

**Supplementary Table S1. Primers used in this study**

| Primer No. | Sequence (5' to 3')                                      | comment                   |
|------------|----------------------------------------------------------|---------------------------|
| 1          | ATGGCAAGCTTCACCCTTCACACAGAAACC                           | cloning of <i>GuCSyGT</i> |
| 2          | TTATCCACTCTTGCTTTTCATGGTTATTATCC                         | cloning of <i>GuCSyGT</i> |
| 3          | TTGGGTCTCGTGACGCCCGCAGCTTCGCGTCGCTGTTTATAGAGCTAGAAATAGCA | <i>GuCSyGT</i> gRNA T1    |
| 4          | TTGGGTCTCCTCAAGCGTGCACTGCACCAGCCGGAATCGAA                | <i>GuCSyGT</i> gRNA T2    |
| 5          | TTGGGTCTCGTTGACCCCGAGAGTTTATAGAGCTAGAAATAGCA             | <i>GuCSyGT</i> gRNA T2    |
| 6          | TTGGGTCTCCACTCTTACGGCTGCACCAGCCGGAATCGAA                 | <i>GuCSyGT</i> gRNA T3    |
| 7          | TTGGGTCTCGGAGTGACCGGGGGTTTATAGAGCTAGAAATAGCA             | <i>GuCSyGT</i> gRNA T3    |
| 8          | TTGGGTCTCCAAACCACCCTCGCGAACTCAGAAGCTGCACCAGCCGGAATCGAA   | <i>GuCSyGT</i> gRNA T4    |
| 9          | CCTCGCAGTTGTACTGCTCCTCTTA                                | target site PCR Fw        |
| 10         | GACCTGAACTCGTCGGTGCGAAGAA                                | target site PCR Rev       |
| 11         | GCCGCCGATTACTTCTTCATTC                                   | qPCR for <i>CYP93E3</i>   |
| 12         | CGCGGATATTGACAAAGTGCTC                                   | qPCR for <i>CYP93E3</i>   |
| 13         | CAGGGGAGCCTAGTAACAATGAC                                  | qPCR for <i>CYP72A566</i> |
| 14         | GCCCTGCCAAGTAAATAGCTTC                                   | qPCR for <i>CYP72A566</i> |
| 15         | GACCCTTGTTTGCAGTGTT                                      | qPCR for <i>GuCSyGT</i>   |
| 16         | GATTGCGAACACGCTAGTGA                                     | qPCR for <i>GuCSyGT</i>   |
| 17         | TCTTCGCAAACTGGCAGTGA                                     | qPCR for $\beta$ -tubulin |
| 18         | CGAGATGTGAGTGGGGCAAA                                     | qPCR for $\beta$ -tubulin |

**Supplementary Table S2. gRNA target sequences**

The underlines indicate PAM sequences.

| No.      | Sequence (5' to 3')             |
|----------|---------------------------------|
| Target-1 | <u>CCC</u> AGCGACGCGAAGCTGCCGGG |
| Target-2 | GTGCACGCTTGACCCCGAGA <u>AGG</u> |
| Target-3 | <u>CCG</u> CCCCGGTCACTCTTTACGGC |
| Target-4 | CTTCTGAGTTCGCGAGGGTGT <u>GG</u> |

**Supplementary Table S3. Estimated concentration (µg/mg-dw) of soyasaponin I and its biosynthetic intermediates soyasaponin III and SBMG in hairy roots**

Estimation was performed based on peak areas from the chromatogram shown in Figure 3, by comparing the peak area of the corresponding authentic standard with known concentrations. n.d., not detected.

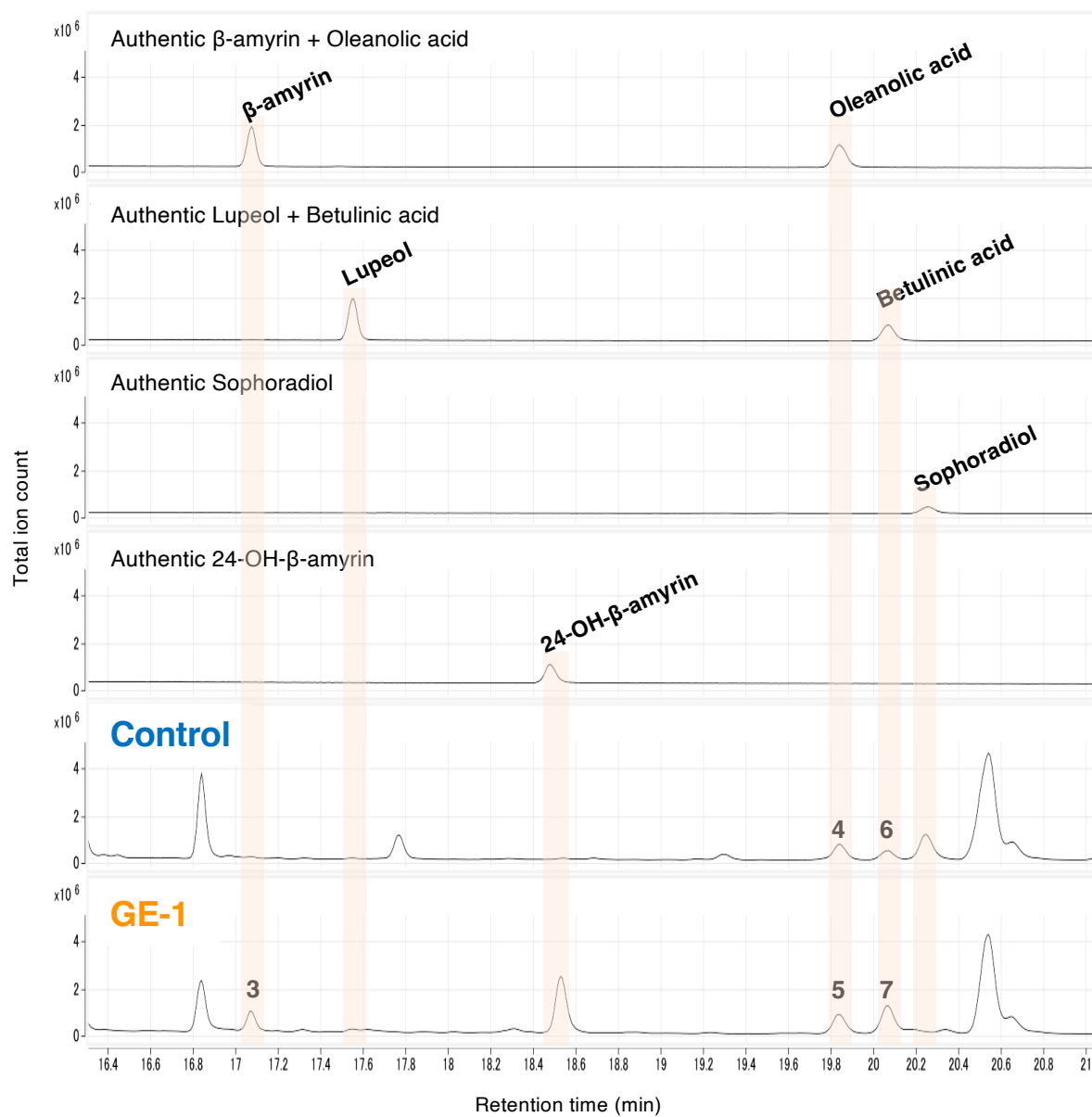
| Compound               | Control | GE-1 | GE-2 |
|------------------------|---------|------|------|
| <b>Soyasaponin I</b>   | 12.3    | n.d. | n.d. |
| <b>Soyasaponin III</b> | 0.229   | n.d. | n.d. |
| <b>SBMG</b>            | 1.23    | n.d. | n.d. |

**Supplementary Table S4. Quantification of sapogenins in acid-hydrolyzed extracts of hairy roots**

Quantification (ng/mg-dw) was performed based on peak areas from the mass chromatogram shown in Figure 4a. The concentrations of triterpenoid sapogenins in hairy roots were determined by comparison with authentic standard curves. n.d., not detected.

| Compound               | Control | GE-1  | GE-2  |
|------------------------|---------|-------|-------|
| <b>β-amyrin</b>        | n.d.    | 75.9  | 59.8  |
| <b>24-OH-β-amyrin</b>  | n.d.    | 302.7 | 501.4 |
| <b>Oleanolic acid</b>  | 398.6   | 372.4 | 355.1 |
| <b>Betulinic acid</b>  | 407.2   | 574.4 | 488.0 |
| <b>Sophoradiol</b>     | 650.7   | n.d.  | n.d.  |
| <b>Soyasapogenol B</b> | 2845.1  | n.d.  | n.d.  |

**a**



**b**

