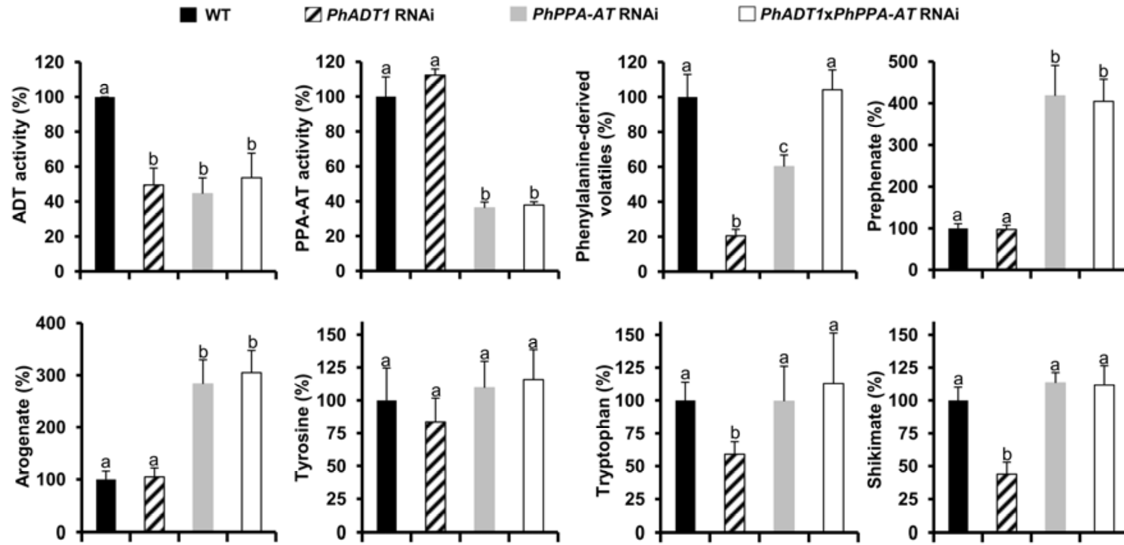
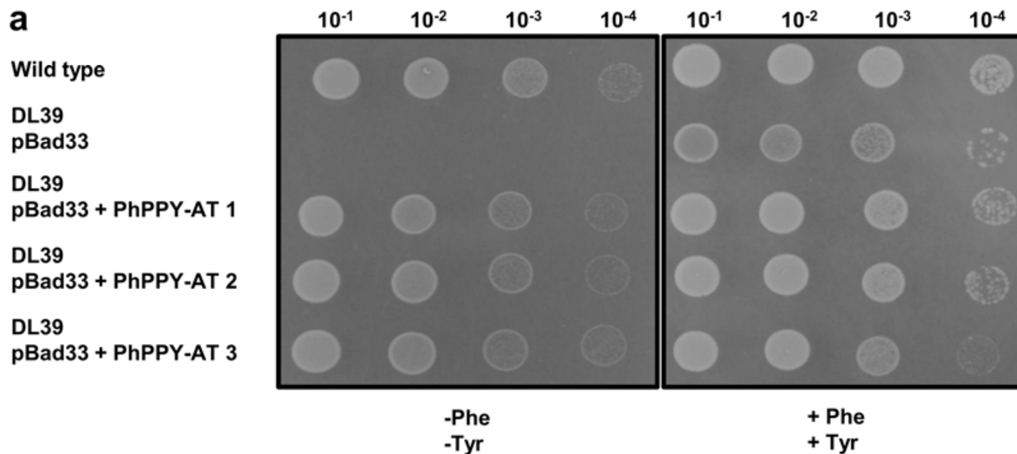
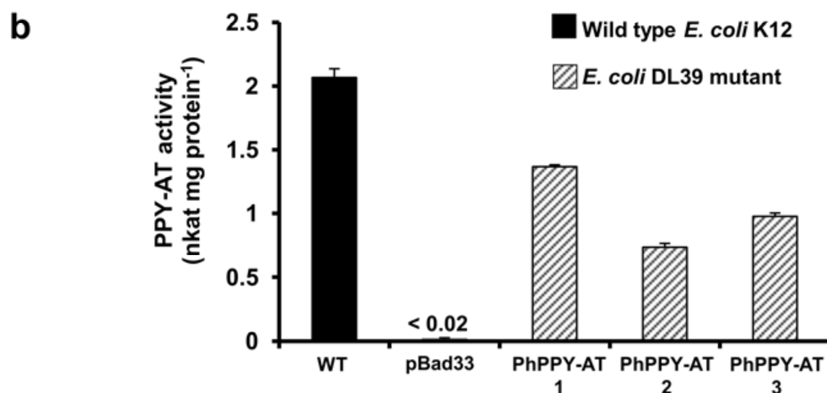


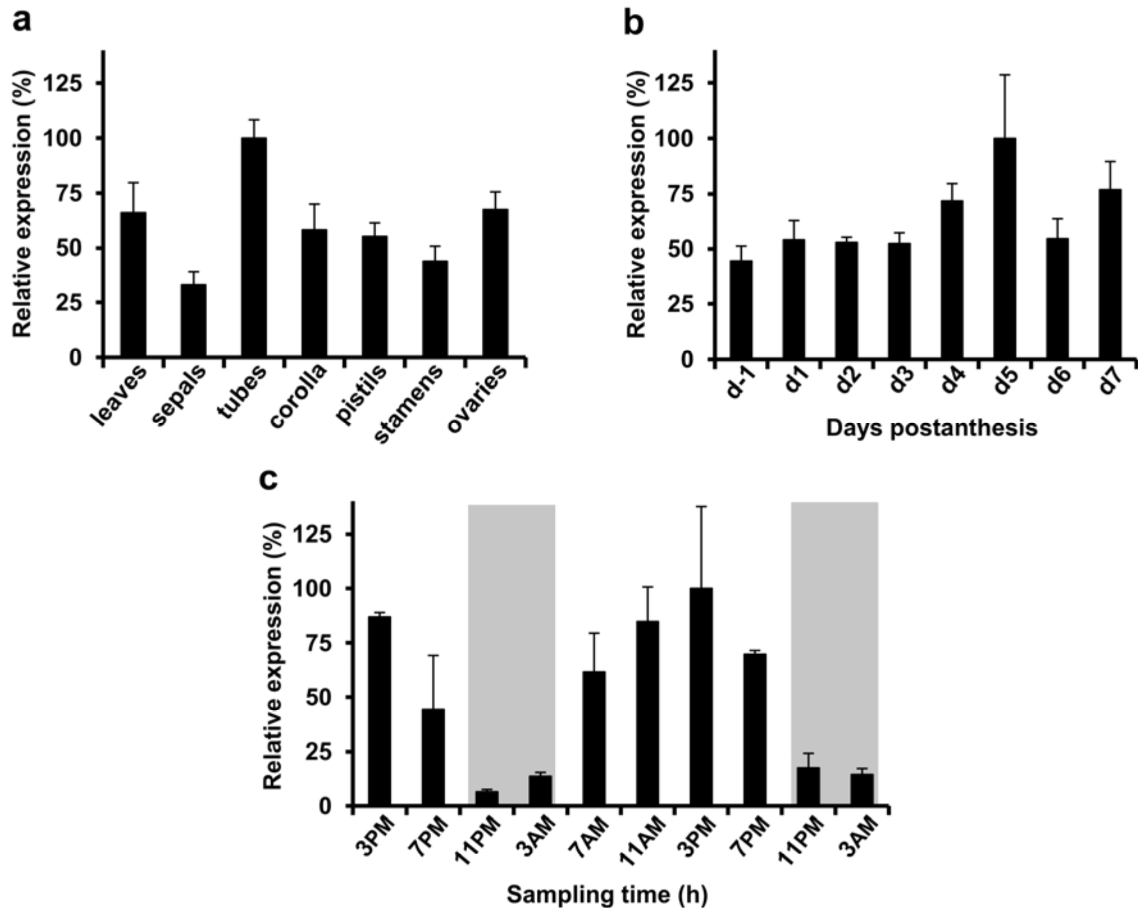
Supplementary Figure S1 | Effect of simultaneous *PhADT1* and *PhPPA-AT* downregulation on the levels of phenylalanine-derived volatiles, tyrosine, tryptophan, aromatic amino acid intermediates, and shikimate in petunia flowers. The levels of plastidial ADT and PPA-AT activity (n = 5 biological replicates), phenylalanine-derived volatiles ($n \geq 5$), prephenate, arogenate, tyrosine, tryptophan and shikimate ($n \geq 6$) in petals of wild-type (WT; black) and the *PhADT1* (striped), *PhPPA-AT* (gray), and *PhADT1*x*PhPPA-AT* (white) RNAi lines harvested at 8 PM, 2 d postanthesis. Data are presented relative to corresponding WT controls set at 100% (687.5 nmol g FW^{-1} h^{-1} for phenylalanine-derived volatiles, 0.10 and 2.60 nkat mg $protein^{-1}$ for plastidial ADT1 and PPA-AT activities, respectively; 3.5, 21.4, 12.6, 16.0, 36.9 nmol g FW^{-1} for prephenate, arogenate, tyrosine, tryptophan, and shikimate, respectively). Data are means ± s.e.m. Columns with the same letters above are not significantly different based on Tukey's test (P < 0.05; One-way ANOVA).





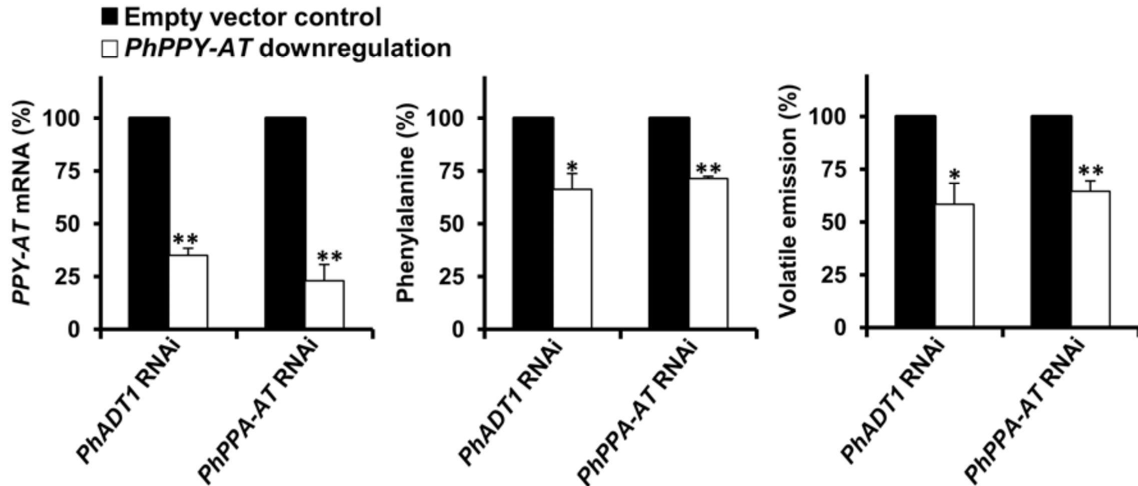
Supplementary Figure S2 | Functional complementation of the phenylalanine auxotrophic *E. coli* mutant DL39 by *PhPPY-AT.*

(**a**) Wild-type *E. coli* (K12), the DL39 mutant transformed with pBad33 alone, and three independent clones of DL39 containing the pBad33-PhPPYAT construct were grown to an OD_{600} = 1 in M9 liquid media, washed, and serially diluted to OD_{600} = 1 x 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. Five μL of cells at each cell density were plated on M9 solid media containing 0.2% arabinose and 50 μg ml^{-1} aspartic acid, valine, leucine and isoleucine, with and without 50 μg ml^{-1} phenylalanine and tyrosine. (**b**) *In vitro* assays measuring PPY-AT activity with glutamate as an amino donor and phenylpyruvate as the keto acid acceptor were performed using crude extracts prepared from cells of wild-type (K12), the DL39 mutant transformed with empty pBad33, and the DL39 mutant harboring pBad33-PhPPYAT. Reactions were carried out at 30°C for 30 min with 74 μg of crude extract proteins. Data are means ± s.e.m. (*n* = 3 independent experiments).



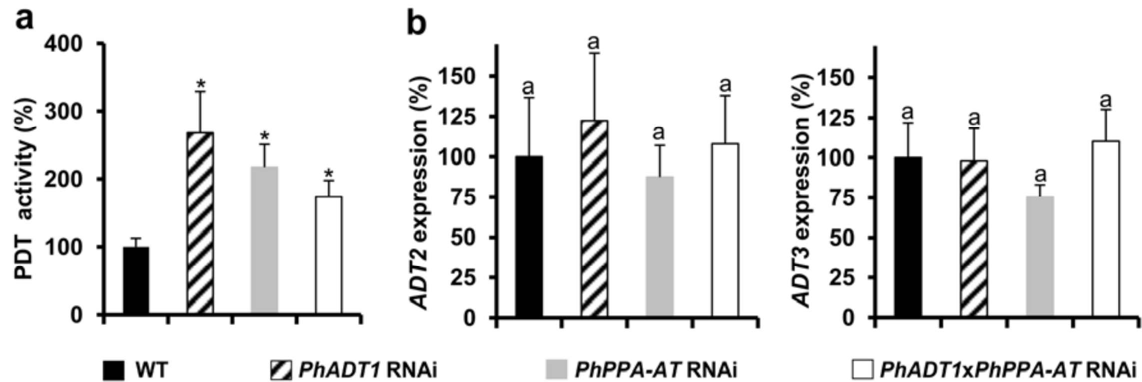
Supplementary Figure S3 | Expression profiles of *PhPPY-AT* in petunia flowers.

(**a**) Tissue specific expression of *PhPPY-AT* presented relative to levels in tubes set as 100%. (**b**) Developmental *PhPPY-AT* expression profile in corolla of petunia flowers from the mature bud stage (d -1) to 7 d postanthesis presented relative to level on d 5, set as 100%. (**c**) Diurnal expression profiles of *PhPPY-AT* in corolla of petunia flowers d 1 to d 3 postanthesis grown under a normal light/dark cycle presented relative to the level at 3 PM on d 2 postanthesis set as 100%. The dark cycles (9 PM to 6 AM) are shown with gray background. Data are means ± s.e.m. (*n* = 4 biological replicates)



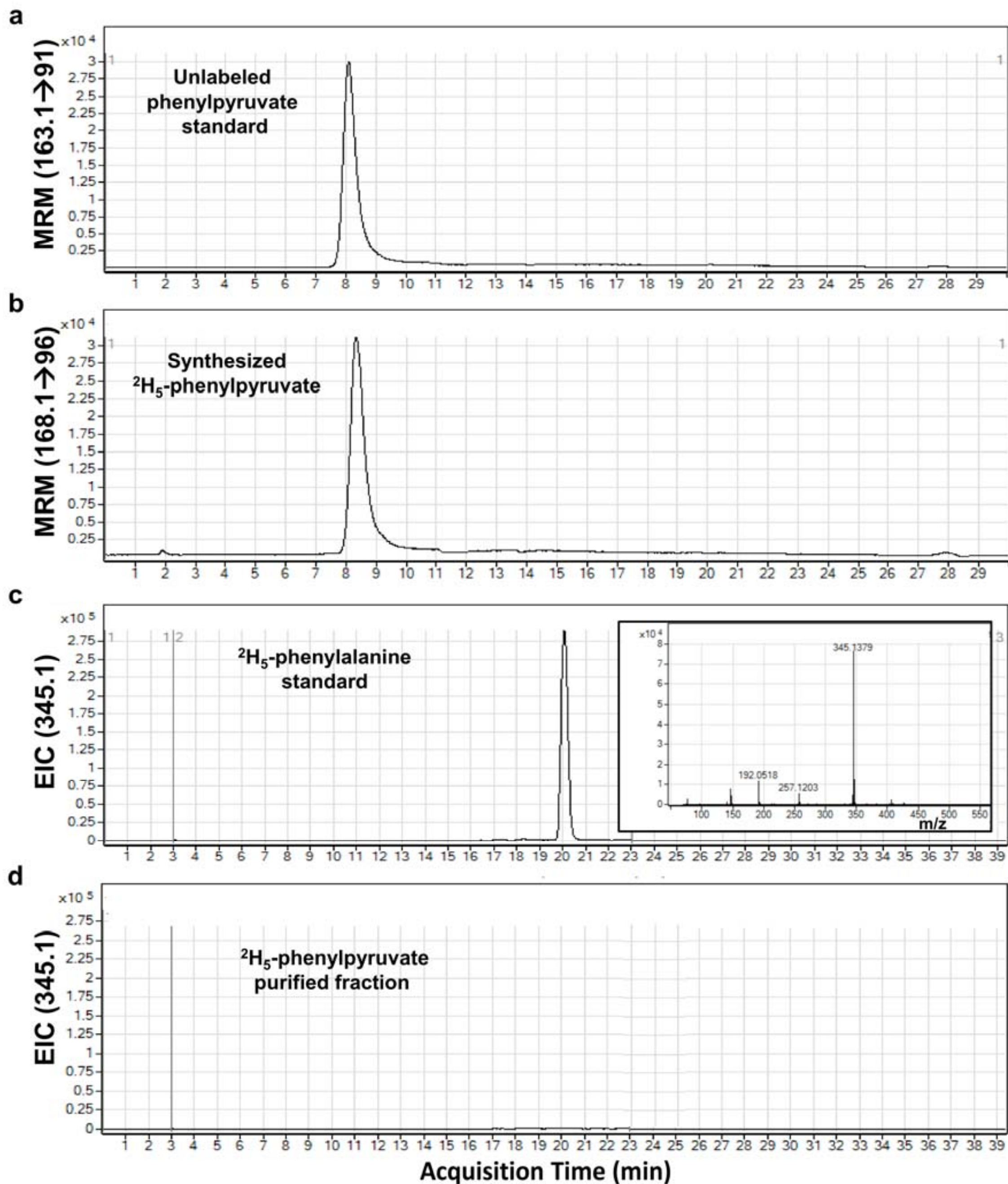
Supplementary Figure S4 | Effect of *PhPPY-AT* downregulation on phenylalanine and phenylalanine-derived volatile levels in petunia flowers of *PhADT1* and *PhPPA-AT* RNAi parental lines.

Downregulation of *PhPPY-AT* in petunia flowers (left panel) significantly decreases the levels of phenylalanine (middle panel) and phenylalanine-derived volatiles (right panels). For each genetic background, black and white columns represent flowers infiltrated with the empty vector or the *PhPPY-AT* RNAi construct, respectively. Data are presented as a percentage relative to the corresponding empty-vector reference. Data are means ± s.e.m. ($n \geq 3$ biological replicates). * $P < 0.05$ and ** $P < 0.01$ as determined by paired two-tailed Student's t-tests.



Supplementary Figure S5 | Effect of simultaneous *PhADT1* and *PhPPA-AT* downregulation on PDT activity, and expression levels of *PhADT2* and *PhADT3* in petunia flowers.

(**a**) PDT activities were determined using triple quadruple LC/MS in plastids isolated from d 1 to d 3 old petunia flowers of wild-type (WT; black) and the *PhADT1* (striped), *PhPPA-AT* (gray), and *PhADT1*x*PhPPA-AT* (white) RNAi lines harvested at 8 PM. Data are presented relative to the level in WT (0.468 pkat mg protein^{-1}) set as 100%. Data are means ± s.e.m. (*n* = 5 biological replicates). * P < 0.05 as determined by unpaired two-tailed Student's t-test between WT and each transgenic line. (**b**) *PhADT2* and *PhADT3* mRNA levels were determined by qRT-PCR with gene-specific primers in corollas of petunia flowers from WT (black) and the *PhADT1* (striped), *PhPPA-AT* (gray), and *PhADT1*x*PhPPA-AT* (white) RNAi lines harvested at 8 PM 2 d postanthesis. Data are presented relative to corresponding levels in WT set as 100%. Data are means ± s.e.m. (*n* = 3 biological replicates). Columns with the same letters above are not significantly different based on Tukey's test (P < 0.05; One-way ANOVA).



Supplementary Figure S6 | Verification of product formation and purity of enzymatically synthesized L-[ring-$^{2}H_{5}$]-Phenylpyruvate. L-[ring-$^{2}H_{5}$]-Phenylpyruvate was synthesized by enzymatic conversion of L-[ring-$^{2}H_{5}$]-phenylalanine by melon CmArAt1. The dominant ion of non-labeled phenylpyruvate (91 m/z) detected by Triple Quadrupole LC/MS in an authentic phenylpyruvate standard (**a**) is shifted in enzymatically synthesized L-[ring-$^{2}H_{5}$]-phenylpyruvate to 96 m/z (**b**). Phenylpyruvate and

L-[ring-$^2H_5$]-phenylpyruvate were monitored in MRM (Multiple Reaction Monitoring) mode and quantified based on a standard calibration curve generated with an authentic non labeled phenylpyruvate standard. (**c**) Authentic standard of L-[ring-$^2H_5$]-phenylalanine after OPA derivatization analyzed by TOF LC/MS. (**d**) OPA derivitization of L-[ring-$^2H_5$]-phenylpyruvate shown in (**b**) to assess the purity of synthesized labeled phenylpyruvate and removal of L-[ring-$^2H_5$]-phenylalanine substrate. L-[ring-$^2H_5$]-phenylalanine derivatized by OPA was monitored in extracted ion chromatogram (EIC) mode of 345.1 m/z. Inserted panel in (**c**) shows mass spectra of L-[ring-2H5]-phenylalanine.

Supplementary Table S1 | Emissions of phenylalanine-derived volatiles in wild-type (WT) and the *PhADT1*, *PhPPA-AT*, and *PhADT1*x*PhPPA-AT* RNAi lines.

Emitted volatiles (nmol g FW^{-1} h^{-1})	WT	*PhADT1* RNAi	*PhPPA-AT* RNAi	*PhADT1*x*PhPPA-AT* RNAi
Benzaldehyde	136.0 ± 20.3[a,c]	15.8 ± 3.2[b]	85.0 ± 15.0[c]	156.8 ± 18.1[a]
Benzyl alcohol	10.0 ± 2.2[a,b]	0.7 ± 0.2[b]	7.6 ± 1.0[a,b]	17.0 ± 5.4[a]
Phenylacetaldehyde	42.4 ± 9.3[a]	3.4 ± 0.9[b]	14.0 ± 2.6[b,c]	39.4 ± 8.3[a,c]
Methylbenzoate	275.2 ± 28.6[a,c]	65.0 ± 10.3[b]	195.0 ± 23.0[c]	289.0 ± 21.0[a]
Phenylethanol	13.1 ± 1.2[a]	0.9 ± 0.5[b]	7.5 ± 0.7[c]	13.9 ± 1.9[a]
Benzylacetate	0.4 ± 0.1[a,b]	0.1 ± 0.0[b]	0.3 ± 0.1[b]	0.7 ± 0.1[a]
Eugenol	3.0 ± 0.5[a]	0.9 ± 0.2[b]	1.9 ± 0.3[a,b]	3.0 ± 1.0[a]
Isoeugenol	194.3 ± 36.6[a]	52.0 ± 13.0[b]	97.2 ± 30.2[a,b]	182.7 ± 35.8[a]
Benzylbenzoate	13.0 ± 3.3[a]	2.1 ± 0.7[b]	5.2 ± 1.1[a,b]	13.1 ± 3.4[a]
Phenylethylbenzoate	0.3 ± 0.1[a]	0.0 ± 0.0[b]	0.1 ± 0.0[b]	0.2 ± 0.0[a,b]
Total emitted volatiles	**687.5 ± 88.9[a]**	**140.7 ± 25.4[b]**	**413.2 ± 45.6[c]**	**715.6 ± 78.4[a]**

Volatiles were collected from 6 PM to 10 PM using six flowers harvested at d 2 postanthesis. Data are means ± s.e.m. (*n* = 5 biological replicates). Means with the same letters within the row are not significantly different based on Tukey's test ($P < 0.05$; One-way ANOVA).

Supplementary Table S2 | Amino donor specificities of petunia PhPPY-AT for forward and reverse reactions.

keto acid substrate	amino donor[c]	PhPPY-AT
Forward		
phenylpyruvate[a]	tyrosine	100[d]
	methionine	12.4 ± 0.45
	glutamate	12.3 ± 0.21
	leucine	10.6 ± 0.45
	tryptophan	6.6 ± 0.32
	histidine	3.0 ± 0.22
	glutamine	0.8 ± 0.04
	aspartate	0.2 ± 0.02
Reverse		
4-hydroxyphenylpyruvate[b]	phenylalanine	5.8 ± 0.19
	glutamate	5.2 ± 0.29
	methionine	5.0 ± 0.23
	leucine	4.6 ± 0.16
	tryptophan	2.7 ± 0.04
	histidine	1.4 ± 0.04
	glutamine	0.3 ± 0.05
	aspartate	0.1 ± 0.06

[a]Phenylpyruvate concentration was 10 mM.

[b]4-hydroxyphenylpyruvate concentration was 4 mM since at higher concentrations inhibition was observed

[c]Amino donor substrate concentration was 10 mM.

[d]PhPPY-AT activity with phenylpyruvate and tyrosine as substrates was 23.6 nkat mg $protein^{-1}$ and set as 100%.

Data are means ± s.e.m. (*n* = 3 independent experiments).

Supplementary Table S3 | Keto acid substrate specificities of petunia PhPPY-AT.

keto acid substrate[a]	amino donor[b]	PhPPY-AT
Phenylpyruvate	L-tyrosine	100[c]
α-ketoglutarate		44.0 ± 1.04
Oxaloacetate		2.1 ± 0.13
Prephenate		n.d.
Pyruvate		n.d.
Phenylpyruvate	L-glutamate	12.2 ± 0.96
Pyruvate		4.2 ± 0.23
Oxaloacetate		2.8 ± 0.40
4-hydroxyphenylpyruvate		2.5 ± 0.08
Prephenate		n.d.

[a]Keto acid and amino donor[b] substrate concentrations were 10 mM.

[c]PhPPY-AT activity with phenylpyruvate and tyrosine as substrates was 23.6 nkat mg $protein^{-1}$ and set as 100%.

Data are means ± s.e.m. (*n* = 3 independent experiments).

n.d., not detectable

Supplementary Table S4 | Catalytic activity of CmArAT1

Reactions : Phenylpyruvate + Tyrosine ↔ Phenylalanine + 4-hydroxyphenylpyruvate Phenylpyruvate + Glutamate ↔ Phenylalanine + α-ketoglutarate		
keto acid substrate[a]	**amino donor**[b]	***Vmax*** **(nmol s^{-1} mg $protein^{-1}$)**
Forward		
Phenylpyruvate	tyrosine[a]	54.59 ± 1.94
Phenylpyruvate	glutamate[a]	1.47 ± 0.02
Reverse		
4-hydroxyphenylpyruvate	phenylalanine[b]	12.35 ± 0.39
a-ketoglutarate	phenylalanine[b]	8.19 ± 0.08

Enzymatic reactions were carried out at 30°C for 10 min in the presence of 5 µg of recombinant CmArAT1. When tyrosine was used as an amino donor, 0.3 µg of CmArAT1 was used and reactions were carried out at 30°C for 3 min.

[a]Keto acid and amino donor[b] substrate concentrations were 10 mM.

Data are means ± s.e.m. (*n* = 3 independent experiments).

Supplementary Table S5 | Amino donor specificity of CmArAT1 with phenylpyruvate as the amino acceptor.

keto acid substrate	amino donor[b]	CmArAT1
phenylpyruvate[a]	tyrosine	100.0[c]
	glutamate	2.7± 0.04
	methionine	2.6 ± 0.07
	leucine	3.8 ± 0.43
	tryptophan	3.5 ± 0.38
	histidine	0.96 ± 0.09
	glutamine	0.15 ± 0.008
	aspartate	n.d.

Enzymatic reactions were carried out at 30°C for 10 min in the presence of 5 µg of recombinant CmArAT1. When tyrosine was used as an amino donor, 0.3 µg of CmArAT1 was used and reactions were carried out at 30°C for 3 min.

[a]Phenylpyruvate and amino donor[b] substrate concentrations were 10 mM.

[c]CmArAT1 activity with phenylpyruvate and tyrosine as substrates was 54.6 nkat mg protein^{-1} and set as 100%.

Data are means ± s.e.m. (n = 3 independent experiments).

n.d., not detectable

Supplementary Table S6 | Sequence information of primers used for qRT-PCR analysis

Primers	**Sequence**
PhADT1 for[16]	5’-TAACTGCGAAGCCATTCCCTGC-3’
PhADT1 rev[16]	5’- CTCTACTGGTAGAACTGCGCG-3’
PhADT2 for[16]	5’-ACGAAGTTGGGTTTGGTCAG-3’
PhADT2 rev[16]	5’- TGCCCCTGCATCTTTTAGTT-3’
PhADT3 for[16]	5’-CAAAATGTGAAGCTATTCCTTGTG-3’
PhADT3 rev[16]	5’-TTCGATCGGTAAAACAGCACG-3’
PhCM1 for[16]	5’-CCTGCTGTTGAAGAGGCTATCA-3’
PhCM1 rev[16]	5’-CAGGGTCACCTCCATTTTCTG-3
PhPPA-AT for[17]	5'-GCAATGACTGGTTGGAGACTTG-3'
PhPPA-AT rev[17]	5'-TCACATCAGGTGCCAGTAGCA-3'
PhUBQ for[16]	5’-GTTAGATTGTCTGCTGTCGATGGT-3’
PhUBQ rev[16]	5’-AGGAGCCAATTAAAGCACTTATCAA-3’
AtTYDC for[38]	5’-GATCCAAGTTTTGAGGTTGTCACTA-3’
AtTYDC rev[38]	5’-ACGGTTACGTTCGTTACATTGG-3’