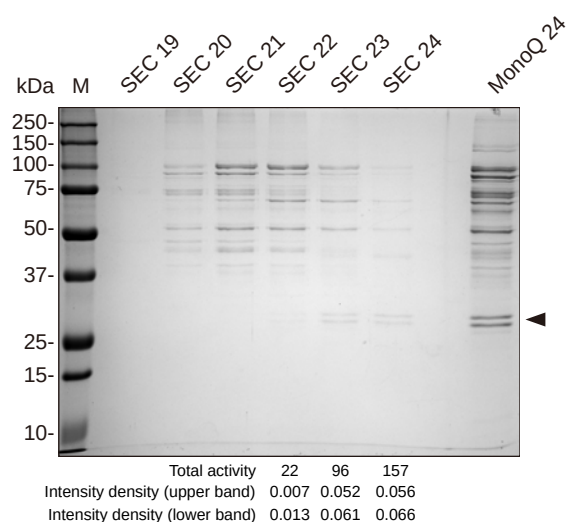


**A peroxisomal heterodimeric enzyme is involved in benzaldehyde synthesis
in plants**

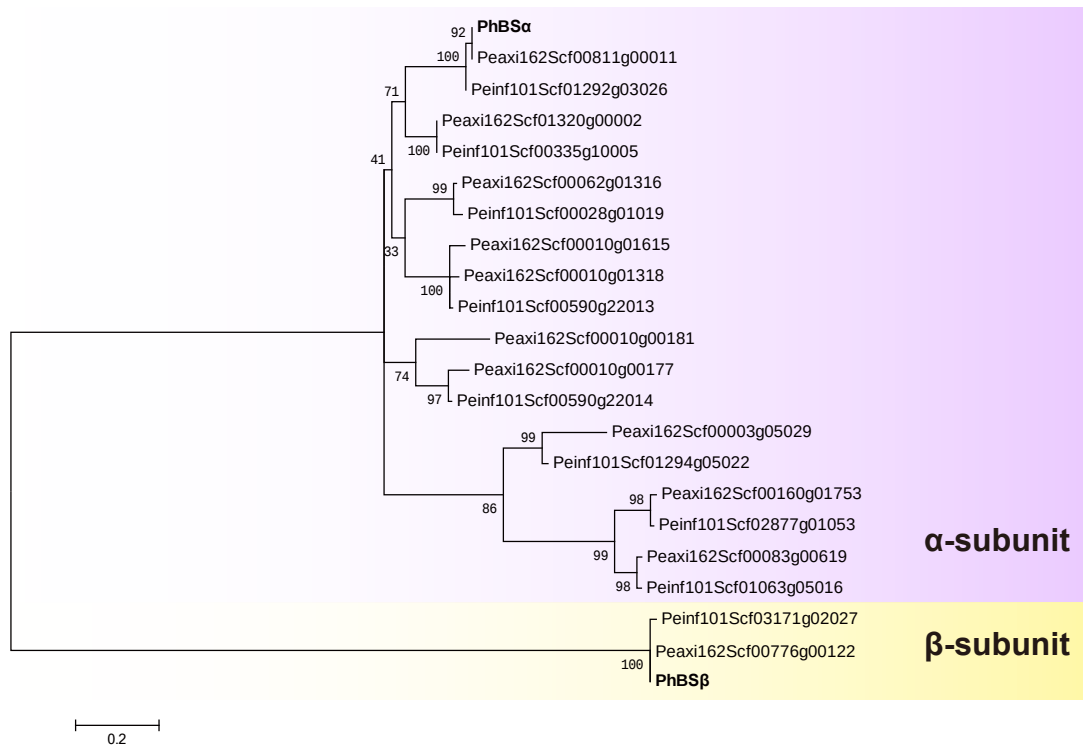
Xing-Qi Huang *et al.*



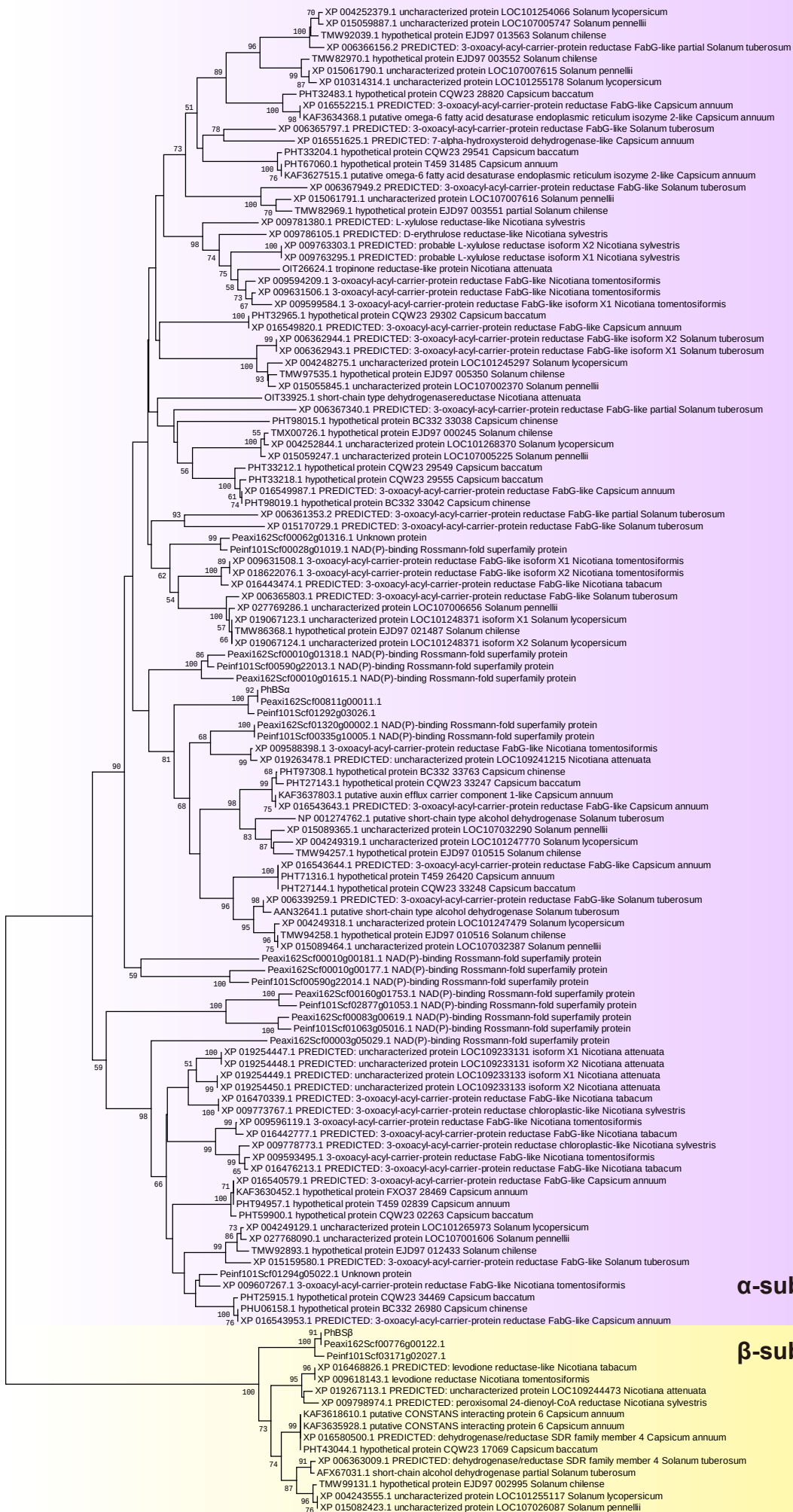
Supplementary Figure 1 | Determination of apparent molecular weight of native PhBS. A small fraction (200 μ L) of crude petunia flower protein extract was loaded onto a Superose 12 10/300 size exclusion column and eluted with buffer C (see Methods for buffer composition). Fractions of 0.5 mL were assayed for benzaldehyde synthase activity, which was plotted against elution volume (blue line). The column was calibrated with the following standard proteins: (1) β -amylase (200 kDa), (2) alcohol dehydrogenase (150 kDa), (3) bovine serum albumin (66 kDa), (4) carbonic anhydrase (29 kDa), and (5) cytochrome *c* (12.4 kDa) and their elution behavior is shown with black line.



Supplementary Figure 2 | SDS-PAGE analysis of fractions from size exclusion chromatography. SEC 19 to SEC 24 are fractions from size exclusion chromatography on a Superose 12 10/300 column of MonoQ fraction 24 (10 μ L of which are loaded in the right lane). Fractions of 0.5 mL were collected from size exclusion chromatography, 20 μ L were used for analysis of BS activity while the rest was precipitated with acetone, re-dissolved in 10 μ L of loading buffer and run on 12% SDS-PAGE. Total BS activity ($\text{pKat}\cdot\text{mg protein}^{-1}$) and intensity density of BS subunits for fractions SEC 22 to SEC 24 are shown below the gel. M, protein molecular weight standards. Triangle indicates the position of BS subunits. The experiment was repeated three times with similar results.



Supplementary Figure 3 | Phylogenetic analysis of benzaldehyde synthase homologs in *Petunia* genus. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model. The tree with the highest log likelihood (-1988.16) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (8 categories (+G, parameter = 1.3343)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 8.33% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

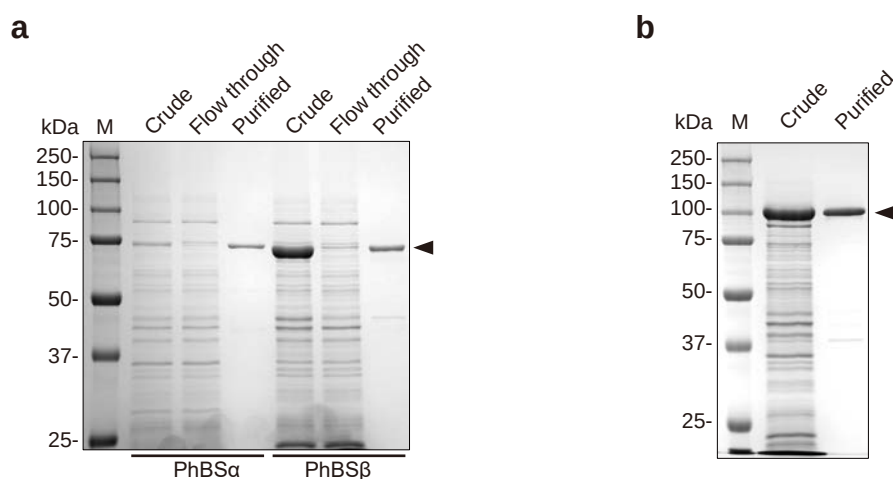


α-subunit

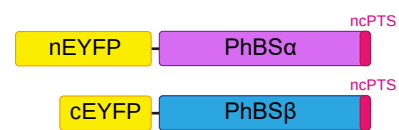
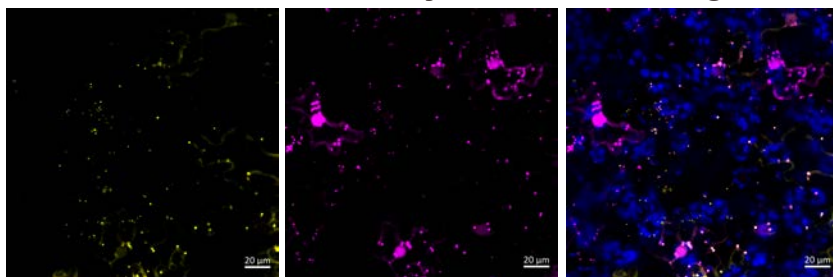
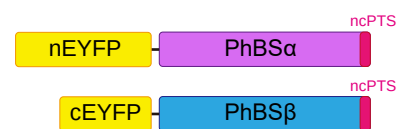
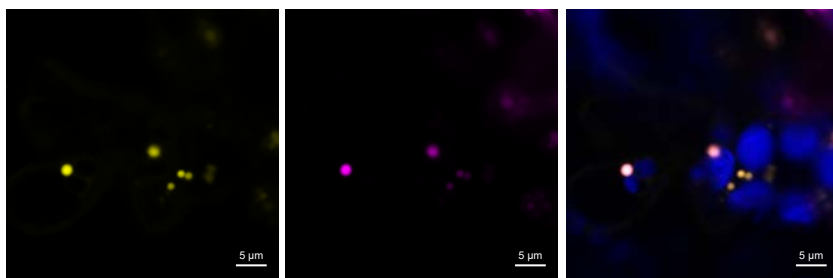
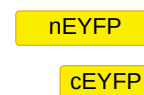
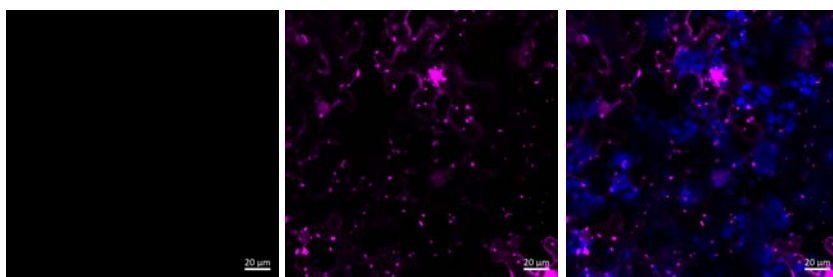
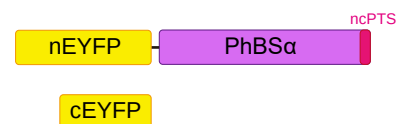
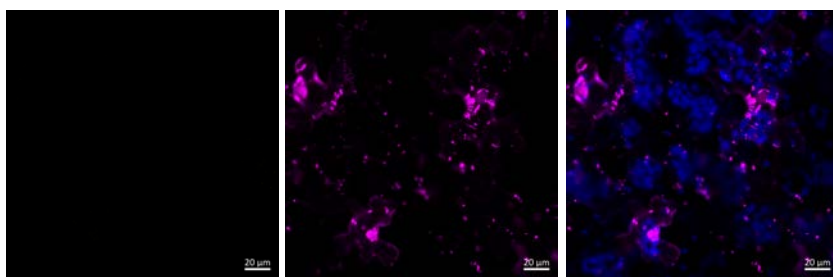
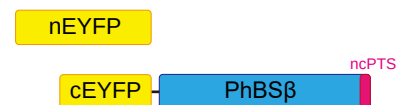
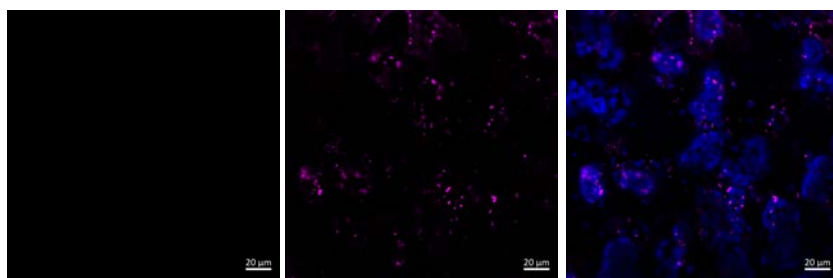
β-subunit

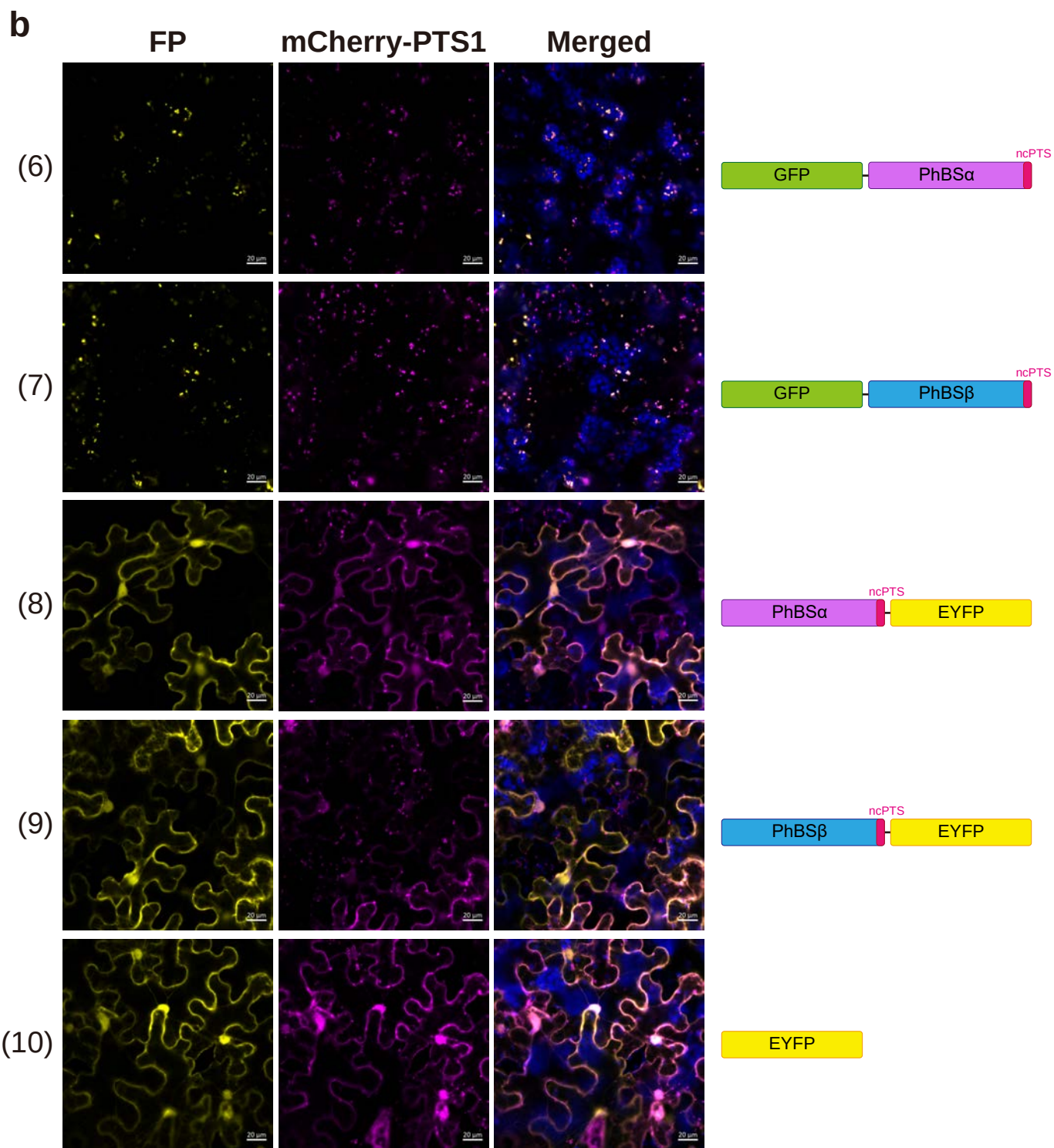
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Supplementary Figure 4 | Phylogenetic analysis of benzaldehyde synthase subunits from Solanaceae family. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 6.0 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of amino acid differences per site. The analysis involved 129 amino acid sequences (Supplementary Data 2).

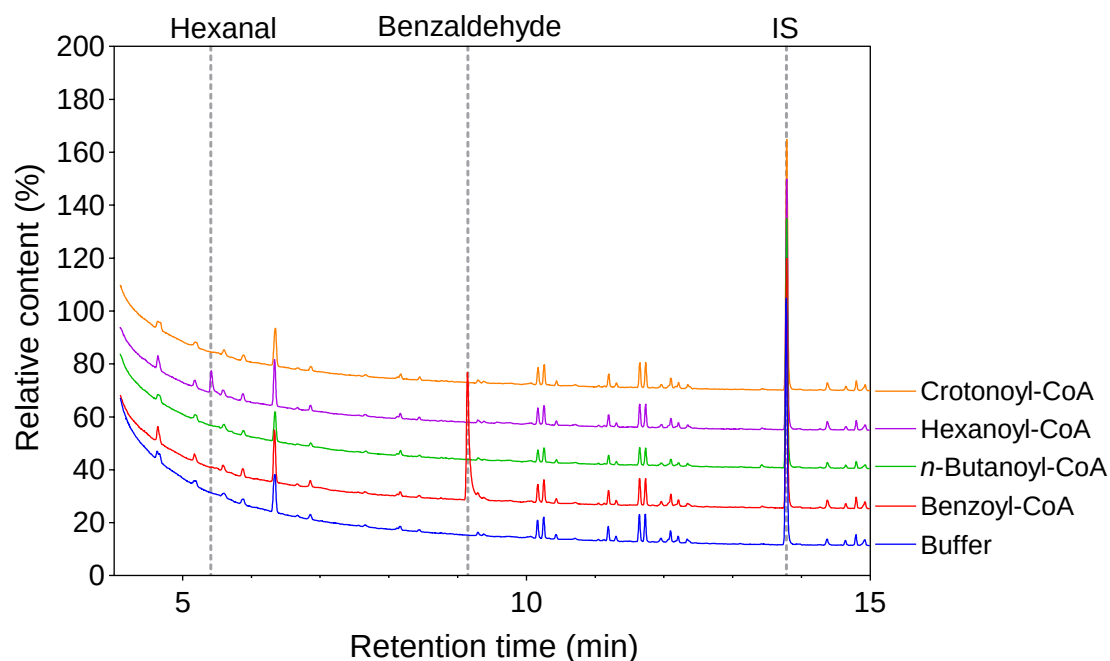


Supplementary Figure 5 | SDS-PAGE gel showing purification of benzaldehyde synthase subunits and Ph4CL1 from *E. coli*. (a) Aliquots of fractions from prokaryotic expression and purification of MBP-tagged PhBS subunits. Crude, total soluble bacterial lysate after IPTG induction; Flow through: fractions passed through amylose resin; Purified, ~ 2 µg of purified protein after elution with 10 mM maltose solution. The experiment was repeated at least six times with similar results. (b) Purification of Ph4CL1. Crude, total soluble bacterial lysate after IPTG induction; Purified, ~ 2 µg of purified protein after elution with maltose solution. The experiment was repeated three times with similar results. M, protein molecular weight standards. Triangle indicates the position of MBP-tagged PhBS subunits (a) and Ph4CL1 protein (b). MBP is ~ 42.5 kDa in size.

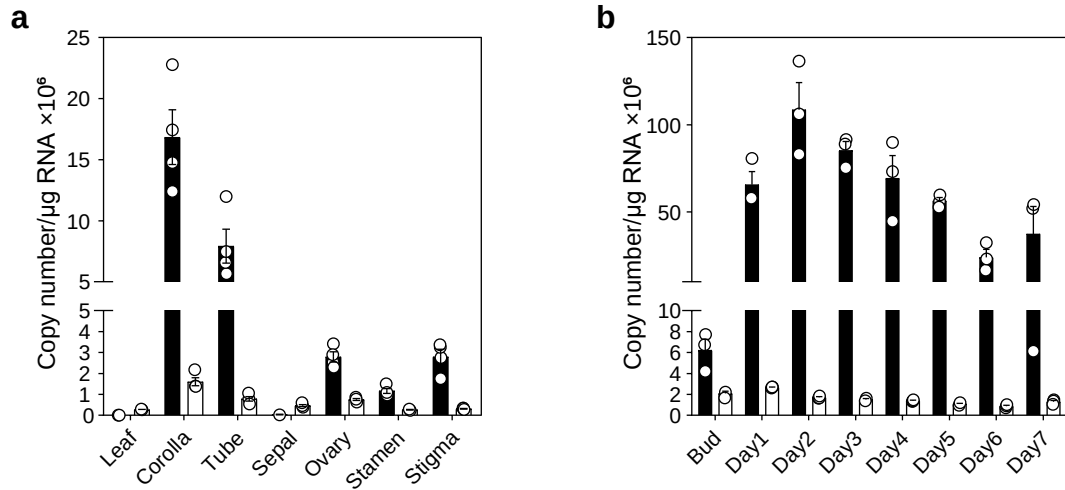
a**FP****mCherry-PTS1****Merged****(1)****(2)****(3)****(4)****(5)**



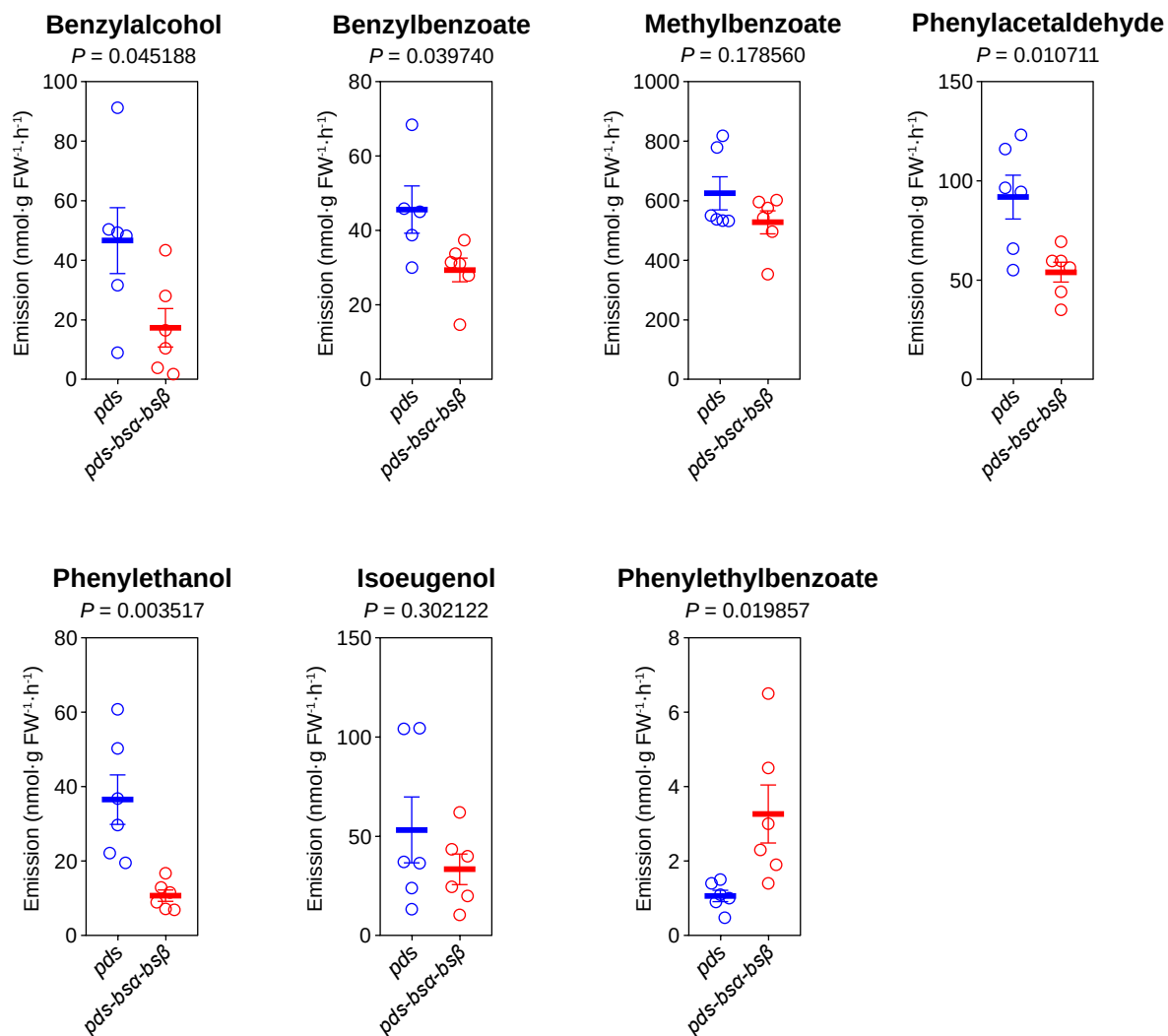
Supplementary Figure 6 | BiFC detection of protein-protein interactions between PhBS subunits and their subcellular localization. (a) Transient BiFC analysis of PhBS α -PhBS β interaction in *N. benthamiana* leaves detected by confocal laser scanning microscopy. The "FP" panels (yellow) represent signals of reconstituted EYFP as a result of protein-protein interactions; the "mCherry-PTS1" panels (magenta) represent signal of peroxisome-targeted mCherry marker protein; the "Merged" panels show merged EYFP and mCherry signals. Blue signals in the "Merged" panels represent chlorophyll autofluorescence. Schematic diagrams of the protein topologies and combinations for each experiment are illustrated on the right as follows (from amino terminus to carboxyl terminus): (1) nEYFP-PhBS α + cEYFP-PhBS β ; (2) nEYFP-PhBS α + cEYFP-PhBS β captured with 63 $\times$ oil immersion objective lens; (3) nEYFP + cEYFP; (4) nEYFP-PhBS α + cEYFP; (5) nEYFP + cEYFP-PhBS β . The experiment was repeated three times with similar results. (b) Subcellular localization of PhBS subunits. PhBS fusion constructs were expressed in *N. benthamiana* leaves and their corresponding transient expression was detected by confocal laser scanning microscopy. The "FP" panels (yellow) represent signals of PhBS fused fluorescent proteins; the "mCherry-PTS1" panels (magenta) represent signal of peroxisome-targeted mCherry marker protein; the "Merged" panels show merged FP and mCherry signals. Blue signals in the "Merged" panels represent chlorophyll autofluorescence. Schematic diagrams of the BS-FP fusion proteins for each experiment are illustrated on the right as follows (from amino terminus to carboxyl terminus): (6) GFP-PhBS α ; (7) GFP-PhBS β ; (8) PhBS α -EYFP; (9) PhBS β -EYFP; (10) EYFP. FP, fluorescent protein; PTS1, classic peroxisomal targeting signal; ncPTS, non-classic PTS. The experiment was repeated three times with similar results. All images were captured using 20 $\times$ objective lens except for (2). Scale bars = 20 μ m, except for (2), in which bars are 5 μ m.



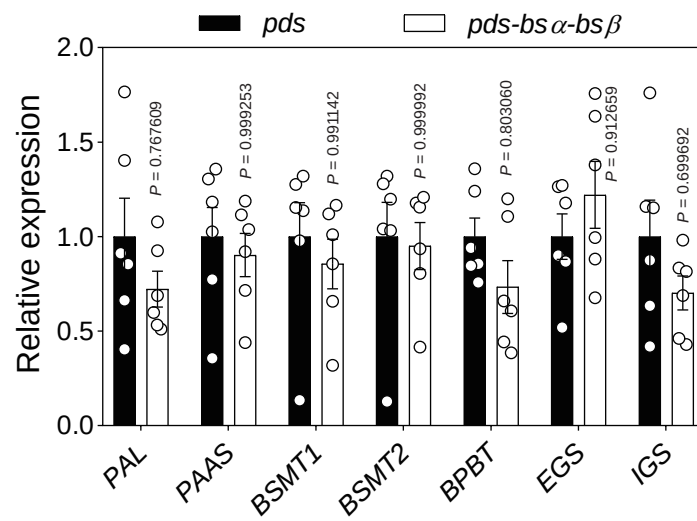
Supplementary Figure 7 | Substrate specificity of purified PhBS. GC-MS analysis of products formed by MBP-tagged PhBS from different short-chain fatty acyl-CoA substrates. Purified PhBS (1:1 ratio between α and β subunits) was incubated with 200 μ M benzoyl-CoA or 1 mM fatty acyl-CoA including *n*-butanoyl-CoA, hexanoyl-CoA, and crotonoyl-CoA. All reactions were carried out at 28°C for 1 hour. Shown are total ion currents (TICs) of scan mode (m/z 35 to 250). The response of internal standard in each run was set as 100%.



Supplementary Figure 8 | Spatial and developmental expression of *PhBSα* and *PhBSβ* in petunia flowers. (a) Tissue-specific expression of *PhBSα* (black bar) and *PhBSβ* (white bar). (b) Developmental expression of *PhBSα* (black bar) and *PhBSβ* (white bar) in petunia corolla from mature buds to day 7 post-anthesis. The *PhBSα* and *PhBSβ* expression was determined by qRT-PCR with gene-specific primers and expressed as copy number of transcripts per microgram of total RNA. Shown are means $\pm$ SE ($n=4$ biological replicates for **a**; $n=3$ biological replicates for **b**).



Supplementary Figure 9 | Effect of *PhBS* downregulation on emission of individual petunia volatiles. Emission rates of individual VOCs are shown in *pds* (control) and *pds-bsa-bsβ* VIGS petunia flowers. Volatiles were collected from 2-day-old flowers from 20:00 till 21:00. Data are means $\pm$ SE ($n=6$ biological replicates). P values, shown on top of each graph, were determined by unpaired two-tailed Student's t -test relative to *pds* control.



Supplementary Figure 10 | Effect of *PhBS* downregulation on expression of scent biosynthetic genes in petunia flowers. Transcript levels were determined by qRT-PCR with gene-specific primers in *pds* (control) and *pds-bsα-bsβ* VIGS flowers at 21:00 and presented relative to the corresponding levels in *pds* control, set as 1. Data are means $\pm$ SE ($n=6$ biological replicates). *P* values were determined by two-way ANOVA multiple comparisons test relative to the *pds* controls. Displayed gene identifiers encode the following proteins¹⁻⁵: *BPBT*, benzoyl-CoA:benzyl alcohol/2-phenylethanol benzoyltransferase; *BSMT*, benzoic acid/salicylic acid carboxyl methyltransferase; *EGS*, eugenol synthase; *IGS*, isoeugenol synthase; *PAAS*, phenylacetaldehyde synthase; *PAL*, phenylalanine ammonia lyase.

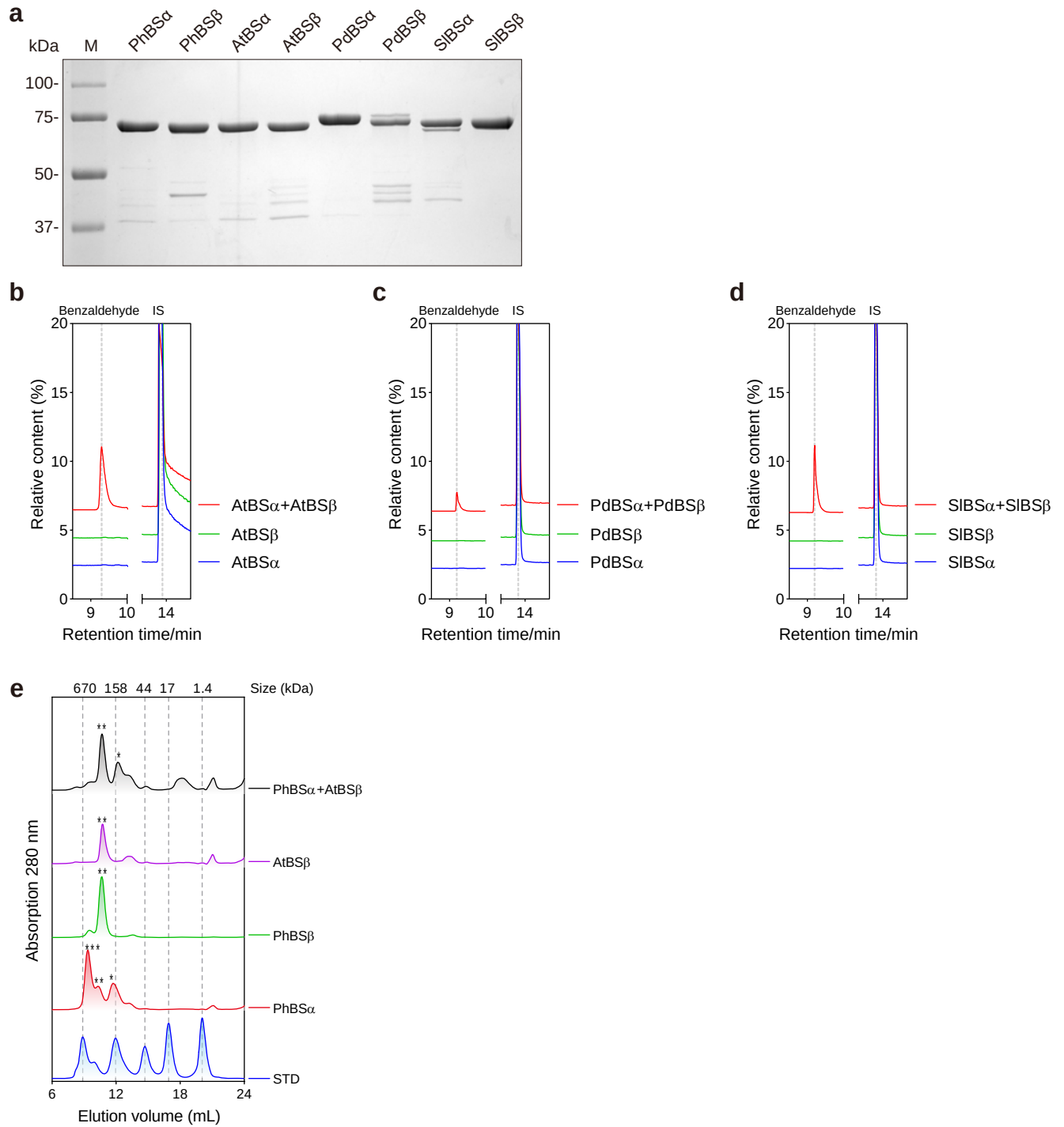
a

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AtBS α	MSNHQTQVLKLLLEPWCELKDKVVLVTGASSGIGREICLDLAKAGCQVIAAARRVDRLNSLCSEIN-----SFSSTG-	71
PdBS α	---MANQVADHLEPWRHLHGKVVMTGASSGLGREFCLDLAKAGCRIVAAARRIDRLQSVCEINQLT VTAPSSSSSSSS	77
SlBS α	-METHAKSVRQLEPWGELAGKVVMTGASSGIGRDFCSDLKAGCRITIAAARRIDRLQSLCDEIN-----SNSNNGS	71
PhBS α	DNN---RAVAVELDVSAANGDATEAAVKEAWGAFGRIDALVNNAGVRGTVHSPLLELTEEECHNTFRTNLTGTWLVSKYVC	146
AtBS α	-----IQAAALELDVSSDAATIQAAREAWDIFGKIDALINNAGIRGNVKSLLDLSEDEWDNVFKTNLKGPVLVSKHVC	145
PdBS α	VNGPGSSRAVAVELDISADGPAIEKSVNKAWDAFGRIDALVNNAGVRGNVSSSLELSEEEWNNVFKTNLTGSWLVSKYVS	157
SlBS α	TKSSQDLRAVAIELDVSAANGSAIEAAVQKAWDAFGRIDGLVNNAGFRGSVLSPLELSEEEWEKIHKTNLRGAWLVTKYVC	151
PhBS α	KFMRDAKQGGSVINISSIAGLNRGQLPGGLAYVSSKGAVNMVTKVMALDELGVDKIRVNSISPGLFKSEITQGLIQKEWLN	226
AtBS α	MLMRDAKRGGSVINISSIAGI-RGMLPGGLAYACSKGGVDTMSRMMALDELGVHKIRVNSIAPGLFKSEITQGLMQKEWLK	224
PdBS α	IRMRDADQGGSIINVSSIAGLHRGYLPGAVAYNCSKAGVNTLAKVMAMELGVHKIRVNAISPGLFRSEITEGLMKKDWLH	237
SlBS α	MHMRAANQGGSIINISSIAGINRGQLPGSLAYTSKEALNSITKVLALDELGPYKIRVNSISPGLFKSEITEGLIQKDWIK	231
PhBS α	NVAMRTIPLRTHGNSADPALTSVVRYLIHDSSEYVSGNLFIVDAGATLPGVPIFSSL	283
AtBS α	NVTERTVPLKVQQ-TVDPGLTSLVRYLIHDSSEQYISGNTYIVDSGATLPGVPIFSSL	280
PdBS α	NVAMKTVPLRTFG-TSDPALTSLVRYLVHDSSEYVSGNVYIVDAGATLPGVPIFSAL	293
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b

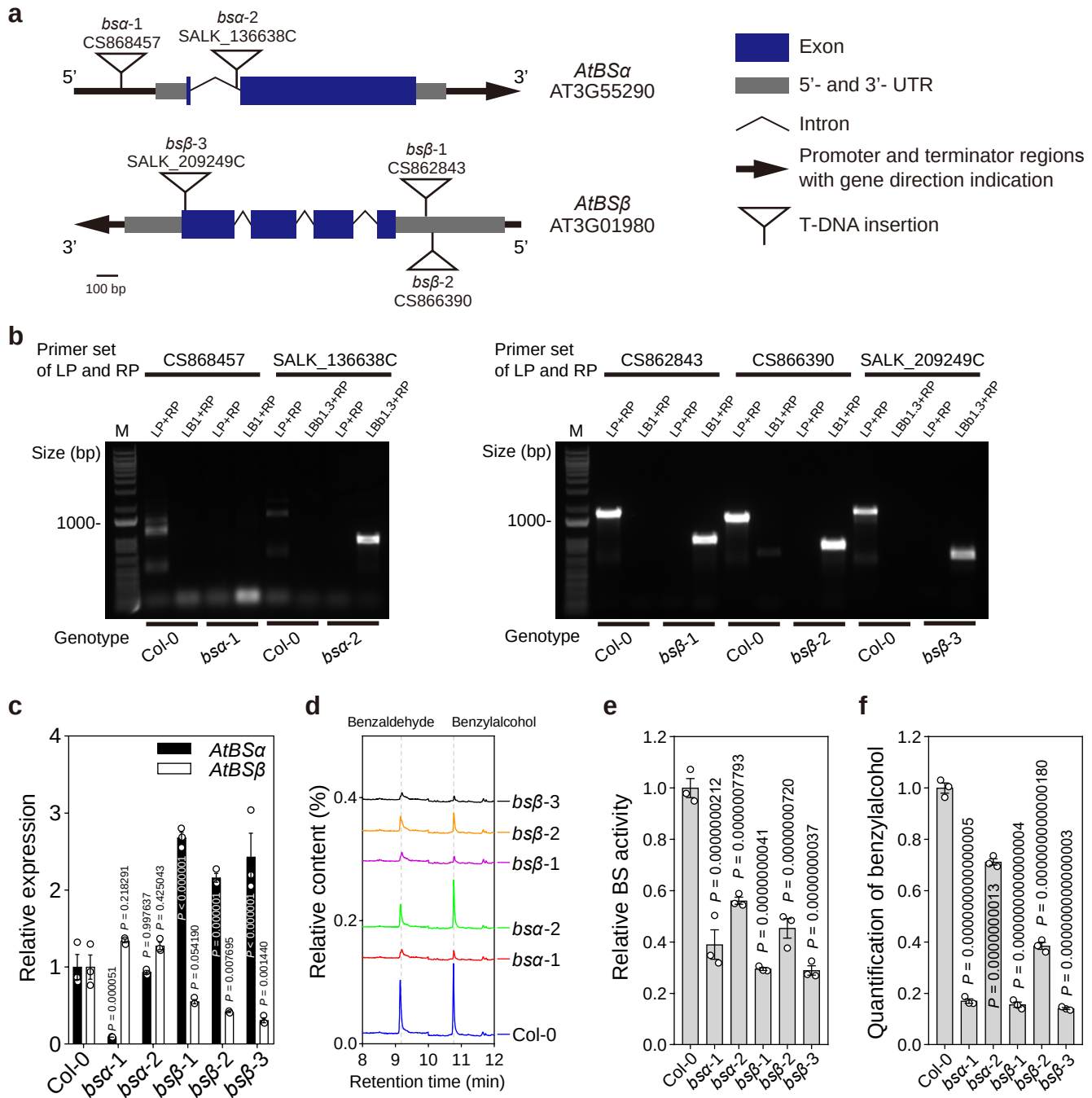
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AtBS β	MEN-PAKRVLMTSNGDEVSRNIAFHAKHGCKLVMMGNEGSLRSIVDKIRDSIEGAFPADVIALDMESDSEVAFHAAVQK	79
PdBS β	MGTSPGKKVLLTSNGDAISHNIAFSLAQRGCLVLMGKESCLRSIQQKIKGSLEGVVPVEVVDVDMEDKREGAFDEAVDK	80
SlBS β	MEN-PGKKVLLTSNGDEICNNIAYHLAQRGCLVLMGNERQLKSVAENIKQSLKGSVAVEVVGLDMTEDRETAFDEAVDK	79
PhBS β	AWKIFGKLDALVNCYSYEGKMQDPLQLIEEEFKKIVKINFAGWYLMKCISKMRDDKSGGSIVFLTSIIIGAERGIYQGA	159
AtBS β	AWELSGHFDAFLNSYTYQGKVQDILQVSQDEFHRITKINLTAPWFLKAVATRMKDHGSGGSIVFMATIASGERALYPGA	159
PdBS β	ACHILGNLDAFVHCYTYEGKMQEHLLEAEDEFKKIMKRNFMASAWFLNNAVGRMRDYGSGGSIIIFLTSIIIGAERGIYPGA	160
SlBS β	AWKIFGKLDALVHCYAYEGKMQDPLQLIDDEFKKIVKINFAGWYLLKCIGNRMRDGKSGGSIVFMTSIIIGAERGIYQGA	159
PhBS β	AAYGSCLGGIQQLVRLAAIELGKHQIRVNGISRGLHLQDEYPKAVGLERAEMTKEAAPLNRLDVKKDLASTVIYLLISD	239
AtBS β	DAYASTSAAIHQLVRASAMSLGKHKIRVNMISRGLHLQDEYTASVGRDRAQKLVKDAAPLGQWLNPDLDLYSTVIYLLISD	239
PdBS β	AANSACSAAVQQLARTSALEIGRYQIRVNAIARGLHLEDEYPMFVGMERAKKLVKEAAPLQRWLDVKNDLASTVIYLLISD	240
SlBS β	AAYGSCAAGIQQLVRLSAIELGKYQIRVNGILRGLHLEDEFPLSVGKERAVKLTKEAAPLNRLDPKKDLASTVIYLLISD	239
PhBS β	DSRYMTGTSIFVDGAQSLVRPRMRSYM	266
AtBS β	GSRFMTGTTVLVDGAQSLTRPRLKSYM	266
PdBS β	GSKYMTGTTIFVDGGQSLTRPRMRSYM	267
SlBS β	DSRYMTGTSIFVDGAQSLVRPRMRSYM	266

Supplementary Figure 12 | Alignment of deduced amino acid sequences for BS subunits from four species. (a) Alignment of amino acid sequences for BS α subunits from *P. hybrida*, *A. thaliana*, *Prunus dulcis* and *S. lycopersicum*. **(b)** Alignment of amino acid sequences for BS β subunits from petunia, Arabidopsis, almond and tomato. Conserved residues are shaded in black, while similar residues are shaded in dark gray.

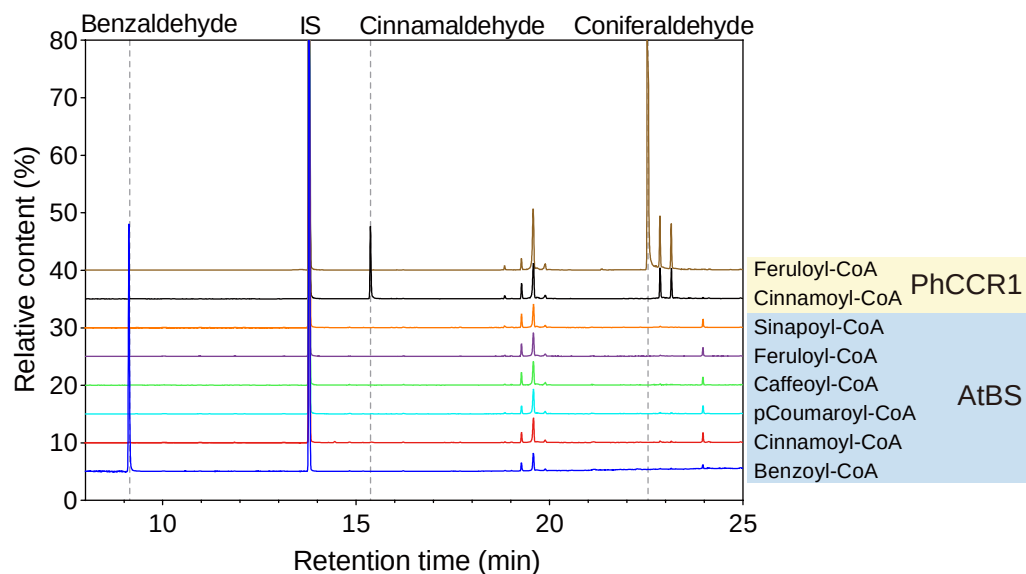


Supplementary Figure 13 | Purification and characterization of BS from phylogenetically distant species. (a) SDS-PAGE analysis of ~2 μ g of purified MBP-tagged BS subunits from petunia, Arabidopsis, almond and tomato. M, protein molecular weight standards. The experiment was repeated at least six times with similar results. (b - d) GC-MS analysis of products formed *in vitro* by Arabidopsis (b), almond (c) and tomato (d) purified recombinant BS proteins in enzymatic assays. The response of internal standard in each run was set as 100%. (e) Gel filtration chromatography of purified recombinant PhBS subunits, AtBS β and PhBS α -AtBS β hybrid. 500 μ L purified proteins (~500 μ g) were loaded onto a Superdex 200 Increase 10/300 GL size exclusion column and eluted with Phosphate Buffered Saline (PBS). The column was calibrated with following standards: bovine thyroglobulin (670 kDa), bovine gamma globulin (158 kDa), chicken ovalbumin (44 kDa), horse myoglobin (17 kDa), and vitamin B-12 (1.4 kDa). Peak labels are as follows: *, dimers; **, tetramers; and ***, multimers. STD, chromatogram of calibration standards.

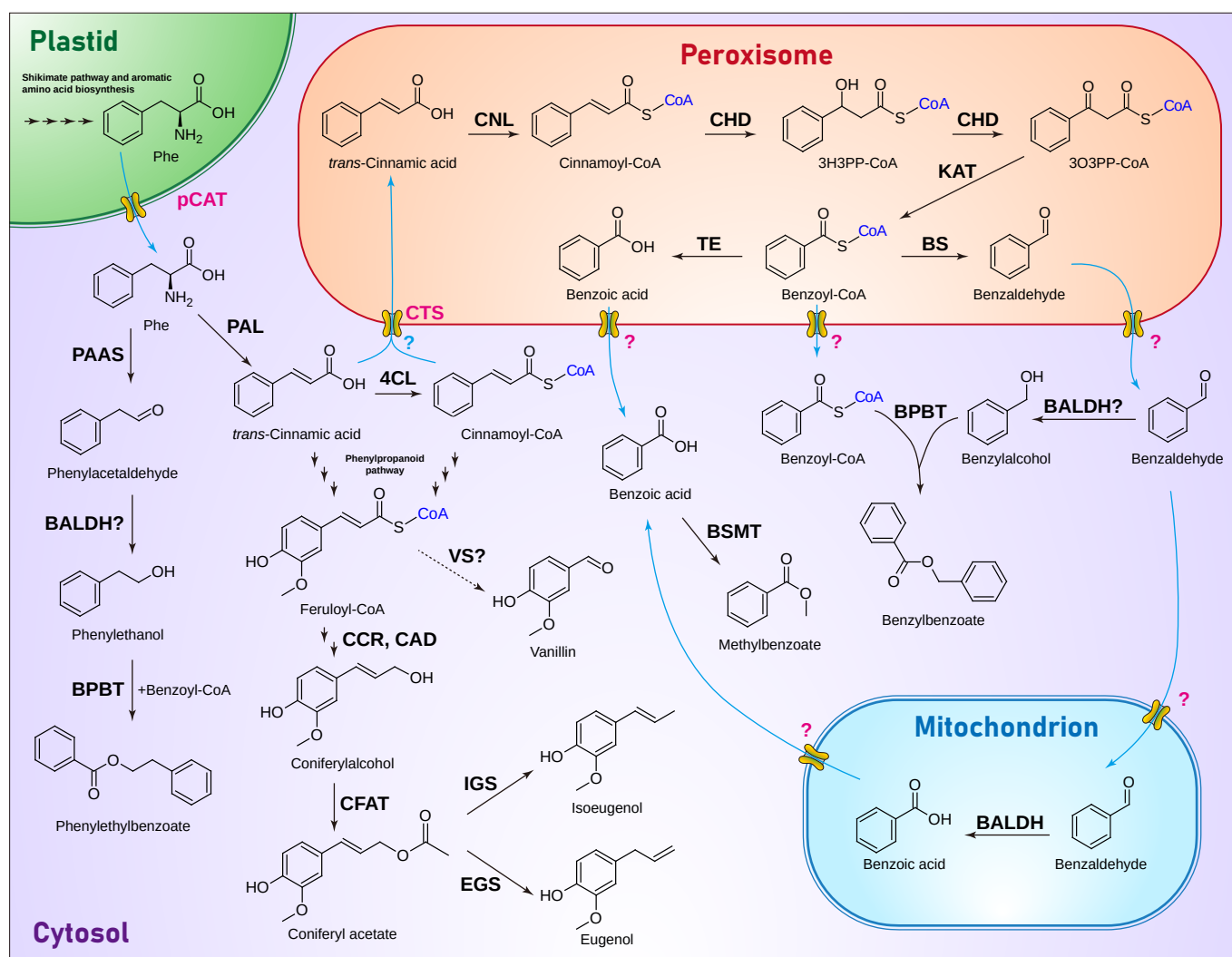
Supplementary Figure 14 | Expression profile of *AtBSα* and *AtBSβ*. Tissue-specific and developmental expression levels of *AtBSα* (**a**) and *AtBSβ* (**b**) transcripts. Data in **a** and **b** were obtained from ePlant (<https://bar.utoronto.ca/eplant/>). Peptide enrichment of *AtBSα* (**c**) and *AtBSβ* (**d**) in different tissues. Data in **c** and **d** were retrieved from Arabidopsis PeptideAtlas project website (<http://www.peptideatlas.org/builds/arabidopsis/>). Column names represent distinct observed peptides of each protein, with asterisk denotes single genome mapping. (**e**) Hierarchical clustering of *AtBS* genes as well as genes involved in β -oxidation and lignin biosynthesis pathways. The plot was generated using ATTED-II Hcluster tool (https://atted.jp/top_draw/#Hcluster). Genes used to build this plot are as follows: *AtBSα* (AT3G55290), *AtBSβ* (AT3G01980), *PAL1* (AT2G37040), *PAL2* (AT3G53260), *PAL3* (AT5G04230), *PAL4* (AT3G10340), *4CL* (AT4G19010), *CCR1* (AT1G15950), *CCR2* (AT1G80820), *C4H* (AT2G30490), *HCT* (AT5G48930), *CAD1* (AT1G72680), *CCoAOMT1* (AT4G34050), *F5H* (AT4G36220), *Peroxidase* (AT5G66390), *PRX52* (AT5G05340), *CCOAMT* (AT1G67980), *KAT1* (AT1G04710), *KAT2* (AT2G33150), *AIM1* (AT4G29010), *MFP2* (AT3G06860), and *AAE12* (AT1G65890). The subcluster containing both *AtBSα* and *AtBSβ* is highlighted with a red box.



Supplementary Figure 15 | Characterization of Arabidopsis *bs* mutants. (a) Schematic diagrams showing the gene structures of *AtBSα* (AT3G55290) and *AtBSβ* (AT3G01980) and T-DNA insertion sites in five *bs* mutant lines. (b) Genotyping of the *bs* mutants by PCR analysis of genomic regions flanking each T-DNA insertion and a corresponding region of the wild-type gene. The experiment was repeated three times with similar results. (c) qRT-PCR analysis of *AtBSα* and *AtBSβ* transcripts in floral tissues of Arabidopsis wild type (ecotype Col-0) and *bs* mutants using gene-specific primers. Expression of each gene in wild type was set to 1. Data are means $\pm$ SE ($n=3$ biological replicates, each replicate contains > 100 fully opened flowers and flower buds). P values were determined by two-way ANOVA multiple comparisons test of each gene relative to the Col-0 controls. (d) GC-MS analysis of BS activities in crude flower extracts from Arabidopsis wild type and *bs* mutants. The response of internal standard in each run was set to 100%. (e) Quantification of apparent BS activities shown in d. BS activity in wild type was set to 1. Data are means $\pm$ SE ($n=3$ biological replicates). (f) Quantification of benzylalcohol formation *in vitro* assays with crude extracts shown in d. Benzylalcohol level produced by a crude extract from wild type was set to 1. Data are means $\pm$ SE ($n=3$ biological replicates). P values in e and f were determined by ordinary one-way ANOVA multiple comparisons test relative to the wild type controls.



Supplementary Figure 16 | Substrate specificity of purified MBP-tagged AtBS. GC-MS analysis of products formed by AtBS from different hydroxycinnamoyl-CoA substrates. Purified AtBS (1:1 ratio between α and β subunits) was incubated with benzoyl-CoA and its structural analogs including cinnamoyl-CoA, *para*-coumaroyl-CoA, caffeoyl-CoA, feruloyl-CoA, and sinapoyl-CoA. All CoA esters except benzoyl-CoA were synthesized using Ph4CL1 and their corresponding free acids. Formation of cinnamaldehyde and coniferaldehyde by purified PhCCR1 was used as a positive control. Shown are combined EICs of mass units 106 (benzaldehyde), 128 (internal standard), 131 (cinnamaldehyde), and 178 (coniferaldehyde). The response of internal standard in each run was set as 100%.



Supplementary Figure 17 | Proposed VOC biosynthetic network in petunia flowers. Enzymes responsible for each biochemical reaction are shown in bold black. Established biochemical reactions are presented by solid black arrows, while unidentified steps are shown by dashed arrows. Transporters involved in metabolite transport between different subcellular locations are shown in bold magenta, with the flow of metabolites indicated by cyan arrows. Cross-organelle transport of metabolites by unknown transporters or diffusion are indicated with magenta question marks. Abbreviations: BALDH, benzaldehyde dehydrogenase; BPBT, benzoyl-CoA:benzyl alcohol/2-phenylethanol benzoyltransferase; BS, benzaldehyde synthase; BSMT, benzoic acid/salicylic acid carboxyl methyltransferase; CAD, cinnamyl alcohol dehydrogenase; CCR, cinnamoyl-CoA reductase; CFAT, coniferyl alcohol acyltransferase; CHD, cinnamoyl-CoA hydratase/dehydrogenase; CNL, cinnamate-CoA ligase; CTS, peroxisomal cinnamic acid/cinnamoyl-CoA transporter COMATOSE. The substrate specificity of CTS is unclear, as indicated by a cyan question mark next to it; EGS, eugenol synthase; IGS, isoeugenol synthase; KAT, 3-ketoacyl thiolase; PAAS, phenylacetaldehyde synthase; PAL, phenylalanine ammonia lyase; pCAT, plastidial cationic amino acid transporter; Phe, phenylalanine; 3H3PP-CoA, 3-hydroxy-3-phenylpropanoyl-CoA; 3O3PP-CoA, 3-oxo-3-phenylpropanoyl-CoA; TE, thioesterase; VS, vanillin synthase.

Supplementary references

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2. Kaminaga, Y. *et al.* Plant phenylacetaldehyde synthase is a bifunctional homotetrameric enzyme that catalyzes phenylalanine decarboxylation and oxidation. *J. Biol. Chem.* **281**, 23357–23366 (2006).
3. Negre, F. *et al.* Regulation of methylbenzoate emission after pollination in snapdragon and petunia flowers. *Plant Cell* **15**, 2992–3006 (2003).
4. Koeduka, T. *et al.* Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 10128–10133 (2006).
5. Koeduka, T. *et al.* The multiple phenylpropene synthases in both *Clarkia breweri* and *Petunia hybrida* represent two distinct protein lineages. *Plant J.* **54**, 362–374 (2008).

Supplementary Table 1. Primers used in this research

Primer name	Sequence (5' to 3')
For VIGS	
PhBS α >pTRV2-PDS/F	ATCCGGTACCGAGCTCGGAGAAGCAAACCTCAGGCACG
PhBS α >pTRV2-PDS/R	AGAATTTTCCTCCAAAAGCACCCC
PhBS β >pTRV2-PDS/F	TGCTTTTGGAGGAAAATTCTGGAAAGAAG
PhBS β >pTRV2-PDS/R	CGAGACGCGTGAGCTCTTGCAATTTTCTTCATATGAATAGCAGTTTACC
For heterologous expression of BS	
PhBS α >pMAL/F	GCGATATCGTCGACGGATCCATGGAGAAGCAAACCTCAGGCAC
PhBS α >pMAL/R	CCTGCAGGGAATTCGGATCCCTACAGGGATGAGAAAATCGGGACAC
PhBS β >pMAL/F	GCGATATCGTCGACGGATCCATGGAAAATTCTGGAAAGAAGGTGTTG
PhBS β >pMAL/R	CCTGCAGGGAATTCGGATCCCTACATGTATGAGCGCATTTCGC
PhBS β >pET32b/F	CTTTAAGAAGGAGATATACATATGGAAAATTCTGGAAAGAAGGTGTTGC
PhBS β >pET32b/R	TCGAGTGC GGCCGAAGCTTCATGTATGAGCGCATTTCGC
AtBS α >pMAL/F	GCGATATCGTCGACGGATCCATGAGCAATCATCAAACCTCAGGTGT
AtBS α >pMAL/R	CCTGCAGGGAATTCGGATCCCTCAGAGAGATGAAAAGATAGGCACACCG
AtBS β >pMAL/F	GCGATATCGTCGACGGATCCATGGAAAATCCGGCGAAGAGAG
AtBS β >pMAL/R	CCTGCAGGGAATTCGGATCCCTACATGTAGGATTTGAGACGGGGT
For Y2H assay	
PhBS α >pGAD/F	CCATGGAGGCCAGTGAATTCATGGAGAAGCAAACCTCAGGCAC
PhBS α >pGAD/R	TGCCCCACCCGGGTGGAATTCCTACAGGGATGAGAAAATCGGGACAC
PhBS β >pGAD/F	CCATGGAGGCCAGTGAATTCATGGAAAATTCTGGAAAGAAGGTGTTG
PhBS β >pGAD/R	TGCCCCACCCGGGTGGAATTCACATGTATGAGCGCATTTCGC
PhBS α >pGBK/F	TATGGCCATGGAGGCCGAATTCATGGAGAAGCAAACCTCAGGCAC
PhBS α >pGBK/R	CGACGGATCCCCGGAATTCCTACAGGGATGAGAAAATCGGGACAC
PhBS β >pGBK/F	TATGGCCATGGAGGCCGAATTCATGGAAAATTCTGGAAAGAAGGTGTTG
PhBS β >pGBK/R	CGACGGATCCCCGGAATTCACATGTATGAGCGCATTTCGC
For subcellular localization and BiFC	
PhBS α >pCNHP-eYFP/F	TATTCTGCCCAAATTCGCGCCATGGAGAAGCAAACCTCAGGCAC
PhBS α >pCNHP-eYFP/R	AGCTCCTCGCCCTTGCTCACCATCAGGGATGAGAAAATCGGGACAC
PhBS β >pCNHP-eYFP/F	TATTCTGCCCAAATTCGCGCCATGGAAAATTCTGGAAAGAAGGTGTTG
PhBS β >pCNHP-eYFP/R	AGCTCCTCGCCCTTGCTCACCATCATGTATGAGCGCATTTCGC
PhBS α >pCNHP-nEYFP-C/F	CCACAACATCGAGGACATGGAGAAGCAAACCTCAGGCAC
PhBS α >pCNHP-nEYFP-C/R	AAAATTTAATGAAACCAGAGTTAACCATGGCTACAGGGATGAGAAAATCGGGACAC
PhBS β >pCNHP-nEYFP-C/F	GGACGAGCTGTACAAGATGGAAAATTCTGGAAAGAAGGTGTTG
PhBS β >pCNHP-nEYFP-C/R	AAAATTTAATGAAACCAGAGTTAACCATGGTCACATGTATGAGCGCATTTCGC
For pathway reconstitution in tobacco	
PhCNL>pCNHP/F	CTGCCCAAATTCGCGATGGACGAGTTACCAAATGTGGAG
PhCNL>pCNHP/R	TTTAATGAAACCAGAGTTAACTACAGACGAGCTGGCAAATCAAG
PhCHD>pCNHP/F	CTGCCCAAATTCGCGATGGCTCAAGTTAAGGTGACGAT
PhCHD>pCNHP/R	TTTAATGAAACCAGAGTTAATTACATGCGTGACCTTGAAGCTG
PhKAT>pCNHP/F	CTGCCCAAATTCGCGATGGAGAAAGCAATTCAAAGGCAAAG
PhKAT>pCNHP/R	TTTAATGAAACCAGAGTTAATTATTTTCGCATCCTTGGATAAGAACTGTTG
PhPAL1>pCNHP/F	CTGCCCAAATTCGCGATGGAGTATGCCAATGAAAACCTGTAATGG
PhPAL1>pCNHP/R	TTTAATGAAACCAGAGTTAATTAGCAGAGTGGAAGAGGAGCAC
PhBS α >pCNHP/F	CTGCCCAAATTCGCGATGGAGAAGCAAACCTCAGGCACGTTTC
PhBS α >pCNHP/R	TTTAATGAAACCAGAGTTAACTACAGGGATGAGAAAATCGGG
PhBS β >pCNHP/F	CTGCCCAAATTCGCGATGGAAAATTCTGGAAAGAAGGTGTTGCTTACT
PhBS β >pCNHP/R	TTTAATGAAACCAGAGTTAATCAGATGTATGAGCGCATTTCGC
For qRT-PCR analysis of gene expression	
AtACT2/F	TGGCTGAGGCTGATGATATTTCAACCAATCG
AtACT2/R	CCAACATATGCATCCTTCTGGTTTCATCCCA
AtBS α /F	CCCATTGGCCATCGAACACACCACAC
AtBS α /R	AGCTCGGGCTTGGAATTCCAGTTGAAC
AtBS β /F	GGAAAATCCGGCGAAGAGAGTGTGATG
AtBS β /R	TGCTATAACATCGGCAGGGAAGGCTCC
PhEF1 α /F	CCTGGTCAAATTTGGAAACGG
PhEF1 α /R	CAGATCGCCTGTCAATCTTGG
PhBS α /F	CACCTCTCCGTTGGAATTGACAGAGGAGGAG
PhBS α /R	CCACCTGGTAATTGCCACGATTAAGACC
PhBS β /F	CTTGACCTCAATTATTGGTGCGGAGAGAGG
PhBS β /R	TCCTGGAGGTGCAAGCCACGAGATATG
BPBT/F	CCATTAGTTTTCTATTGTTACATGGC
BPBT/R	CACCTCGACAATACAAAAGACACC
BSMT1/F	AAGGCACTCAATGTCTATTTTCGG
BSMT1/R	GAATAAGATAATTCAATATGTGCGTGAAC
BSMT2/F	TGCATCTCATAGGTGGTCGAG
BSMT2/R	AGAGAGATCTGAAAGGACCCCT
PAL/F	ACTCTGTTAATGACAACCCCTTGAT
PAL/R	CTGAAAATTTCCACCATGCA
EGS/F	ATCGCAACATCTCCAATTCCA

EGS/R	CAAACGATGGCTCGATCACA
IGS/F	TGATGCAAAGGCTGTGTTGAA
IGS/R	TTGCTGCCCTTGGATCATCT
For verification of Arabidopsis T-DNA insertion lines	
LB1	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC
LBb1.3	ATTTTGCCGATTCGGAAC
SAIL_1152_B06/LP	ACATGTCACAATGATTTTCGTATCC
SAIL_1152_B06/RP	CCCTCAATGGAATCTCTAATCTTG
SAIL_198_H07/LP	ACATGTCACAATGATTTTCGTATCC
SAIL_198_H07/RP	GGAGGAAAGAAGACAAAATGAGAG
SALK_209249/LP	GATTAAGTACGTACCTGGGGAATG
SALK_209249/RP	TATGTTTGTGATGATTGGGTTTC
SAIL_840_G05.C/LP	ATATGTTTGGTATTTGTTGATGCG
SAIL_840_G05.C/RP	GCTCATATTGTTTCTCTGTGTGG
SALK_136638.19.15.x/LP	TCGGTATTATTGAACCATTGATTG
SALK_136638.19.15.x/RP	TGATGAAACGTCTAACTCAAGAGC

F: forward primer; R: reverse primer.