

Supplementary data:

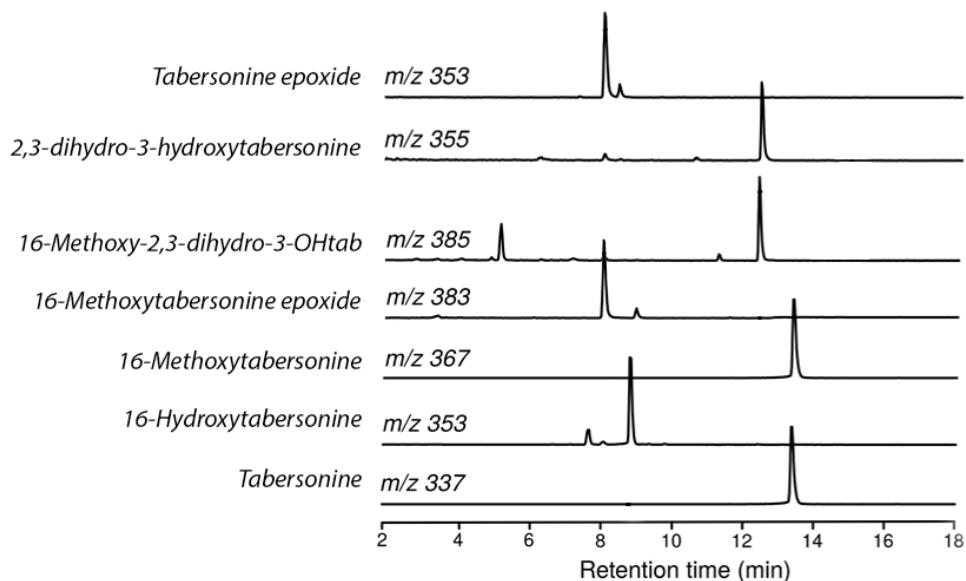


Figure 1. UPLC-MS chromatograms of the natural products produced by the yeast strains.

Table S1: Primers list

Gene	Forward primer	Reverse primer
T16H2	CTGAGAACTAGTCGGTCATGGAGTTGTATTATTTCCA CC	CTGAGAACTAGTCTAATATTACCTTGAGAGAA GAAGC
16OMT	CTGAGAACTAGTCGGTCATGGATGTTCAATCTGAGGAG T	CTGAGAACTAGTCAAGGATAAACCTCAATGAGA C
T3O	CTGAGAACTAGTCGGTCATGGAGTTCATGAATCTTCT CCC	CTGAGAACTAGTCATGCATAGGACGTAGCGA
T3R	CTGAGATCTAGATCGGTATGCCAATGGCTCAAAGTC	CTGAGATCTAGATTAGGGTATTGAAAGTGTTC C
T16H2_helix	CTGAGAACTAGTATGGAGTTGTATTATTTCCACCTTG	GCCAGATTCTTAGAGTTGGCTAA
EROMT (fusion primer)	ACTCTAAAGAAATCTGGCATGGATGTTCAATCTGAGGA GTTCC	CTGAGAACTAGTCAAGGATAAACCTCAATGAGA CTCC
pTEF1	CCAGTCGATTATCATGTCGCTACCACACACCATAGCTT CAAAATG	CTGAGAACTAGTTGTAATTAAAACCTAGATTAG ATTGCTATGC
pPGK1	TAGCGAACATGATAATCGACTGGAGACCGAATTTTC GAAGAA	CTGAGAACTAGTTCTAGATGTTATATTGTTG AAAAAGTAGATAATTAC
pTDH3	TAGCGAACATGATAATCGACTGGCTATTCGAGGACCT TGTCACCTT	CTGAGAACTAGTCTAGATTGTTGTTATGTGTG TTTATTGAAAC

pACT1	CCAGTCGATTATCATGTCGCTAGTGAAGATGTGGCTGC AAGATT	CTGAGATCTAGAGCGGCCGCTTAATTCAAGTAAA TTTCGATCTGG
CPR <i>C. roseus</i>	GGATCCCCCTCGAGTTAACGTAATGGATTCTCCT CAGAAAAGTTAACG	GAATTGCTAGCTCTAGAGCGATGCCACTTAC CAAACATCACGTAAGTATCTACC
ARG3	CATTACGCTCCTCGTATTAC	GTAAGAAATTGGACAACCTCGAAGG
tCPS1	GAGCTCTTAGTCATTGTATGGTC	ACTAGTGCGCAATGATTGAATAGTCAAAG
HIS5	TCTAGAAATAGATTAATTAAACAGTATATGTACAG	GTCGACTGATATTAGCTATATGTACGTTAG
URA3	GATTGGTAATCTCCGAAC	TTTGTGAGTTAGTATAACATGC
IDP1	GAGCTCCAACAATAAGGTATATATATTGATAACAG	ACTAGTCGAATTACGTAGCCCAATC
PRM5	TCTAGAAAACTTTATGATATTGCAATATTTTTAA GC	CTCGAGTATAATAAGACACGGACGCAC
SAM2	CTGAGAACTAGTATGTCCAAGAGCAAACCTTCTTATT ACC	CTGAGAACTAGTTAAAATTCCAATTCTTGGTT TTCC



Figure S2: Fusion of the T16H2 transmembrane helix to the N-terminal end of 16OMT (ER_16OMT). Alignment of the first 55 residues of T16H2 with ER_16OMT. The red rectangle highlights the added sequence including the predicted transmembrane helix identified in T16H2.

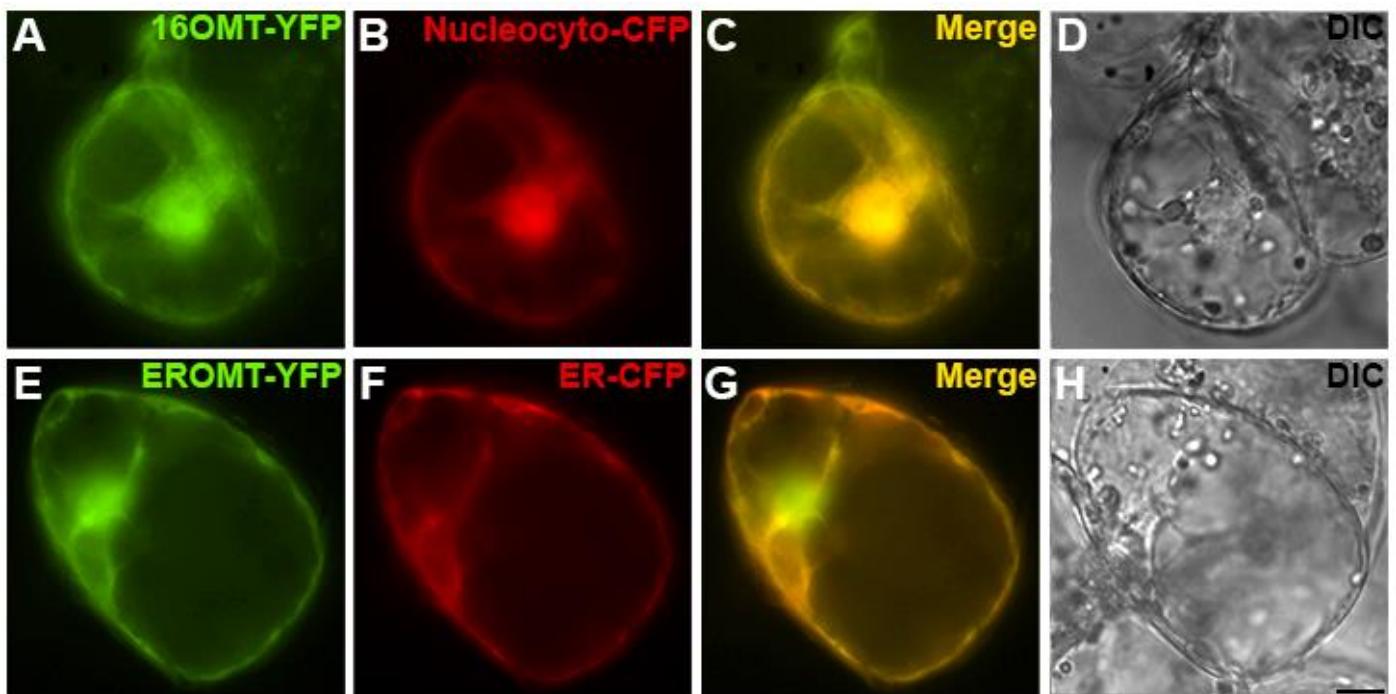


Figure S3: Subcellular localization of 16OMT and EROMT in *C. roseus* cells. Cells were transformed transiently with 16OMT-YFP (A-D) and EROMT-YFP (E-H) expressing vectors in combination with CFP-nucleocytosolic or CFP-ER marker (second column). Co-localization of the two fluorescence signals appeared in the merged image (C, G). The morphology is observed with differential interference contrast (DIC). Bar: 10 μ m.

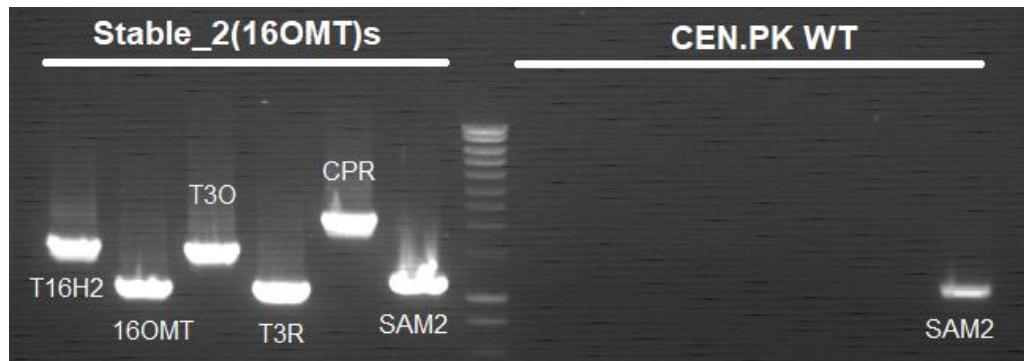


Figure S4: Phusion PCR amplification of the integrated genes from the vindoline's pathway using genomic DNA from the designed stable yeast (Stable_2(16OMT)s) and genomic DNA from wild type CEN.PK (CEN.PK WT). The yeast gene SAM2 (S-adenosylmethionine synthetase) was used as positive control. T16H2: tabersonyne-16-hydroxylase, 16OMT: tabersonine-16-O-methyltransferase, T3O: tabersonine 3-oxygenase, T3R: tabersonine 3-reductase, CPR: optimized *C. roseus* CPR, SAM2: S-adenosylmethionine synthetase.

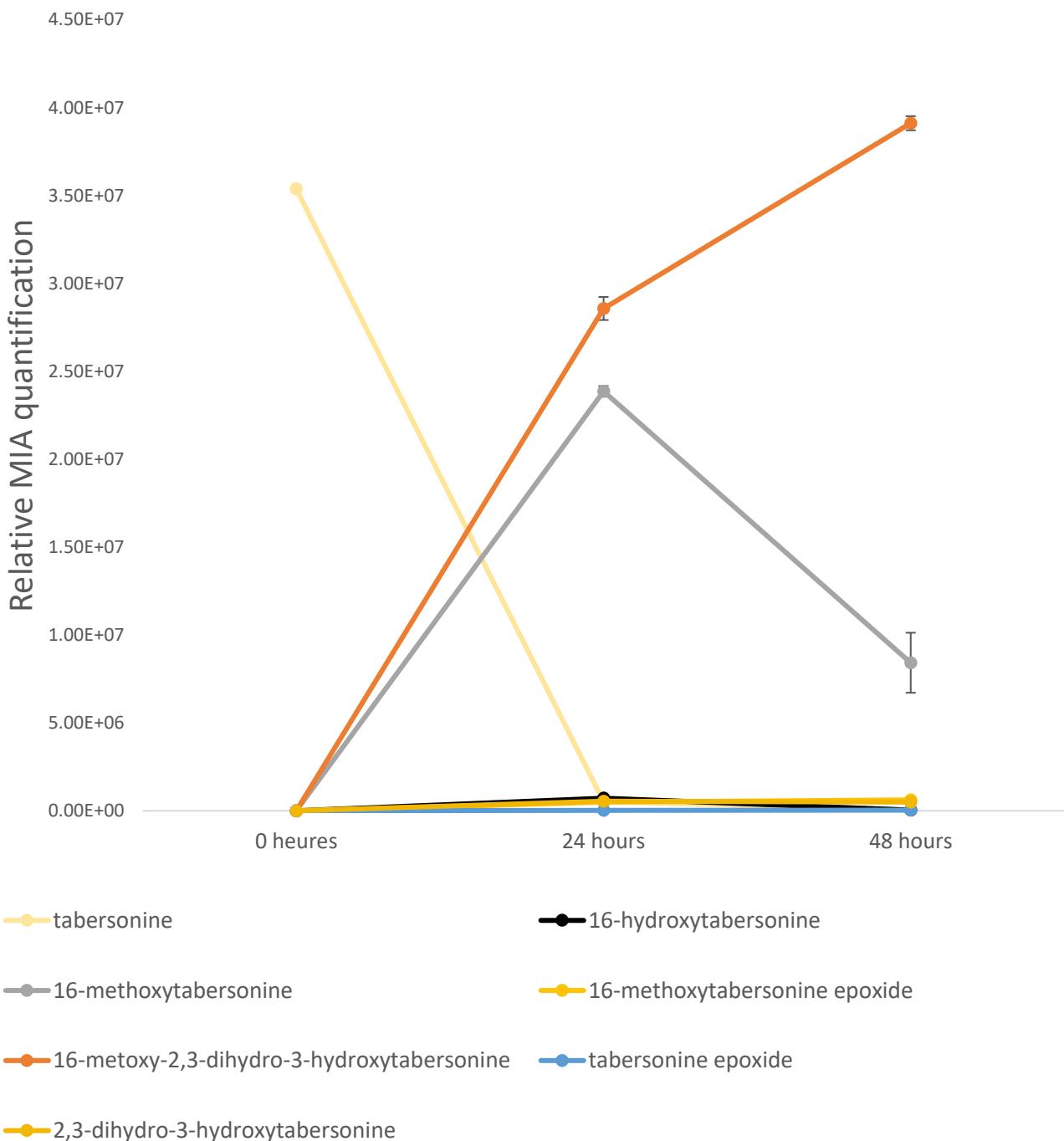


Figure S5: Evolution of the accumulation of vindoline and vindorosine biosynthetic intermediates in the stable_2(16OMT)s yeast strain fed with tabersonine. Alkaloids were quantified by UPLC-MS in the yeast culture medium before and 24 and 48 hours post-feeding with tabersonine (250 μ M). Error bars correspond to the standard error of biological replicates ($n = 3$).