# Supporting Information

## Runguphan et al. 10.1073/pnas.0903393106

### *SI Text*

**Verification of Transferred DNA (T-DNA) Integration by Genomic DNA Analysis.** The genomic DNA from transformed hairy roots was isolated (Qiagen Dneasy kit) and then subjected to PCR amplification using T-DNA specific primers with STR primers serving as a positive control (Fig. S1). Specifically, primers for PCR amplification were designed to amplify the complete STR gene (STR_for: CCATGGCAAACTTTTCTGAATCTAAATCC and STR_rev: GGTCACCCTAGCTAGAAACATAAGAATTTC), a 500-bp region of the CaMV 35S promoter (CaMV 35S_for: TAGAGGACCTAACAGAAC and CaMV 35S_rev: CCGTGTTCTCTCCAAATG), an 800-bp region of the antibiotic resistance NPTII gene (NPTII_for: atggcaattaccttatccgc and NPTII_rev: tcagaagaactcgtcaagaag) and a 550-bp region of the PDK intron (PDK_int_for: aatagtaattctagctggtttg and PDK_int_rev: ttcttgtacaaagtggtctag).

Primers for real-time RT-PCR of transformed hairy roots for tryptophan decarboxylase (TDC_for: TACTTGGATGGAATCGAACG and TDC_rev: ACAAATGTGGTTGCTTGACC, amplicon size 106 bp) anthranilate synthase, alpha subunit (ASα_f: GAACGTTTGCCCTTCTCTTC and ASα_for_rev: CAGCGGACTTGATAAGACGA. amplicon size 105 bp), strictosidine synthase (STR_for: GTCCAAGATGGCCGAGTTAT and STR_rev: TATGTCCTCCCACACAATGG, amplicon size 140 bp), strictosidine glucosidase (SGD_for: ATATTGATGCTCGGGAAAGG and SGD_rev: AGACGGCTTCCTACAAGAGC, amplicon size 109 bp) and Orca3 (ORCA3_for: ATCCAGGATTTGGTTGGGTA and ORCA3_rev: CTCTAACGGGTCCGGAAATA, amplicon size 143 bp). (See Fig. S1.)

**Assessment of Alkaloid Production Rescue by Addition of Tryptamine.** Ten root tips from hairy roots transformed with pTDCi were subcultured in 50 mL of Gamborg's B5 liquid media with tryptamine as described in *Materials and Methods*. Samples were ionized by ESI with a Micromass LCT Premier TOF Mass Spectrometer. The LC was performed on Acquity Ultra Performance BEH C18, 1.7 $\mu$m, 2.1 $\times$ 100 mm column on a gradient of 10–60% acetonitrile/water (0.1% TFA) over 13 min at a flow rate of 0.6 mL/min. The capillary and sample cone voltages were 1,300 and 60 V, respectively. The desolvation and source temperature were 300 and 100 °C. The cone and desolvation gas flow rates were 60 and 800 L/hour. (See Figs. S2 and Fig. S3.)

**Assessment of Alkaloid Mutasynthesis.** See Figs. S4–S7 and Table S1.

**Assessment of the Stability of Alkaloid Production Suppression in Subsequent Subcultures.** Ten root tips from hairy roots transformed with pTDCi were subcultured every 2–3 weeks in 50 mL of Gamborg's B5 liquid media and grown at 26 °C in the dark at 125 rpm. Eighteen-day-old hairy roots were processed as described in *Materials and Methods*. (See Fig. S8.)

**Morphology of 2-Week-Old TDCi and Wild-Type Hairy Roots.** See Fig. S9 *a* and *b*.

**Expression Levels of Secologanin Biosynthetic Genes SLS and G10H.** Primers for real-time RT-PCR of transformed hairy roots for secologanin synthase (SLS) (SLS_for: GGATTGGGCATGGTTTACTC and SLS_rev: CCATGGGTTTAGACAAGGCT, amplicon size 140 bp) and geraniol-10-hydroxylase (G10H) (G10H_for: TGCTTGGACCTGTTTGTAGC and G10H_rev: TCCTCTGCCGATTACTTGTG, amplicon size 132 bp). (See Fig. S10.)

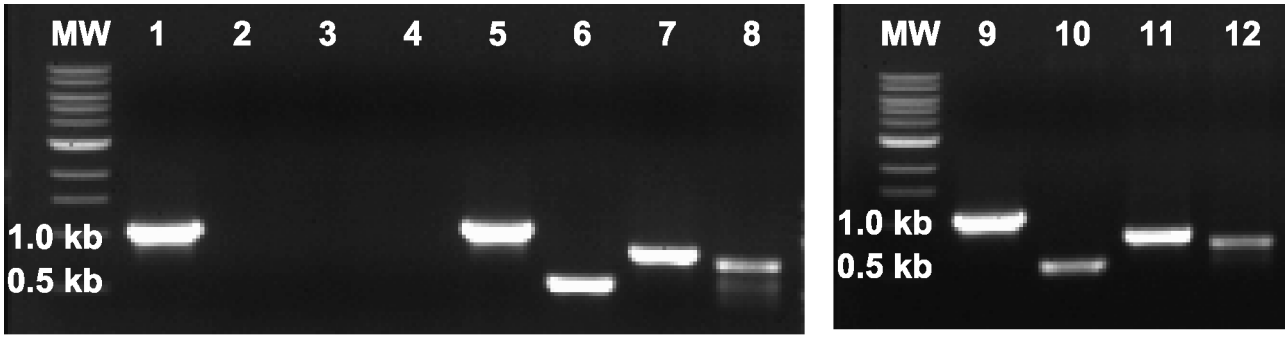



**Fig. S1.** 1% agarose gel of PCR amplification of TDCi and wild-type hairy root genomic DNA. Lane 1: template, wild type; primers, STR. Lane 2: template, wild type; primers, CaMV35S. Lane 3: template, wild type; primers, NPTII. Lane 4: template, wild type; primers, PDK intron. Lane 5: template, TDCi cell line 3; primers, STR. Lane 6: template, TDCi cell line 3; primers, CaMV35S. Lane 7: template, TDCi cell line 3; primers, NPTII. Lane 8: template, TDCi cell line 3; primers, PDK intron. Lane 5: template, TDCi cell line 15; primers, STR. Lane 6: template, TDCi cell line 15; primers, CaMV35S. Lane 7: template, TDCi cell line 15; primers, NPTII. Lane 8: template, TDCi cell line 15; primers, PDK intron

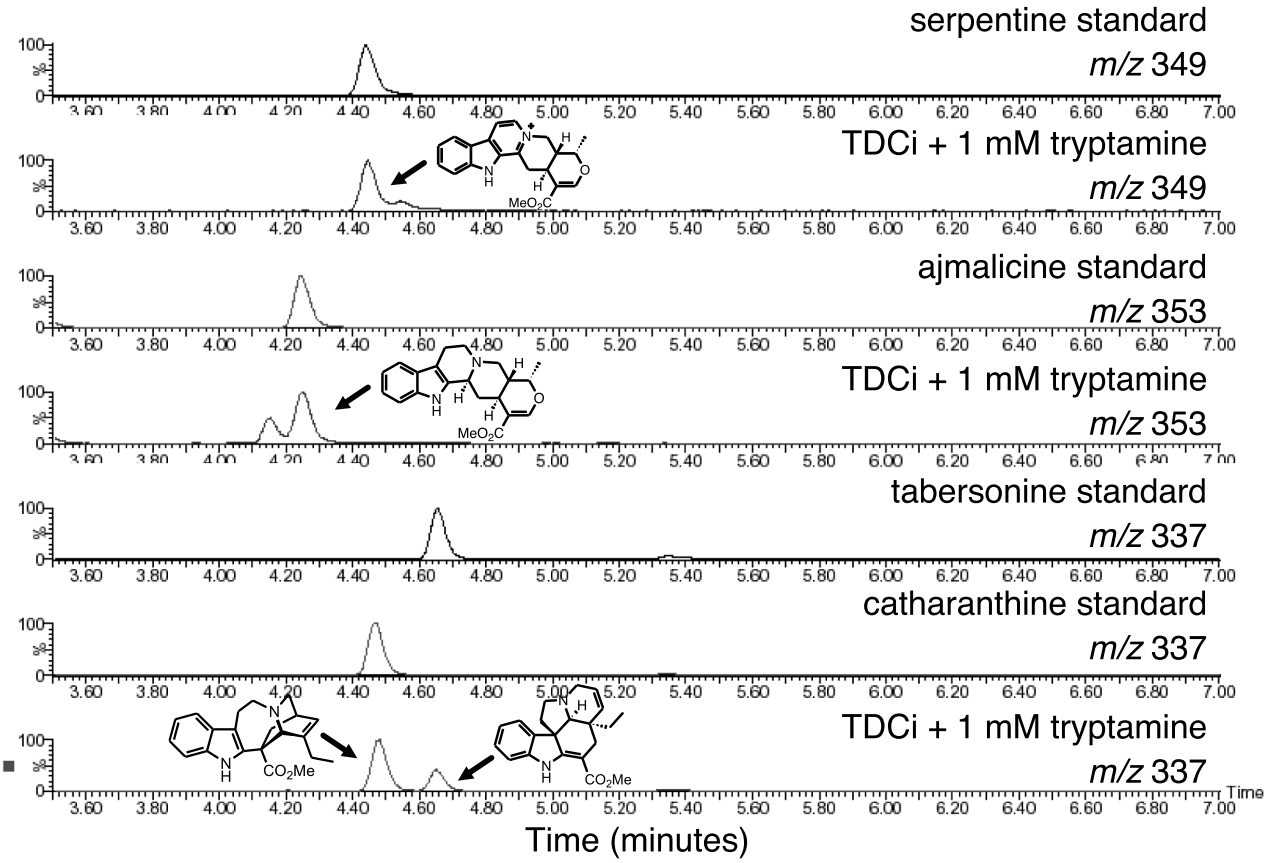



**Fig. S2.** Extracted LC-MS chromatograms showing the presence of ajmalicine, serpentine, catharanthine and tabersonine. These compounds are only present when tryptamine is added to the culture media. Results using a concentration of 1.0 mM tryptamine are shown. Authentic standards of ajmalicine **3**, serpentine **4**, catharanthine **5**, and tabersonine **6** are used to validate the structural assignments of these compounds.

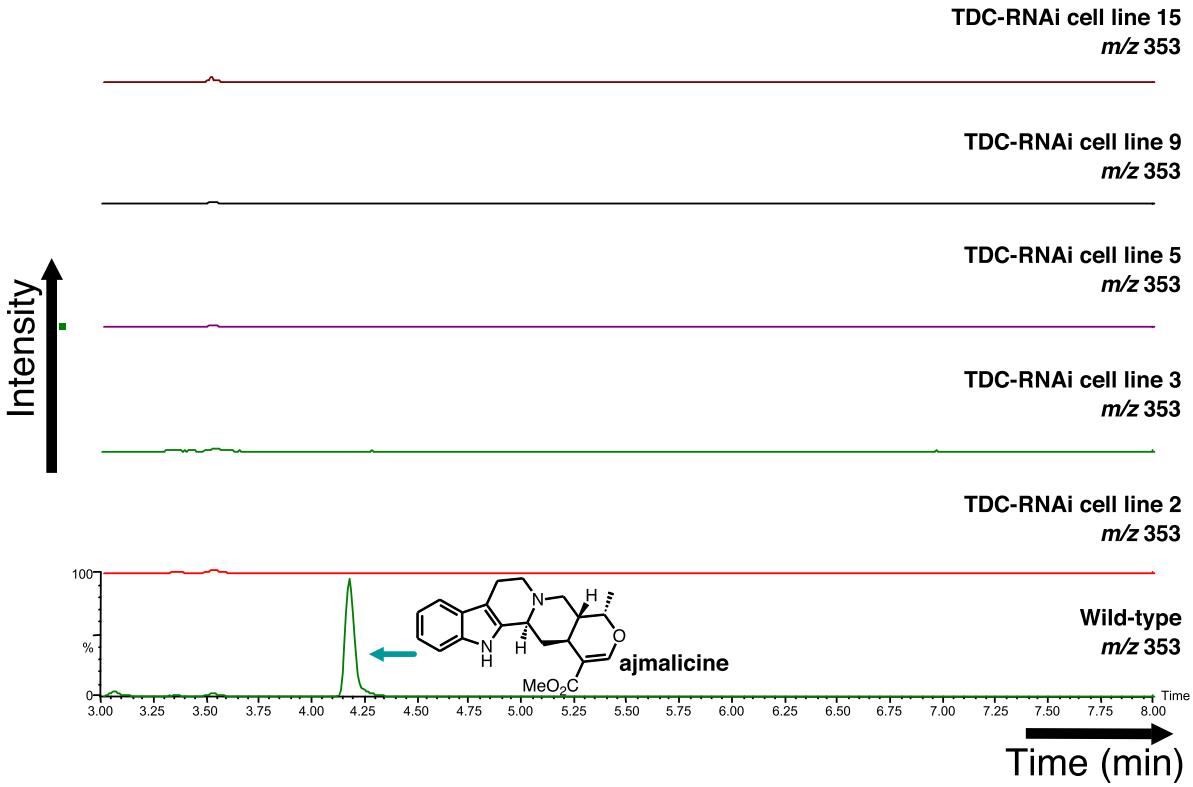



**Fig. S3.** Extracted LC-MS chromatograms showing the absence of A. ajmalicine, B. serpentine, C. catharanthine and tabersonine in five silenced lines. Wild-type lines are shown for comparison. Note that wild-type lines do not produce catharanthine 5 at *m/z* 337; only tabersonine is observed in wild-type lines at this mass. (*D*) Total ion counts for five silenced lines.

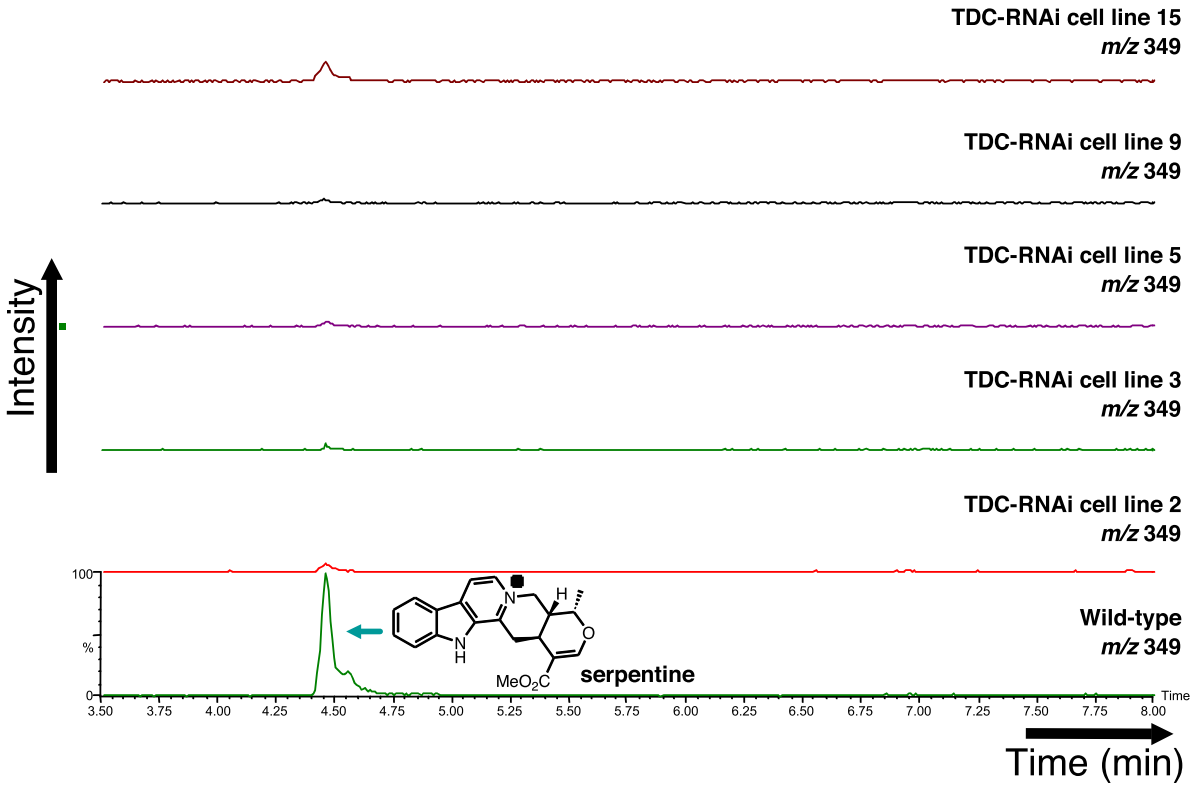
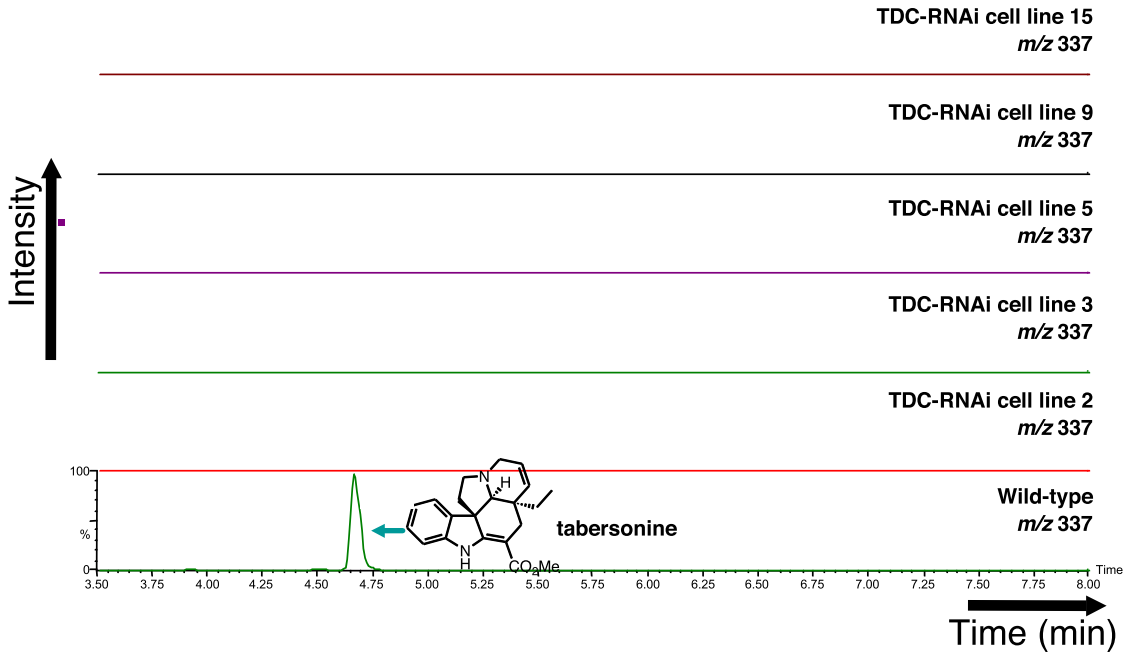



Fig. S3 (continued).

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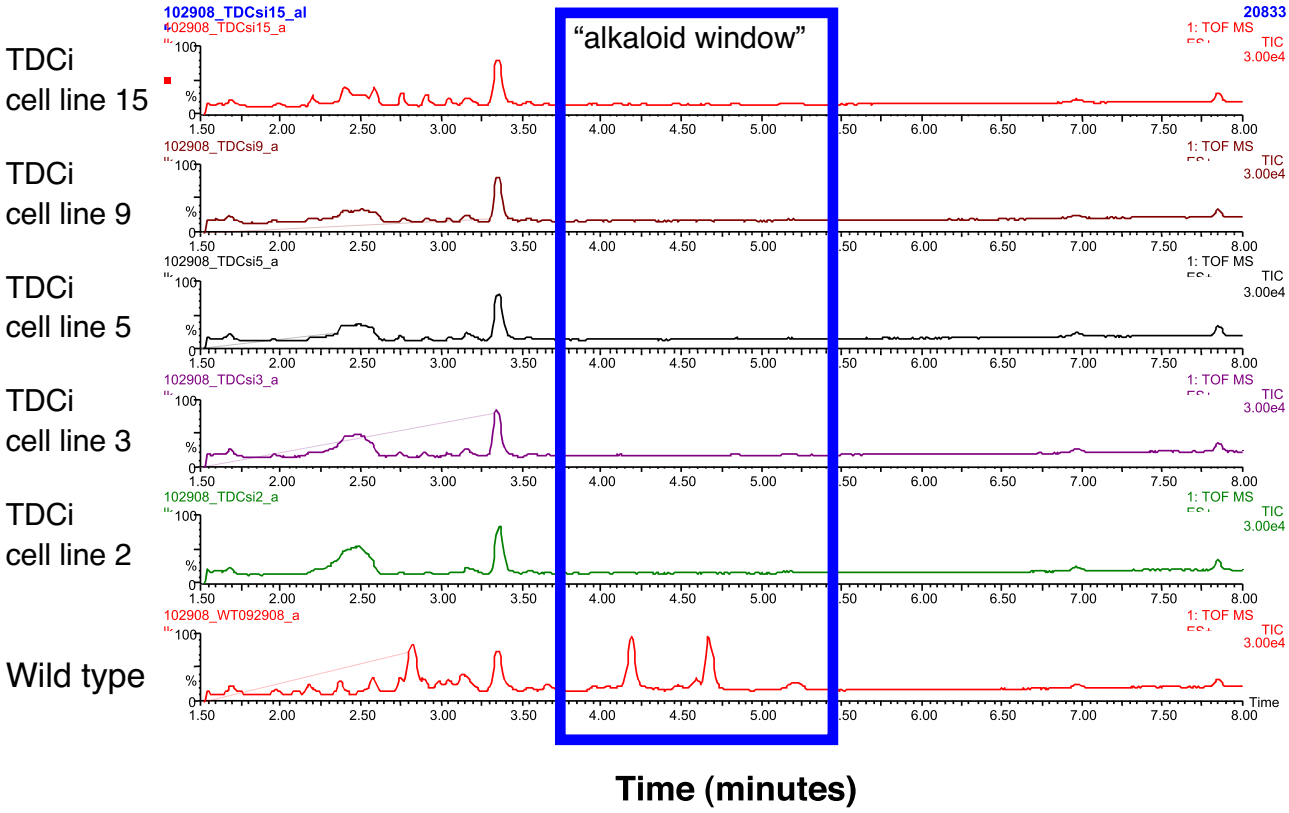


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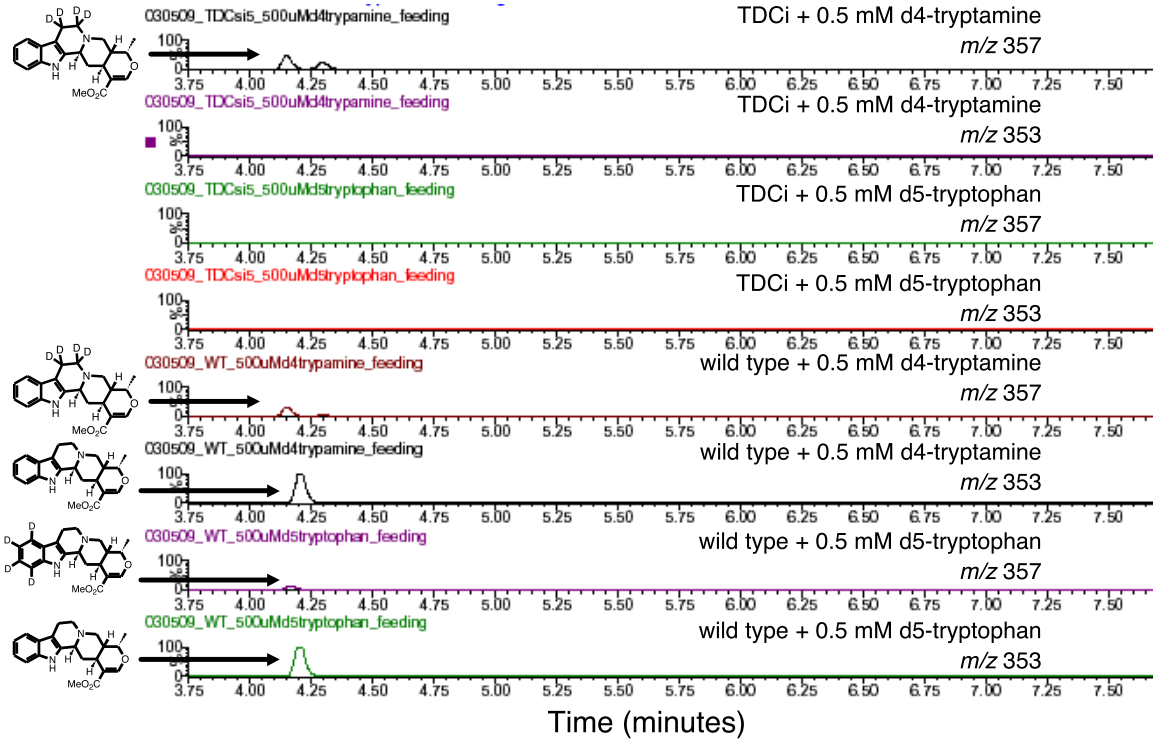
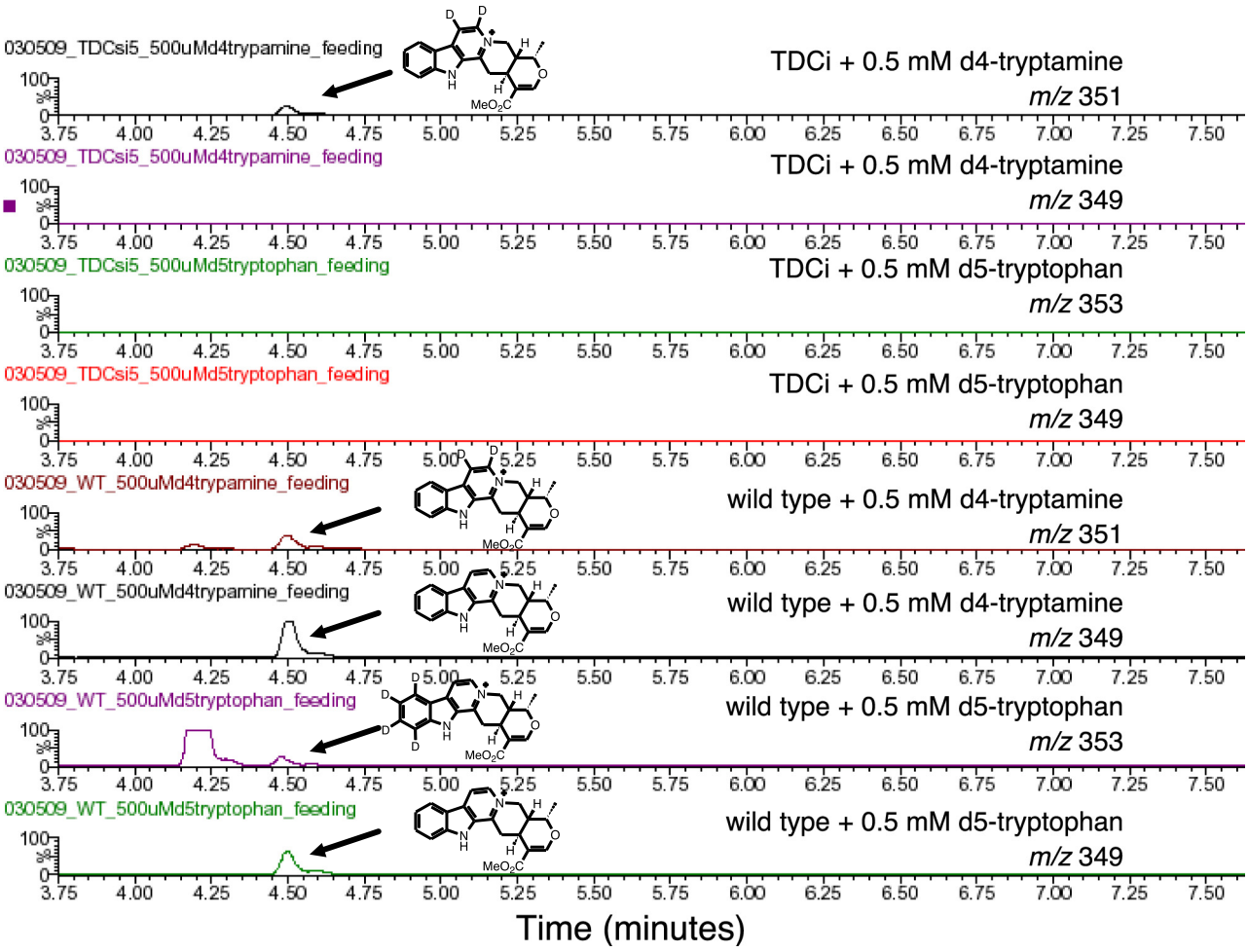



**Fig. S4.** Extracted LC-MS chromatograms showing the presence of deuterated ajmalicine (*A*), serpentine (*B*), and catharanthine and tabersonine (*C*). In the tryptophan decarboxylase suppressed line, these compounds are only present when $\alpha,\alpha,\beta,\beta$-d4-tryptamine (CDN Isotopes) is added to the culture media. In the wild-type line, deuterated alkaloids are present when either $\alpha,\alpha,\beta,\beta$-d4-tryptamine (CDN Isotopes) or 2′,4′,5′,6′,7′-d5-tryptophan (CDN isotope) is added to the culture media. Results using a concentration of 0.5 mM d4-tryptamine or 0.5 mM d5-tryptophan are shown.

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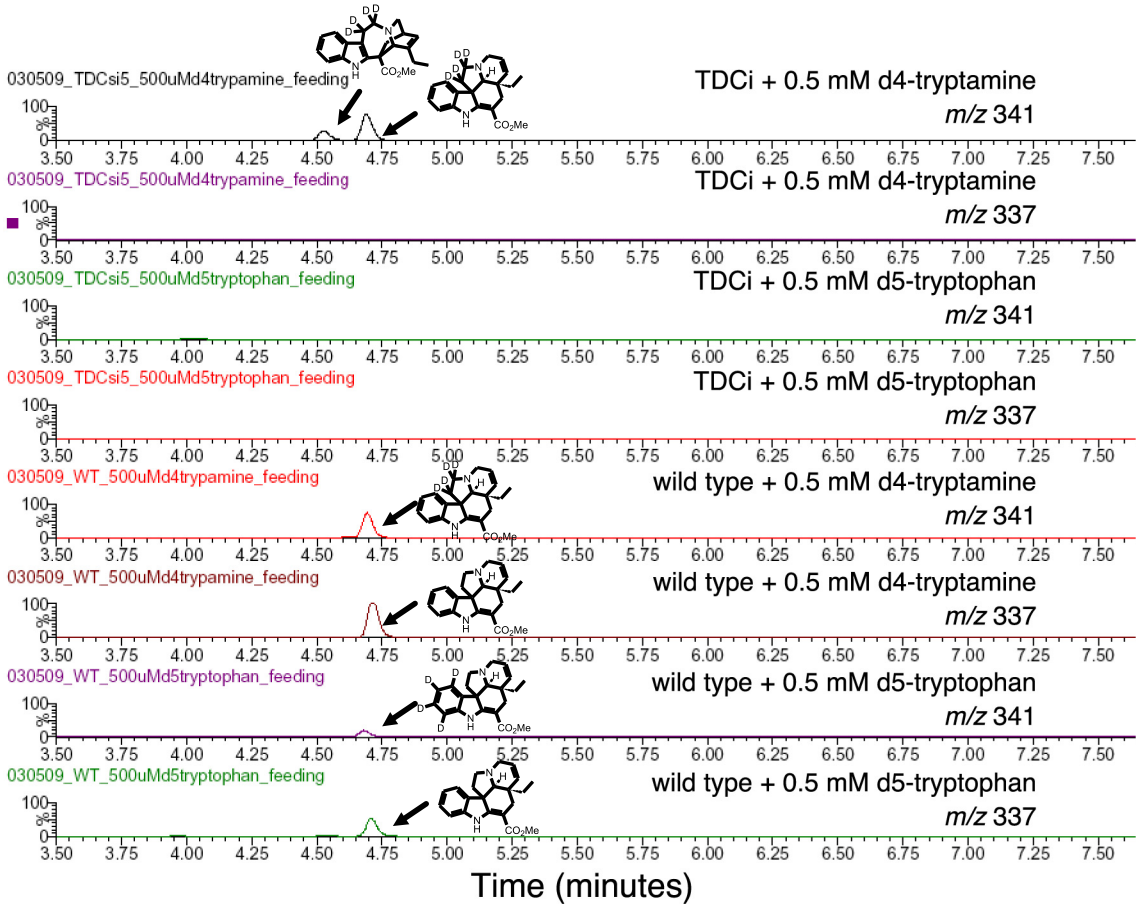



**Fig. S4 (continued).**

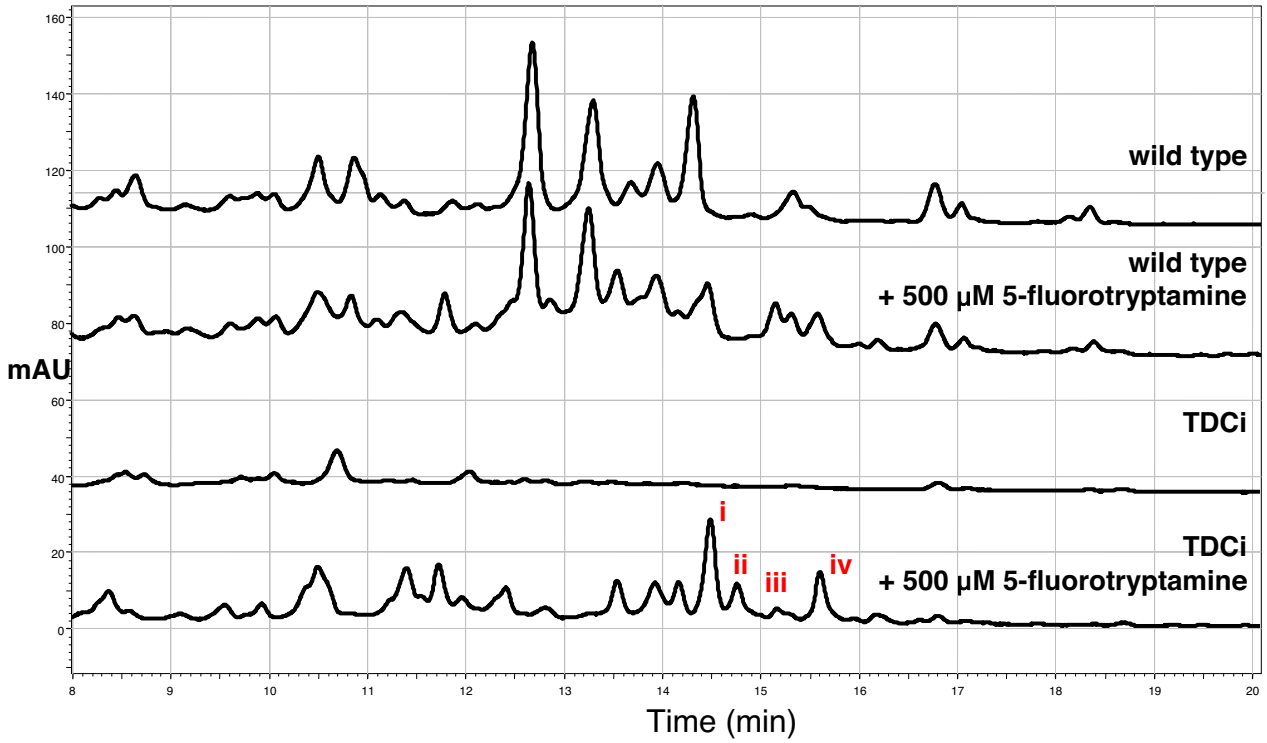



**Fig. S5.** Metabolite production in wild-type and TDCi line as evidenced by high performance liquid chromatography analysis of *C. roseus* hairy root extracts. The alkaloid extracts were run on a Hibar RT 250–4 prepacked reverse phase column using a gradient of 10–60% acetonitrile/water (0.1% TFA) over 25 min at 1 mL/min. Alkaloids were monitored at 280 nm. (*i*) Fluoro-ajmalicine **3**. (*ii*) Fluoro-catharanthine **5**. (*iii*) Fluoro-tabersonine **6**. (*iv*) Fluoro-serpentine **4**.

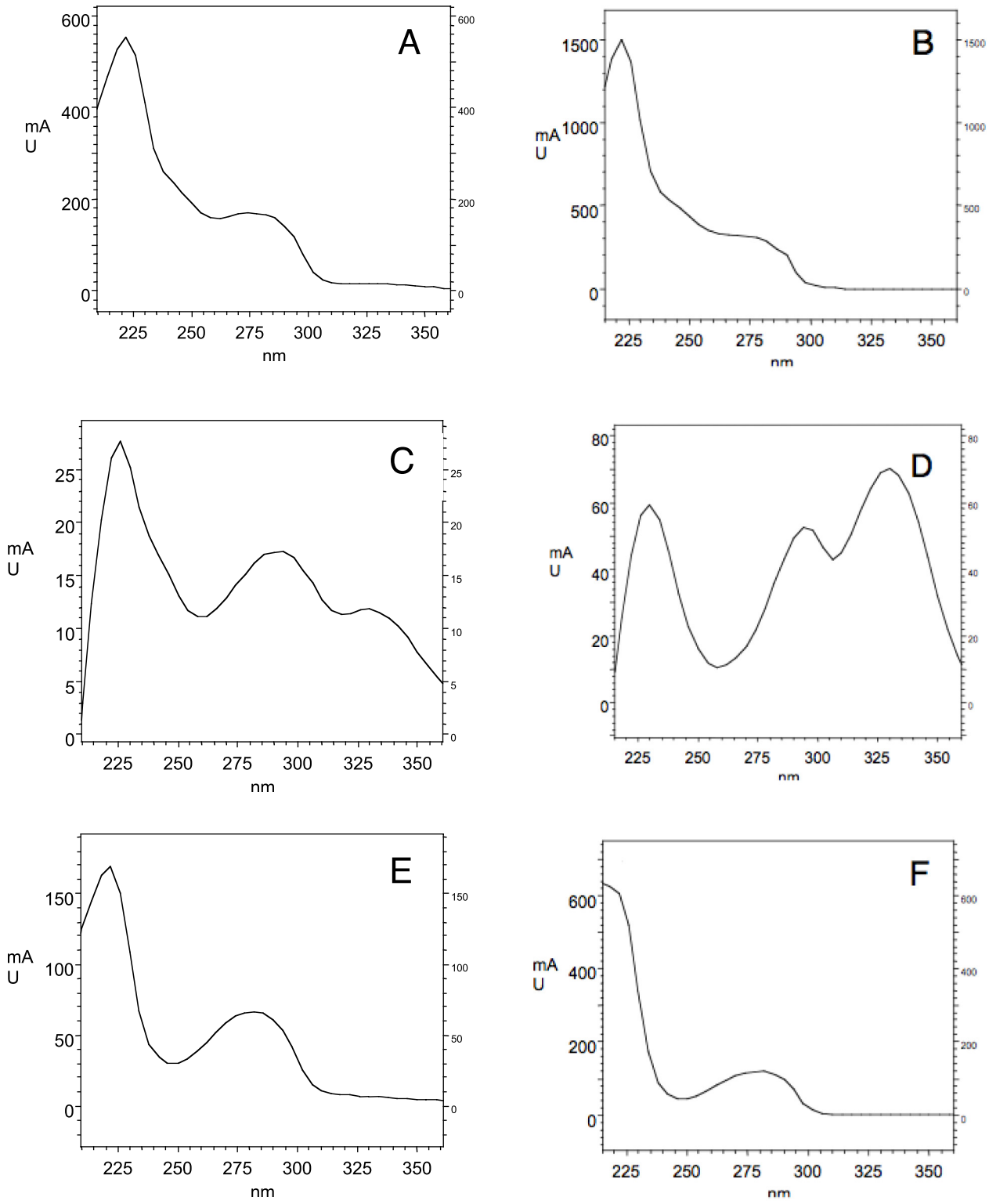



**Fig. S6.** UV spectra of alkaloid standards and selected unnatural alkaloids from TDCi hairy root fed with 5-fluorotryptamine (0.5 mM). (*A*) 5-fluoroajmalicine (fraction *i* in Fig. S4). (*B*) Ajmalicine standard. (*C*) 5-fluorotabersonine (fraction iii in Fig. S4). (*D*) Tabersonine standard. (*E*) 5-fluorocatharanthine (fraction *ii* in Fig. S4). (*F*) Catharanthine standard. (*G*) 5-fluoroserpentine (fraction *iv* in Fig. S4). (*H*) serpentine standard.

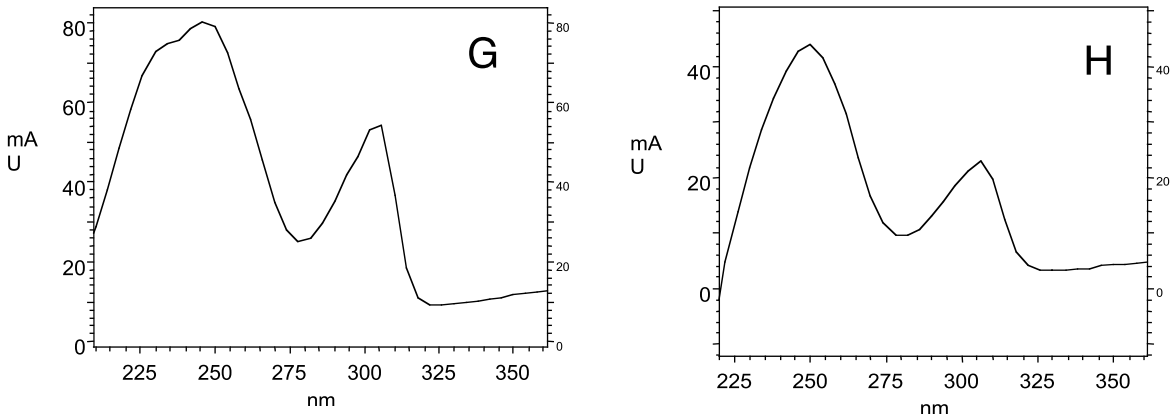



**Fig. S6 (continued).**

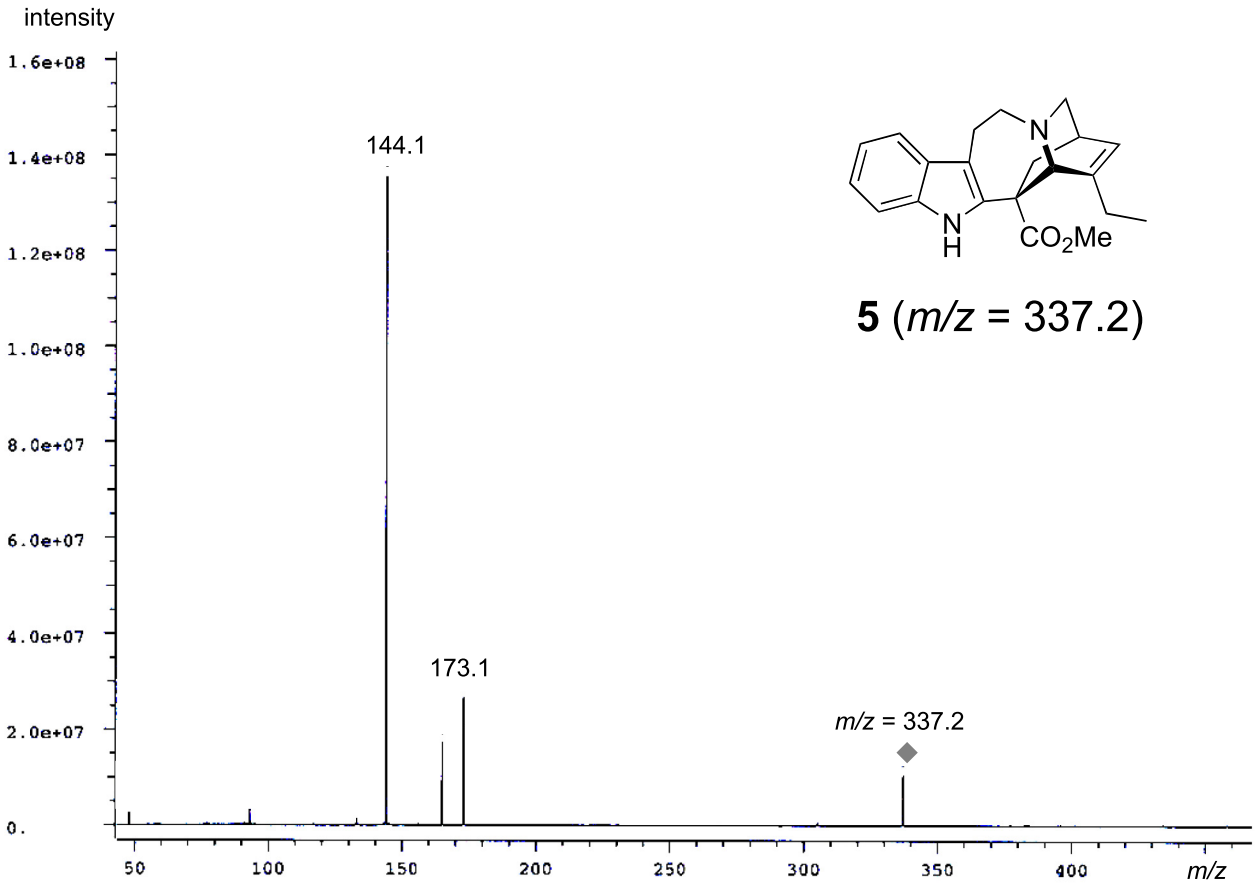
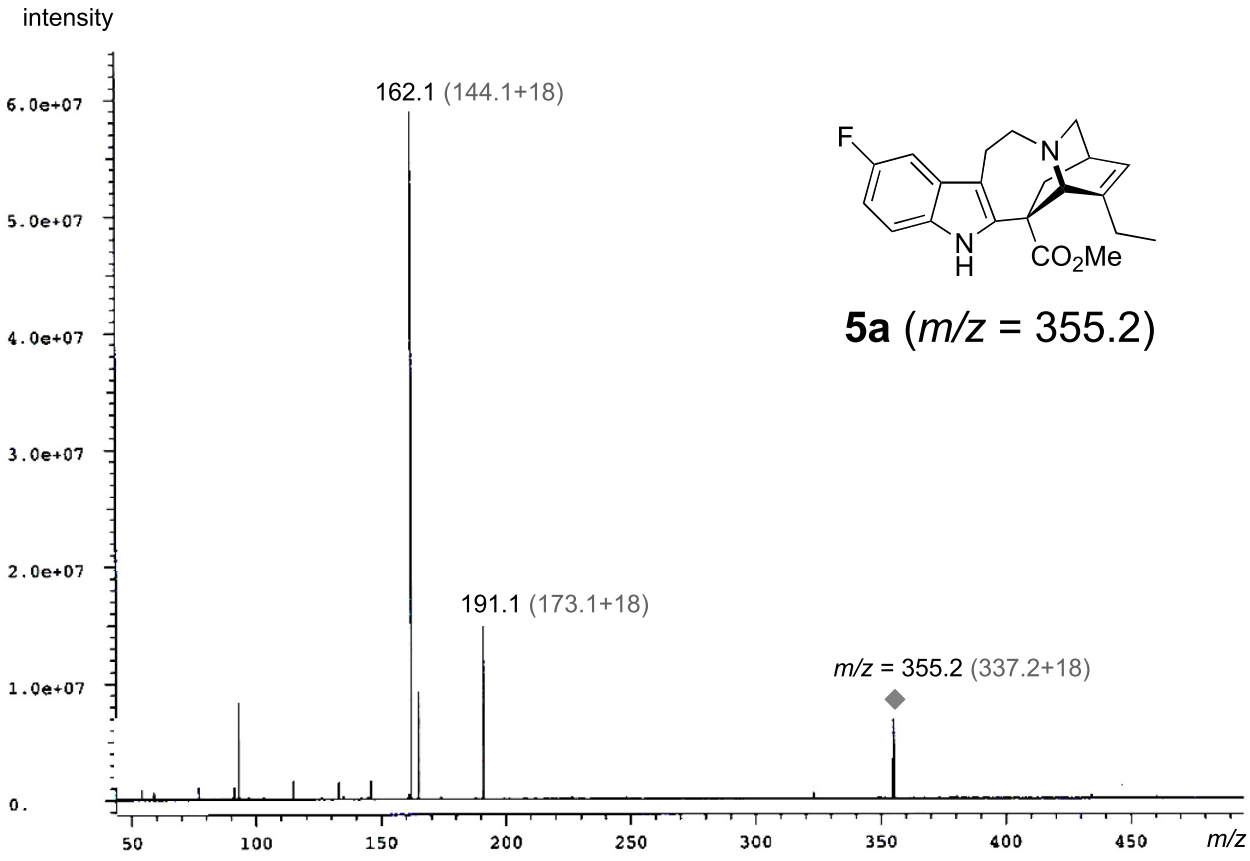



**Fig. S7.** MS/MS analysis for catharanthine **5** standard (*A*), fluorinated catharanthine **5a** (*B*), tabersonine **6** standard (*C*), and fluorinated tabersonine (*D*). (*A*) Catharanthine is identified as the *m/z* = 337.2 ion showing the fragments *m/z* = 173.1 and 144.1. (*B*) Fluorinated catharanthine is identified as the *m/z* = 355.2 (337.2 + 18) ion showing the fragments *m/z* = 191.1 (173.1 + 18) and 162.1 (144.1 + 18). (*C*) Tabersonine is identified as the *m/z* = 337.2 ion showing the fragments *m/z* = 305.2, 277.2, 249.1, 228.1, 196.1 and 168.1. (*D*) Fluorinated tabersonine is identified as the *m/z* = 355.2 (337.2 + 18) ion showing the fragments *m/z* = 323.2 (305.2 + 18), 295.2 (277.2 + 18), 267.1 (249.1 + 18), 246.1 (228.1 + 18), 214.1 (196.1 + 18), and 186.1 (168.1 + 18).

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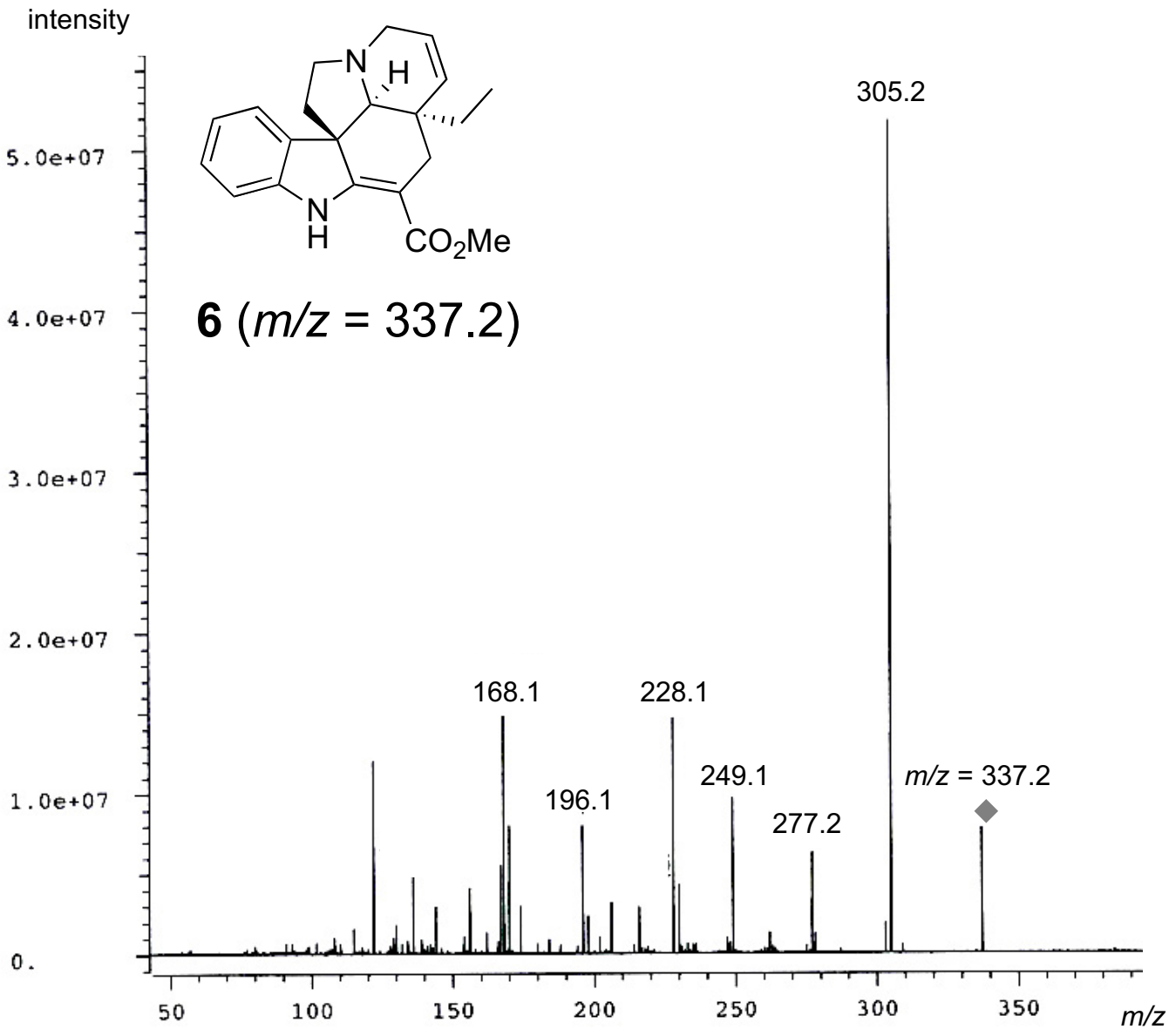


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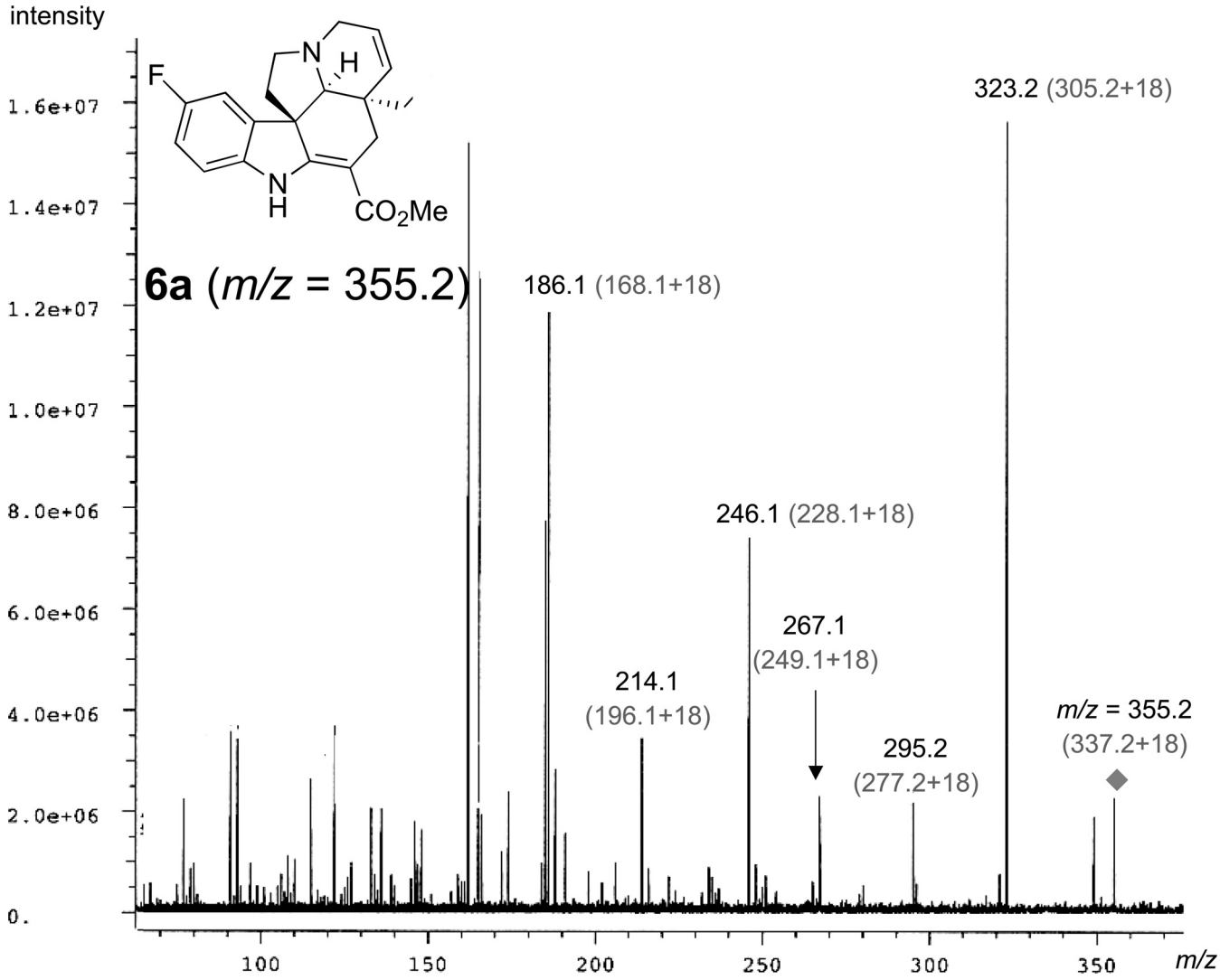


**Fig. S7 (continued).**

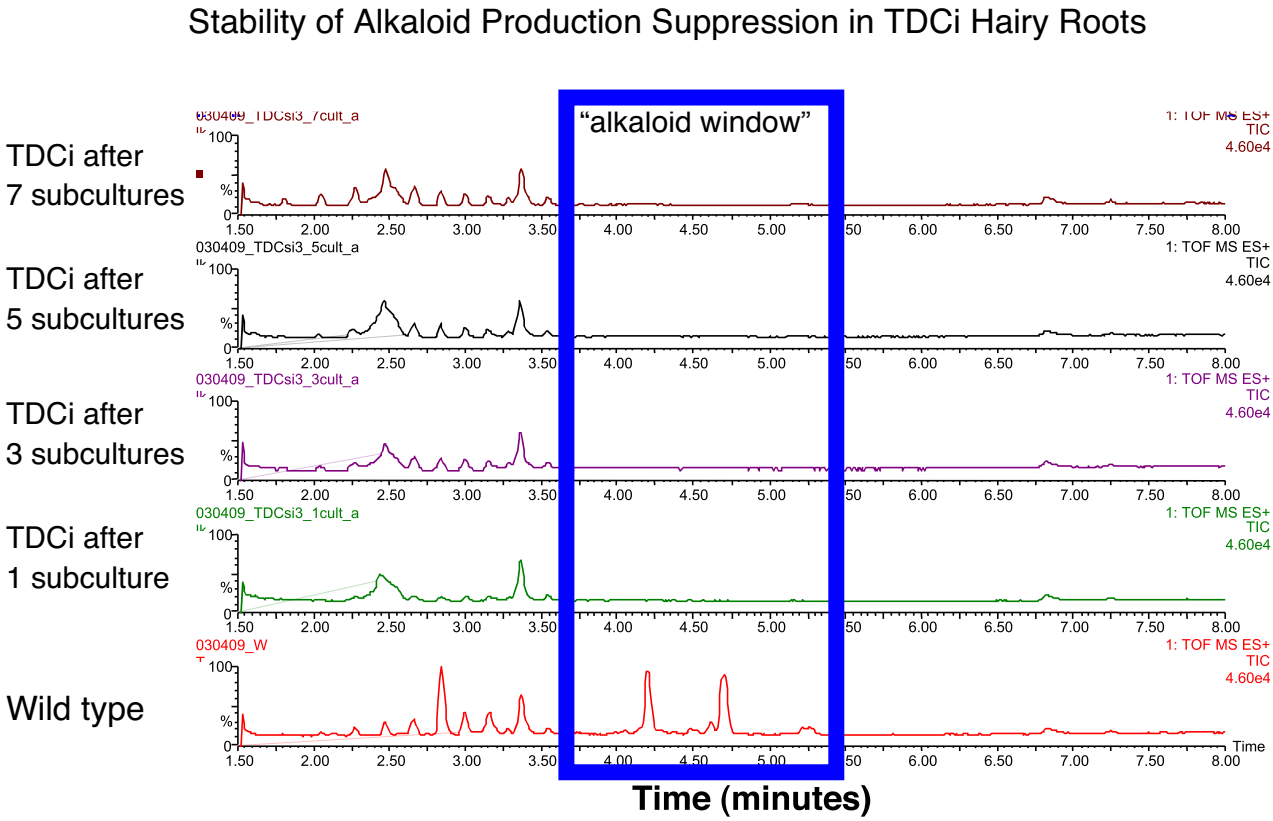


**Fig. S8.** Metabolite production in TDCi hairy roots after 1, 3, 5 and 7 subcultures as evidenced by LC-MS analysis of *C. roseus* extracts. ''Alkaloid window'' represents the range of retention time that natural *C. roseus* alkaloids usually elute.

**Fig. S9.** (*a*) Two-week-old wild-type hairy roots in solid Gamborg's B5 media.

**Fig. S9 (continued).** (*b*) Two-week-old TDCi hairy roots in solid Gamborg's B5 media.

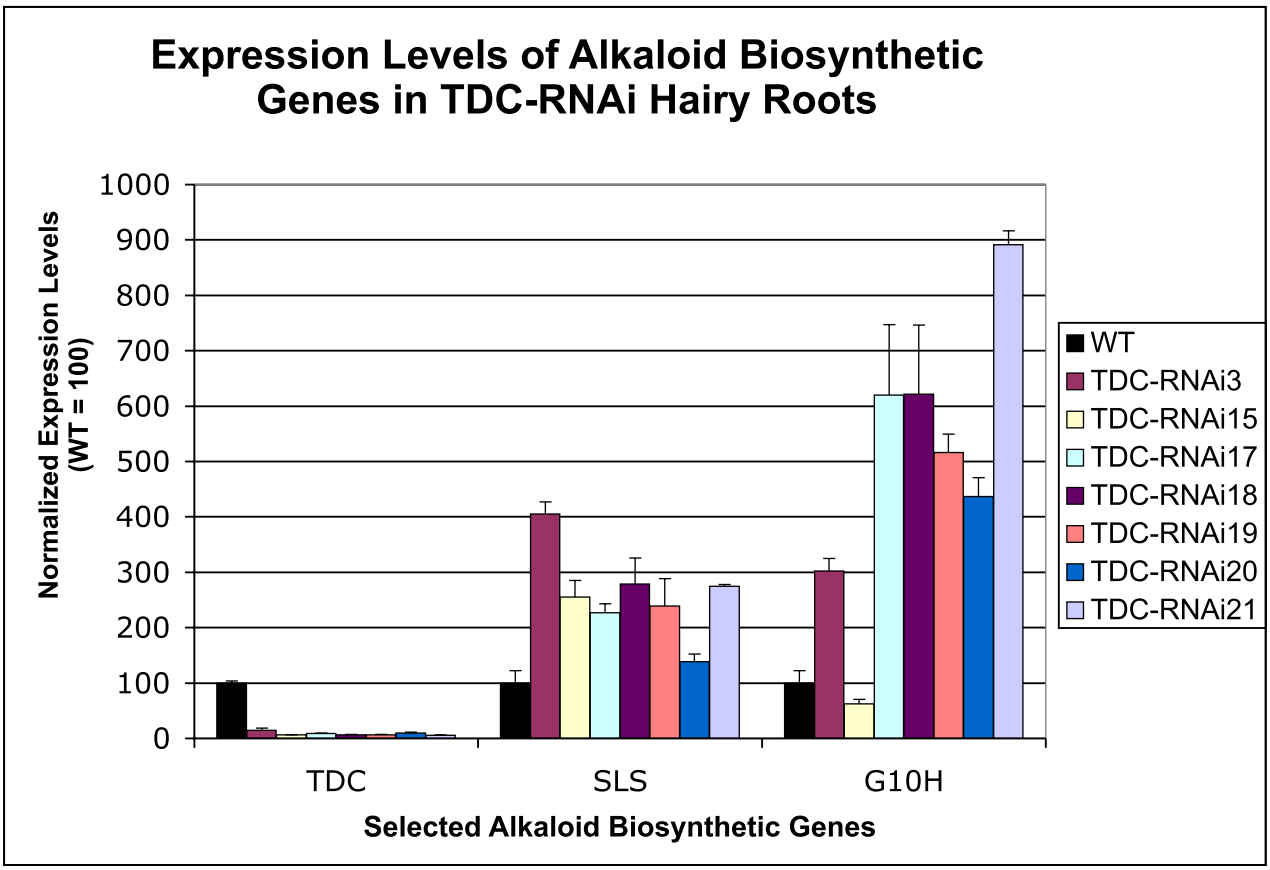


**Fig. S10.** Expression levels of SLS and G10H in 7 different TDCi lines measured by RT PCR. Expression levels of the silenced lines are normalized to the expression levels in the wild-type hairy root line. TDC (tryptophan decarboxylase) expression levels are shown for comparison. SLS (secologanin synthase), G10H (geraniol-10-hydroxylase).

## Other Supporting Information Files

Table S1