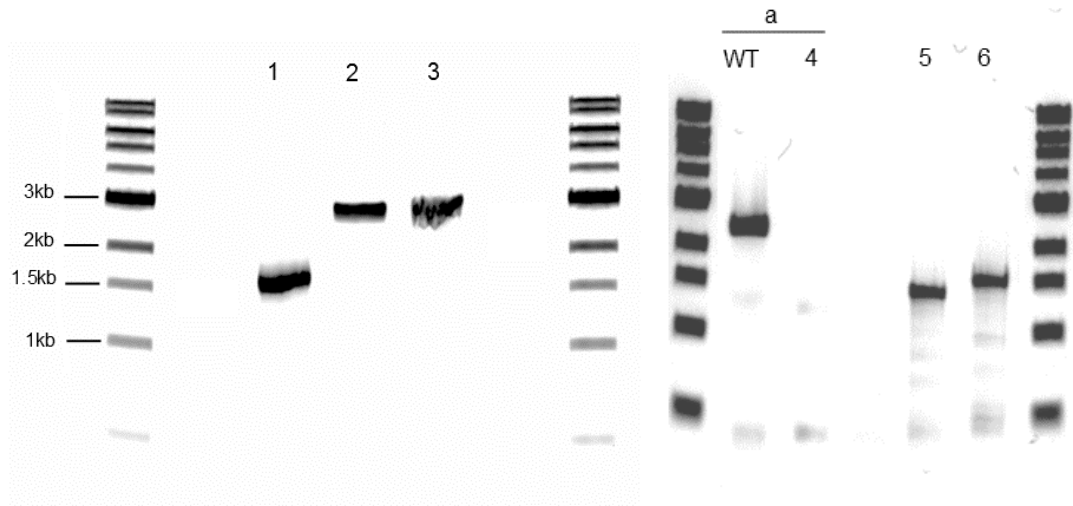
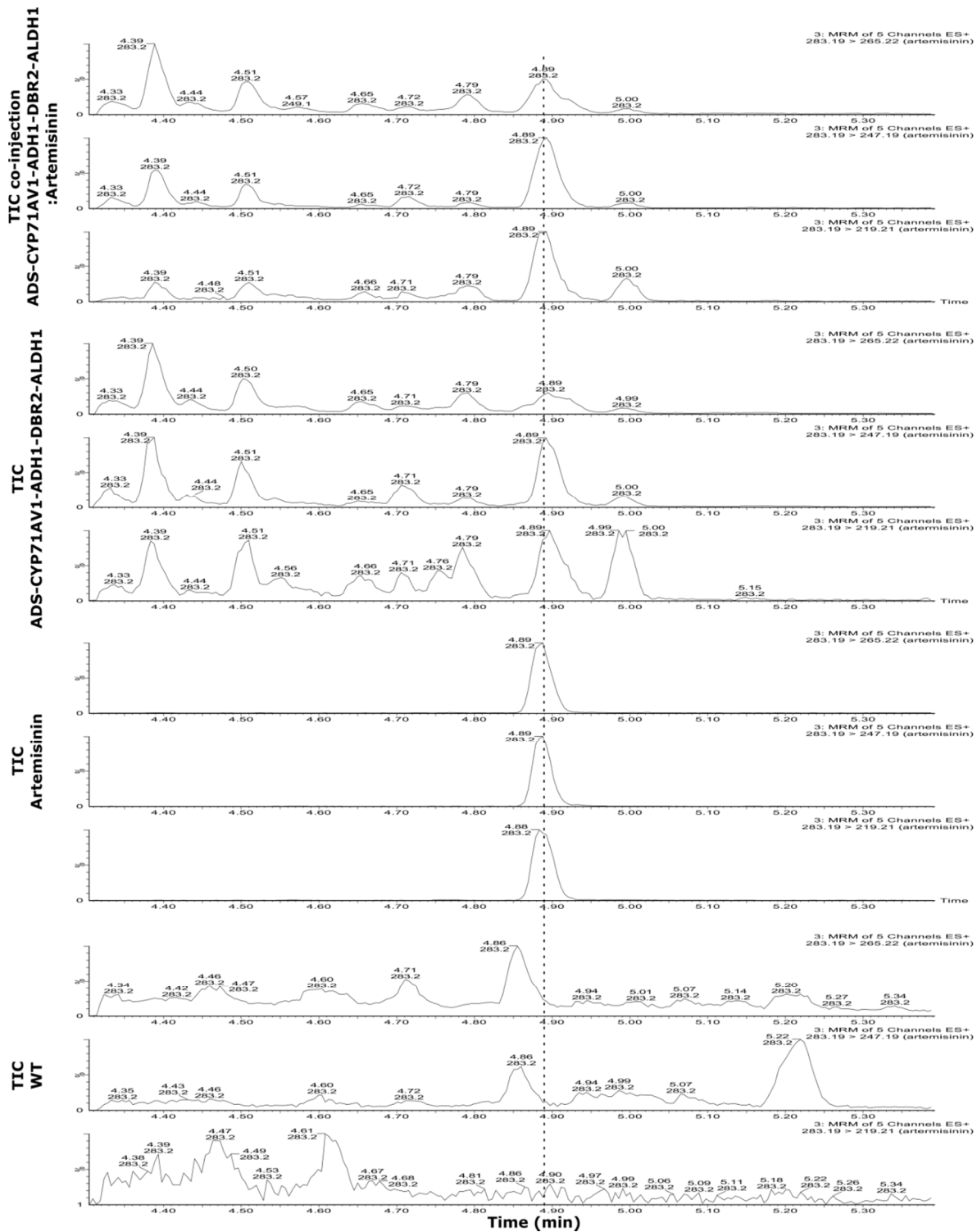


Supplemental data



Supplemental figure 1. Transgenic moss line expressing the five genes via PCR using gene-specific primers of 1. *ADS* (1641bp), 2. *CYP71AV1-LP4/2A-ADHI* (2709bp) and 3. *DBR2-LP4/2A-ALDH1* (2748bp). Primer pair 'a' only bind at the DNA region specific to the wild type (WT) Pp108 locus, giving a band of 2580 bp if the WT locus is intact. This band is absent in the shown transformed line (4). Primer pair 5 and 6 binds at the recombinant region upstream and downstream the 5' and 3' Pp108 genomic locus giving sizes of 1442 and 1525 bp respectively, indicating insertion in the targeted genome locus. The PCR products were sequence-verified.



Supplemental figure 2. UPLC-MRM-MS analysis of artemisinin detection in three transition states (from bottom: WT *P. patens*, transgenic *P.patens*, artemisinin standard and co-injection of transgenic *P. patens*: artemisinin standard (1:1)).

Supplemental Table 1. Primers used

Primer Sequence	Comment
1 TCAGAATTAGATTGACATATATGTTGAAAATGGATCAAAG	Forward primer for HR 5' flanking region
2 ATTCCATTCTTGGTCAGATGAGTTTACTCTTTC	Reverse primer for HR 5' flanking region
3 TAATTCCTTCTTTTTGAGGTATATATTATCTTAGCATGG	Forward primer for HR 3' flanking region
4 ACGAAGGCCGTTCTTCCCTGG	Reverse primer for HR 3' flanking region
5 CCCTGTTGTTTGGTGTACTTCTGCAGGTCGAAGCTAAATGGGCTAACGAAGGC	Forward primer for ADS
6 GGCGTCTCGCATATCTCATTAAAGCAGGACTCAGATGGACATCGGGTAAACCAG	Reverse primer for ADS
7 TAATGAGCATTGCATGTCTAAGTTATAAAAAATTACCAC	Forward primer for ZmUBI promoter
8 AAATAATTATAAAACATACTTGTATTATTATAATAGATAGGTAAGGTTAGAGC	Reverse primer for ZmUBI promoter
9 GTCTCGCATATCTCATTAAAGCAGGAC	Forward primer for OCS terminator
10 GTTACCCGGCCCGCTCCTCAAAAAGAAAGAATTA	Reverse primer for OCS terminator
11 CTAATCCAAAAATGTCAAAGATACAGTCTCAGAAG	Forward primer for G418 selection cassette
12 ACTGGATTTTGGTTTTAGGAATTAGAAATTTTATTGATAGAAG	Reverse primer for G418 selection cassette
13 GGCCCGAGGTCATTCATATGC	Forward primer for rice actin promoter
14 GTGCCATTGCTTTGAGGATAGATTTTATTCTAGAGGATCCCCGATATCTTCTACC	Reverse primer for rice actin promoter
15 ATGAAATCTATCTCAAAGCAATGGCAC	Forward primer for CYP71ADH1-LP4/2A-ADH1
16 AGTAGCAACTTCGCTCTGCTGCATTTGATCAAACTTAATAAGGATTTTCACGCAGTCAGG	Reverse primer for CYP71ADH1-LP4/2A-ADH1
17 AGCGGCCGATCGTTCAAAC	Forward primer for NOS terminator
18 GAGACTGTATCTTTGACATTTTGGAGTATTAGCATTCTTCTGAAAGGAATTCTCATG	Reverse primer for NOS terminator
19 TACTCCAAAAATGTCAAAGATACAGTCTCAGAAG	Forward primer for Hyg selection cassette
20 AGTTTTGATCTTGAAAGATCTTTTATCTTTAGAGTTAAGAACTCTT	Reverse primer for Hyg selection cassette
21 ACAACCAAGCGGCTTGAAACAATAG	Forward primer for AtiUBI promoter
22 GGAGAACAGAGTAGGTTTTTCGGACATCTTTTGTGTTTCGCTTCTCTCACGTAGAAAC	Reverse primer for AtiUBI promoter
23 ATGTCCGAAAAACCTACTCTGTCTCC	Forward primer for DBR2-LP4/2A-ALDH1
24 AGCAAGCAAGAGATGGGATTCTTGATAAGAGTCTCTTCACGCCACGGACTGTCATAG	Reverse primer for DBR2-LP4/2A-ALDH1
25 AGAGACTCTTATCAAGAATCCCATCTCTTGC	Forward primer for Arabidopsis terminator
26 ACTGTATCTTTGACATTTTGGAGTAGAGTTTGGTACGTCACAACTTAAATCATTTTAC	Reverse primer for Arabidopsis terminator
27 CCAATCCGACCTCAAACAGAGC	Forward primer for 5' Pp108 locus
28 TGCTGCATTATGGATGGAAG	Reverse primer for 3' Pp108 locus
29 TGTGTTGAGCATATAAGAAACCCTTAGTATGTA	Forward primer at Tcamv terminator
30 CAGTGACGACAAATCGTTGG	Reverse primer between OCS terminator and 3' 108 locus downstream

Supplemental Table 2. Optimized MRM transition settings for UPLC-MRM-MS measurement of artemisinin, artemisinic acid, dihydroartemisinic acid, artemisinic alcohol and dihydroartemisinic alcohol.

	Parent (<i>m/z</i>)	Daughter (<i>m/z</i>)	Cone voltage	Collision voltage
Artemisinin	283.19	265.22	12	14
		247.19	12	8
		219.21	12	8
Artemisinic acid	235.16	217.21	18	14
		199.25	18	16
		189.22	18	10
Dihydroartemisinic acid	237.16	163.17	16	28
		107.12	16	26
		81.10	16	18
Artemisinic alcohol	221.16	203.27	14	20
		147.09	14	10
Dihydroartemisinic alcohol	223.22	205.27	14	24
		109.13	14	14
		95.07	14	12