Supplementary Material

Directed biosynthesis of new to nature alkaloids in a heterologous *Nicotiana benthamiana* expression host

Marianna Boccia[1], Dagny Grzech[1], Adriana Lopes[2], Sarah E. O'Connor[1*], Lorenzo Caputi[1*]

[1] Max-Planck Institute for Chemical Ecology, Department of Natural Product Biosynthesis; Hans-Knoll Strasse 8, 07745, Jena, Germany.

[2]Univ Ribeirao Preto UNAERP, Unidade Biotecnol, Av Costabile Romano 2201, BR-14096900 Ribeirao Preto, SP - Brazil

*** Correspondence:**
Corresponding Author
lcaputi@ice.mpg.de

oconnor@ice.mpg.de

Figure S1. Vinblastine biosynthetic pathway starting from the precursor strictosidine.

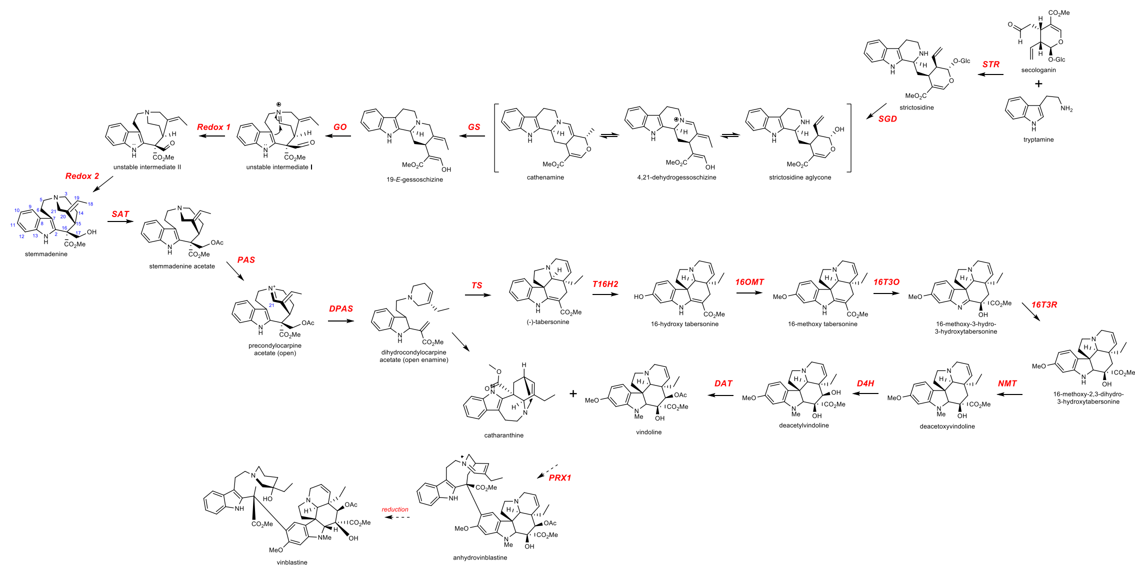

Figure S2. UHPLC-MS chromatograms in MRM mode of 4-fluoro-alstonine produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrTHAS, CrAS. Panel A: MRM chromatogram of alstonine, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 4-fluoro-alstonine produced by co-infiltrating with 4-fluoro-strictosidine. Panel C: MRM chromatogram of the empty vector negative control co-infiltrated with 4-fluoro-strictosidine. Panel D: Fragmentation spectrum of 4-fluoro-alstonine product. 4-fluoro-alstonine=$C_{21}H_{20}O_2N_2F$, Observed *m/z*=367.1444, Theoretical *m/z*=367.1452, Δppm=-2.17. Horizontal axis for panels A, B and C represents the retention time in minutes.

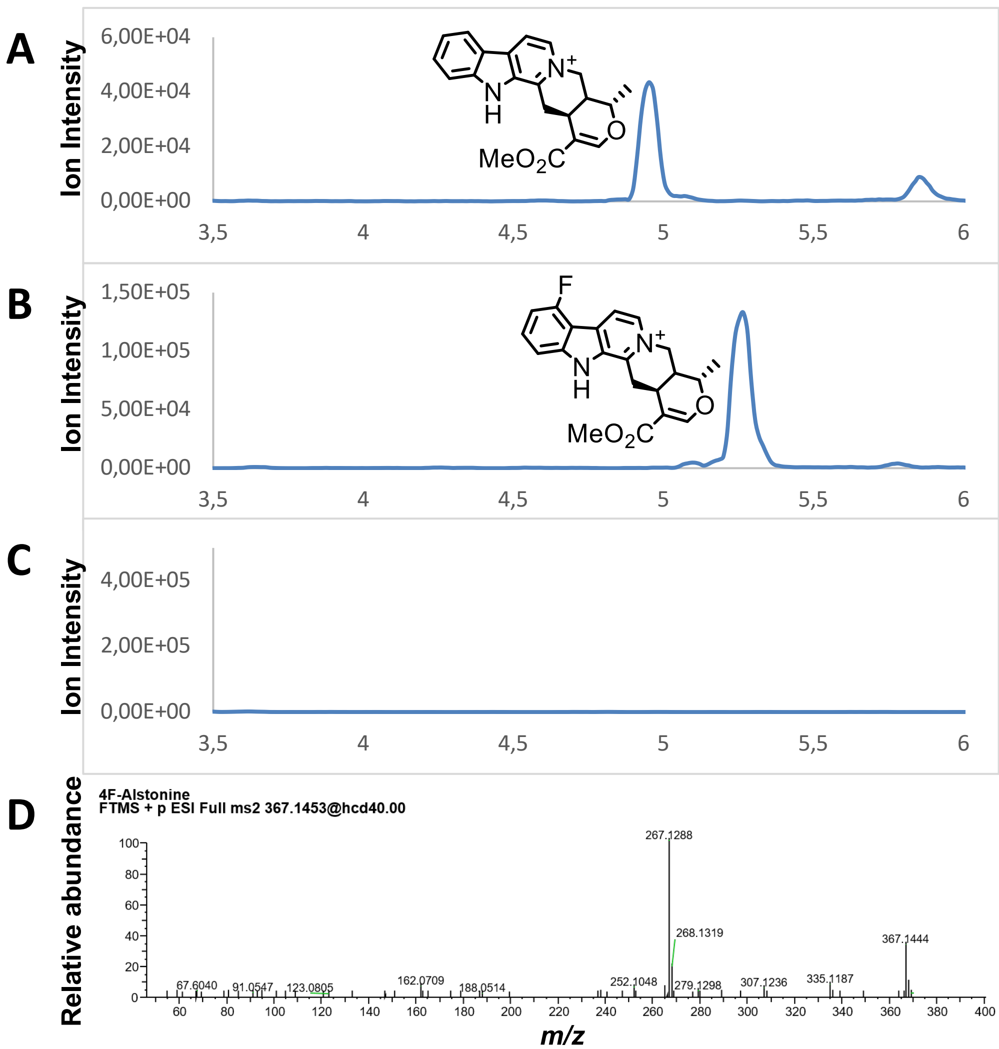

Figure S3. UHPLC-MS chromatograms in MRM mode of 5-fluoro-alstonine produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrTHAS, CrAS. Panel A: MRM chromatogram of alstonine, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 5-fluoro-alstonine produced by co-infiltrating with 5-fluoro-strictosidine. Panel C: MRM chromatogram of the empty vector negative control co-infiltrated with 5-fluoro-strictosidine. Panel D: Fragmentation spectrum of 5-fluoro-alstonine product. 5-fluoro-alstonine=$C_{21}H_{20}O_2N_2F$, Observed *m/z*=367.1452, Theoretical *m/z*=367.1452, Δppm=-0.12. Horizontal axis for panels A, B and C represents the retention time in minutes.

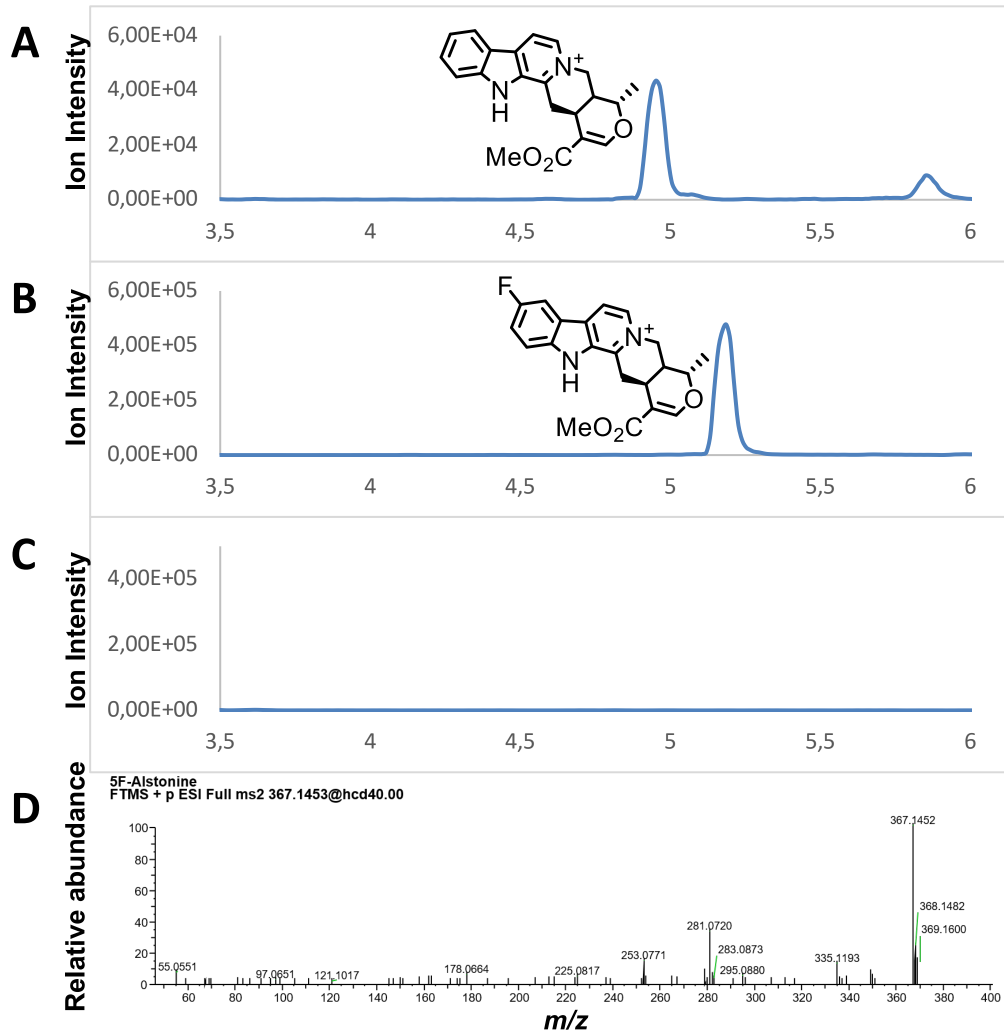

Figure S4. UHPLC-MS chromatograms in MRM mode of 6-fluoro-alstonine produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrTHAS, CrAS. Panel A: MRM chromatogram of alstonine, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 6-fluoro-alstonine produced by co-infiltrating with 6-fluoro-strictosidine. Panel C: MRM chromatogram of the empty vector negative control co-infiltrated with 6-fluoro-strictosidine. Panel D: Fragmentation spectrum of 6-fluoro-alstonine product. 6-fluoro-alstonine=$C_{21}H_{20}O_2N_2F$, Observed *m/z*=367.1449, Theoretical *m/z*=367.1452, Δppm=-0.87. Horizontal axis for panels A, B and C represents the retention time in minutes.

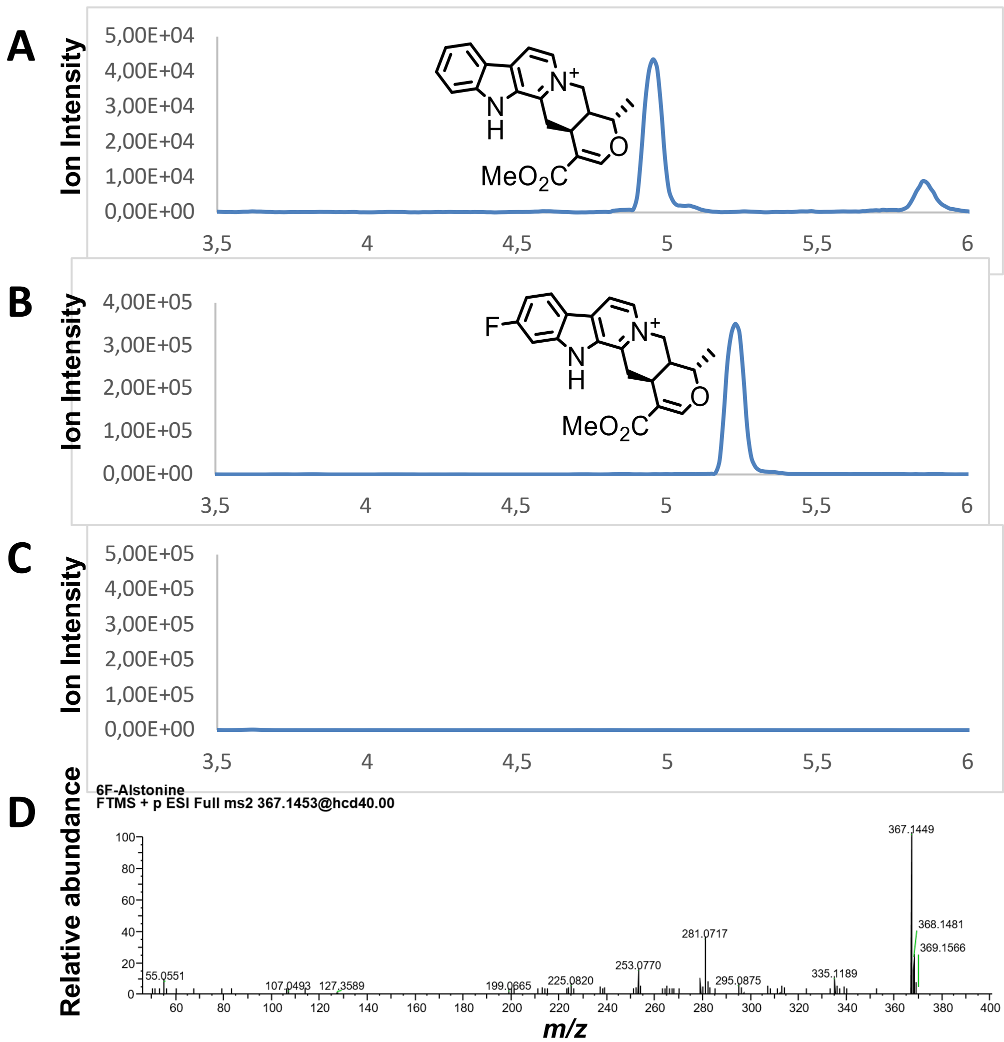

Figure S5. UHPLC-MS chromatograms in MRM mode of 7-fluoro-alstonine produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrTHAS, CrAS. Panel A: MRM chromatogram of alstonine, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 7-fluoro-alstonine produced by co-infiltrating with 7-fluoro-strictosidine. Panel C: MRM chromatogram of the empty vector negative control co-infiltrated with 7-fluoro-strictosidine. Panel D: Fragmentation spectrum of 7-fluoro-alstonine product. 7-fluoro-alstonine=$C_{21}H_{20}O_2N_2F$, Observed *m/z*=367.1451, Theoretical *m/z*=367.1452, Δppm=0.27. Horizontal axis for panels A, B and C represents the retention time in minutes.

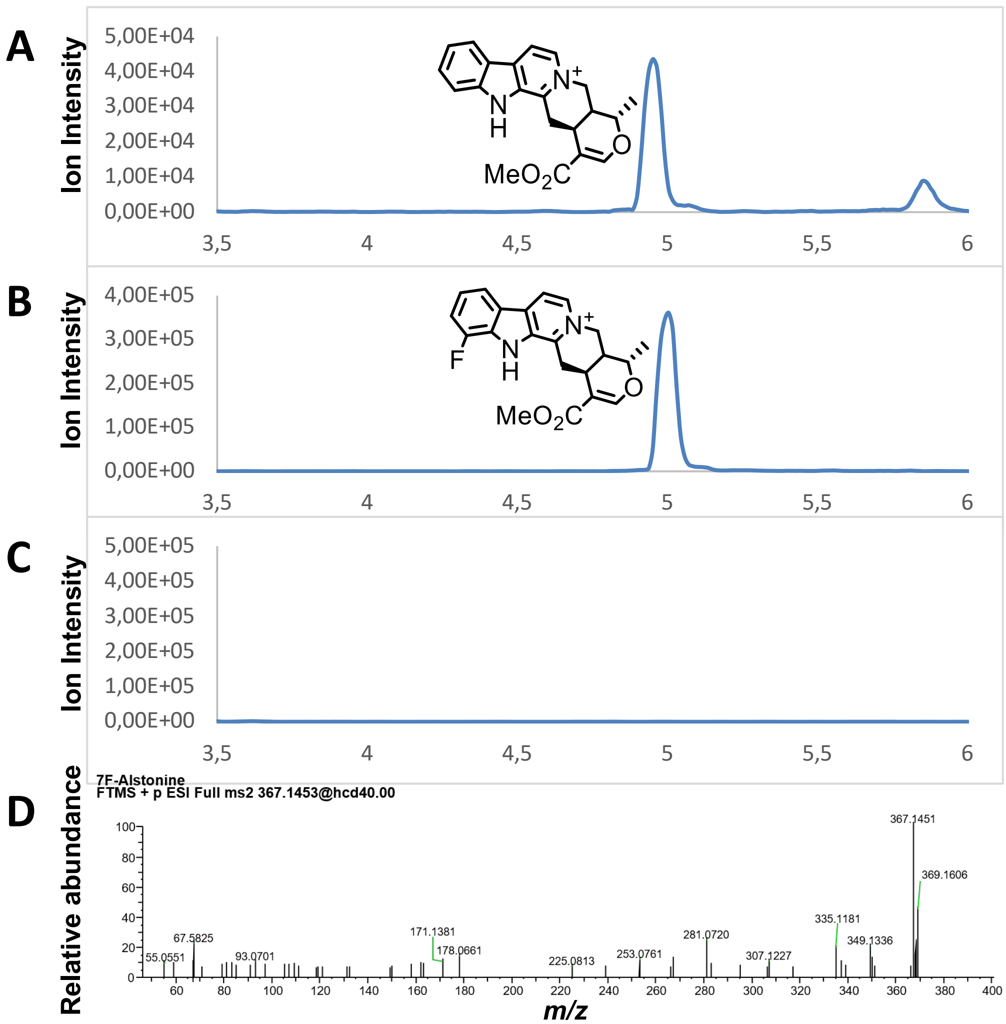

Figure S6. UHPLC-MS chromatograms in MRM mode of 7-chloro-alstonine produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrTHAS, CrAS. Panel A: MRM chromatogram of alstonine, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 7-chloro-alstonine produced by co-infiltrating with 7-chloro-strictosidine. Panel C: MRM chromatogram of the empty vector negative control co-infiltrated with 7-chloro-strictosidine. Panel D: Fragmentation spectrum of 7-chloro-alstonine product. 7-chloro-alstonine=$C_{21}H_{20}O_2N_2Cl$, Observed *m/z*=383.1153, Theoretical *m/z*=383.1157, Δppm=-1.05. Horizontal axis for panels A, B and C represents the retention time in minutes.

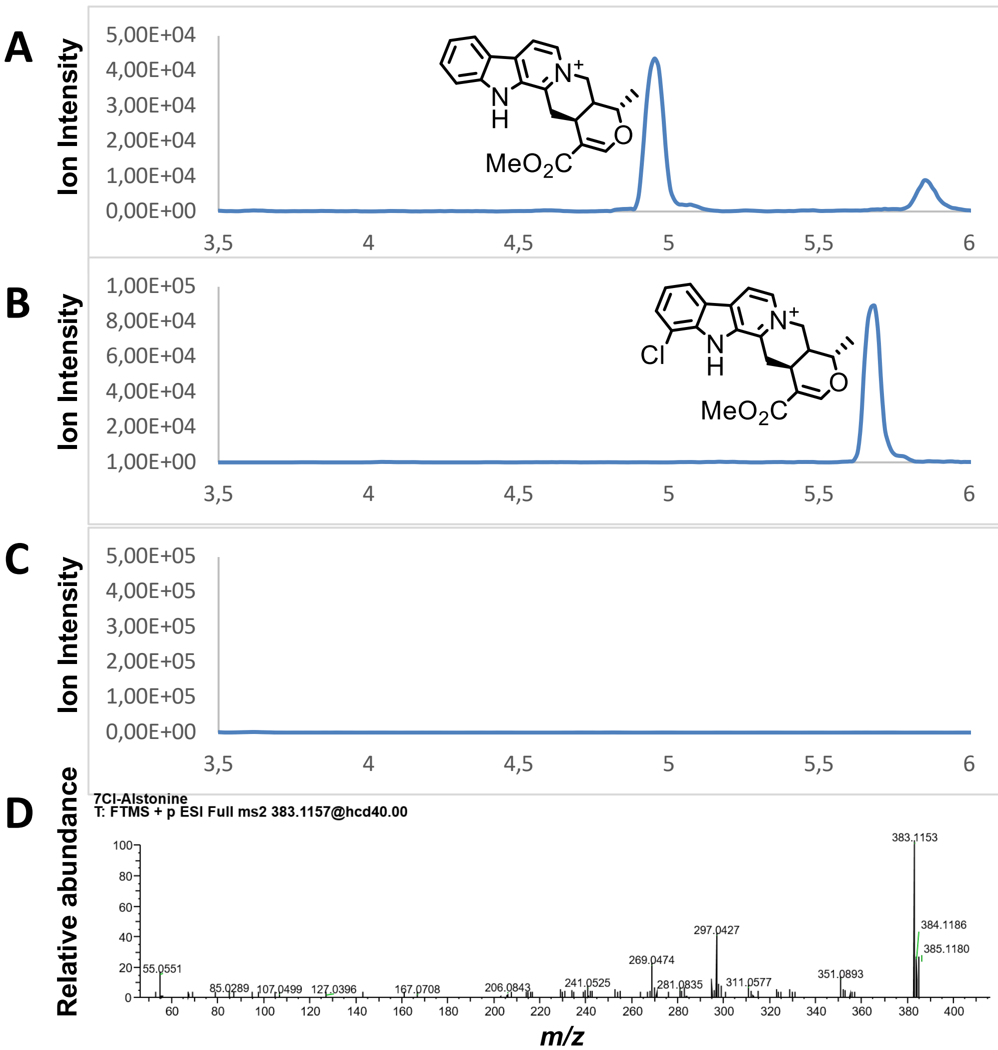

Figure S7. Comparison of the fragmentation spectra of serpentine standard (panel A) and alstonine produced in *N. benthamiana* (panel B) by co-infiltrating the genes CrSGD, CrTHAS, CrAS. Serpentine=$C_{21}H_{21}O_3N_2$, Observed *m/z*=349.1547, Theoretical *m/z*=349.1546, Δppm=0.07. Alstonine=$C_{21}H_{21}O_3N_2$, Observed *m/z*=349.1544, Theoretical *m/z*=349.1546, Δppm=-0.89. The data are in agreement with what reported in the literature (Kumar S., Singh A. Bajpai V., Srivastava M., Singh B.P. and Kumar B., Structural characterization of monoterpene indole alkaloids in ethanolic extracts of Rauwolfia species by liquid chromatography with quadrupole time-of-flight mass pectrometry, *J. Pharm. Anal.*, 2016, 363-373.)

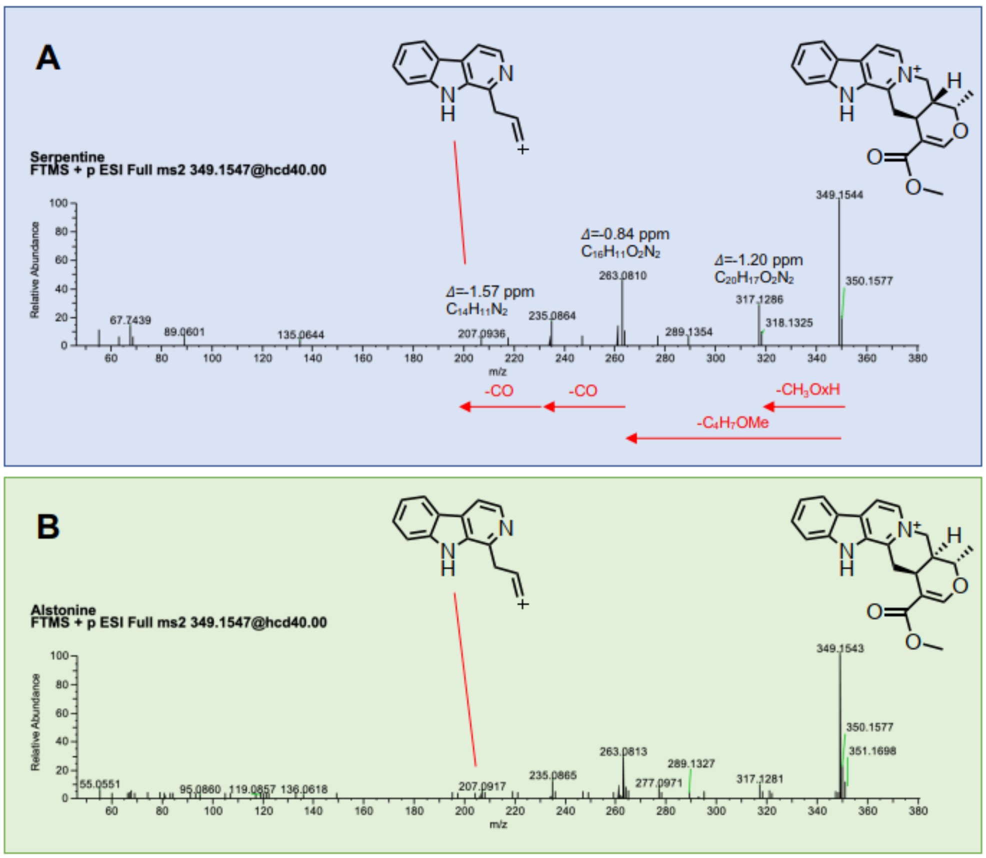

Figure S8. UHPLC-MS chromatograms in MRM mode of methyl and methoxy-tetrahydroalstonine produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrTHAS, CrAS. Panel A: MRM chromatogram of tetrahydroalstonine, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 6-methyl-tetrahydroalstonine produced by co-infiltrating with 6-methyl-strictosidine. Panel C: MRM chromatogram of 5-methoxy-tetrahydroalstonine produced by co-infiltrating with 5-methoxy-strictosidine. Panel D: MRM chromatogram of the empty vector negative control co-infiltrated with 5-methoxy- or 6-methyl-strictosidine. *unknown compound. Horizontal axis represents the retention time in minutes.

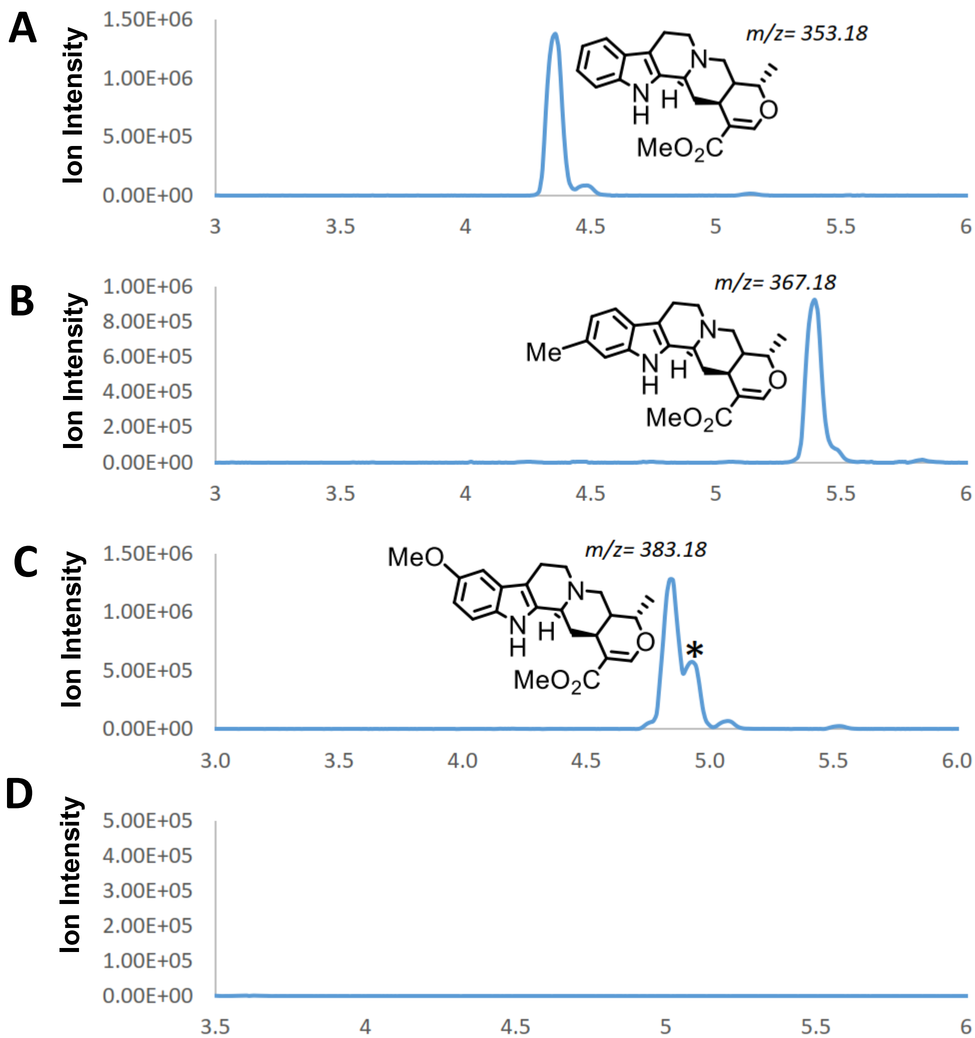

Figure S9. Fragmentation spectrum of stemmadenine acetate standard. Molecular Formula= $C_{23}H_{28}O_4N_2$, $[M+H]^+$ Observed *m/z*=397.2115, Theoretical *m/z*=397.2121, Δppm=-1.60.

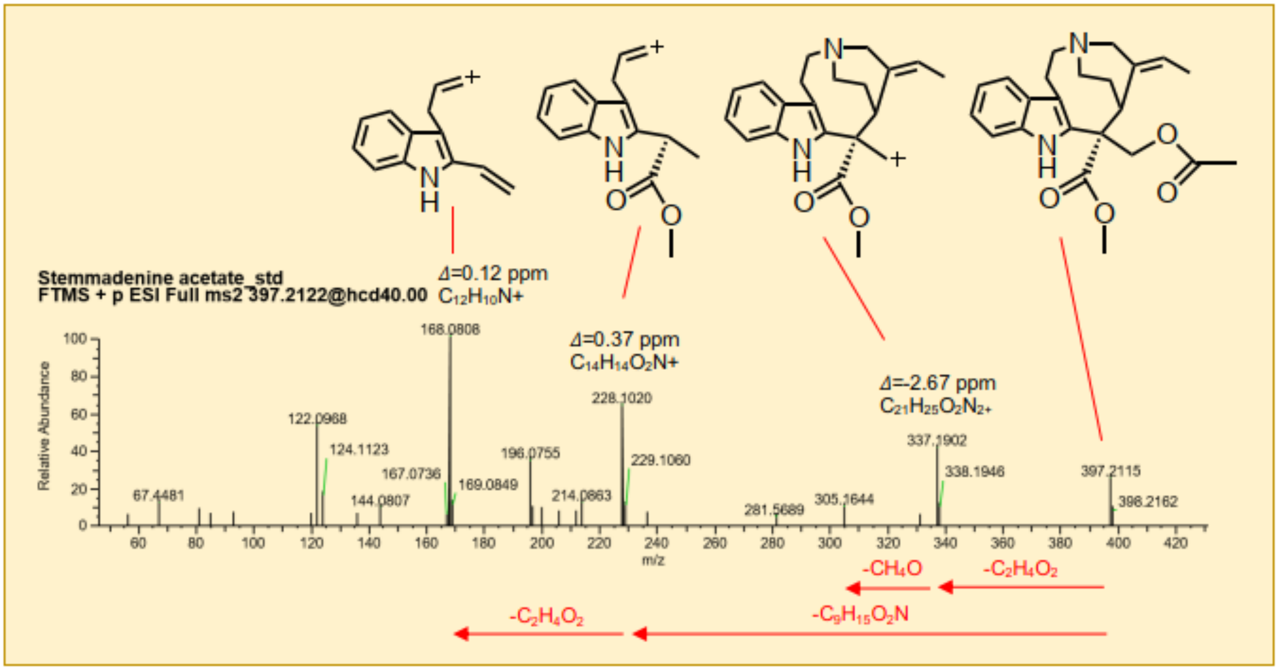

Figure S10. UHPLC-MS chromatograms in MRM mode of 4-fluoro-stemmadenine acetate produced in *N. benthamiana* leaves by co-infiltrating the genes CrSGD, CrGS, CrGO, CrRedOx1, CrRedOx2, CrSAT. Panel A: MRM chromatogram of stemmadenine acetate, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 4-fluoro-stemmadenine acetate, produced by co-infiltrating with 4-fluoro-strictosidine. Panel C: MRM chromatogram of the empty vector negative control co-infiltrated with 4-fluoro-strictosidine. Panel D: Fragmentation spectrum of 4-fluoro-stemmadenine acetate product. 4-fluoro-stemmadenine acetate=$C_{23}H_{28}O_4N_2F$, Observed *m/z*=415.2018, Theoretical *m/z*=415.2027, Δppm=-2.29. Horizontal axis for panels A, B and C represents the retention time in minutes.

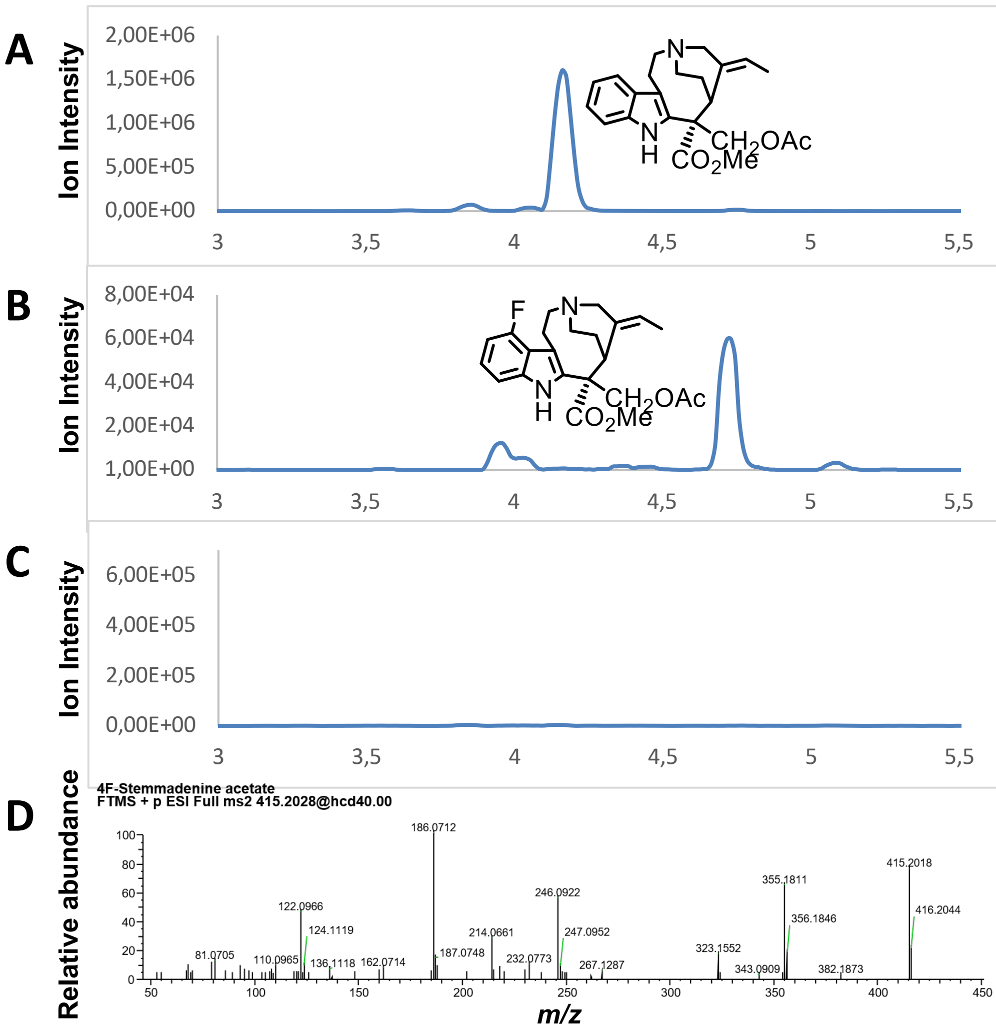

Figure S11. UHPLC-MS chromatograms in MRM mode of 5-fluoro-stemmadenine acetate produced in *N. benthamiana* leaves by co-infiltrating the genes CrSGD, CrGS, CrGO, CrRedOx1, CrRedOx2, CrSAT. Panel A: MRM chromatogram of stemmadenine acetate, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 5-fluoro-stemmadenine acetate, produced by co-infiltrating with 5-fluoro-strictosidine. Panel C: MRM chromatogram of the empty vector negative control co-infiltrated with 5-fluoro-strictosidine. Panel D: Fragmentation spectrum of 5-fluoro-stemmadenine acetate product. 5-fluoro-stemmadenine acetate=$C_{23}H_{28}O_4N_2F$, Observed *m/z*=415.2023, Theoretical *m/z*=415.2027, Δppm=0.96. Horizontal axis for panels A, B and C represents the retention time in minutes.

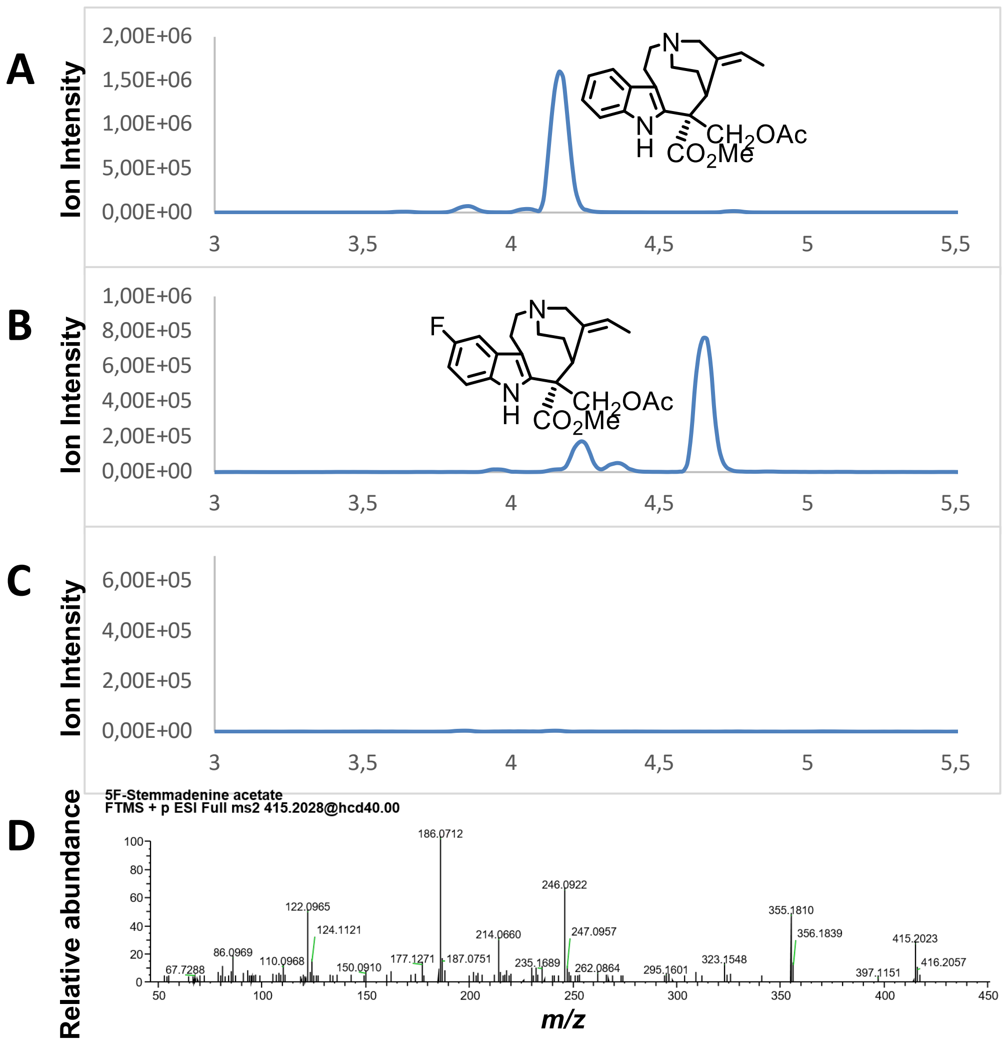

Figure S12. UHPLC-MS chromatograms in MRM mode of 6-fluoro-stemmadenine acetate produced in *N. benthamiana* leaves by co-infiltrating the genes CrSGD, CrGS, CrGO, CrRedOx1, CrRedOx2, CrSAT. Top panel: MRM chromatogram of stemmadenine acetate, produced by co-infiltrating with strictosidine. Middle panel: MRM chromatogram of 6-fluoro-stemmadenine acetate, produced by co-infiltrating with 6-fluoro-strictosidine. Bottom panel: MRM chromatogram of the empty vector negative control co-infiltrated with 6-fluoro-strictosidine. Panel D: Fragmentation spectrum of 6-fluoro-stemmadenine acetate product. 6-fluoro-stemmadenine acetate=$C_{23}H_{28}O_4N_2F$, Observed *m/z*=415.2018, Theoretical *m/z*=415.2027, Δppm=-1.62. Horizontal axis for panels A, B and C represents the retention time in minutes.

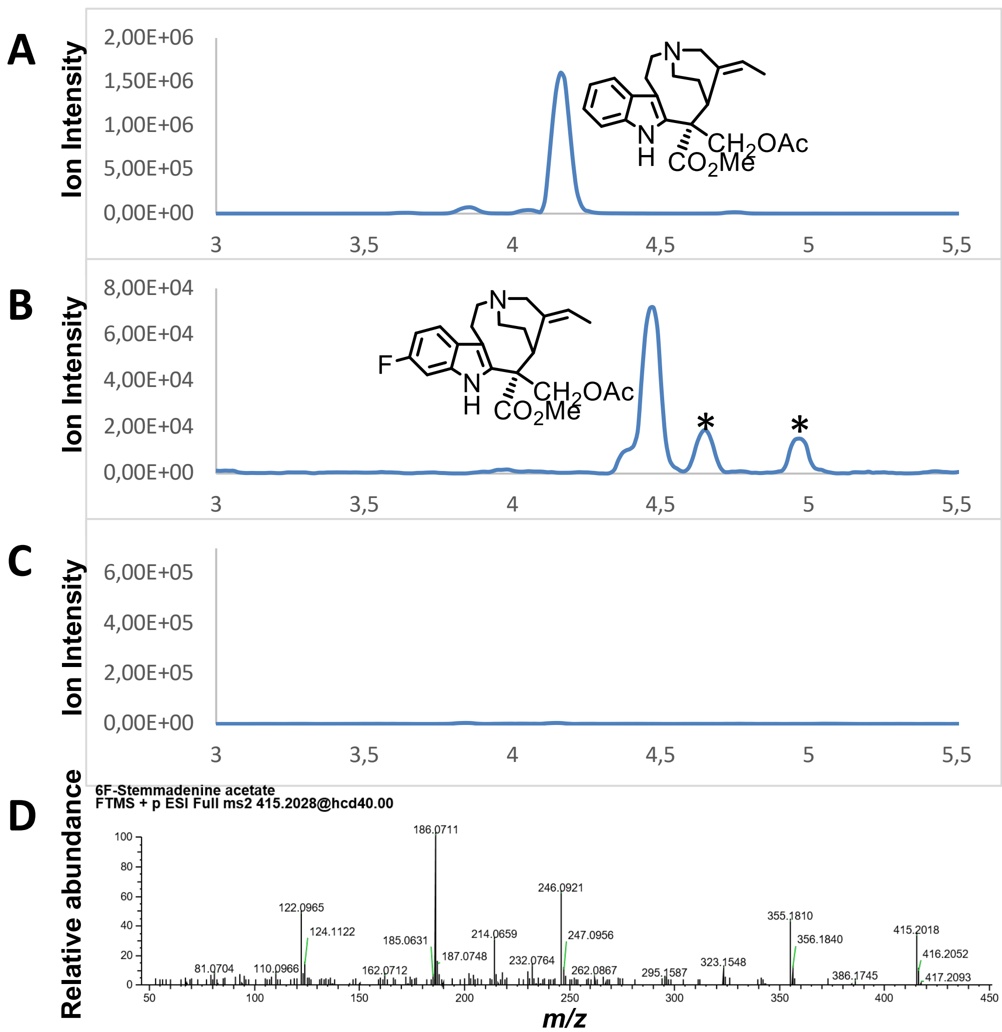

Figure S13. UHPLC-MS chromatograms in MRM mode of 7-fluoro-stemmadenine acetate produced in *N. benthamiana* leaves by co-infiltrating the genes CrSGD, CrGS, CrGO, CrRedOx1, CrRedOx2, CrSAT. Panel A: MRM chromatogram of stemmadenine acetate, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 7-fluoro-stemmadenine acetate, produced by co-infiltrating with 7-fluoro-strictosidine. Panel C: MRM chromatogram of the empty vector negative control co-infiltrated with 7-fluoro-strictosidine. Horizontal axis for panels A, B and C represents the retention time in minutes.

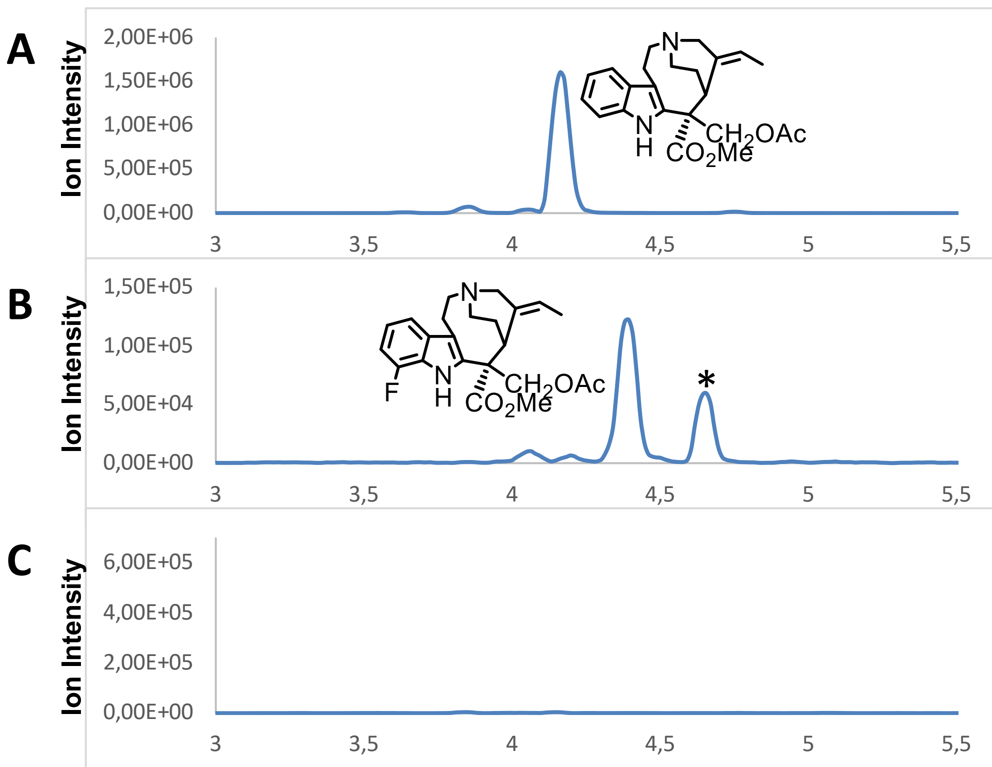

Figure S14. UHPLC-MS chromatograms in MRM mode of the shunt product fluoro-akuammicine produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrGS, CrGO, CrRedOx1, CrRedOx2, CrSAT. Panel A: MRM chromatogram of akuammicine, produced by co-infiltrating with strictosidine. Panels B and C: MRM chromatogram of 4, 5, 6, and 7-fluoro-akuammicine, produced by co-infiltrating with 4, 5, 6, 7-fluoro-strictosidine, respectively. Panel D: MRM chromatogram of the empty vector negative control co-infiltrated with 5-fluoro-strictosidine. Horizontal axis represents the retention time in minutes.

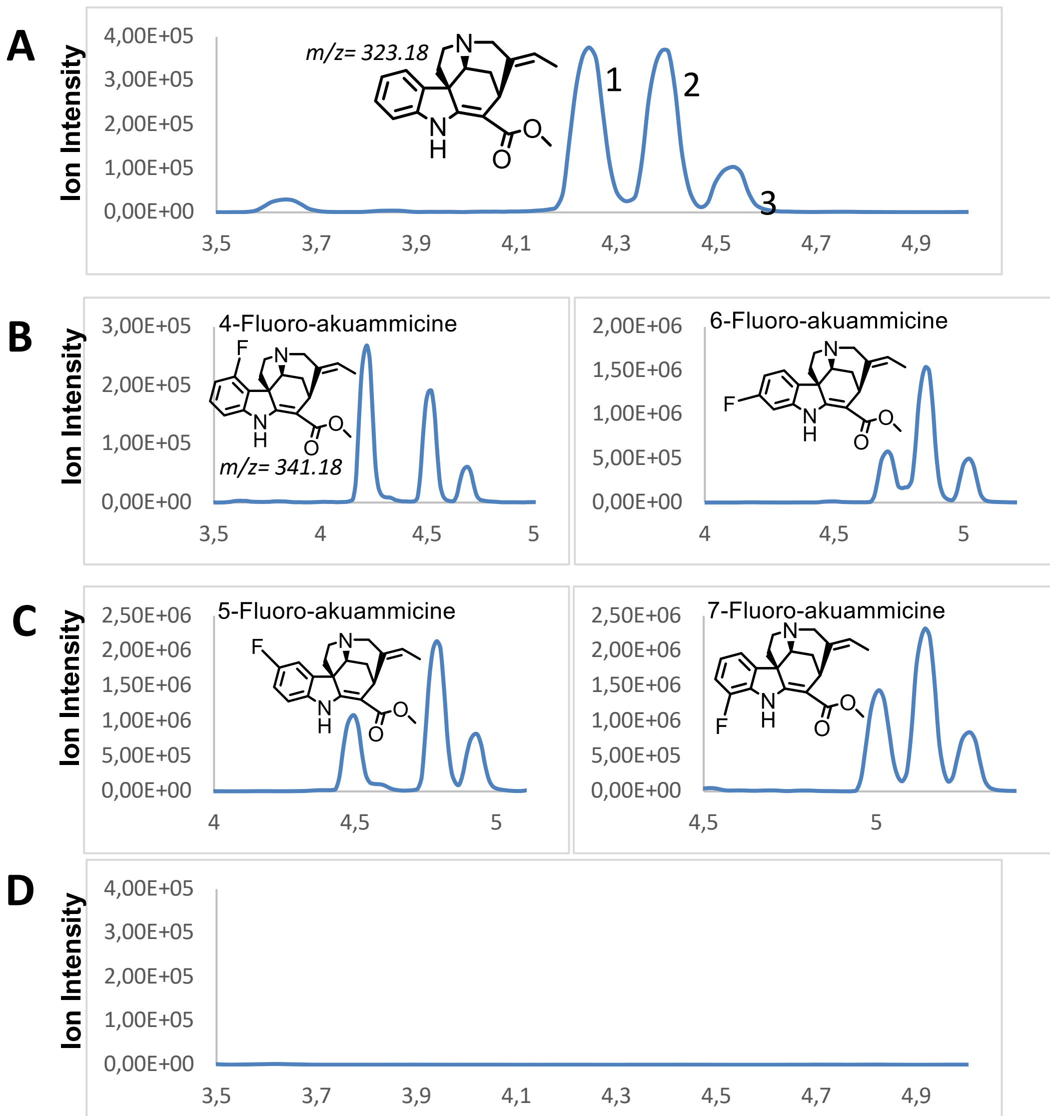

Figure S15. UHPLC-MS chromatograms in MRM mode of fluoro-precondylocarpine acetate produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrGS, CrGO, CrRedOx1, CrRedOx2, CrSAT. Panel A: MRM chromatogram of precondylocarpine acetate, produced by co-infiltrating with strictosidine. Panels B and C: MRM chromatogram of 4, 5, 6, and 7-fluoro- precondylocarpine acetate, produced by co-infiltrating with 4, 5, 6, 7-fluoro-strictosidine, respectively. Panel D: MRM chromatogram of the empty vector negative control co-infiltrated with 5-fluoro-strictosidine. Horizontal axis represents the retention time in minutes.

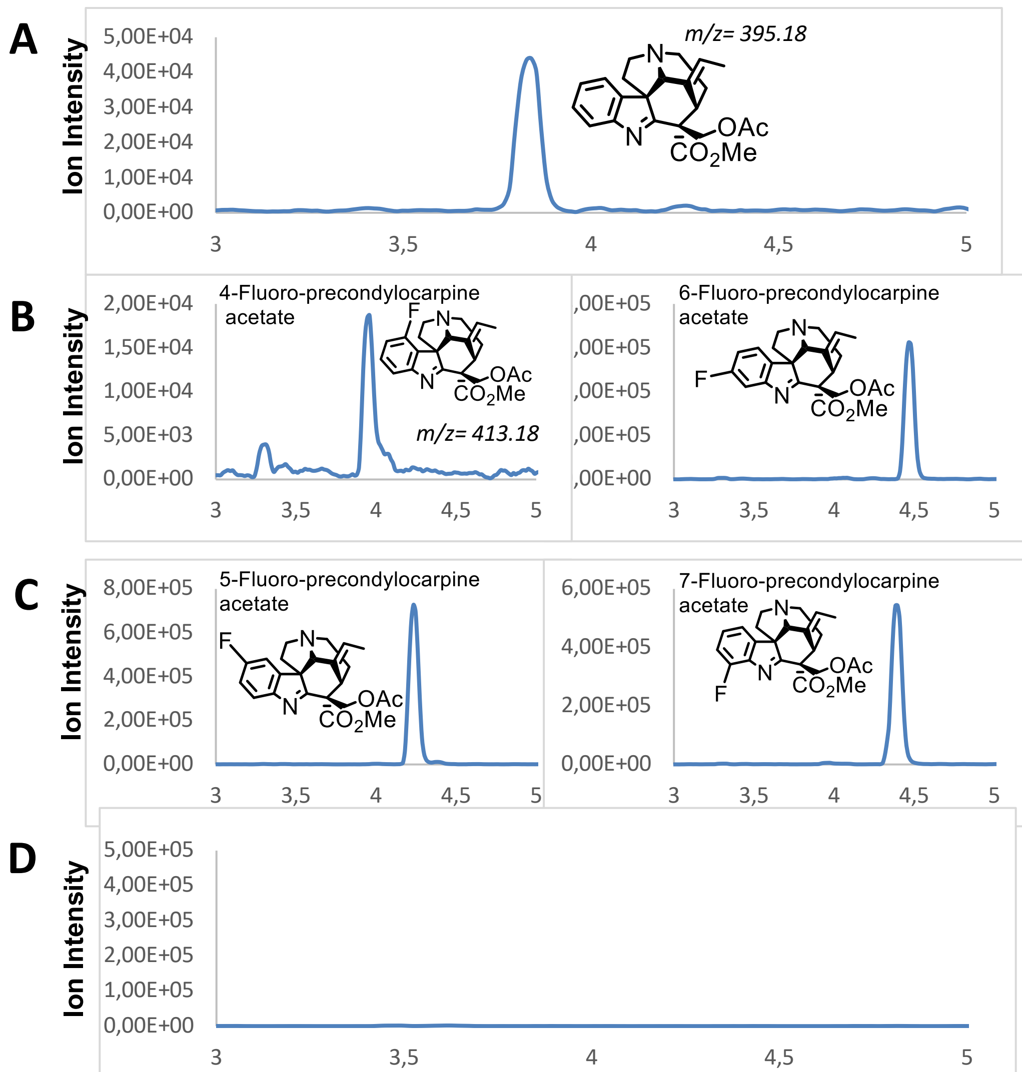

Figure S16. UHPLC-MS chromatograms in MRM mode of the shunt product 6-methyl-akuammicine produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrGS, CrGO, CrRedOx1, CrRedOx2, CrSAT. Panel A: MRM chromatogram of akuammicine, produced by co-infiltrating with strictosidine. Panel B: MRM chromatogram of 6-methyl-akuammicine, produced by co-infiltrating with 6-methyl-strictosidine, respectively. Panel 3: MRM chromatogram of 7-methyl-akuammicine, produced by co-infiltrating with 7-methyl-strictosidine, respectively. Panel D: MRM chromatogram of the empty vector negative control co-infiltrated with 6-methyl-strictosidine. #1; #2; #3 akuammicine isomers. Horizontal axis represents the retention time in minutes.

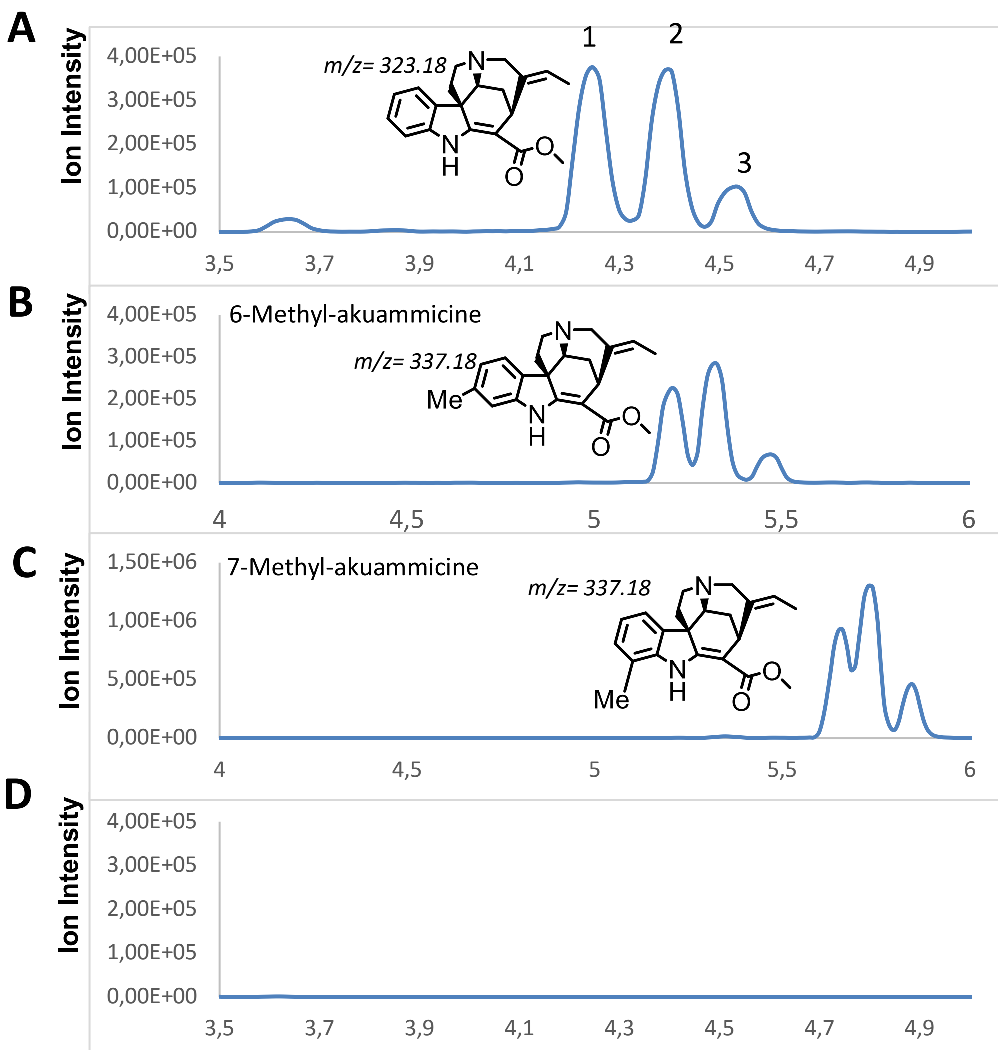

Figure S17. UHPLC-MS chromatograms in MRM mode of alkaloids produced in *N. benthamiana* leaves produced by co-infiltrating the genes CrSGD, CrGS, CrGO, CrRedOx1, CrRedOx2, CrSAT and chloro-strictosidine as substrate. Panel A: MRM chromatogram of stemmadenine acetate (left) and 6-Chloro-stemmadenine acetate (right), produced by co-infiltrating with strictosidine and 6-chloro-strictosidine, respectively. Panel B: MRM chromatogram of akuammicine and 6-chloro-akuammicine, produced by co-infiltrating with strictosidine and 6-chloro-strictosidine, respectively. Panel C: MRM chromatogram of MRM trace of precondylocarpine acetate (left) and 6-chloro-precondylocarpine acetate (right), produced by co-infiltrating with strictosidine and 6-chloro-strictosidine, respectively. Horizontal axis represents the retention time in minutes.

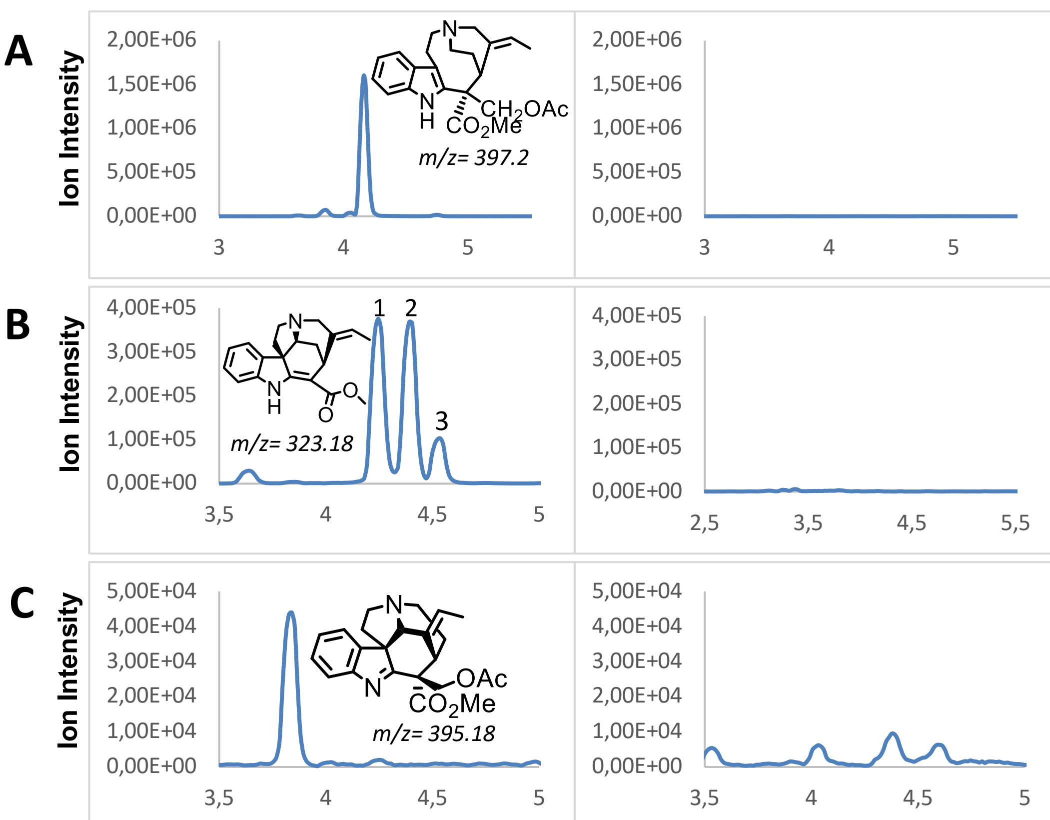

Table S1. Tryptamine analogs

Compound	*Company*
4-Fluoro tryptamine	Sigma Aldrich
5-Fluoro tryptamine	Sigma Aldrich
6-Fluoro tryptamine	Sigma Aldrich
7-Fluoro tryptamine	Sigma Aldrich
4-Methoxy tryptamine	Sigma Aldrich
5-Methoxy tryptamine	Sigma Aldrich
6-Methoxy tryptamine	Sigma Aldrich
7-Methoxy tryptamine	Sigma Aldrich
5-Chloro tryptamine	Alfa Aesar
6-Chloro tryptamine	Cayman chemical
7-Chloro tryptamine	Sigma Aldrich
5-Methyl tryptamine	Sigma Aldrich
6-Methyl tryptamine	BIOSYNTH
7-Methyl tryptamine	Sigma Aldrich
6-Hydroxy tryptamine	Sigma Aldrich
7-Bromo tryptamine	Fluorochem
6-Hydroxy tryptamine	TRC

Table S2. Sequences of the genes used in this study. Genbank accession numbers: CrSGD (AF112888), CrGS (MF770507), CrGO (MF770508), CrRedox1 (MF770509), CrRedox2 (MF770510), CrSAT (MF770511), CrTHAS (KM524258.1), CrAS (CYP71AY1), SlUbi10p and SlUbi10t (SOLYC01G096290.2).

CrSGD	AGGTCTCTAATGGGTTCTAAGGATGATCAATCACTTGTCGTGGCTATCAGTCCTGCCG CCGAGCCTAACGGGAACCACTCAGTTCCTATACCGTTTGCATATCCGAGCATACCTAT ACAGCCGCGAAAACATAATAAACCTATAGTACACAGGCGGGACTTTCCTTCTGACTT TATTCTTGGAGCTGGCGGTTCCGCGTACCAATGCGAAGGCGCTTACAACGAGGGAAA TAGAGGACCAAGCATTTGGGACACATTTACTAATAGATACCCTGCAAAGATTGCAGA CGGCAGTAACGGAAACCAGGCAATTAACAGCTATAACTTATACAAAGAGGACATAA AGATAATGAAACAGACTGGGCTTGAGAGCTACCGATTCTCTATCTCTTGGTCCCGAGT TCTGCCTGGTGGAAACTTGAGTGGCGGGGTTAACAAGGACGGAGTGAAATTTTACCA CGATTTCATTGACGAACTACTGGCAAACGGTATTAAGCCTTTCGCCACCTTGTTTCAT TGGGACCTGCCTCAGGCGTTAGAAGATGAATACGGTGGATTTCTCAGCGACCGCATA GTTGAGGACTTCACAGAATACGCTGAGTTCTGTTTCTGGGAGTTTGGCGATAAGGTTA AGTTCTGGACAACATTTAACGAGCCTCACACATACGTCGCTTCAGGGTACGCTACGG GAGAGTTCGCCCCCGGCAGGGGCGGAGCTGACGGAAAAGGTGAGCCAGGAAAGGAG CCTTACATTGCTACCCACAACCTTTTGCTCTCACATAAGGCAGCAGTAGAGGTTTACA GAAAGAACTTCCAAAAGTGCCAGGGCGGAGAGATCGGCATAGTCTTAAACTCTATGT GGATGGAACCCCTTAACGAGACTAAGGAAGATATCGACGCACGCGAGCGTGGACTC GACTTTATGCTTGGTTGGTTTATTGAACCTCTCACTACAGGAGAGTATCCTAAGAGTA TGCGGGCACTCGTTGGTAGTCGGTTGCCTGAGTTCTCCACAGAGGTGTCAGAGAAGC TCACTGGCTGTTACGACTTCATTGGTATGAACTACTACACTACAACATACGTAAGCAA CGCCGATAAGATCCCTGACACCCCTGGATATGAAACCGACGCAAGAATCAACAAGA ACATATTCGTGAAGAAGGTAGACGGTAAAGAGGTTAGAATAGGCGAGCCATGTTAC GGTGGTTGGCAACACGTGGTGCCTTCCGGTCTATATAACCTACTTGTGTATACCAAAG AAAAGTATCACGTACCCGTTATATACGTTAGCGAGTGCGGCGTAGTAGAAGAGAACA GGACAAATATTTTGTTGACTGAGGGCAAGACAAATATCCTTTTGACTGAGGCCAGAC ATGACAAGCTTCGAGTGGACTTCTTGCAGTCACACCTAGCCTCAGTTCGGGACGCAA TAGACGACGGAGTCAACGTTAAGGGGTTCTTCGTATGGAGTTTCTTTGATAATTTTGA GTGGAACCTTGGTTACATCTGTAGATACGGTATAATTCACGTAGACTACAAGACATTC CAGCGATACCCTAAAGACTCCGCTATTTGGTATAAGAACTTTATCTCAGAGGGCTTCG TGACTAACACCGCAAAGAAACGTTTTAGGGAAGAGGACAAGCTGGTAGAACTTGTTA AGAAACAGAAGTATTAAGCTTAGAGACCT
CrGS	ATGGCCGGAGAAACAACCAAACTCGACCTTTCAGTGAAGGCTGTGGGATGGGGTGCT GCAGATGCATCTGGTGTCCTTCAGCCCATCAAGTTCTATAGAAGAGTCCCTGGTGAAC GGGATGTGAAGATTAGAGTTTTGTACTCTGGTGTTTGCAATTTCGATATGGAAATGGT CAGAAACAAGTGGGGTTTCACCAGATATCCTTATGTGTTTGGACATGAAACTGCCGG TGAGGTGGTAGAAGTTGGCAGCAAAGTAGAGAAATTCAAGGTTGGAGACAAGGTAG CTGTGGGATGTATGGTCGGATCTTGTGGTCAATGTTATAATTGTCAAAGTGGAATGGA GAATTACTGCCCAGAGCCCAATATGGCTGATGGATCTGTTTACCGTGAGCAAGGGGA ACGATCCTATGGGGGTTGTTCAAATGTGATGGTTGTTGATGAAAAGTTCGTCCTCCGA TGGCCCGAAAACTTGCCTCAAGATAAAGGGGTTGCTCTCCTTTGTGCTGGGGTTGTTG TTTATAGCCCAATGAAACATTTGGGACTCGATAAGCCAGGAAAGCATATTGGGGTTT TCGGGCTGGGAGGTCTTGGTTCTGTTGCTGTTAAGTTTATTAAGGCTTTTGGTGGTAA GGCTACTGTTATTAGTACATCAAGGCGTAAGGAGAAGGAAGCCATTGAAGAACATGG TGCTGATGCTTTTGTTGTCAACACTGACTCTGAGCAATTGAAGGCTCTGGCAGGTACT ATGGATGGTGTTGTGGACACCACCCCAGGTGGCCGCACTCCTATGTCACTTATGCTCA ATTTGCTCAAGTTTGACGGCGCGGTTATGCTCGTAGGTGCACCGGAGTCGCTATTTGA GCTCCCTGCGGCACCTCTCATTATGGGAAGGAAAAAGATAATCGGAAGTTCCACTGG AGGCCTCAAAGAGTACCAAGAAATGCTTGATTTCGCAGCCAAACATAACATTGTATG TGATACTGAAGTTATTGGGATTGACTATCTCAGCACTGCTATGGAACGTATCAAGAAT TTGGATGTCAAGTACCGTTTTGCGATTGACATTGGAAATACATTGAAATTTGAGGAAT AA

CrGO	ATGGAGTTTTCTTTCTCCTCACCAGCTCTCTACATAGTTTATTTCTTGCTTTTCTTTGTG GTAAGGCAATTATTGAAACCCAAAAGTAAGAAAAAATTGCCACCAGGTCCAAGAAC ACTACCCTTAATTGGAAACCTTCATCAACTCTCGGGACCTTTACCTCATCGTACCCTA AAAAATTTGTCCGATAAACATGGTCCTTTGATGCACGTGAAAATGGGCGAACGTTCG GCAATTATAGTATCAGATGCAAGAATGGCAAAAATAGTTCTTCATAATAACGGTTTA GCCGTTGCAGATCGGTCAGTAAATACTGTCGCAAGTATTATGACTTATAATAGTTTGG GTGTTACCTTTGCTCAGTATGGAGATTACTTAACAAAATTACGTCAAATCTATACTTT GGAACTTTTAAGTCAGAAAAAAGTTCGATCTTTCTACAGTTGTTTTGAAGATGAACTC GATACTTTTGTTAAGTCAATTAAGTCTAACGTTGGACAACCTATGGTTTTGTACGAGA AAGCTTCTGCTTATTTGTATGCTACCATTTGTAGAACTATTTTTGGGAGTGTTTGCAAG GAAAAGGAGAAAATGATTAAGATAGTGAAGAAAACGTCGTTACTGTCTGGAACACC ACTTAGATTGGAGGATCTTTTTCCTTCTATGAGCATTTTTTGTAGATTTAGTAAAACTT TGAATCAATTGAGAGGACTTTTGCAAGAAATGGATGATATTTTGGAAGAAATCATAG TTGAAAGGGAAAAGGCTTCTGAAGTCTCAAAAGAGGCAAAGGATGATGAAGATATG CTTAGTGTTCTGTTGAGGCATAAATGGTACAATCCAAGTGGTGCTAAATTCAGAATCA CTAATGCAGACATCAAAGCCATTATCTTTGAGTTGATCTTAGCAGCAACCCTAAGTGT AGCAGATGTTACAGAATGGGCTATGGTTGAAATTCTAAGGGATCCCAAAAGTTTGAA AAAAGTGTACGAAGAAGTACGAGGTATTTGTAAAGAAAAGAAAAGAGTGACAGGAT ACGACGTTGAAAAAATGGAATTTATGCGTCTTTGTGTGAAGGAATCAACAAGAATAC ATCCAGCTGCTCCTCTTTTAGTTCCTAGAGAATGCAGAGAAGATTTTGAAGTAGATGG ATACACAGTTCCAAAAGGGGCTTGGGTTATAACAAATTGCTGGGCTGTTCAAATGGA TCCTACAGTATGGCCCGAACCAGAGAAGTTTGATCCAGAAAGATATATTCGTAACCC AATGGATTTCTATGGAAGTAACTTTGAATTGATACCATTTGGGACAGGTAGAAGAGG ATGTCCTGGTATTTTATATGGAGTGACAAATGCTGAGTTTATGTTGGCTGCTATGTTTT ATCATTTTGATTGGGAAATAGCAGATGGTAAAAAACCAGAAGAAATTGATTTGACTG AGGACTTTGGTGCTGGTTGCATTATGAAGTATCCTTTGAAATTGGTTCCTCATCTTGTT AACGATTAA
CrRedOx1	ATGGCTGATCGCGTGAAAACCGTAGGATGGGCAGCTCACGACAGCTCCGGCTTCCTC TCTCCCTTCCAATTCACTCGAAGGGCAACAGGTGAAGAAGATGTGAGGTTGAAGGTG TTGTACTGTGGTGTCTGTCACTCAGACCTTCATAACATCAAGAACGAAATGGGATTCA CCTCCTACCCTTGTGTCCCCGGGCATGAGGTTGTGGGGGAAGTGACGGAAGTGGGGA ACAAAGTAAAGAAATTCATAATTGGTGATAAAGTTGGGGTTGGATTATTCGTTGACT CATGTGGCGAATGCGAACAATGTGTGAATGATGTAGAAACCTATTGTCCCAAATTGA AAATGGCTTATTTATCCATTGATGATGATGGAACTGTGATTCAAGGAGGGTACTCAA AGGAAATGGTCATCAAAGAACGCTACGTTTTCCGGTGGCCGGAAAATCTTCCTCTAC CCGCCGGTACACCGCTTCTGGGTGCCGGTAGTACAGTTTATAGTCCAATGAAATACTA TGGACTTGATAAGTCAGGACAACATCTAGGAGTTGTTGGCCTTGGTGGACTTGGTCAT TTAGCTGTTAAATTTGCAAAGGCTTTTGGACTTAAAGTTACTGTCATTAGTACCTCTC CTAGCAAGAAGGATGAAGCCATCAATCACCTTGGTGCTGATGCATTTTTAGTTAGCA CTGATCAAGAGCAAACCCAGAAAGCAATGAGCACAATGGATGGCATAATTGACACA GTATCAGCTCCTCATGCATTGATGCCATTGTTTTCCCTATTGAAACCAAATGGGAAGC TAATTGTTGTTGGTGCACCAAATAAACCAGTTGAATTAGATATTCTATTTCTTGTCAT GGGAAGGAAAATGCTCGGAACATCTGCTGTTGGGGGAGTGAAGGAGACACAAGAGA TGATCGATTTTGCAGCAAAACATGGTATAGTTGCAGATGTAGAAGTTGTGGAAATGG AGAATGTGAACAATGCAATGGAGCGGCTTGCGAAGGGGGATGTTAGATATAGATTTG TTCTTGATATTGGGAATGCAACAGTAGCTGTCGGTTAA

CrRedOx2	ATGGAAAAGCAAGTTGAGATCCCTGAAGTAGAATTGAATTCAGGACATAAAATGCCA ATTGTGGGATACGGAACATGCGTGCCAGAACCCATGCCACCGTTGGAAGAACTAACC GCAATCTTCTTAGATGCAATAAAGGTTGGTTACCGGCACTTCGACACGGCTTCGAGTT ACGGCACAGAGGAGGCACTTGGTAAAGCCATAGCTGAGGCTATAAACAGCGGTTTG GTTAAAAGTAGGGAGGAATTCTTCATCAGTTGTAAGCTGTGGATTGAAGATGCAGAT CATGACCTTATCTTGCCTGCCCTCAACCAGTCACTTCAGATTCTTGGGGTTGATTATTT GGATCTATATATGATACACATGCCGGTGAGAGTGAGGAAAGGTGCTCCCATGTTCAA TTATTCAAAAGAGGATTTCCTTCCATTTGACATACAAGGTACATGGAAGGCCATGGA AGAGTGCAGCAAACAAGGATTGGCCAAGTCTATTGGTGTCAGCAACTACTCTGTTGA AAAACTCACTAAACTCCTAGAAACCTCCACTATTCCCCCCGCCGTTAATCAGGTTGAG ATGAATGTAGCTTGGCAACAGAGAAAATTGCTGCCATTTTGCAAAGAGAAAAACATT CATATAACATCATGGTCCCCACTCCTATCTTATGGTGTCGCTTGGGGAAGTAATGCTG TCATGGAAAATCCTGTTCTCCAACAAATTGCGGCTTCCAAAGGCAAAACTGTGGCAC AGGTGGCACTAAGATGGATATACGAGCAAGGAGCAAGTCTCATTACAAGGACTTCCA ACAAGGACAGAATGTTTGAAAATGTTCAAATTTTTGATTGGGAACTCAGTAAAGAAG AATTGGATCAAATTCATGAAATCCCACAACGTAGGGGTACTTTAGGTGAAGAATTTA TGCATCCAGAAGGACCGATCAAATCCCCAGAGGAGCTTTGGGATGGAGACTTGTAA
CrSAT	ATGCACCCCAGATGCAGATATTGTCAGAGGAACTGATTCAACCATCATCTCCGACAC CCCAAACCTTGAAAACCCATAAACTTTCCCATCTTGATCAAGTTTTATTAACATGTCA TATCCCTATTATTCTCTTTTATCCAAATCAATTGGACTCAAATCTCGATCGAGCCCAA AGATCTGAGAATCTAAAACGATCTTTATCAACAGTGTTAACTCAATTTTACCCTTTAG CCGGAAGAATCAATATAAATTCTTCCGTAGATTGTAATGATTCCGGAGTCCCTTTTCT TGAAGCTCGAGTTCATTCCCAACTCTCAGAAGCAATTAAAAATGTCGCCATAGATGA ACTCAATCAATACCTGCCATTCCAACCTTATCCCGGTGGGGAGGAAAGTGGGTTGAA AAAAGATATCCCCTTAGCTGTAAAAATCAGTTGTTTCGAATGTGGCGGAACAGCAAT TGGGGTCTGTATTTCCCACAAGATTGCCGATGCGTTGTCCTTGGCTACCTTCCTCAATT CATGGACCGCAACATGCCAGGAAGAGACTGATATTGTTCAACCTAATTTCGATCTGG GATCCCATCATTTTCCGCCTATGGAAAGCATCCCAGCACCTGAATTTCTACCGGATGA AAATATTGTGATGAAACGGTTCGTGTTCGATAAAGAAAAATTAGAAGCTCTAAAAGC CCAATTAGCTTCCTCTGCAACAGAAGTGAAGAATTCAAGTCGGGTACAAATTGTTATT GCTGTTATATGGAAGCAATTCATTGACGTGACCCGGGCAAAATTCGATACCAAGAAC AAATTAGTAGCAGCTCAAGCAGTGAATTTGAGATCAAGAATGAATCCACCATTTCCT CAATCTGCTATGGGGAATATAGCCACAATGGCATATGCCGTTGCAGAGGAGGATAAG GATTTTTCAGATCTTGTCGGTCCATTGAAAACCAGTCTTGCAAAAATTGATGATGAAC ATGTTAAAGAATTACAAAAAGGAGTAACATATTTGGATTATGAAGCTGAACCACAAG AATTGTTCTCTTTTAGTAGTTGGTGCAGGCTTGGGTTTTATGATTTGGATTTTGGATGG GGAAAGCCTGTTTCTGTTTGTACAACAACTGTGCCTATGAAGAATTTGGTATATTTGA TGGATACAAGAAATGAAGATGGAATGGAAGCATGGATCAGCATGGCTGAAGATGAG ATGTCTATGCTTTCCTCTGATTTTCTTTCACTTCTGGACACTGATTTTAGCAATTGA

CrTHAS	ATGGCAATGGCTTCAAAGTCACCTTCTGAAGAAGTATATCCAGTGAAGGCATTTGGT TTGGCTGCTAAGGATTCTTCTGGGCTTTTCTCTCCATTCAACTTCTCAAGAAGGGCCA CAGGGGAACACGATGTGCAGCTCAAAGTATTATACTGTGGGACTTGCCAATATGACA GGGAAATGAGCAAAAACAAATTTGGATTTACAAGCTATCCTTATGTTTTAGGGCATG AAATTGTGGGTGAGGTAACTGAAGTTGGCAGCAAGGTGCAGAAATTCAAAGTCGGG GACAAAGTGGGCGTAGCAAGCATAATTGAAACTTGTGGCAAATGTGAAATGTGTACA AATGAAGTTGAAAATTACTGTCCAGAAGCAGGATCAATAGACAGCAATTACGGGGC ATGTTCAAATATAGCAGTGATAAACGAGAATTTTGTCATCCGTTGGCCTGAAAATCTT CCTTTGGATTCTGGTGTTCCTCTTCTATGTGCAGGAATCACGGCTTATAGTCCCATGA AACGTTATGGACTTGATAAACCTGGAAAACGTATCGGCATAGCCGGTCTAGGAGGAC TTGGACATGTAGCTCTTAGATTTGCCAAAGCTTTTGGGGCTAAGGTGACAGTGATTAG TTCTTCACTTAAGAAAAAACGTGAAGCCTTTGAGAAATTCGGAGCAGATTCTTTCTTG GTCAGCAGTAATCCAGAAGAAATGCAGGGTGCAGCAGGAACATTGGATGGGATCAT AGACACTATACCAGGGAATCACTCTCTTGAGCCACTCCTTGCTTTATTGAAGCCTCTT GGGAAGCTTATCATTTTAGGTGCACCAGAAATGCCCTTTGAGGTTCCCGCTCCTTCCC TGCTTATGGGTGGAAAAGTAATGGCTGCCAGTACTGCTGGGAGTATGAAGGAAATAC AAGAGATGATTGAATTTGCAGCAGAACACAACATAGTAGCAGATGTGGAGGTTATCT CTATTGACTATGTGAACACTGCAATGGAGCGCCTTGATAACTCTGATGTGAGATATCG TTTCGTGATTGATATAGGGAACACTCTGAAATCAAATTAA
CrAS	ATGGATCAGCTGATGAACTTCTCTCTCACCTCTCCCATTTTCCTTCTTCTCTCCTCCTT ATTTCTCATCATCTTACTTAACAAACTGATGAGAGGTAACAAAATCCAAAAGGGCAA AAAACTACCACCAGGTCCAAAAAAAATTGCCATAATAGGGAACCTCCATCAAATGGT AGGTTCACTTCCTCATCGTGTTCTAAACAATTTAGCAGAAAAATATGGACCGATCATG CACTTGCGAATCGGCCAACTTTCAGCCGTTATTATATCATCAGCAGAAAAAGCGAAA GAAATATTAAACACTCATGGTGTTCGTGTCGCGGACAGGCCACAAACTACTGTGGCT AAAATCATGTTGTATAATAGTTTAGGTGTCACTTTTGCTCCCTATGGTGATTACCTAA AACAATTGCGTCAAATTTATGCGATGGAACTTCTTAGCCCTAAAACAGTGAAGTCCTT TTGGACAATTATGGACGATGAGCTTTCCACAATGATAACCTCAATTAAATCTGAAGTT GGTCAACCAATGATTTTACATGATAAAATGATGACTTATTTATATGCTATGCTTTGTA GAGCTACAGTTGGTAGCGTATGTAATGGACGAGAGACTCTAATTATGGCAGCTAAAG AAACGTCAGCTCTTTCTGCTTCTATTAGGATTGAAGATTTATTTCCTTCGGTGAAAAT ACTTCCTGTAATTAGTGGATTGAAGTCAAAATTAACTAACTTGTTGAAAGAACTTGAT ATTGTTCTTGAGGATATTATTAGTGCTCGTGAGAAGAAATTATTAAGCCAGCCCCAGC AGCCATTAATGTTGGATGAAGAAGATATGTTGGGAGTTCTCCTCAAGTACAAAAATG GAAAGGGAAATGATACTAAATTTAGAGTCACCAACAATGACATCAAGGCCATCGTTT TTGAACTGATTTTGGCTGGAACTCTAAGTTCAGCAGCTATAGTAGAATGGTGTATGTC AGAATTGATGAAAAACCCTGAATTATTGAAGAAGGCACAAGATGAAGTTAGGCAAG TTCTAAAGGGTAAGAAAACAATAAGTGGAAGTGATGTTGGTAAATTGGAATATGTTA AAATGGTGGTTAAGGAATCAGTGAGATTACATCCTCCAGCTCCCCTTTTATTTCCAAG AGAATGTAGGGAAGAATTTGAGATAGATGGAATGACCATACCTAAAAAATCTTGGGT GATTATAAACTACTGGGCAATTGGAAGAGATCCCAAAATTTGGCCCAATGCTGATAA GTTTGAGCCTGAGAGATTCAGTAATAACAACATTGATTTCTATGGAAGCAATTTTGAG TTGATACCATTTGGTGCTGGAAGAAGGGTTTGTCCTGGAATATTATTTGGAACAACTA ATGTTGAGCTTTTGCTTGCTGCATTTCTCTTTCATTTTGATTGGGAACTTCCTGGAGGC ATGAAACCAGAAGAATTAGATATGAATGAGTTATTTGGTGCTGGTTGCATTAGAGAA AATCCTTTGTGTCTCATTCCTAGCATCTCAACTGTAGTTGAAGGAAACTAA

SlUbi10_Pr	GGAGGTCAACTACCCCAATTTAAATTTTATTTGATTAAGATATTTTTATGGACCTACT TTATAATTAAAAATATTTTCTATTTGAAAAGGAAGGACAAAAATCATACAATTTTGGT CCAACTACTCCTCTCTTTTTTTTTTTGGCTTTATAAAAAAGGAAAGTGATTAGTAATA AATAATTAAATAATGAAAAAAGGAGGAAATAAAATTTTCGAATTAAAATGTAAAAG AGAAAAAGGAGAGGGAGTAATCATTGTTTAACTTTATCTAAAGTACCCCAATTCGAT TTTACATGTATATCAAATTATACAAATATTTTATTAAAATATAGATATTGAATAATTT TATTATTCTTGAACATGTAAATAAAAATTATCTATTATTTCAATTTTTATATAAACTAT TATTTGAAATCTCAATTATGATTTTTTAATATCACTTTCTATCCATGATAATTTCAGCT TAAAAAGTTTTGTCAATAATTACATTAATTTTGTTGATGAGGATGACAAGATTTCGGT CATCAATTACATATACACAAATTGAAATAGTAAGCAACTTGATTTTTTTTCTCATAAT GATAATGACAAAGACACGAAAAGACAATTCAATATTCACATTGATTTATTTTTATATG ATAATAATTACAATAATAATATTCTTATAAAGAAAGAGATCAATTTTGACTGATCCA AAAATTTATTTATTTTTACTATACCAACGTCACTAATTATATCTAATAATGTAAAACA ATTCAATCTTACTTAAATATTAATTTGAAATAAACTATTTTTATAACGAAATTACTAA ATTTATCCAATAACAAAAAGGTCTTAAGAAGACATAAATTCTTTTTTTGTAATGCTCA AATAAATTTGAGTAAAAAAGAATGAAATTGAGTGATTTTTTTTTAATCATAAGAAAA TAAATAATTAATTTCAATATAATAAAACAGTAATATAATTTCATAAATGGAATTCAAT ACTTACCTCTTAGATATAAAAAATAAATATAAAAATAAAGTGTTTCTAATAAACCCG CAATTTAAATAAAATATTTAATATTTTCAATCAAATTTAAATAATTATATTAAAATAT CGTAGAAAAAGAGCAATATATAATACAAGAAAGAAGATTTAAGTACAATTATCAACT ATTATTATACTCTAATTTTGTTATATTTAATTTCTTACGGTTAAGGTCATGTTCACGAT AAACTCAAAATACGCTGTATGAGGACATATTTTAAATTTTAACCAATAATAAAACTA AGTTATTTTTAGTATATTTTTTTGTTTAACGTGACTTAATTTTTCTTTTCTAGAGGAGC GTGTAAGTGTCAACCTCATTCTCCTAATTTTCCCAACCACATAAAAAAAAAAATAAAG GTAGCTTTTGCGTGTTGATTTGGTACACTACACGTCATTATTACACGTGTTTTCGTATG ATTGGTTAATCCATGAGGCGGTTTCCTCTAGAGTCGGCCATACCATCTATAAAATAAA GCTTTCTGCAGCTCATTTTTTCATCTTCTATCTGATTTCTATTATAATTTCTCTGAATTG CCTTCAAATTTCTCTTTCAAGGTTAGAATTTTTCTCTATTTTTTGGTTTTTGTTTGTTTA GATTCTGAGTTTAGTTAATCAGGTGCTGTTAAAGCCCTAAATTTTGAGTTTTTTTCGGT TGTTTTGATGGAAAATACCTAACAATTGAGTTTTTTCATGTTGTTTTGTCGGAGAATG CCTACAATTGGAGTTCCTTTCGTTGTTTTGATGAGAAAGCCCCTAATTTGAGTGTTTTT CCGTCGATTTGATTTTAAAGGTTTATATTCGAGTTTTTTTCGTCGGTTTAATGAGAAGG CCTAAAATAGGAGTTTTTCTGGTTGATTTGACTAAAAAAGCCATGGAATTTTGTGTTT TTGATGTCGCTTTGGTTCTCAAGGCCTAAGATCTGAGTTTCTCCGGTTGTTTTGATGAA AAAGCCCTAAAATTGGAGTTTTTATCTTGTGTTTTAGGTTGTTTTAATCCTTATAATTT GAGTTTTTTCGTTGTTCTGATTGTTGTTTTTATGAATTTTGCAG

SlUbi10_Te	GCTTGTTGTGGTTGTCTGGTTGCGTCTGTTGCCCGTTGTCTGTTGCCCATTGTGGTGGT TGTGTTTGTATGATGGTCGTTAAGGATCATCAATGTGTTTTCGCTTTTTGTTCCATTCT GTTTCTCATTTGTGAATAATAATGGTATCTTTATGAATATGCAGTTTGTGGTTTCTTTT CTGATTGCAGTTCTGAGCATTTTGTTTTTGCTTCCGTTTACTATACCACTTACAGTTTG CACTAATTTAGTTGATATGCGAGCCATCTGATGTTTGATGATTCAAATGGCGTTTATG TAACTCGTACCCGAGTGGATGGAGAAGAGCTCCATTGCCGGTTTGTTTCATGGGTGG CGGAGGGCAACTCCTGGGAAGGAACAAAAGAAAAACCGTGATACGAGTTCATGGGT GAGAGCTCCAGCTTGATCCCTTCTCTGTCGATCAAATTTGAATTTTTGGATCACGGCA GGCTCACAAGATAATCCAAAGTAAAACATAATGAATAGTACTTCTCAATGATCACTT ATTTTTAGCAAATCAGCAATTGTGCATGTCAAATGATTTCGGTGTAAGAGAAAGAGT TGATGAATCAAAATATCTGTAGCTGGATCAAGAATCTGAGGCAGTTGTATGTATCAA TGATCTTTCCGCTACAATGATGTTAGCTATCCGAGTCAAATTGTTGTAGAATTGCATA CTTCGGCATCACATTCTGGATGACATAATAAATAGGAAGTCTTCAGATCCCTAAAAA ATTGAGAGCTAATAACATTAGTCCTAGATGTAACTGGGTGACAACCAAGAAAGAGAC ATGCAAATACTACTTTTGTTTGAAGGAGCATCCCTGGTTTGACATATTTTTTCTGAAT ATCAAACTTTGAAACTCTACCTAGTCTAATGTCTAACGACAGATCTTACTGGTTTAAC TGCAGTGATATCTACTATCTTTTGGAATGTTTTCTCCTTCAGTTATACATCAAGTTCCA AGATGCAGGTGTGCTTGATTGATGTACATGGCTGTGAGAAGTGCATCCTGATGTTCA GATGATGGTTCATTCTAATGTCTTTTCCTTCAATCAGTTTTCTCAGTCTGACTTAGCTT GTTTCATCTGCATGTTTGAATGTTCGTTTACTCATAGTAATTGCATTTTTGTAGCAGAA CATATCATTGGTCATGGTTTCAACTGTGCGCGAGTCTTATGCTTATTCAAACTAGGAA AGCCTCCGTCTAGAGGGTACACGAGTTGTTGCTCTGTGTGCGTCAGTCCATAGTATTA ATCTTGCTAGTTGTAGTATATTGTTTATGTGGACTCGGAATTCATCATATGCTCCTTCT TTGCATCAAGTAAGGCAAGGTAATGTATAGAAGCTTTTTAACTCTTTCATGGAAGCTG GCCTTTGCCAGCATACCATCCAGAAGATATCAACCCTGCATCTTGGCTGCCG

Table S3. Sequences of forward and reverse primers used in this investigation. Underlined sequences are the cloning overhangs.

Gene	Forward primer	Reverse primer
CrSGD	TTGCGGTCTCTAATGGGTTCTAAGGATGATCAA	GCAAGGTCTCTAAGCTTAATACTTCTGTTTCTTAA
CrGS	TTGCGGTCTCTAATGGCCGGAGAAACAACCAAA	GCAAGGTCTCTAAGCTTATTCCTCAAATTTCAATG
CrGO	TTGCGGTCTCTAATGGAGTTTTCTTTCTCCTCA	GCAAGGTCTCTAAGCTTAATCGTTAACAAGATGAGGAA
CrRedOx1	TTGCGGTCTCTAATGCTGATCGCGTGAAAACCGT	GCAAGGTCTCTAAGCTTAACCGACAGCTACTGTTG
CrRedOx2	TTGCGGTCTCTAATGGAAAAGCAAGTTGAGATC	GCAAGGTCTCTAAGCTTACAAGTCTCCATCCCAAA
CrSAT	TTGCGGTCTCTAATGCGGGTCTCTAATGCACCCCAGATGCAGATAT	GCAAGGTCTCTAAGCTCAATTGCTAAAATCAGT
CrTHAS	TTGCGGTCTCTAATGGCAATGGCTTCAAAGTCAC	GCAAGGTCTCTAAGCTTAATTTGATTTCAGAGTGTTC
CrAS	TTGCGGTCTCTAATGGATCAGCTGATGAACTTCTCTC	GCAAGGTCTCTAAGCTTAGTTTCCTTCAACTACAGTT
SlUbi10_Pr	TGGTCTCGGGAGGTCAACTACCCCAATTTAAAT	TGGTCTCGCATTCTGCAAAATTCATAAAAACAACAA
SlUbi10_Te	GTGGTCTCGGCTTGTTGTGGTTGTCTGGTTGCGTC	GTGGTCTCGAGCGCGGCAGCCAAGATGCAGGGTTGATAT

Table S4. MRM transitions, collision energy and retention times of the compounds analyzed in this study.

	Quantifier ion		*Qualifier ion 1*	*Qualifier ion 2*	
Compound	*Q1 m/z*	*CE (eV)*	*Q2 m/z*	*Q2 m/z*	*Retention time*
Serpentine	349.18	55	205.9	262.9	4.95
4-Fluoro alstonine	367.18	55	223.9	280.9	5.20
5-Fluoro alstonine	367.18	55	223.9	280.9	5.18
6-Fluoro alstonine	367.18	55	223.9	280.9	5.23
7-Fluoro alstonine	367.18	55	223.9	280.9	5.05
7-Chloro alstonine	383.18	55	239.9	296.9	5.68
Stemmadenine acetate	397.2	41	168.2	227.9	4.16
4-Fluoro stemmadenine acetate	415.20	41	186.2	245.2	4.71
5-Fluoro stemmadenine acetate	415.20	41	186.2	245.2	4.64
6-Fluoro stemmadenine acetate	415.20	41	186.2	245.2	4.47
7-Fluoro stemmadenine acetate	415.20	41	186.2	245.2	4.39
Tetrahydroalstonine	353.20	25	144.2	122.2	4.77
6-methyl tetrahydroalstonine	367.2	25	158.0	235.0	5.39
7-methoxy tetrahydroalstonine	383.2	25	174.0	251.0	4.84
Precondylocarpine acetate	395.2	22	168.9	227.0	3.85
4-fluoro precondylocarpine acetate	413.2	22	186.9	245.0	3.95
5-fluoro precondylocarpine acetate	413.2	22	186.9	245.0	4.21
6-fluoro precondylocarpine acetate	413.2	22	186.9	245.0	4.46
7-fluoro precondylocarpine acetate	413.2	22	186.9	245.0	4.38
Akuammicine	323.2	20	291.1	182.1	4.22
6-Methyl akuammicine	337.2	20	305.1	196.1	5.20
7-Methyl akuammicine	337.2	20	305.1	196.1	5.60
4-fluoro akuammicine	341.2	20	309.1	200.1	4.21
5-fluoro akuammicine	341.2	20	309.1	200.1	4.49
6-fluoro akuammicine	341.2	20	309.1	200.1	4.70
7-fluoro akuammicine	341.2	20	309.1	200.1	5.00