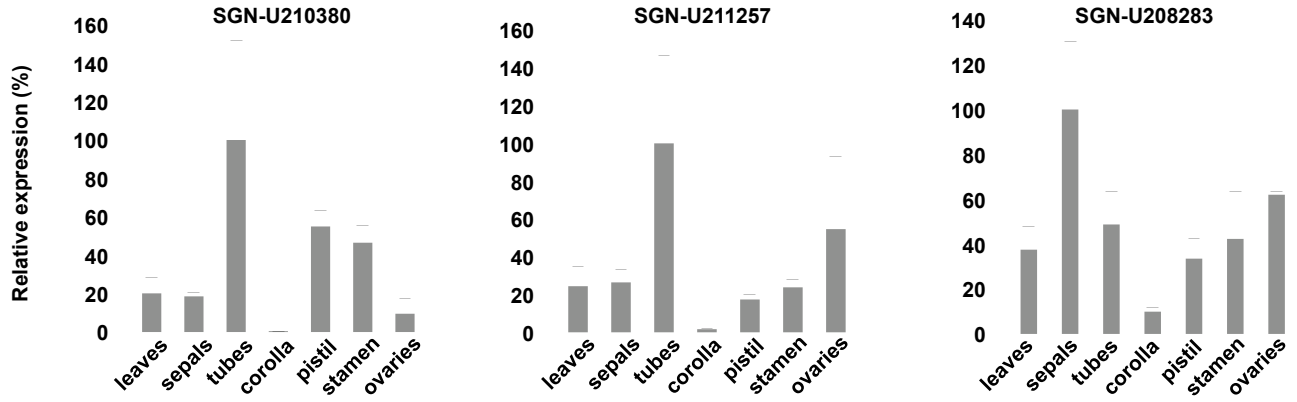
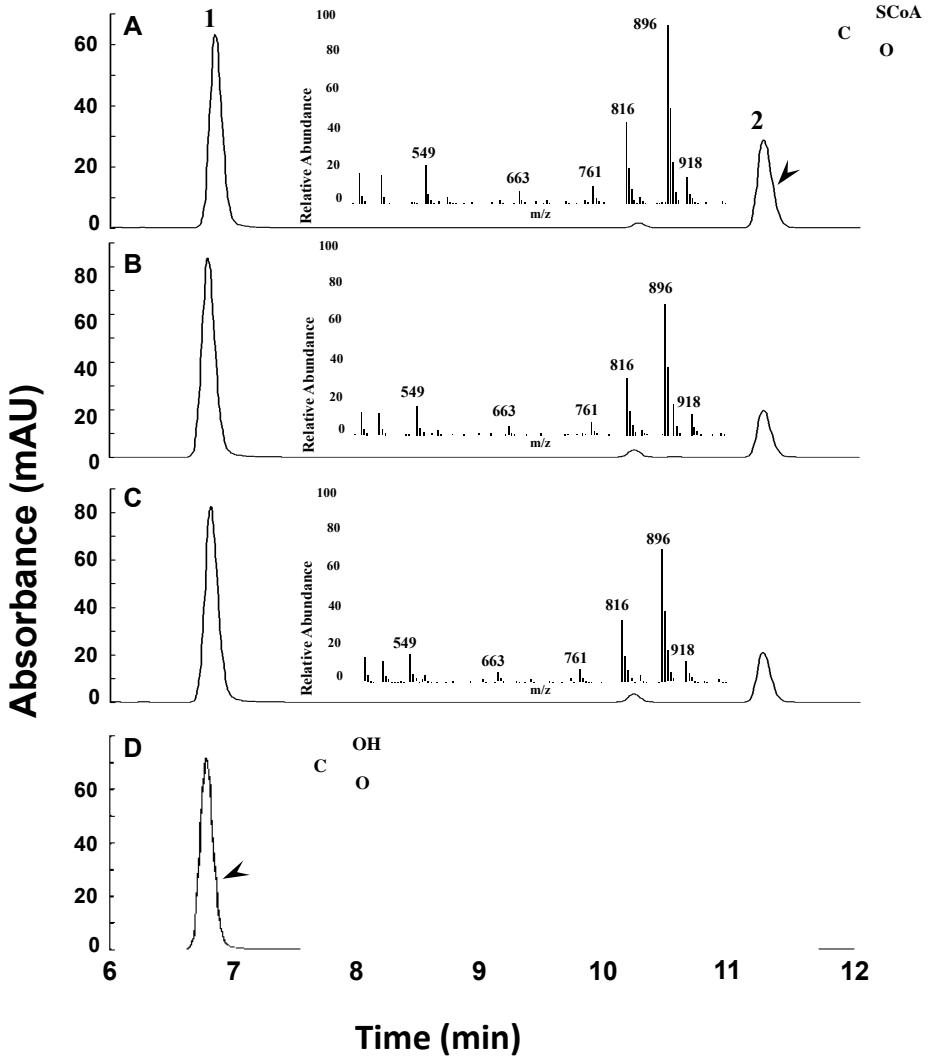
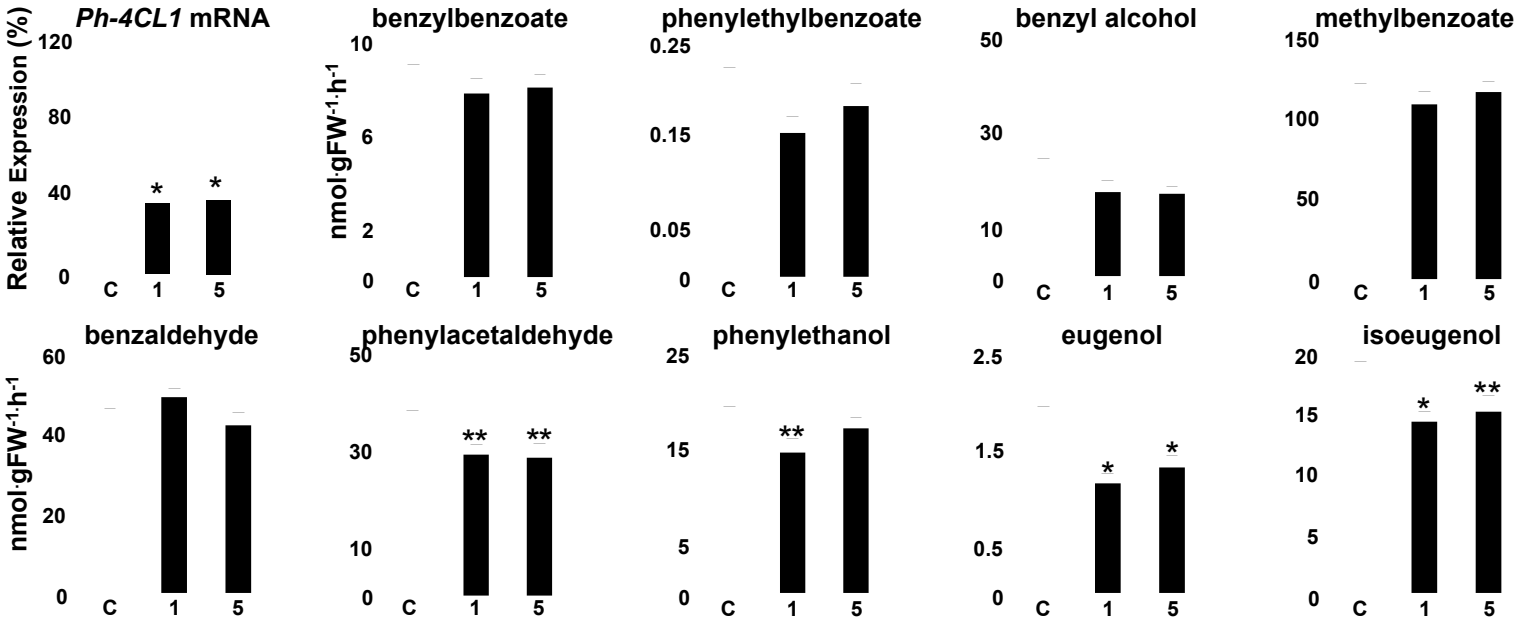


Supplemental Figure 1. Tissue-specific Expression Profiles of Putative 4CL Candidates.

SGN-U210380; *SGN-U211257*; *SGN-U208283* transcript levels were determined by qRT-PCR relative to the reference gene *(elongation factor 1-alpha*), in leaves and floral tissues harvested on day 2 postanthesis at 3 PM. Data are means ± SE (*n* = 3 biological replicates). Tissue-specific expression of *SGN-U210380*; *SGN-U211257* and *SGN-U208283* is shown relative to the tissue with the highest transcript level.

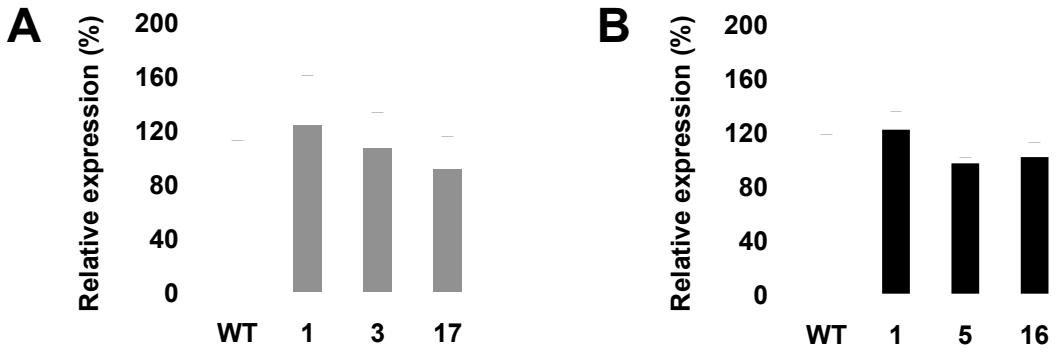


Supplemental Figure 2. LC-MS Analysis of Product Formed by Recombinant Ph-CNL and Ph-4CL1 from *trans*-Cinnamic Acid.
(**A**) Reaction catalyzed by Ph-CNL
(**B**) Reaction catalyzed by Ph-4CL1.
(**C**) Reaction catalyzed by *Nicotiana tobacco* 4CL used for formation of cinnamoyl-CoA standard.
(**D**) Reaction catalyzed by boiled Ph-CNL.
1, *trans*-cinnamic acid; 2, cinnamoyl-CoA. Inserts with numbered peaks in A-C represent mass spectra of cinnamoyl-CoA. Retention time and mass spectra of Ph-CNL and Ph-4CL1 produced cinnamoyl-CoA are identical to those formed by *N. tobacco* 4CL. m/z, mass-to-charge ratio.



Supplemental Figure 3. Effect of *Ph-4CL1*-RNAi Suppression on on *Ph-4CL1* Expression and Emission of Benzenoid/Phenylpropanoid Compounds in Corollas of Petunia Flowers.

Ph-4CL1 mRNA levels were determined by qRT-PCR in corollas of control (C, white bars) and two independent *Ph-4CL1* RNAi lines (1and 5, black bars) harvested at 3 PM, 2 d postanthesis (upper left corner panel). Expression values for transgenic lines are shown as percentage of *Ph-4CL1* expression in control petals which is set at 100%. Data are means ± SE (*n* = 3 biological replicates).
Scent collections were performed from detached flowers 2 d postanthesis for 12 h starting at 8 PM. Emission rates are expressed on a per hour basis assuming a constant emission rate over the 12-h period. Data are means ± SE (n ≥ 7 biological replicates). * P < 0.01, ** P < 0.05 by Student's *t* test of transgenics relative to control.



Supplemental Figure 4. Expression of *Ph-4CL1 and Ph-CNL* in *Ph-CNL*-RNAi and *Ph-4CL1*-RNAi Lines, Respectively.

Ph-4CL1 transcript levels in *Ph-CNL*-RNAi lines (A) and *Ph-CNL* mRNA expression in *Ph-4CL1*-RNAi lines (B) were determined by qRT-PCR. Data are means ± SE (*n* = 3 biological replicates). Expressions of *Ph-CNL* and *Ph-4CL1* are shown relative to the corresponding transcript levels in corollas of wild type flowers collected on day 2 postanthesis at 3 PM.

Supplemental Table 1. Accession Numbers of Protein Sequences Used for Phylogenetic Analysis in Figure 3.

Clade	Protein	Accession Number	Database
I	At2g47240 LACS1	NP_182246.1	NCBI
I	At1g49430 LACS2	NP_175368.2	NCBI
I	At1g64400 LACS3	NP_176622.1	NCBI
I	At4g23850 LACS4	NP_194116.1	NCBI
I	At4g11030 LACS5	NP_192841.1	NCBI
I	At3g05970 LACS6	NP_566265.1	NCBI
I	At5g27600 LACS7	NP_198112.2	NCBI
I	At2g04350 LACS8	NP_178516.1	NCBI
I	At1g77590 LACS9	NP_177882.1	NCBI
I	At4g14070 AAE15	NP_193143.2	NCBI
I	At3g23790 AAE16	NP_189021.2	NCBI
II	At5g23050 AAE17	NP_197696.2	NCBI
II	At1g55320 AAE18	NP_175929.3	NCBI
II	At5g36880 ACS	NP_198504.1	NCBI
III	At4g03400	NP_192249.1	NCBI
III	At2g46370 JAR1	NP_566071.1	NCBI
III	At5g13370	NP_196841.2	NCBI
III	At5g13360	NP_196840.1	NCBI
III	At5g13350	NP_196839.1	NCBI
III	At5g13380	NP_196842.2	NCBI
III	At5g54510 GH3.6	NP_200262.1	NCBI
III	At4g27260 GH3.5	NP_194456.1	NCBI
III	At4g37390 GH3.2	NP_195455.1	NCBI
III	At1g59500 GH3.4	NP_176159.1	NCBI
III	At2g23170 GH3.3	NP_179898.1	NCBI
III	At2g14960	NP_179101.1	NCBI
III	At2g47750 GH3.9	NP_182296.1	NCBI
III	At1g28130 GH3.17	NP_174134.1	NCBI
III	At1g48670	NP_175300.1	NCBI
III	At1g48660	NP_175299.1	NCBI
III	At5g51470	NP_199960.1	NCBI
III	At1g23160	NP_173729.1	NCBI
III	At5g13320 PBS3	NP_196836.1	NCBI
IV	At1g62940 ACOS5	NP_176482.1	NCBI
IV	Pp1s96_224	Pp1s96_224V6.1	Phytozome
IV	Pp1s325_20	Pp1s325_20V6.1	Phytozome
IV	At1g65060 At4CL3	NP_176686.1	NCBI
IV	At3g21230 At4CL4	NP_188760.3	NCBI
IV	At1g51680 At4CL1	NP_001077697.1	NCBI

Clade	Protein	Accession Number	Database
IV	At3g21240 At4CL2	NP_188761.1	NCBI
IV	Ph 4CL1	JN120849	NCBI
IV	Os 4CL3 Os02g0177600	NP_001046069.1	NCBI
IV	Os 4CL4 Os06g0656500	NP_001058252.1	NCBI
IV	Os 4CL5 Os08g0448000	NP_001061935.1	NCBI
IV	Os 4CL2 Os02g0697400	NP_001047819.1	NCBI
IV	Os 4CL1 Os08g0245200	NP_001061353.1	NCBI
IV	Pt6s18490	POPTR_0006s18490.1	Phytozome
IV	Pt6s18510	POPTR_0006s18510.1	Phytozome
IV	Pt18s10210	POPTR_0018s10210.1	Phytozome
IV	Pt3s18720	POPTR_0003s18720.1	Phytozome
IV	Pt19s07600	POPTR_0019s07600.1	Phytozome
IV	Pt1s07400	POPTR_0001s07400.1	Phytozome
IV	Zm 4CL	NP_001105258.1	NCBI
IV	Sb 4CL1	XP_002452704.1	NCBI
IV	Sb 4CL2	XP_002451647.1	NCBI
IV	Gm 4CL1	NP_001237750.1	NCBI
IV	Gm 4CL2	P31687.2	NCBI
IV	Gm 4CL3	NP_001237270.1	NCBI
IV	Ps 4CL1	ABR17998.1	NCBI
IV	Pp1s185_66	Pp1s185_66V6.1	Phytozome
IV	Pp1s20_346	Pp1s20_346V6.1	Phytozome
IV	Pp1s71_170	Pp1s71_170V6.1	Phytozome
IV	Pp1s167_96	Pp1s167_96V6.1	Phytozome
IV	Nt 4CL1	O24145.1	NCBI
IV	Nt 4CL2	O24146.1	NCBI
IV	Ri 4CL2	AAF91309.1	NCBI
V	At4g05160	NP_192425.1	NCBI
V	At1g20500	NP_173474.5	NCBI
V	At1g20490	NP_173473.2	NCBI
V	At5g38120	NP_198628.2	NCBI
V	At1g20510 OPCL1	NP_564115.1	NCBI
V	At1g20480	NP_173472.1	NCBI
V	At5g63380	NP_201143.1	NCBI
V	At4g19010	NP_193636.1	NCBI
VI	At1g21530 AAE10	NP_001077573.1	NCBI
VI	At1g21540 AAE9	NP_173573.1	NCBI
VI	At1g77240 AAE4	NP_177848.1	NCBI
VI	At5g16370 AAE5	NP_197141.1	NCBI
VI	At5g16340 AAE6	NP_197138.1	NCBI
VI	At1g75960 AAE8	NP_177724.1	NCBI
VI	At3g16910 AAE7/ACN1	NP_188316.1	NCBI

Clade	Protein	Accession Number	Database
VI	Ps ACN1	ABK25037.1	NCBI
VI	Pp1s220_60	Pp1s220_60V6.1	Phytozome
VI	At2g17650 AAE2	NP_179356.1	NCBI
VI	At1g20560 AAE1	NP_564116.1	NCBI
VI	Pp1s7_313	Pp1s7_313V6.1	Phytozome
VI	At1g76290	NP_177756.1	NCBI
VI	Pt16s03410	POPTR_0016s03410.1	Phytozome
VI	Pt4s08030	POPTR_0004s08030.1	Phytozome
VI	Pt6s03470	POPTR_0006s03470.1	Phytozome
VI	Pt6s03460	POPTR_0006s03460.1	Phytozome
VI	Pt17s02150	POPTR_0017s02150.1	Phytozome
VI	Pt17s02130	POPTR_0017s02130.1	Phytozome
VI	Gm CNL2	XP_003538370.1	NCBI
VI	Gm CNL3	XP_003552891.1	NCBI
VI	Gm CNL1	XP_003544957.1	NCBI
VI	Gm CNL4	XP_003518359.1	NCBI
VI	Ph CNL	JN120848	NCBI
VI	Cb BZL	JN135247	NCBI
VI	At1g65880 BZO1	NP_176763.1	NCBI
VI	At1g65890 AAE12	NP_176764.1	NCBI
VI	At1g68270	NP_176994.1	NCBI
VI	At1g66120 AAE11	NP_176786.1	NCBI
VI	Zm CNL	NP_001147787.1	NCBI
VI	Sb CNL	XP_002468582.1	NCBI
VI	Os CNL2 Os03g0130100	NP_001048852.1	NCBI
VI	Os CNL1 Os09g0555800	NP_001063895.1	NCBI
VII	At3g48990 AAE3	NP_190468.1	NCBI
VII	At3g16170 AAE13	NP_566537.1	NCBI
VII	At1g30520 AAE14	NP_174340.2	NCBI

Supplemental Table 2. Activities of enzymes involved in benzenoid biosynthesis in petunia petals of control and *PhCNL*-RNAi Lines

		RNAi-*CNL* lines		
	Control	**1**	**3**	**17**
PAL	150.454 ± 17.793	158.15 ± 10.499	147.13 ± 20.86	142.847 ± 21.218
BALDH	26.165 ± 2.4	26.477 ± 1.098	26.449 ± 0.718	30.155 ± 2.054
IGS	0.064 ± 0.003	0.08 ± 0.01	0.087 ± 0.005	0.076 ± 0.007
EGS	0.241± 0.023	0.268 ± 0.028	0.282 ± 0.017	0.31 ± 0.017
BSMT	4.284 ±0.134	4.856 ± 0.361	4.873 ± 0.244	4.962 ± 0.3
BPBT	3.859 ± 0.183	3.332 ± 0.206	3.258 ± 0.3	2.44 ± 0.429
PAAS	0.021 ± 0.005	0.016 ± 0.002	0.02 ± 0.006	0.018 ± 0.004
4CL1	45.485 ± 3.056	59.564 ± 7.907	46.053 ± 8.173	57.419 ± 12.955

Petal crude extracts were prepared from day 2 flowers postanthesis collected at 8 PM. Data are means ± SE (n =3 to 6 biological replicates) and expressed in pkat/mg protein. Activities were not significantly different between genotypes ($P > 0.05$ by Student's t test). BALDH, benzaldehyde dehydrogenase; BPBT, benzoyl-CoA:benzyl alcohol/phenylethanol benzoyltransferase; BSMT, S-adenosyl-L-Met:benzoic/salicylic acid carboxyl methyltransferase; 4CL1, 4-coumarate:CoA ligase; EGS, eugenol synthase; IGS, isoeugenol synthase; PAAS, phenylacetaldehyde synthase; PAL, phenylalanine ammonia lyase

Supplemental Table 3. Subcellular fractionation of petunia petal crude extracts. Activities of marker enzymes, Ph-4CL1 and Ph-CNL.

Marker	Crude extract	Cytosolic fraction	Peroxisomal fraction
Catalase[1]	57.9 ± 1.9 (100% of total)	15.0 ± 9.0 (26.0% of total)	457.5 ± 53.5 (1.6% of total)
Fumarase[1]	342.1 ± 33.8 (100% of total)	155.4 ± 7.1 (50.4% of total)	261.5 ± 53.3 (0.2% of total)
Alcohol dehydrogenase[2]	125.4 ± 13.3 (100% of total)	309.6 ± 64.7 (86.9% of total)	n.d.
Chlorophyll[3]	0.158 ± 0.004	< 0.01	< 0.01
4CL[2*]	20.0 ± 4.8 (100% of total)	30.4 ± 2.4 (152.0% of total)	n.d.
CNL[2**]	9.9 ± 0.3 (100% of total)	7.6 ± 0.1*** (76.8% of total)	82.8 ± 7.3 (2.4% of total)

[1]Specific activity in nkat mg^{-1} [2]Specific activity in pkat mg^{-1} [3]Units are ng x gFW^{-1}
*Activity toward ferulic acid **Activity toward cinnamic acid n.d. - not detected
***Note that Ph-4CL1 contributes to cytosolic cinnamate CoA ligase activity

Supplemental Table 4. Substrate specificity of PhCNL with short, medium and long chain fatty acids

substrate	%
trans-cinnamic acid	100
p-coumaric acid	76
octanoic acid	n.d
hexanoic acid	n.d.
buryric acid	n.d.
propanoic acid	n.d.
myristic acid	n.d.
palmitic acid	n.d.

Acyl Coa synthetase activity of the affinity purified recombinant Ph-CNL was determined by a coupled assay with myokinase, pyruvate kinase and lactate dehydrogenase measuring AMP formation. Values represent relative AMP formation by monitoring NADH oxidation (A_{340nm}), normalized to the reaction containing Ph-CNL and *trans*-cinnamic acid, the preferred substrate for this enzyme, which was set as 100% (29.19 ± 11 nkat mg^{-1} protein, n = 3). As positive control for fatty acids, petunia petal crude extract was analyzed for acyl CoA synthetase activity toward hexanoic acid which was 58.1 ± 3.8 nkat mg^{-1} protein (n = 3).

n.d., not detected, activity below detection level.