

Supplementary Materials for
Solanoeclepin B, a hatching factor for potato cyst nematode

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Sci. Adv. **9**, eadf4166 (2023)
DOI: 10.1126/sciadv.adf4166

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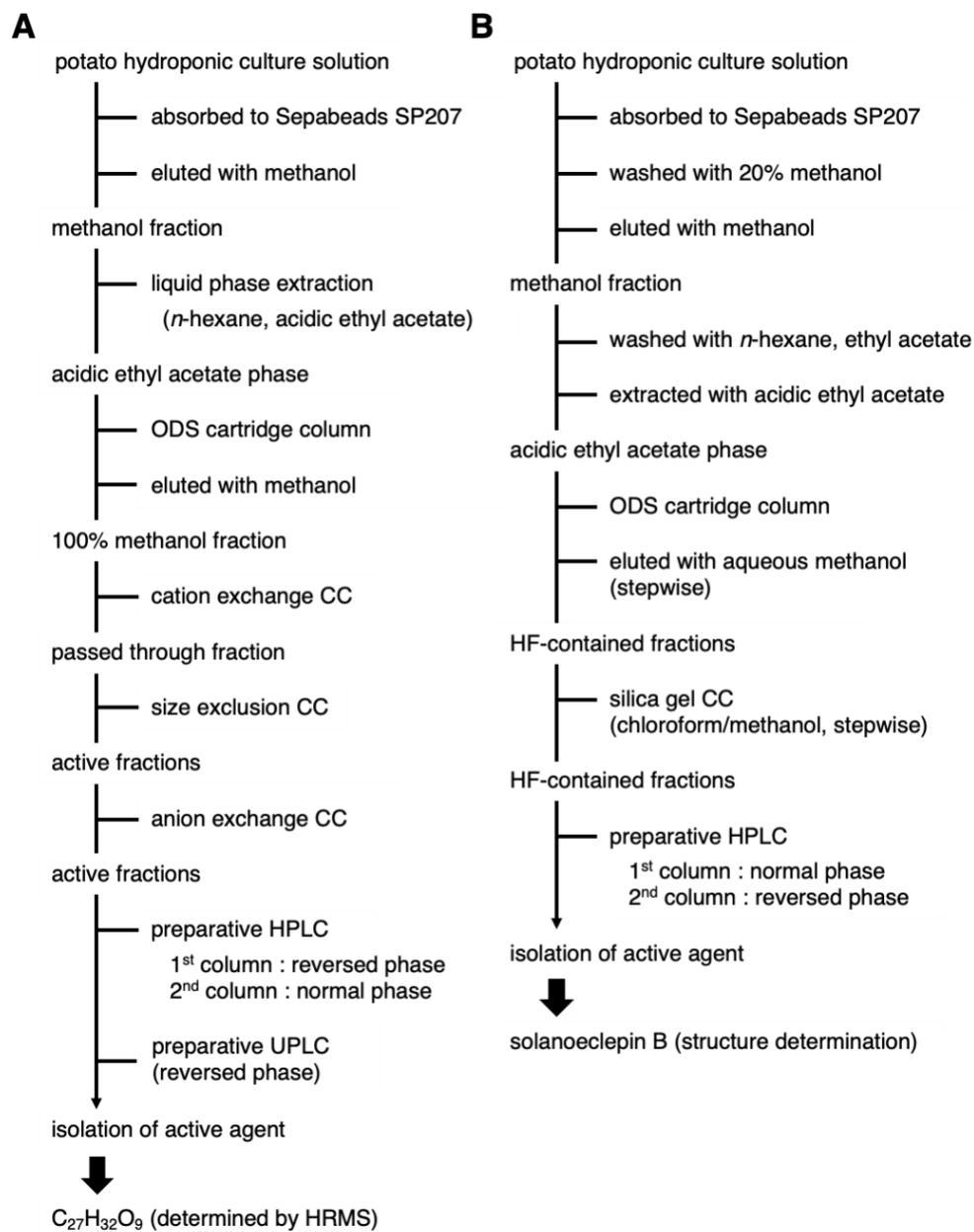


Fig. S1. Purification scheme of HF. (A) Hatching assay-guided purification and (B) UPLC-MS/MS detection-based purification.

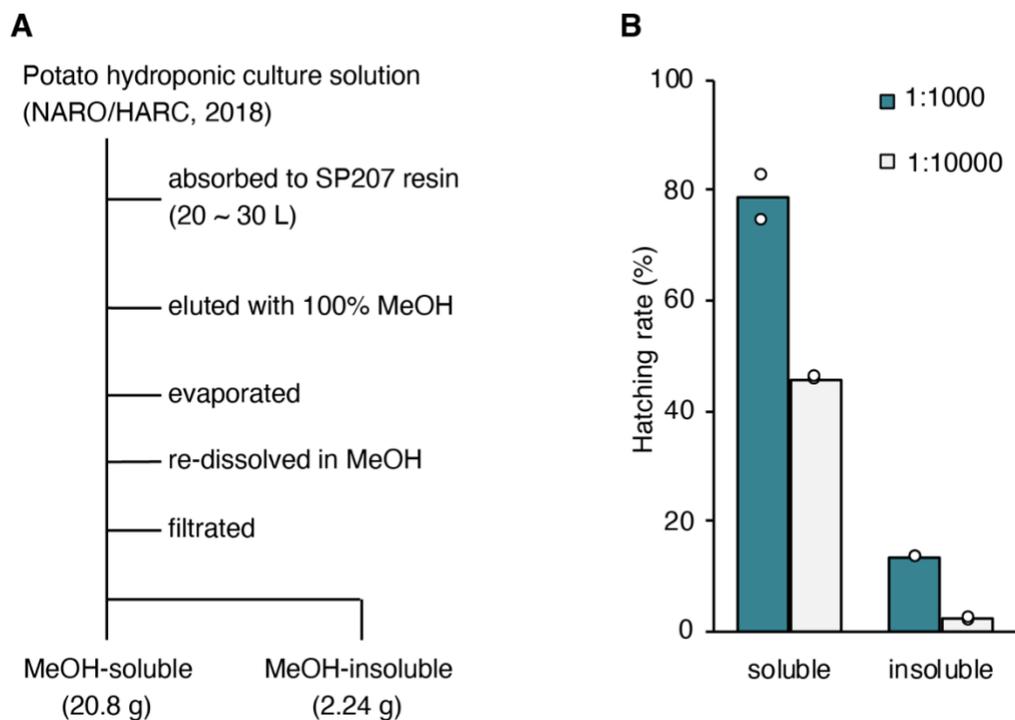


Fig. S2. The concentration of HFs from potato hydroponic culture solution. (A) Schematic concentration process using Sepabeads SP207 resin. (B) HS activity of the methanol eluents from Sepabeads SP207 resin. The hatching assay was conducted with *Globodera rostochiensis* eggs. The assay results at two different dilution rates are shown. The values indicate the means of two biological replicates; white dots are individual measurements.

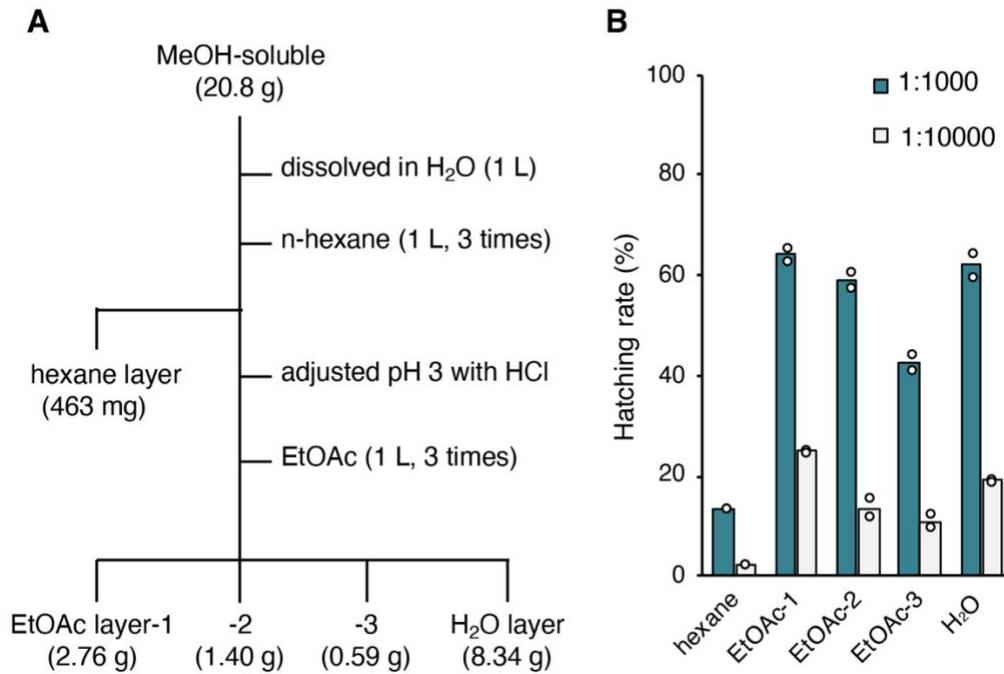


Fig. S3. Liquid-phase extraction of HFs. (A) Schematic of liquid-phase extraction process. (B) HS activity of *n*-hexane, ethyl acetate, and aqueous phase. Hatching assay was conducted with *Globodera rostochiensis* eggs. The assay results at two different dilution rates are shown. The values indicate the means of two biological replicates; white dots are individual measurements.

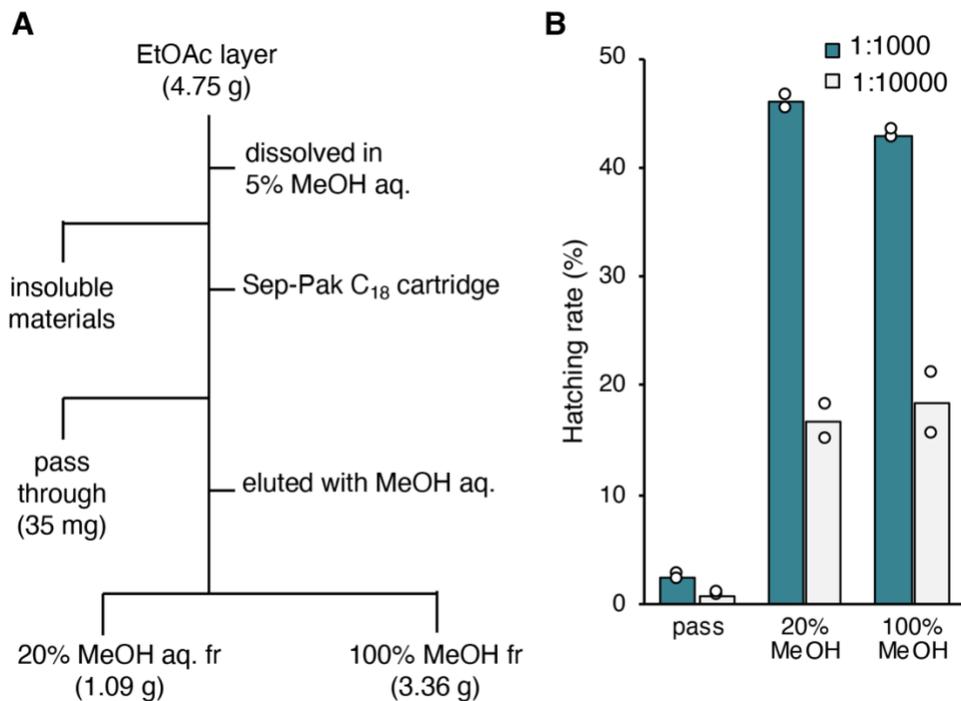


Fig. S4. The separation of HFs using ODS column chromatography. (A) Schematic of ODS column chromatography process. (B) HS activity of each separated fraction. Hatching assay was conducted with *Globodera rostochiensis* eggs. The assay results at two different dilution rates are shown. The values indicate the means of two biological replicates; white dots are individual measurements.

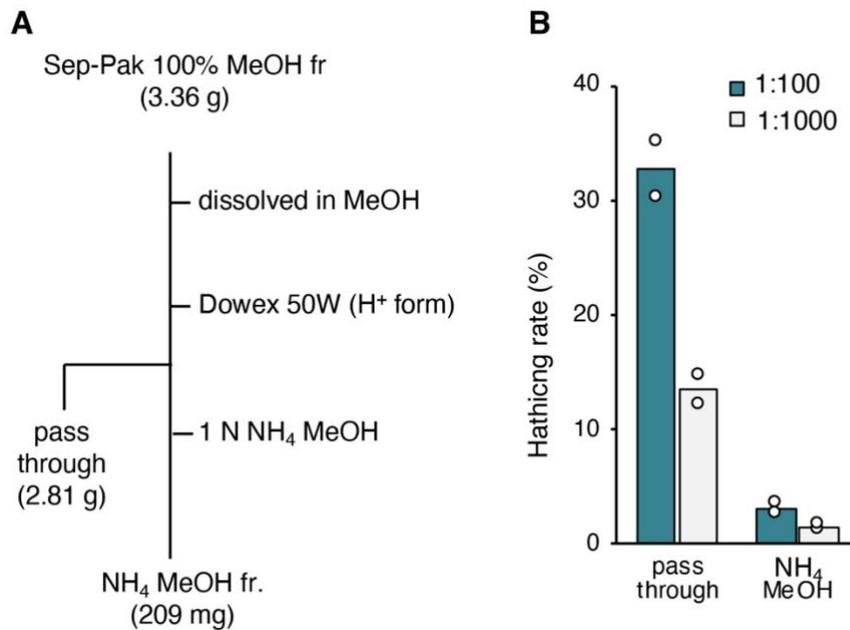


Fig. S5. The separation of HFs using strong cation exchange (SCX) column chromatography. (A) Schematic of SCX column chromatography process. (B) HS activity of each separated fraction. The hatching assay was conducted with *Globodera rostochiensis* eggs. The assay results at two different dilution rates are shown. The values indicate the means of two biological replicates; white dots are individual measurements.

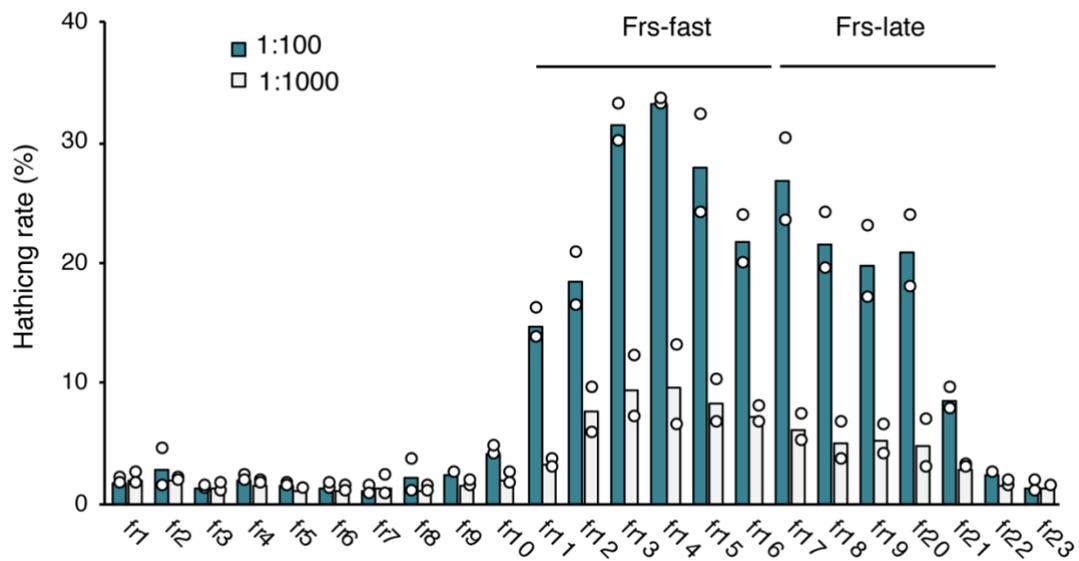


Fig. S6. The separation of HFs using size exclusion column chromatography. HS activity of each separated fraction. The hatching assay was conducted with *Globodera rostochiensis* eggs. The assay results at two different dilution rates are shown. The values indicate the means of two biological replicates; white dots are individual measurements.

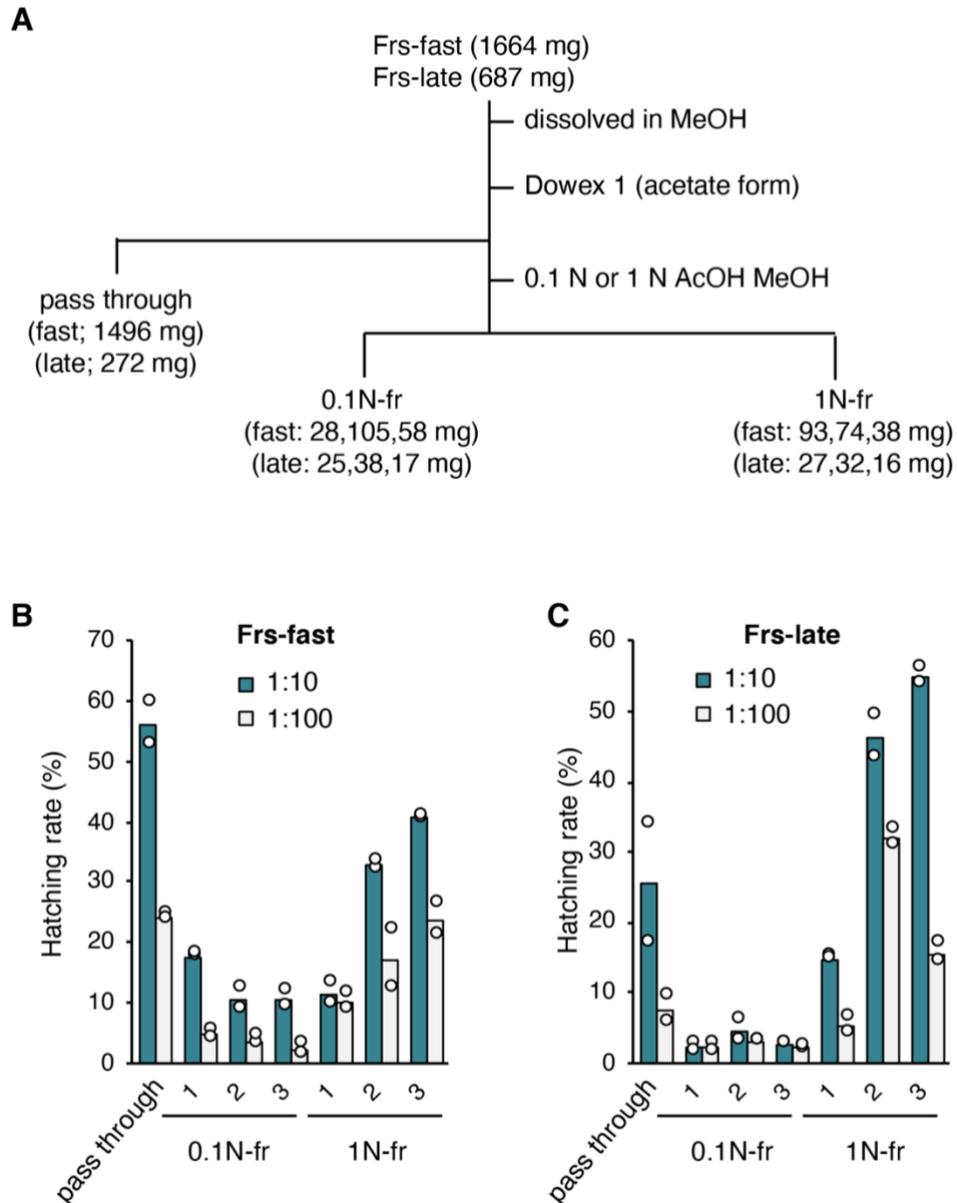


Fig. S7. The separation of HFs using strong anion exchange (SAX) column chromatography. (A) Schematic of SAX column chromatography process. (B,C) HS activity of each separated fraction derived from Frs-fast (B) and Frs-late (C). Hatching assay was conducted with *Globodera rostochiensis* eggs. The assay results at two different dilution rates are shown. The values indicate the means of two biological replicates; white dots are individual measurements.

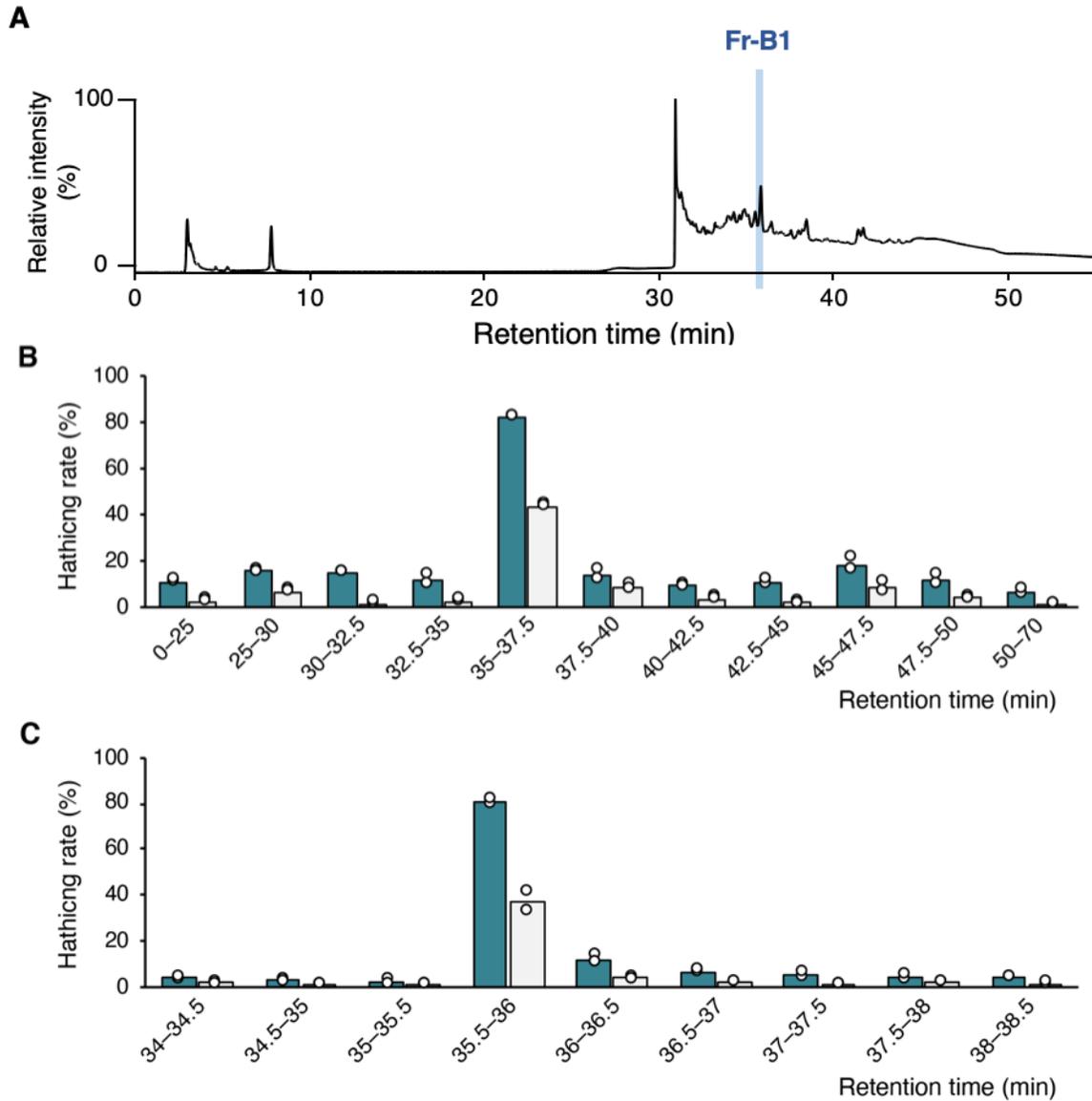


Fig. S8. The separation of HF in Fr-B using preparative NP-HPLC. (A) A chromatogram of UV absorbance at 270 nm. (B, C) HS activity of fractions collected at different retention times. The hatching assay was conducted with *Globodera rostochiensis* eggs. The hatching rates (%) are shown for two dilutions. Fractions were grouped and tested at first (B) and then each fraction around the Rt of 35–37.5 min was tested independently for HS activity (C). The assay results at two different dilution rates are shown. The values indicate the means of two biological replicates; white dots are individual measurements.

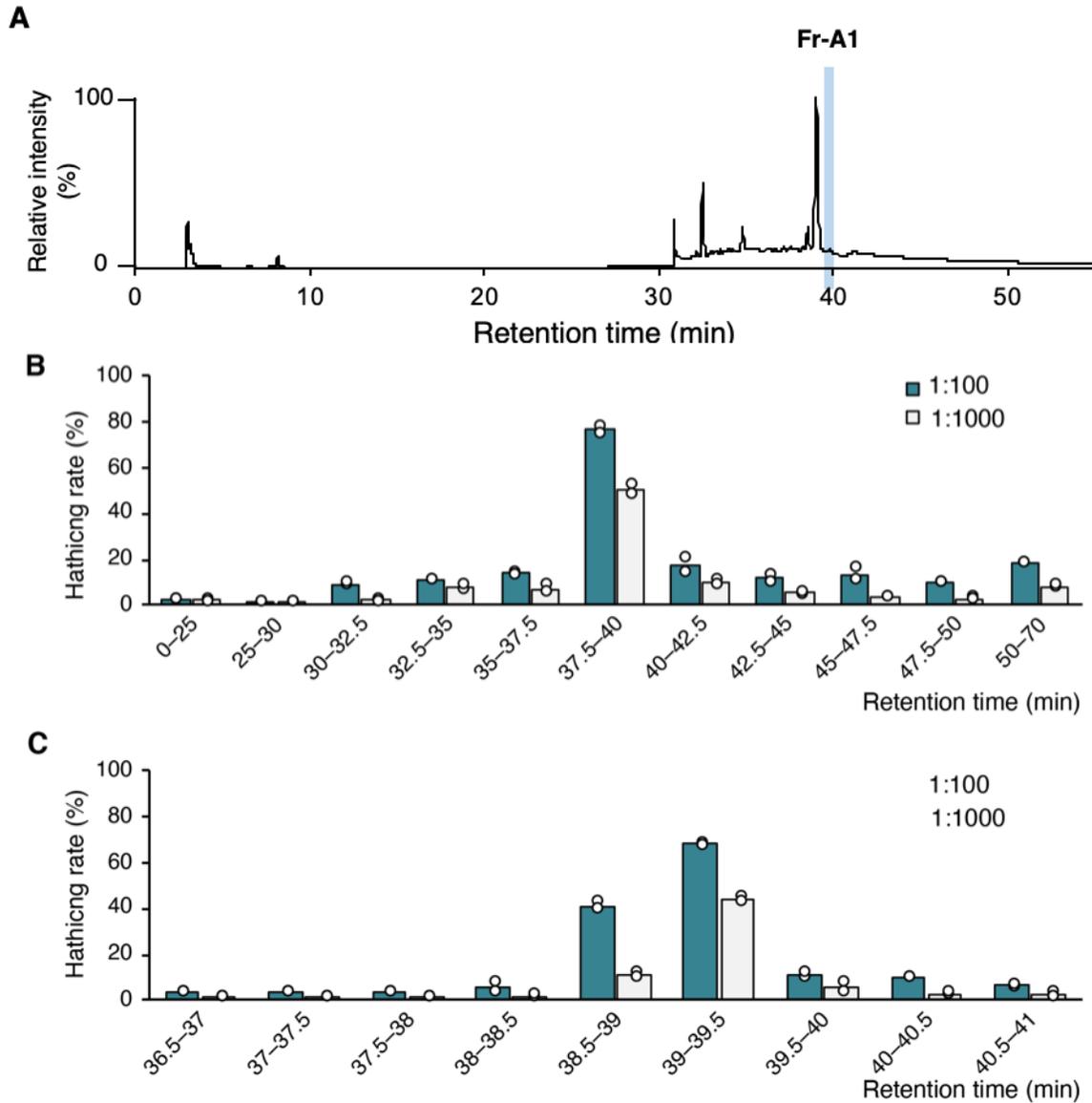


Fig. S9. Separation of HF in Fr-A is shown in Fig. 1B using preparative NP-HPLC. (A) A chromatogram of UV absorbance at 270 nm is shown. (B, C) HS activity of fractions collected during each retention time. The hatching assay was conducted with *Globodera rostochiensis* eggs. The hatching rates (%) are shown for two dilution series. First, fractions were grouped and tested (B) and then each fraction in the group of 36.5–41 min was observed for HS activity and tested independently (C). The assay results at two different dilution rates are presented. The values represent the means of two biological replicates, and white dots are individual measurements.

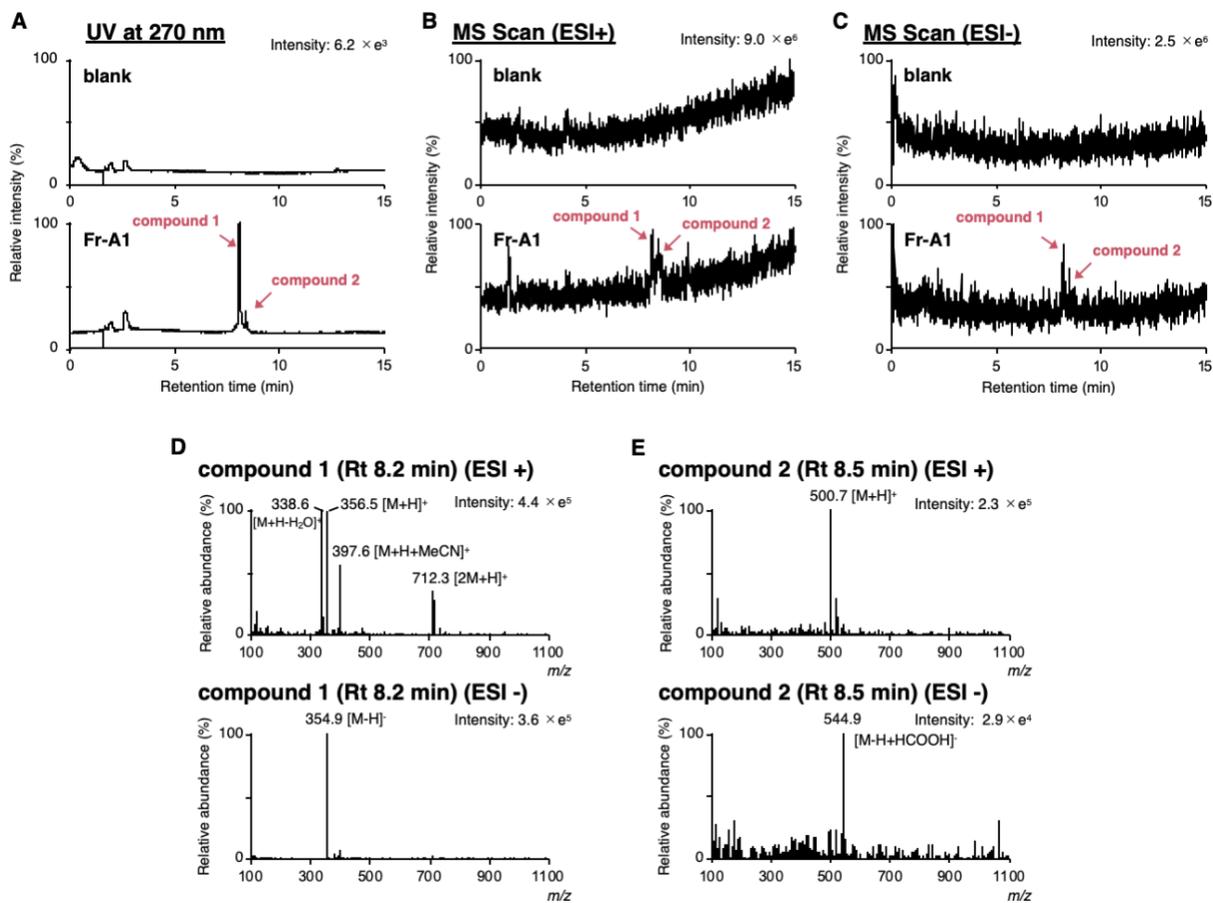


Fig. S10. UPLC analysis of Fr-A1. (A) Chromatograms of UV absorbance at 270 nm are shown. (B, C) Chromatograms obtained by positive (B) and negative (C) ESI mass spectrometry scan mode with the range of m/z 100–1100. (D, E) Positive and negative ESI mass spectra obtained at compound 1 (Rt 8.2 min) (D) and compound 2 (Rt 8.5 min) (E).

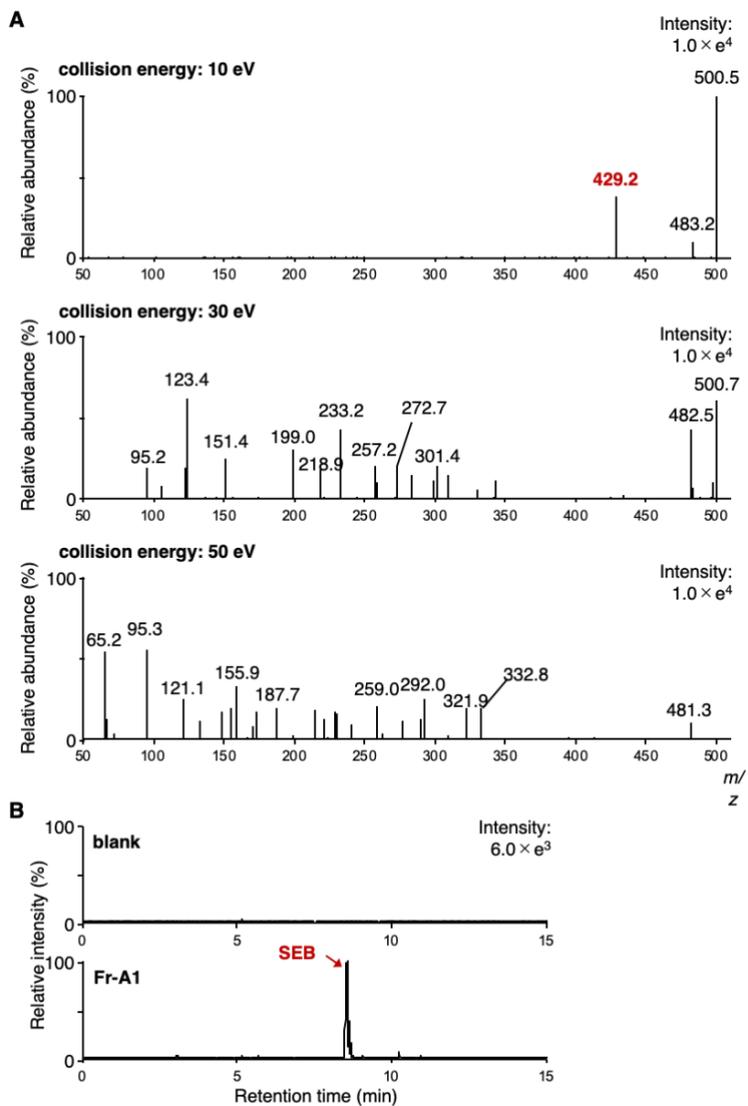


Fig. S11. Method for detecting SEB using LC-MS/MS analysis. (A) Product ion spectra at 10, 30, and 50 eV are shown. (B) MRM chromatograms of Fr-A1. The MRM transition of m/z 501 > 429 was selected.

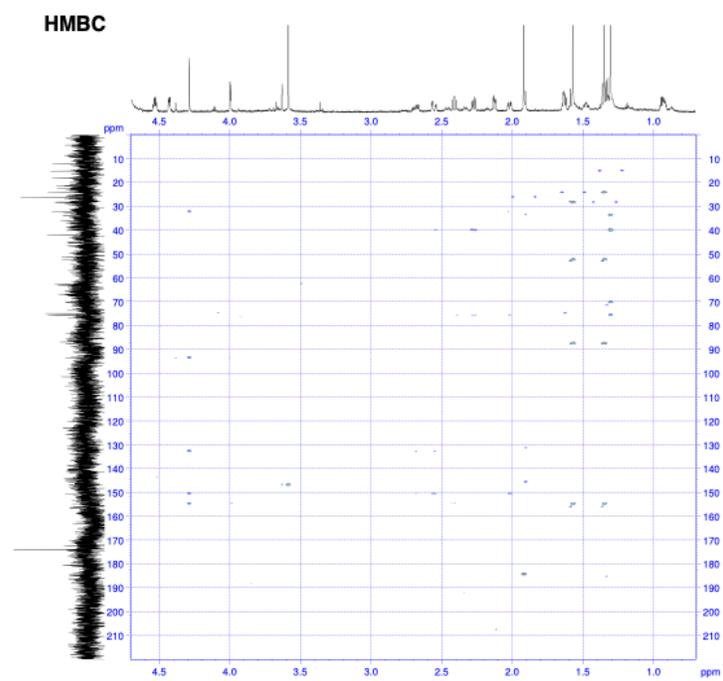
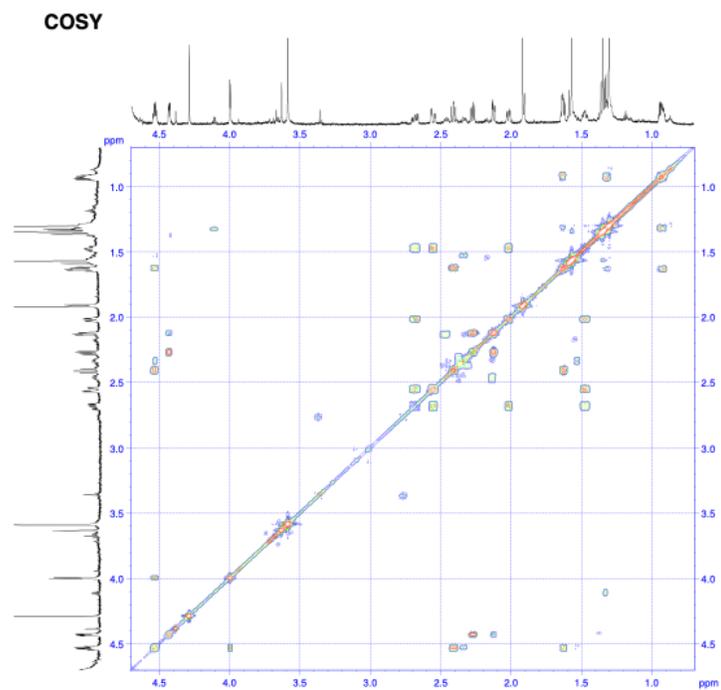


Fig. S12. (Continued)

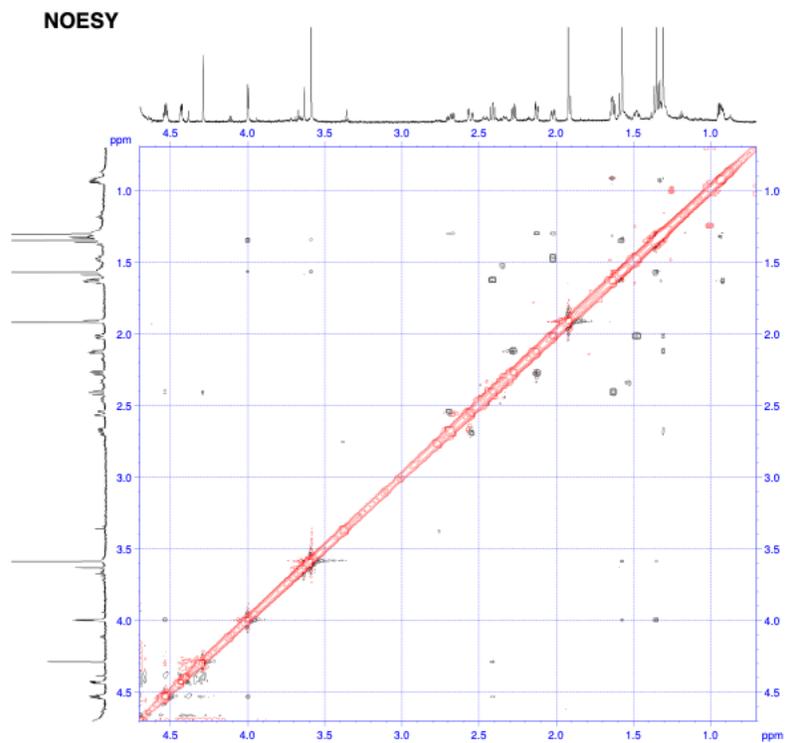


Fig. S12. (Continued) NMR spectra of solanoeclepin B (SEB)

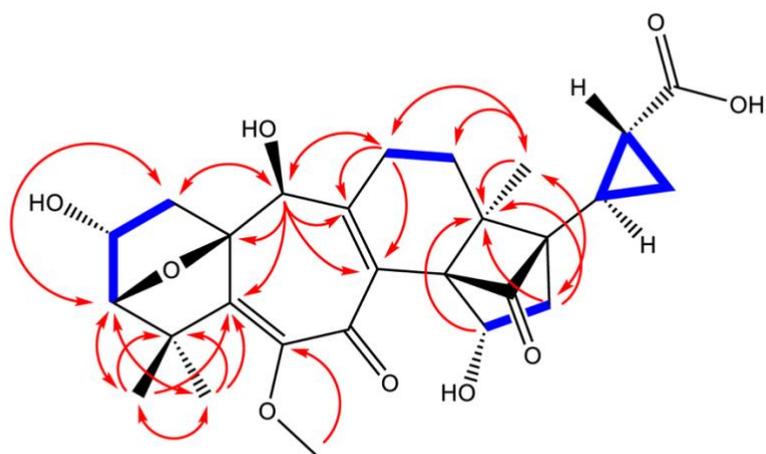
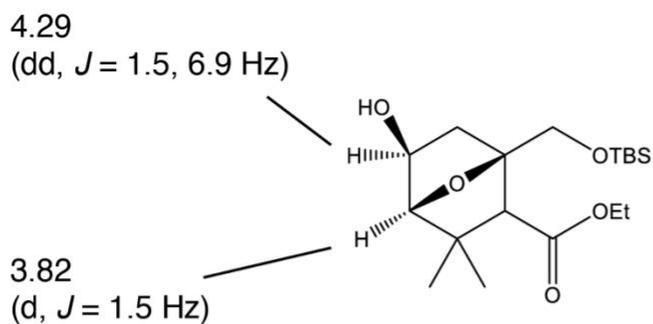
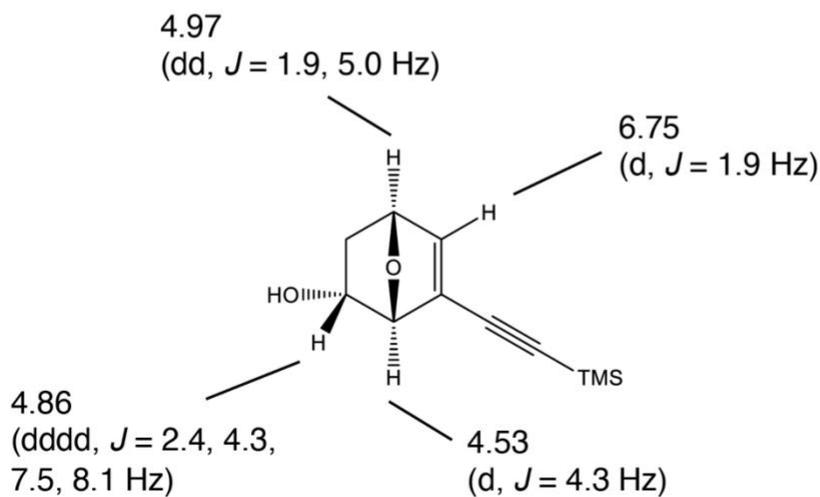


Fig. S13. Observed COSY (blue bold bond) and HMBC (red arrow) for SEB.



compound a
 (+)-(1*R*,2*S*,4*R*,5*S*)-1-(tert-Butyldimethylsilyloxymethyl)-5-hydroxy-3,3-dimethyl-7-oxabicyclo[2.2.1]heptane-2-carboxylic acid ethyl ester



compound b
 (±)-6-[(Trimethylsilyl)ethynyl]-7-oxabicyclo[2.2.1]hept-5-en-2-endo-ol

Fig. S14. ^1H chemical shifts and coupling constants of structurally related compounds.

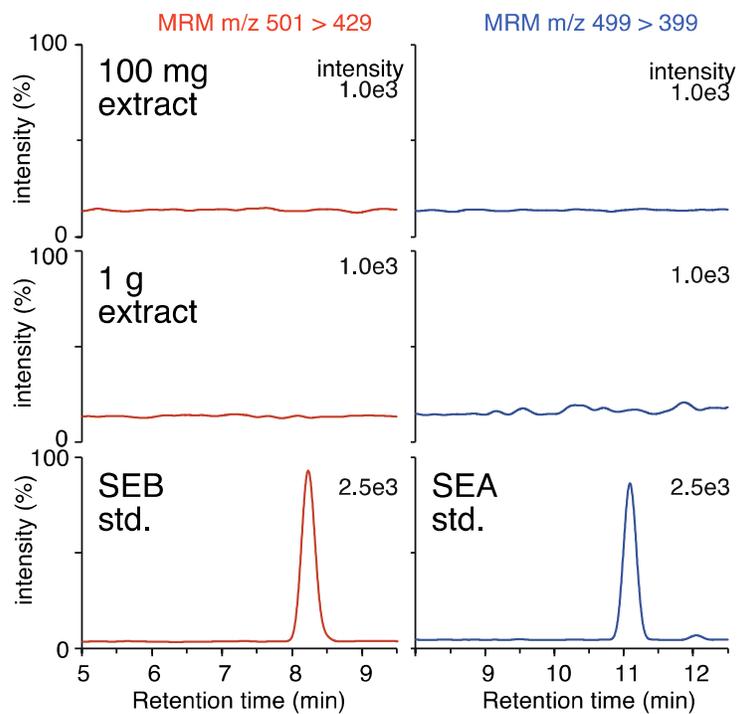


Fig. S15. MRM-LC-MS/MS analysis of the extract of tomato hairy roots. The extract was prepared with 100 mg and 1 g of FW tomato hairy roots, respectively. Fresh tomato hairy roots (100 mg or 1 g) were extracted and purified with Oasis® MAX.

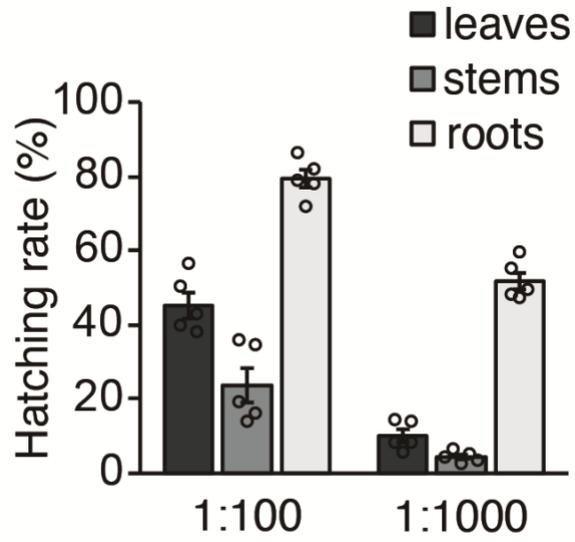
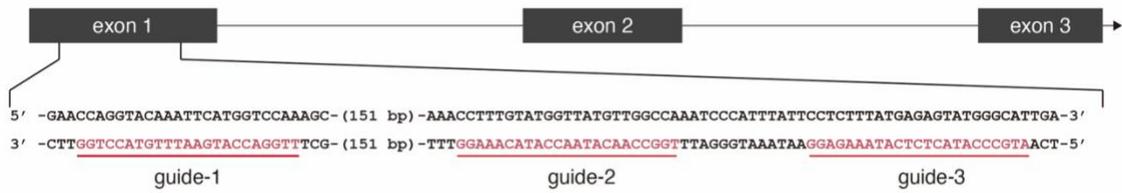
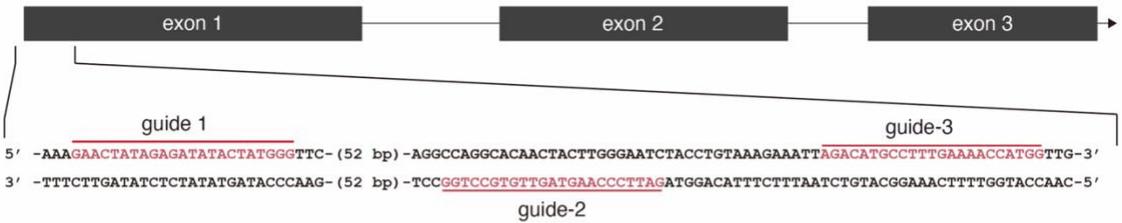


Fig. S16. HS activity of extracts of various parts of the tomato. The hatching assay was conducted with *Globodera rostochiensis* eggs. The assay results at two different dilution rates are shown. The values indicate the means of five biological replicates; white dots are individual measurements.

SOLA1_Solyc06g067870



SOLA2_Solyc06g067860



SOLA3_Solyc12g042980

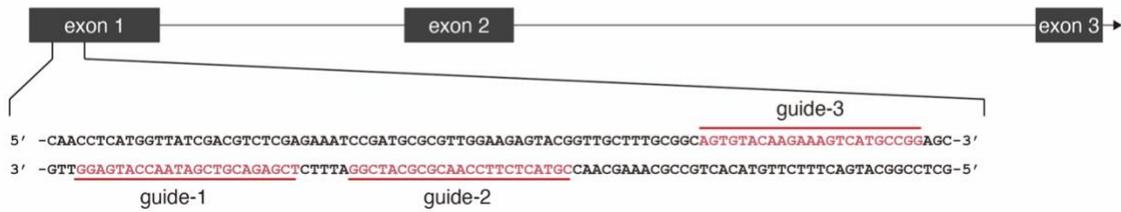


Fig. S17. Gene structures and target sites for CRISPR/Cas9-mediated genome editing. Each target region is shown by red letters.

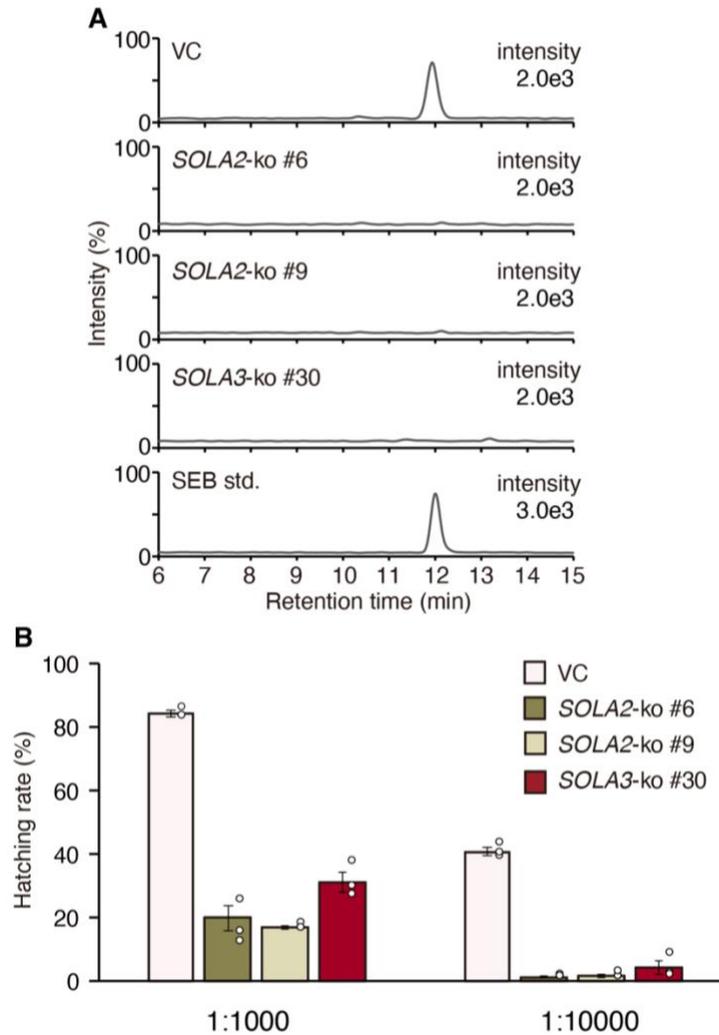


Fig. S19. Analysis of candidate *DOX* gene-disrupted tomato hairy roots. (A) UPLC-MS/MS chromatograms of partially purified culture medium of vector control or candidate gene-disrupted tomato hairy roots. (B) The hatching rate of *Globodera rostochiensis* eggs treated with partially purified culture medium of vector control (VC) or candidate gene-disrupted tomato hairy roots. The sample solutions were prepared at the same concentration as the original hairy root culture medium. The assay results at two different dilution rates are shown. Bars indicate the mean hatching rate induced by samples derived from each tomato hairy root lines. Error bars show the standard error (n = 3); white dots are individual measurements.

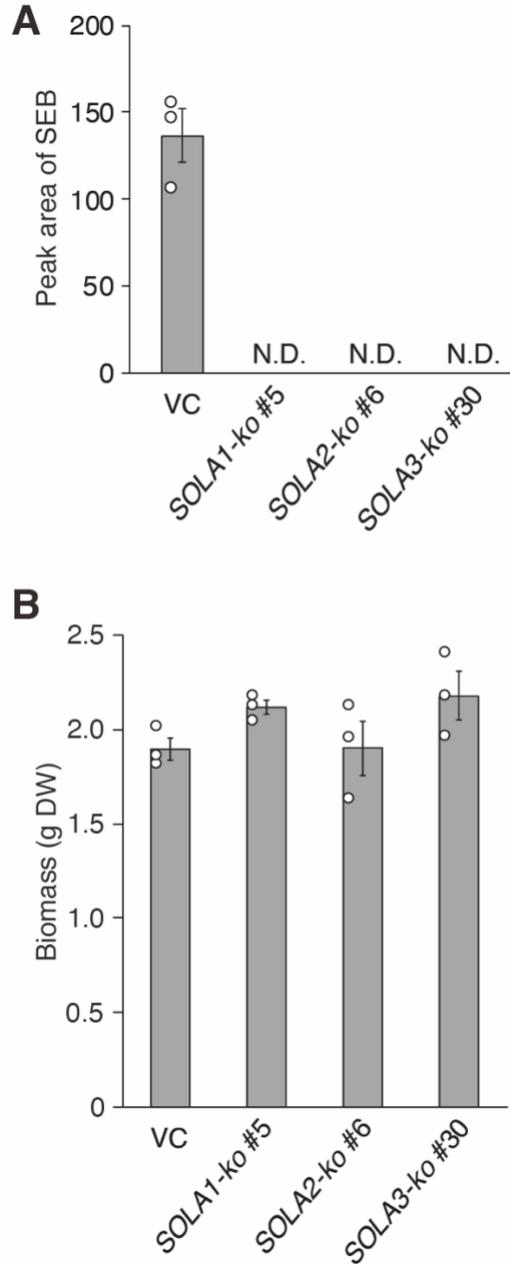


Fig. S20. SEB production and growth rate of *SOLA1–3*-knockout tomato hairy roots. (A) After 2 weeks of cultivation, the culture medium was collected and subjected to UPLC-MS/MS. Chromatograms were recorded in the MRM mode at m/z 501 > 429. Data are presented as the mean \pm SE ($n = 3$ biologically independent flasks). (B) The fresh weight of hairy roots after 2 weeks of culture.

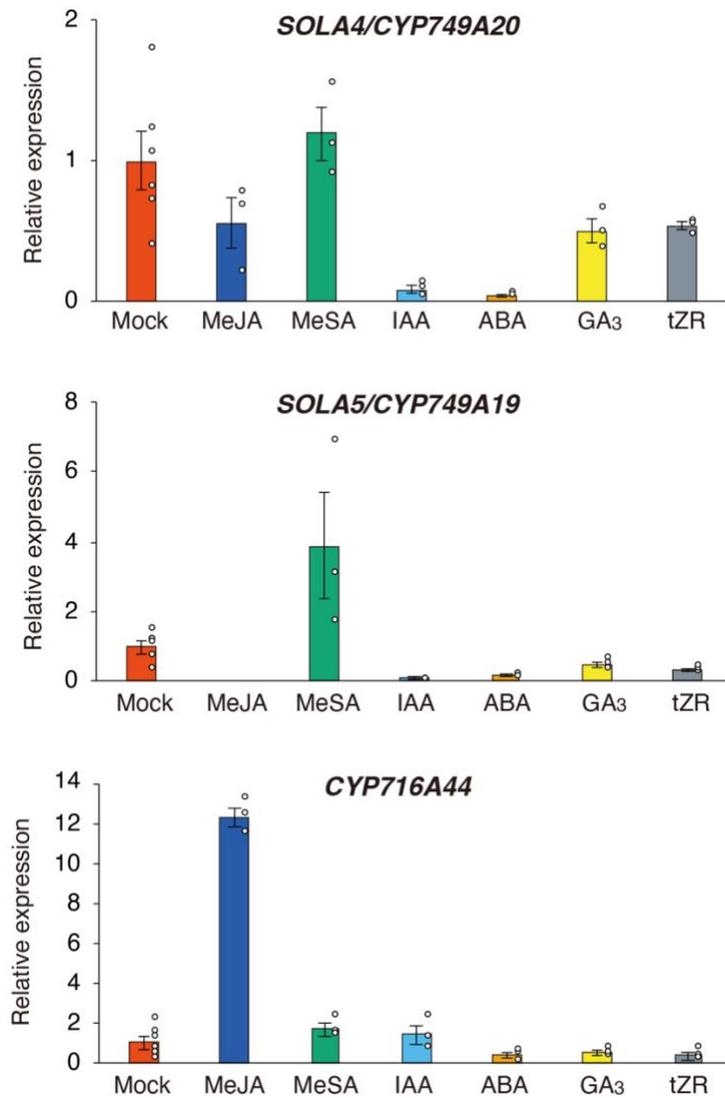


Fig. S21. Analysis of transcription levels of candidate genes analyzed by real-time quantitative RT-PCR in hairy roots treated with each phytohormone. Error bars represent means \pm SE (n = 3 biologically independent flasks).

SOLA4-ko_#19

55 bp guide 1 guide 2 57 bp guide 3
5'- TACATT//TCTCCTATGGACACTCTTAAAAATCGTTTATTTCAGTATGGTGGATG//TTCCTCATGG-AAATACCAAAGATATATC -3' WT
5'- TAC----- (72 bp) -----TCTTAAAAATCGTTTATTTCAGT-TGGTGGATG//TTCCTCATGGAAATACCAAAGATATATC -3' -72 (8/8)

SOLA4-ko_#31

55 bp guide 1 guide 2 57 bp guide 3
5'- TACATT//TCTCCTATGGACACTCTTAAAAATCGTTTATTTCAGTATGGTGGATG//TTCCTCATGGAAATACCAAAGATATATC -3' WT
5'- TACATT//TCTCCTATGGACACTCTTAAAAATCGTTTATTTCAGTA----- (74 bp) -----GGAAATACCAAAGATATATC -3' -74 (8/8)

SOLA5-ko_#19

 guide 1 guide 2 guide 3
5'- TCCCAAGAAGCTTTTCGGAG-ACGGGGTTGCGTCATCTAAAGGCGAAAAATGGGTAAAAATGAGGAACTAGCTAACCATGTTTTTCATGGAGTAAGCCTAA -3' WT
5'- TCCCAAGAAGCTTTTCGGAG-ACGGGGTTGCGTCATCTAAAGGCGAAAAATGGGTAAAAATGAGGAACTAGCTAACCATG---TTCATGGAGTAAGCCTAA -3' -23 (6/8)
5'- TCCCAAGAAGCTTTTCGGAGTACGGGGTTGCGTCATCTAAAGGCGAAAAATGGGTAAAAATGAGGAACTAGCTAACCATG-----GTAAGCCTAA -3' -32 (2/8)

SOLA5-ko_#23

 guide 1 guide 2 guide 3
5'- TCCCAAGAAGCTTTTCGGAG-ACGGGGTTGCGTCATCTAAAGGCGAAAAATGGGTAAAAATGAGGAACTAGCTAACCATGTTTTTCATGGAGTAAGCCTAA -3' WT
5'- TCCCAAGAAGCTTTTCGGAGAACGGGGTTGCGTCATCTAAAGGCGAAAAATGGGTAAAAATGAGGAACTAGCTAACCATG---TCATGGAGTAAGCCTAA -3' -2 (6/8)
5'- TCCCAAGAAGCTTTTCGGAGTACGGGGTTGCGTCATCTAAAGGCGAAAAATGGGTAAAAATGAGGAACTAGCTAACCATG-TTTTCATGGAGTAAGCCTAA -3' 0 (1/8)
5'- TCCCAAGAAGCTTTTCGGAGTACGGGGTTGCGTCATCTAAAGGCGAAAAATGG-----CTAGCTAACCATG-TTTTCATGGAGTAAGCCTAA -3' -15 (1/8)

CYP716A44-ko_#4

 guide 1 13 bp guide 2 92 bp guide 3
5'- TGAAGCATTGCAACGTTACGTGGTA//AACCCAAC-GTCACCTTTGCTACGGGATGGGAAAATAAAGAGCAAG//GCTGACCCATT-TGATGTTTTGGCTTCTGG-3' WT
5'- TGAAGCATT-----GGTA//AACCCAACGGTCACCTTTGCTTCGGGATGGGAAAATAAAGAGCAAG//GCTGACCCATTGTGATGTTTTGGCTTCTGG-3' -11 (3/6)
5'- TGAAGCATT----- (69 bp) -----GCAA- (100 bp) -CGTTATGATGTTTTGGCTTCTGG-3' -168 (3/6)

Fig. S23. Genotyping of sequences surrounding the target sites for CRISPR/Cas9-mediated gene disruption. WT; wild-type sequence. gRNA target sequences are indicated by red letters. The number of deleted and inserted and sequence frequencies in the cloned PCR products are indicated to the right of the sequence.

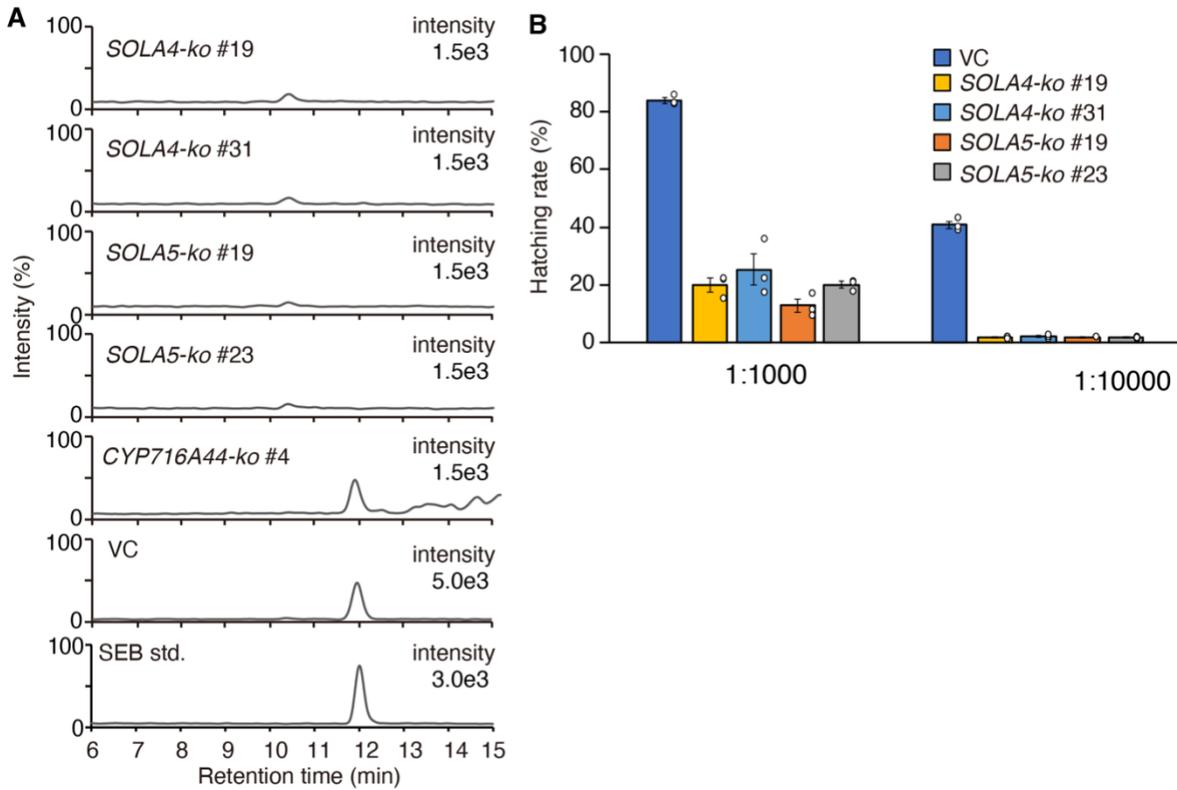


Fig. S24. Analysis of candidate *CYP* gene-disrupted tomato hairy roots. (A) MRM-LC-MS/MS chromatograms of partially purified culture medium of vector control or candidate gene-disrupted tomato hairy roots. (B) The hatching rate of *Globodera rostochiensis* eggs treated with partially purified culture medium of vector control (VC) or candidate gene-disrupted tomato hairy roots. Bars indicate the mean hatching rate induced by samples derived from each tomato hairy root line. Error bars indicate the standard error ($n = 3$); white dots are individual measurements. The sample solutions were prepared at the same concentration as the original hairy root culture medium. The numbers at the bottom of the graph indicate the dilution rate.

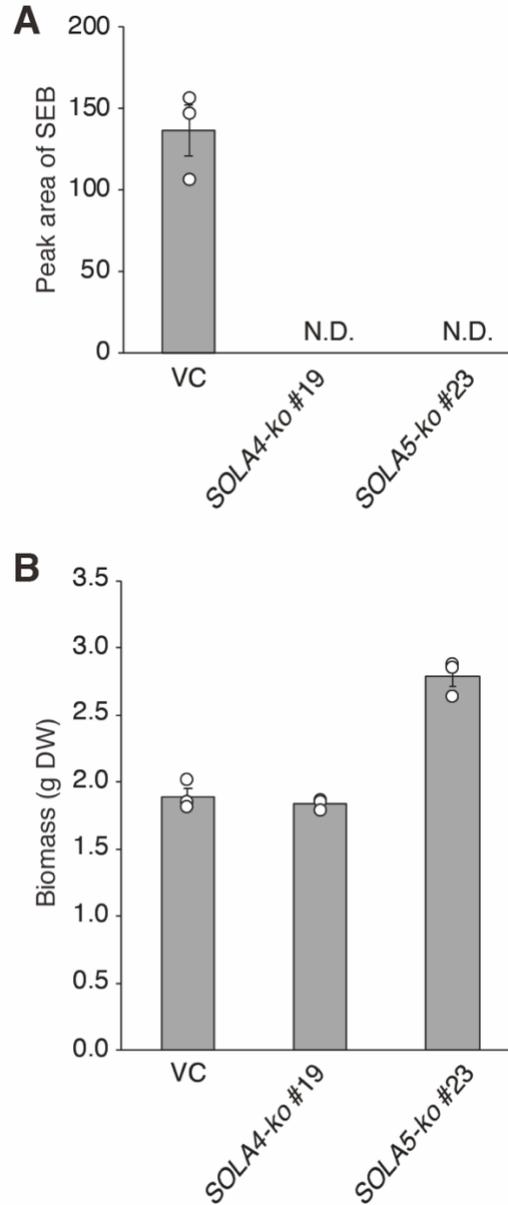


Fig. S25. SEB production and growth rate of *SOLA4*- and *SOLA3*-knockout tomato hairy roots. (A) After 2 weeks of cultivation, the culture medium was collected and subjected to UPLC-MS/MS. Chromatograms were recorded in the MRM mode at m/z 501 > 429. Data are presented as the mean \pm SE ($n = 3$ biologically independent flasks). (B) The fresh weight of hairy roots after 2 weeks of culture. Data are presented as the mean \pm SE ($n = 3$ biologically independent flasks).

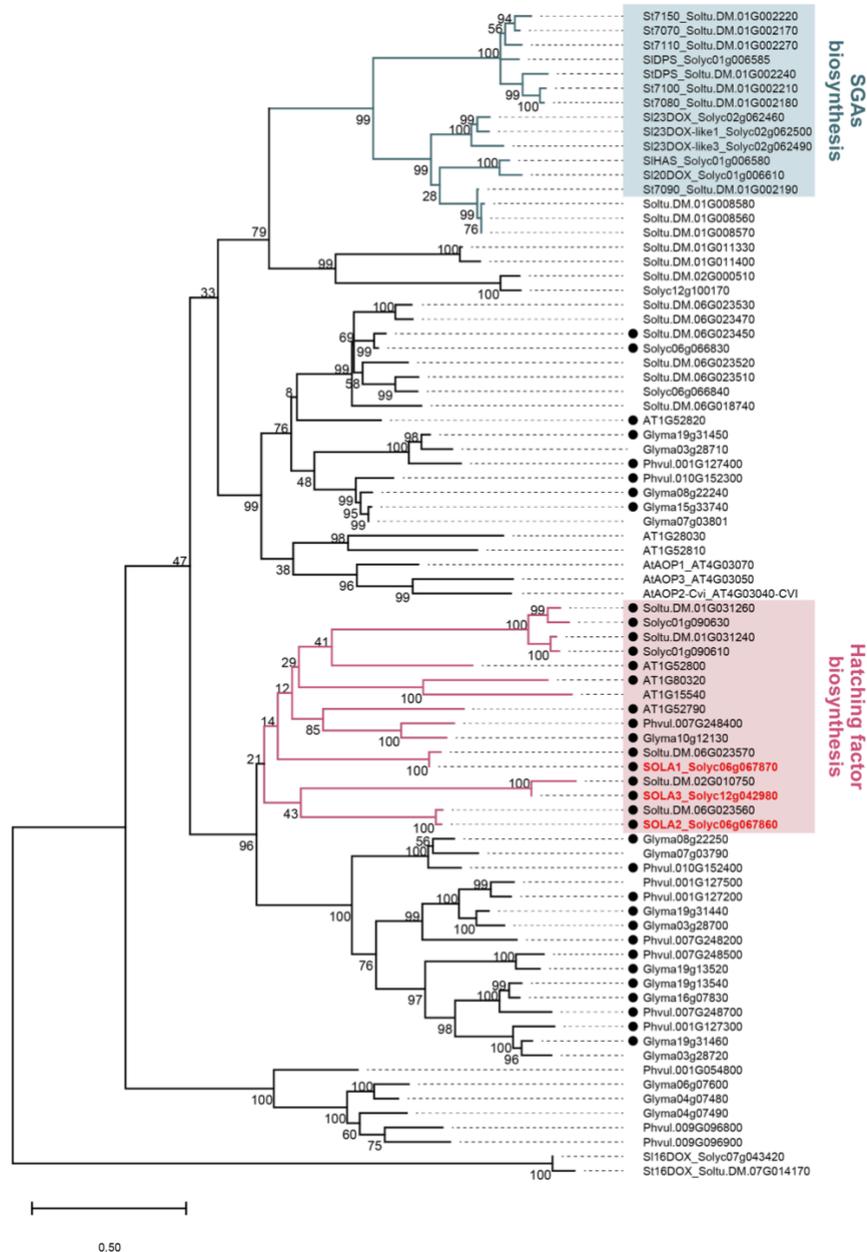


Fig. S27. Phylogenetic tree of the *DOXC20* clade genes. The amino acid sequences of the *DOXC20* genes were obtained from *Solanum lycopersicum* (Solyc_), *S. tuberosum* (Soltu.DM._), *Glycine max* (Glyma_), *Phaseolus vulgaris* (Phvul._), *Arabidopsis thaliana* (At_). The amino acid sequences of two *DOXC41* genes, *Sl16DOX* and *St16DOX*, were used for the outgroups. A phylogenetic tree was generated using the maximum-likelihood method in MEGA X. Bootstrap values based on 1000 replicates are shown at the branching points

Table S1. Ion formulas of HF-500 estimated by positive mode ESI-HRMS analysis.

<i>m/z</i> (measured)	Estimated ion formula	<i>m/z</i> (calculated)	Error (ppm)	Ion type
501.2118	C ₂₇ H ₃₃ O ₉	501.2119	0.3	[M+H] ⁺
	C ₂₈ H ₂₉ N ₄ O ₅	501.2132	2.9	-
	C ₂₄ H ₂₅ N ₁₀ O ₃	501.2106	-2.4	-
523.1940	C ₂₇ H ₃₂ NaO ₉	523.1939	-0.2	[M+Na] ⁺
	C ₂₈ H ₂₉ N ₄ O ₅	523.1952	2.3	-
	C ₂₄ H ₂₅ N ₁₀ NaO ₃	523.1925	-2.8	-
539.1681	C ₂₇ H ₃₂ KO ₉	539.1678	-0.5	[M+K] ⁺

Table S2. Ion formulas of HF-500 estimated by negative mode ESI-HRMS analysis.

<i>m/z</i> (measured)	Estimated ion formula	<i>m/z</i> (calculated)	Error (ppm)	Ion type
499.1969	C ₂₇ H ₃₁ O ₉	499.1974	0.9	[M-H] ⁻
	C ₂₈ H ₂₈ N ₄ O ₅	499.1987	3.6	-
545.2031	C ₂₈ H ₃₃ O ₁₁	545.2028	-0.6	[M-H+HCOOH] ⁻
559.2160	C ₂₉ H ₃₅ O ₁₁	559.2185	4.4	[M-H+CH ₃ COOH] ⁻

Table S3. Assignments of ^{13}C and ^1H chemical shifts of SEB and synthetic SEA.

position	solanoeclepin B				solanoeclepin A		
	δ_{C}	δ_{H}	multiplicity	coupling constant	δ_{H}	multiplicity	coupling constant
1	41.85	1.63	1H ^a		2.46	1H, d	$J = 17.2$ Hz
		2.41	1H, dd	$J = 12.3, 10.5$ Hz	2.55	1H, d	$J = 17.2$ Hz
2	74.85	4.53	1H, ddd	$J = 14.6, 4.3, 4.3$ Hz			
3	87.62	4.00	1H, d	$J = 4.8$ Hz	4.01	1H, s	
4	52.38						
5	154.68						
6	146.81						
7	- ^c						
8	150.55						
9	132.68						
10	93.48						
11	32.21	2.56	1H, dd	$J = 20.0, 5.4$ Hz	2.42	1H, dd	$J = 19.8, 5.0$ Hz
		2.67	1H, ddd	$J = 20.1, 11.6, 5.7$ Hz	2.53	1H, ddd	$J = 19.8, 6.0, 5.8$ Hz
12	33.65	1.47	1H, ddd	$J = 12.9, 12.9, 5.8$ Hz	1.71–1.67	1H, m	
		2.02	1H, dd	$J = 14.0, 5.5$ Hz	1.89	1H, dd	$J = 14.3, 4.6$ Hz
13	39.9						
14	- ^c						
15	66.88	4.43	1H, dd	$J = 7.8, 3.0$ Hz	4.27	1H, dd	$J = 7.4, 3.0$ Hz
16	39.76	2.13	1H, dd	$J = 12.6, 3.0$ Hz	1.97	1H, dd	$J = 12.6, 3.0$ Hz
		2.27	1H, dd	$J = 12.4, 7.6$ Hz	2.1	1H, dd	$J = 12.6, 7.4$ Hz
17	- ^c						
18	15.08	1.30	3H, s		1.14 ^b	3H, s	
19	75.6	4.29	1H, s		4.38	1H, s	
20	- ^c						
21	17.99	1.32	1H, m		1.42-1.31	1H ^a	
22	11.99	0.95-0.91	2H, m		1.03-0.96	2H, m	
23	21.4	1.63	1H ^a		1.42-1.31	1H ^a	
24	- ^c						
25, 26	28.31	1.35	3H, s		1.15 ^b	3H, s	
		1.57	3H, s		1.28	3H, s	
27	62.54	3.59	3H, s		3.42	3H, s	

a: overlapping

b: unable to distinguish

c: could not detect

Table S4. HS activities of SEA and SEB for *Globodera rostochiensis* eggs.

	10 ng/ml			1 ng/ml			100 pg/ml			10 pg/ml			1 pg/ml			100 fg/ml		
	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean
SEB	61.6	62.5	62.0	73.9	76.7	75.3	79.3	81.1	80.2	62.1	66.9	64.5	29.1	27.5	28.3	14.1	10.4	12.3
SEA	87.1	84.0	85.5	87.3	90.1	88.7	88.1	89.9	89.0	82.8	83.1	83.0	59.6	48.8	54.2	24.6	25.1	24.8

Table S5. HS activities of SEA and SEB for *Globodera pallida* eggs.

	10 ng/ml			1 ng/ml			100 pg/ml			10 pg/ml			1 pg/ml			100 fg/ml		
	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean	rep.-1	rep.-2	mean
SEB	87.7	88.7	88.2	84.3	89.8	87.1	67.7	67.7	67.7	22.1	27.0	24.6	1.3	1.3	1.3	0.3	0.0	0.1
SEA	66.5	53.8	60.1	62.1	60.3	61.2	65.9	56.0	61.0	39.2	35.3	37.3	8.4	11.2	9.8	1.1	1.0	1.1

Table S6. List of tomato *DOX* genes specifically expressed in roots.

Gene ID	DOX clade	Gene name	Bud	Flower	Leaf	Root	Fruits stage			
							1cm	2cm	3cm	Mature green
<i>Solyc01g108860</i>	<i>DOXC21</i>		0	0	0	1345	0	0	0	1
<i>Solyc01g090610</i>	<i>DOXC20</i>		0	0	0	648	0	0	0	0
<i>Solyc06g066830</i>	<i>DOXC20</i>		0	0	0	282	0	0	0	0
<i>Solyc12g042980</i>	<i>DOXC20</i>	<i>SOLA3</i>	0	0	0	180	0	0	0	0
<i>Solyc06g067860</i>	<i>DOXC20</i>	<i>SOLA2</i>	0	0	0	152	0	0	0	0
<i>Solyc11g072200</i>	<i>DOXC41</i>		0	0	0	139	0	0	0	0
<i>Solyc06g067870</i>	<i>DOXC20</i>	<i>SOLA1</i>	0	0	2	128	0	0	0	0
<i>Solyc01g090630</i>	<i>DOXC20</i>		0	0	0	94	0	0	0	0
<i>Solyc02g071470</i>	<i>DOXC52</i>		0	0	2	87	0	1	1	8
<i>Solyc09g089830</i>	<i>DOXC22</i>		0	0	0	83	0	0	0	0
<i>Solyc06g083910</i>	<i>DOXC38</i>		1	0	0	75	0	0	0	0
<i>Solyc02g070080</i>	<i>DOXC38</i>		0	0	0	67	0	0	0	0
<i>Solyc09g089680</i>	<i>DOXC31</i>		1	3	1	43	0	0	0	2
<i>Solyc11g010400</i>	<i>DOXC38</i>		0	0	0	41	0	0	0	0
<i>Solyc03g116260</i>	<i>DOXC22</i>		0	1	1	38	0	0	0	0
<i>Solyc01g067620</i>	<i>DOXC54</i>		3	15	0	36	0	1	1	0
<i>Solyc06g073580</i>	<i>DOXC38</i>		2	0	0	26	0	0	0	0
<i>Solyc06g069900</i>	<i>DOXC22</i>		0	0	0	20	0	0	0	0
<i>Solyc01g006580</i>	<i>DOXC20</i>		0	0	0	19	0	0	0	0
<i>Solyc09g089790</i>	<i>DOXC31</i>		0	1	0	18	0	0	0	0
<i>Solyc06g060070</i>	<i>DOXC53</i>		5	1	0	17	0	0	0	0
<i>Solyc03g120970</i>	<i>DOXC3</i>		2	1	2	14	2	1	1	1
<i>Solyc02g071440</i>	<i>DOXC52</i>		4	2	0	12	0	1	0	0
<i>Solyc02g071410</i>	<i>DOXC52</i>		1	1	0	12	0	0	1	1
<i>Solyc02g071490</i>	<i>DOXC52</i>		2	1	0	11	1	2	2	2
<i>Solyc09g089780</i>	<i>DOXC31</i>		0	0	0	8	0	0	0	0
<i>Solyc09g089810</i>	<i>DOXC31</i>		0	0	0	7	0	0	0	0
<i>Solyc07g045040</i>	<i>DOXC55</i>		0	0	0	6	0	0	0	0
<i>Solyc03g025490</i>	<i>DOXC17</i>		0	0	0	5	0	0	0	0
<i>Solyc09g089800</i>	<i>DOXC31</i>		0	0	0	5	0	0	0	0
<i>Solyc07g054870</i>	<i>DOXC37</i>		0	0	1	5	0	0	0	0

Table S7. List of tomato *CYP* genes specifically expressed in roots.

Gene ID	CYP	bud	flower	leaf	root	1cm fruit	2cm fruit	3cm fruit	Mature green fruit	Breaker	Breaker 10 d
<i>Solyc05g011970</i>	<i>CYP749A20</i>	8	7	2	409	2	2	2	2	2	26
<i>Solyc05g011940</i>	<i>CYP749A19</i>	0	0	0	79	0	0	0	0	0	0
<i>Solyc05g021390</i>	<i>CYP716A44</i>	3	0	0	71	0	0	0	0	0	0
<i>Solyc12g042480</i>	<i>CYP736A74</i>	4	8	3	57	3	4	5	15	6	3
<i>Solyc10g007960</i>	<i>CYP74C3</i>	0	0	0	52	1	0	0	0	0	0
<i>Solyc02g084930</i>	<i>CYP722C1</i>	1	1	5	52	0	0	0	0	0	0
<i>Solyc06g065430</i>	<i>CYP716A43</i>	1	0	0	47	0	0	0	0	0	0
<i>Solyc01g109140</i>	<i>CYP74D1</i>	0	0	0	47	0	0	0	13	0	0
<i>Solyc05g047680</i>	<i>CYP78A77</i>	0	1	6	45	0	1	0	0	0	0
<i>Solyc03g095310</i>	<i>CYP714A17</i>	1	7	4	45	11	6	7	4	1	1
<i>Solyc08g075320</i>	<i>CYP707A8</i>	0	1	0	40	0	0	0	0	3	2
<i>Solyc07g055350</i>	<i>CYP72A182</i>	0	0	0	34	0	0	0	0	0	0
<i>Solyc02g082070</i>	<i>CYP71BL1</i>	0	1	0	28	0	0	0	0	0	0
<i>Solyc04g079660</i>	<i>CYP82D38</i>	4	2	5	27	1	0	1	2	2	2
<i>Solyc02g069600</i>	<i>CYP716C6</i>	0	0	0	27	0	0	0	0	0	0
<i>Solyc12g006860</i>	<i>CYP734A8</i>	2	7	2	26	1	1	1	0	0	0
<i>Solyc04g078270</i>	<i>CYP81B40</i>	0	1	0	26	0	0	0	0	0	0
<i>Solyc10g039210</i>	<i>CYP82M2</i>	0	0	0	24	0	0	0	0	0	0
<i>Solyc08g062950</i>	<i>CYP711A21</i>	1	3	5	20	8	10	7	7	2	0
<i>Solyc10g017510</i>	<i>CYP71BE18</i>	0	0	0	20	1	1	1	0	0	0
<i>Solyc03g111880</i>	<i>CYP71AU33</i>	1	0	0	20	0	0	0	0	0	0
<i>Solyc09g098770</i>	<i>CYP76B12</i>	0	0	1	18	0	0	0	0	0	0
<i>Solyc02g065190</i>	<i>CYP76B17</i>	0	0	4	18	0	1	0	0	0	0
<i>Solyc01g008650</i>	<i>CYP71D185</i>	0	0	0	16	0	0	0	0	0	0
<i>Solyc01g094140</i>	<i>CYP704A63</i>	0	0	0	16	0	0	0	3	1	0
<i>Solyc06g074420</i>	<i>CYP94C29</i>	3	2	1	14	6	3	2	0	0	0
<i>Solyc02g092250</i>	<i>CYP71BE17</i>	1	1	1	13	0	1	1	1	1	0
<i>Solyc07g055530</i>	<i>CYP72A174</i>	3	2	3	12	6	5	5	4	3	4
<i>Solyc10g083700</i>	<i>CYP76A21</i>	0	2	0	11	2	5	3	2	0	0
<i>Solyc03g114940</i>	<i>CYP78A75</i>	1	1	3	11	1	0	0	0	0	0
<i>Solyc02g094860</i>	<i>CYP735A20</i>	0	5	0	10	5	5	1	1	0	0
<i>Solyc12g088970</i>	<i>CYP82C22</i>	0	0	0	10	0	0	0	0	0	0
<i>Solyc10g018150</i>	<i>CYP712G1</i>	0	0	0	10	0	0	0	0	0	0
<i>Solyc04g078340</i>	<i>CYP81C9</i>	0	1	0	7	0	1	0	0	0	0
<i>Solyc06g082730</i>	<i>CYP714G9</i>	0	0	0	7	0	0	0	0	0	0
<i>Solyc10g009310</i>	<i>CYP78A78</i>	0	0	0	6	0	0	0	0	0	0
<i>Solyc01g096280</i>	<i>CYP78A74</i>	0	2	2	5	0	0	0	0	0	0
<i>Solyc03g112040</i>	<i>CYP71AU32</i>	0	0	0	5	0	1	0	2	0	4
<i>Solyc01g008640</i>	<i>CYP71D186</i>	0	0	0	5	0	0	0	0	0	0
<i>Solyc01g094130</i>	<i>CYP704A64</i>	0	0	0	5	0	0	0	0	0	0
<i>Solyc08g080430</i>	<i>CYP87A21</i>	0	0	0	4	0	0	0	0	0	0
<i>Solyc03g121510</i>	<i>CYP90D19</i>	0	0	0	4	0	0	0	0	0	0
<i>Solyc10g081550</i>	<i>CYP82M3</i>	0	0	0	4	0	0	0	0	0	0
<i>Solyc06g076800</i>	<i>CYP86A33</i>	0	0	1	3	0	0	0	0	0	0
<i>Solyc03g019870</i>	<i>CYP720A1</i>	0	0	0	3	0	0	0	0	0	0
<i>Solyc02g090300</i>	<i>CYP76B24</i>	1	0	0	3	1	0	0	0	0	0
<i>Solyc04g080650</i>	<i>CYP722A1</i>	0	0	0	3	1	0	0	0	0	0
<i>Solyc10g007890</i>	<i>CYP72A194</i>	0	0	0	2	0	0	0	0	0	0
<i>Solyc09g008910</i>	<i>CYP76A19</i>	0	0	0	2	0	0	0	0	0	0
<i>Solyc06g073570</i>	<i>CYP82W1</i>	0	0	0	2	0	0	0	0	0	0
<i>Solyc02g094110</i>	<i>CYP94B17</i>	0	0	0	2	0	0	0	0	0	0
<i>Solyc07g055970</i>	<i>CYP718A6</i>	0	0	0	2	0	0	0	0	0	0
<i>Solyc04g011920</i>	<i>CYP96A51</i>	0	0	0	1	0	0	0	0	0	0
<i>Solyc07g055490</i>	<i>CYP72A176</i>	0	0	0	1	0	0	0	0	0	0
<i>Solyc02g014730</i>	<i>CYP86B11</i>	0	0	0	1	0	0	0	0	0	0
<i>Solyc04g011940</i>	<i>CYP96A49</i>	0	0	0	1	0	0	0	0	0	0
<i>Solyc04g054250</i>	<i>CYP736A67</i>	0	0	0	1	0	0	0	0	0	0

Table S8. Primer sequences used in this paper.

Primer No.	Usage	Primer sequence (5' → 3')
Primer-1	For qRT-PCR of <i>SOLA1</i> (Fw)	GGTTATGTTGGCCAAATCCCATT
Primer-2	For qRT-PCR of <i>SOLA1</i> (Rv)	TGCTAGCAATGCTTCGCTAAAATCA
Primer-3	For qRT-PCR of <i>SOLA2</i> (Fw)	ACTCGAGACCTTCAATGGATAACTC
Primer-4	For qRT-PCR of <i>SOLA2</i> (Rv)	GCACCCTATCATTGCTCCATCC
Primer-5	For qRT-PCR of <i>SOLA3</i> (Fw)	ACCTTGAGGAGTGGCACAACC
Primer-6	For qRT-PCR of <i>SOLA3</i> (Rv)	CTTCCCTTAGCTCCGGCATGA
Primer-7	For qRT-PCR of <i>Solyc01g090610</i> (Fw)	CACCAAGTACAGAGCAGCACC
Primer-8	For qRT-PCR of <i>Solyc01g090610</i> (Rv)	ACCGTGAGAAGCCTTTATCTG
Primer-9	For qRT-PCR of <i>Solyc01g090630</i> (Fw)	ACGTTGATGTCCCTCCCTCC
Primer-10	For qRT-PCR of <i>Solyc01g090630</i> (Rv)	AACACTTGGTGCTTGTCTGG
Primer-11	For qRT-PCR of <i>SOLA4</i> (Fw)	TGCATGAATCCGACACGAAC
Primer-12	For qRT-PCR of <i>SOLA4</i> (Rv)	TTGCCATTGCGGATGAAGTG
Primer-13	For qRT-PCR of <i>SOLA5</i> (Fw)	CCCAAGAAGCTTTTCGGAGAC
Primer-14	For qRT-PCR of <i>SOLA5</i> (Rv)	ACGTTGAGCATTGTCTCAC
Primer-15	For qRT-PCR of <i>CYP716A44</i> (Fw)	ATCTTGCTGAGCTTCCACAC
Primer-16	For qRT-PCR of <i>CYP716A44</i> (Rv)	AAATCATGGTGCGTGGTAGC
Primer-17	For qRT-PCR of <i>Ubiquitin 3</i> (Fw)	CACCAAGCCAAAGAAGATCAAGC
Primer-18	For qRT-PCR of <i>Ubiquitin 3</i> (Rv)	TCAGCATTAGGGCAGCTCTTACG
Primer-19	For construction of CRISPR/Cas9 vector of <i>SOLA1</i>	TTGGGTCTCGTGCAGTTGGACCATGAATTTGTACCGTTTTAGAGCTAGAAATAGCA
Primer-20		TTGGGTCTCGATAACCATAACAAGTTTTAGAGCTAGAAATAGCA
Primer-21		TTGGGTCTCCTTATGTTGGCCACTGCACCAGCCGGGAATCGAA
Primer-22		TTGGGTCTCCAAACTTGTATGGTTATGTTGGCCACTGCACCAGCCGGGAATCGAA
Primer-23	For construction of CRISPR/Cas9 vector of <i>SOLA2</i>	TTGGGTCTCGTGCAGAACTATAGAGATATACTATGTTTTAGAGCTAGAAATAGCA
Primer-24		TTGGGTCTCGAGTAGTTGTGCCGTTTTAGAGCTAGAAATAGCA
Primer-25		TTGGGTCTCCTACTTGGGAATCTGCACCAGCCGGGAATCGAA
Primer-26		TTGGGTCTCCAAACTGGTTTTCAAAGGCATGTCTCTGCACCAGCCGGGAATCGAA
Primer-27	For construction of CRISPR/Cas9 vector of <i>SOLA3</i>	TTGGGTCTCGTGCAGTCGAGACGTGCATAACCATGGTTTTAGAGCTAGAAATAGCA
Primer-28		TTGGGTCTCGTCCAACGCGCATGTTTTAGAGCTAGAAATAGCA
Primer-29		TTGGGTCTCCTGGAAGAGTACGCTGCACCAGCCGGGAATCGAA
Primer-30		TTGGGTCTCCAAACGCATGACTTTCTTGTACTCTGCACCAGCCGGGAATCGAA
Primer-31	For construction of CRISPR/Cas9 vector of <i>SOLA4</i>	TTGGGTCTCGTGCAGATATCTTTGGTATTTCCATGGTTTTAGAGCTAGAAATAGCA
Primer-32		TTGGGTCTCGGAAGTCAAAGTCTTTAGAGCTAGAAATAGCA
Primer-33		TTGGGTCTCCCTTCTCATCAGTCTGCACCAGCCGGGAATCGAA
Primer-34		TTGGGTCTCCAAACATGATTGATATTTCTCATGACTGCACCAGCCGGGAATCGAA
Primer-35	For construction of CRISPR/Cas9 vector of <i>SOLA4</i>	TTGGGTCTCGTGCAGCAAGAAGCTTTTCGGAGACGGTTTTAGAGCTAGAAATAGCA
Primer-36		TTGGGTCTCGTGGGTAAAAATGGTTTTAGAGCTAGAAATAGCA
Primer-37		TTGGGTCTCCCCATTTTTCGCTGCACCAGCCGGGAATCGAA
Primer-38		TTGGGTCTCCAAACTGTTTTTCATGGAGTAAGCCTGCACCAGCCGGGAATCGAA
Primer-39	For construction of CRISPR/Cas9 vector of <i>CYP716A44</i>	TTGGGTCTCGTGCAGTCCCCTAGCAAAGTGACGTTGTTTTAGAGCTAGAAATAGCA
Primer-40		TTGGGTCTCCTTGCATGCTTCTGCACCAGCCGGGAATCGAA
Primer-41		TTGGGTCTCGGCAACGTTACGTGTTTTAGAGCTAGAAATAGCA
Primer-42		TTGGGTCTCCAAACATTTGATGTTTTGGCTTCTGCTGCACCAGCCGGGAATCGAA
Primer-43	For genotyping of <i>SOLA1</i> -ko (Fw)	GGGTTCTCTAACAGTTACCCACAA
Primer-44	For genotyping of <i>SOLA1</i> -ko (Rv)	CCATTAGGCCACATGAAATTGGT
Primer-45	For genotyping of <i>SOLA2</i> -ko (Fw)	AAGAAGCCTATATAAAACCAAGCAA
Primer-46	For genotyping of <i>SOLA2</i> -ko (Rv)	TGGCCACATGAGGTTGGAAA
Primer-47	For genotyping of <i>SOLA3</i> -ko (Fw)	TGGCTTTCCAAGTGAACCAA
Primer-48	For genotyping of <i>SOLA3</i> -ko (Rv)	ACCAGTATTTATGAACGTTGTCAAT
Primer-49	For genotyping of <i>SOLA4</i> -ko (Fw)	TTCCCTACACATCATTATCAAAGC
Primer-50	For genotyping of <i>SOLA4</i> -ko (Rv)	TTGTGCCCTCTAGCTCCAT
Primer-51	For genotyping of <i>SOLA5</i> -ko (Fw)	CTCTACTGGCATGGACTGCAA
Primer-52	For genotyping of <i>SOLA5</i> -ko (Rv)	TTCCAAGTTTCGAGCATTGTCT
Primer-53	For genotyping of <i>CYP716A44</i> -ko (Fw)	TTGTGGTGCATCAGCCAATAAA
Primer-54	For genotyping of <i>CYP716A44</i> -ko (Rv)	TTGTGTTGATGATACTTTCCCTCA