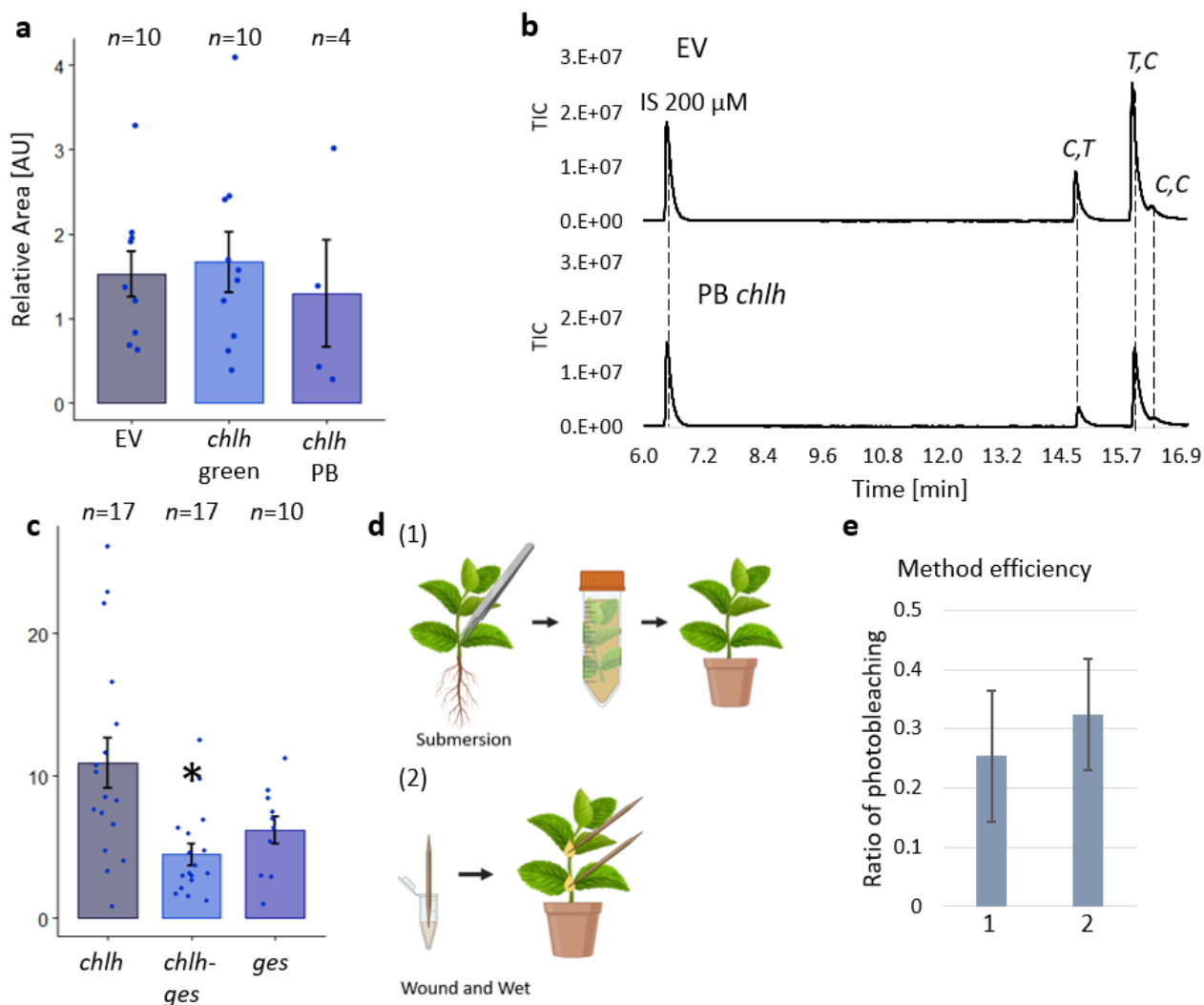


**Supplemental Information for**

**In vivo characterization of key iridoid biosynthesis pathway genes in catnip (*Nepeta cataria*)**

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### Supplemental Figure 1

**a** Total normalized nepetalactone content for plants infected with empty-pTRV2 vector (EV); green tissue from plants infected with pTRV2-ChlH (*chlh*); photo-bleached tissue from plants infected with pTRV2-ChlH (PB *chlh*). Normalized nepetalactone content is calculated by dividing the nepetalactone peaks areas by the internal standard (200  $\mu$ M camphor). Error bars are standard error (EV  $n=10$ ; *chlh* green  $n=10$ ; *chlh* PB  $n=4$ ). **b** A representative MS spectrum of EV and PB *chlh* samples. C,T is (*S*)-*cis,trans* nepetalactone, T,C is (*S*)-*trans-cis* nepetalactone and C,C is (*S*)-*cis-cis* nepetalactone. IS is internal standard (200  $\mu$ M camphor). **c** Total normalized nepetalactone content for PB *chlh* tissue, photo-bleached tissue from plants infected with the double silencing vector for *chlh* and *ges* (*chlh-ges*); green tissue from plants in which *ges* was silenced alone (*ges*). Error bars represent standard error (*chlh*  $n=17$ ; *chlh-ges*  $n=17$ ; *ges*  $n=10$ ). Asterisk indicates statistically significant difference (t-test  $P$ -value  $<0.05$ ). **d** Methods reported and used for infection. Method 1 (submersion) was reported in (Palmer and O'Connor, 2020);

method **2** (Wound and wet) was used in this paper. E) Ratio of photobleaching efficiency from methods 1 (submersion) and 2 (wound and wet). Ratios were calculated by counting the number of plants displaying the photobleached phenotype divided by the total number of infected plants. Error bars are standard deviation.

## Supplemental Tables

| Code  | Gene                 | Sense | Sequence                               |
|-------|----------------------|-------|--|
| LP056 | NECA_E-N_VIGS_CHLH   | F     | TAAGGTTACCGAATTACCAATGACATGAAGGCCAC    |
| LP057 | NECA_E-N_VIGS_CHLH   | R     | TACCGGATCCCCATGGAGCTCACAATTTGAGGGCCAC  |
| LP058 | NECA_B-X_VIGS_GES1   | F     | GCCTCCATGGGGATCTCGCCTTTGTAGCGAGGTGG    |
| LP059 | NECA_B-X_VIGS_GES1   | R     | ATGCCCGGGCCTCGATTTGAGTACTTCGGGTCTGGG   |
| LP069 | Neca_EcoR1_VIGS_GES  | F     | TAAGGTTACCGAATTAATCCAACGGCTGGGAATCGG   |
| Lp070 | Neca_Nco1_VIGS_GES   | R     | GGTGGATCCCCCATGTCGCTACAAAGGCGAGGTGC    |
| Lp071 | Neca_EcoR1_VIGS_GES  | R     | GCCTTCTAGAGAATTTGCTACAAAGGCGAGGTGC     |
| Lp072 | Neca_EcoR1_VIGS_ISY  | F     | TAAGGTTACCGAATTATGCTGCCGCAGGCGGACACCC  |
| Lp073 | Neca_EcoR1_VIGS_ISY  | R     | GCCTTCTAGAGAATTTGGTCTGCAAGCAGATGTGCTTC |
| Lp091 | Neca_EcoR1_VIGS_MLP1 | F     | TAAGGTTACCGAATTACCAAATTATCCCCAAGGCTT   |
| Lp092 | Neca_EcoR1_VIGS_MLP1 | R     | GCCTTCTAGAGAATTTGCCTTTTCAAATTCTCCTGAA  |
| Lp131 | Neca_RLK_VIGS_EcoR1  | F     | TAAGGTTACCGAATTGATGCTACATTTGTGCAGAGAGG |
| lp132 | Neca_RLK_vigs_EcoR1  | R     | GCCTTCTAGAGAATTCGAATGTGACTCATACT       |
| lp228 | qRT_PCR_NC_CHLH_1    | F     | TCAGACCACGCAAGAAGTCC                   |
| lp229 | qRT_PCR_NC_CHLH_1    | R     | CCAAGTAGCCCAACAAGCTCA                  |
| lp230 | qRT_PCR_NC_CHLH_2    | F     | GAGCTTGTGGGCTACTTGGT                   |
| lp231 | qRT_PCR_NC_CHLH_2    | R     | CTTCTCCACAGCAGCCTTGA                   |
| lp232 | qRT_PCR_NC_GESA_1    | F     | CGCTTCCCAAGGACCTCAAT                   |
| lp233 | qRT_PCR_NC_GESA_1    | R     | TCGTTGTTGTGGACTACGGG                   |
| lp234 | qRT_PCR_NC_GESA_2    | F     | GTGTAACCGACCAAAACGCC                   |
| lp235 | qRT_PCR_NC_GESA_2    | R     | CGAGATCATCCCAAAGGCGA                   |
| lp236 | qRT_PCR_NC_ACT1_1    | F     | TCGTGTTGGTCTGAAGAGC                    |
| lp237 | qRT_PCR_NC_ACT1_1    | R     | GAGAGAACGGCCTGGATAGC                   |
| lp238 | qRT_PCR_NC_ACT1_2    | F     | GCTATCCAGGCCGTTCTCTC                   |
| lp239 | qRT_PCR_NC_ACT1_2    | R     | CTCACACCATCACCGGAGTC                   |
| lp240 | qRT_PCR_NC_UBQ9_1    | F     | AGGTCGAGAGCTCGGATACT                   |
| lp241 | qRT_PCR_NC_UBQ9_1    | R     | AGCCTTTGCTGATCTGGTGG                   |
| lp242 | qRT_PCR_NC_UBQ9_2    | F     | GAGGATGGGAGAACCTTGGC                   |
| lp243 | qRT_PCR_NC_UBQ9_2    | R     | CTCAAACGCAGCACCAGATG                   |
| lp248 | qRT_PCR_NC_MLPL_1    | F     | ATGAACAAGTCCCTGATCCTGT                 |
| lp249 | qRT_PCR_NC_MLPL_1    | R     | TGACATGTGTGGTTCATGCCA                  |
| lp250 | qRT_PCR_NC_MLPL_2    | F     | GCAGCAGCAAAAATGGTGGA                   |
| lp251 | qRT_PCR_NC_MLPL_2    | R     | TGACATGTGTGGTTCATGCCA                  |
| lp252 | qRT_PCR_NC_RLK_1     | F     | GTCGGGTGAAGTCCCAGATG                   |
| lp253 | qRT_PCR_NC_RLK_1     | R     | ATCACTTTCGGCATGAGGCA                   |
| lp254 | qRT_PCR_NC_RLK_2     | F     | TGCCTCATGCCGAAAGTGAT                   |
| lp255 | qRT_PCR_NC_RLK_2     | R     | TTTTTCGCAGCCCTCTCCTT                   |
| lp272 | qrt_per_ncisy_3      | F     | GGTGCGGTTTTGAGGTTTCC                   |
| lp273 | qrt_per_ncisy_3      | R     | GCGGCCCATATTTGATGCTC                   |
| lp274 | qrt_per_ncisy_4      | F     | TTCAACGTCAGCAATGGGGA                   |
| lp275 | qrt_per_ncisy_4      | R     | TTGCTGGCCTTCTCGTATC                    |

**Supplemental Table 1.** List of primers used in this study.

*ges*

| Bucket                  | p-Value  | Average Ratio<br>"ges"/"chlh" | Fold Change<br>"ges"/"chlh" | Max. Intensity |
|-------------------------|----------|-------------------------------|-----------------------------|----------------|
| 3.85 min : 360.14251 Da | 0.018729 | 0.766                         | -1.274                      | 192677         |
| 3.33 min : 568.20113 Da | 0.008756 | 0.466                         | -2.093                      | 21343          |
| 4.69 min : 348.17900 Da | 0.000358 | 0.458                         | -2.127                      | 14842          |

*isy*

| Bucket                  | p-Value  | Average Ratio<br>"isy"/"chlh" | Fold Change<br>"isy"/"chlh" | Max. Intensity |
|-------------------------|----------|-------------------------------|-----------------------------|----------------|
| 4.23 min : 346.16340 Da | 0.005941 | 8.612                         | 9.545                       | 103129         |
| 3.97 min : 430.11185 Da | 0.016313 | 0.344                         | -2.623                      | 61906          |
| 3.91 min : 430.14828 Da | 0.04477  | 0.251                         | -3.593                      | 14039          |
| 4.54 min : 448.10203 Da | 0.020802 | 0.464                         | -1.944                      | 14011          |
| 4.02 min : 444.16400 Da | 0.008218 | 0.393                         | -2.297                      | 13504          |
| 3.75 min : 240.10021 Da | 0.003275 | 0.43                          | -2.096                      | 11595          |
| 4.20 min : 362.15717 Da | 0.029325 | 5.736                         | 6.357                       | 11556          |

*mpl*

| Bucket                  | p-Value  | Average Ratio<br>"mpl"/"chlh" | Fold Change<br>"mpl"/"chlh" | Max. Intensity |
|-------------------------|----------|-------------------------------|-----------------------------|----------------|
| 3.97 min : 430.11185 Da | 0.009426 | 0.413                         | -2.7                        | 61906          |
| 2.80 min : 342.09567 Da | 0.028005 | 0.108                         | -10.347                     | 43052          |
| 1.28 min : 190.04802 Da | 0.045702 | 0.469                         | -2.38                       | 17403          |
| 4.99 min : 186.12587 Da | 0.046783 | 2.38                          | 2.132                       | 14188          |
| 3.67 min : 296.05367 Da | 0.002605 | 0.498                         | -2.242                      | 14090          |
| 4.54 min : 448.10203 Da | 0.002748 | 0.45                          | -2.481                      | 14011          |
| 3.49 min : 438.17443 Da | 0.018737 | 0.492                         | -2.269                      | 10520          |

**Supplemental Table 2.** Significantly up or downregulated metabolites identified in semi-targeted bucket correlation analyses of QToF LC-MS samples.