SUPPORTING INFORMATION FOR:

High Titer Heterologous Production of Lyngbyatoxin in *E. coli*, a Protein Kinase C Activator from an Uncultured Marine Cyanobacterium

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Supplementary Table 1: ^{1}H (300 MHz) NMR data for ILV, **2**

	δ_H (multiplicity, J=Hz)	
No.	conformer A[†]	conformer B[†]
2	7.11 (s)	6.94 (s)
5	6.95 (dd, 7.4, 0.8)	6.44 (dd, 7.4, 0.8)
6	7.06 (t, 7.7, 7.7)	6.95 (t, 7.8, 7.8)
7	7.28 (dd, 8.0, 1.2)	6.88 (dd, 8.2, 1.0)
8	2.88 (dd, 14.3, 1.6)	3.04 (dd, 14.3, 1.6)
	3.03 (dd, 14.3, 1.6)	3.09 (m)
9	4.24 (m)	2.24 (m)
12	3.09 (m)	4.49 (d, 10.2)
14	3.22 (dd, 11.2, 6.9)	3.46 (dd, 11.2, 9.1)
	not observed*	3.62 (dd, 11.2, 4.6)
15	2.31 (dquin, 11.0, 6.6x4)	2.55 (dquin, 10.2, 6.6x4)
16	0.9 (d, 6.5)	0.61 (d, 6.7)
17	1.25 (d, 6.7)	0.89 (d, 6.4)
18	2.71 (s)	2.89 (s)

*Signal was obscured by the solvent peak.

[†]Two conformational states are observed for ILV.

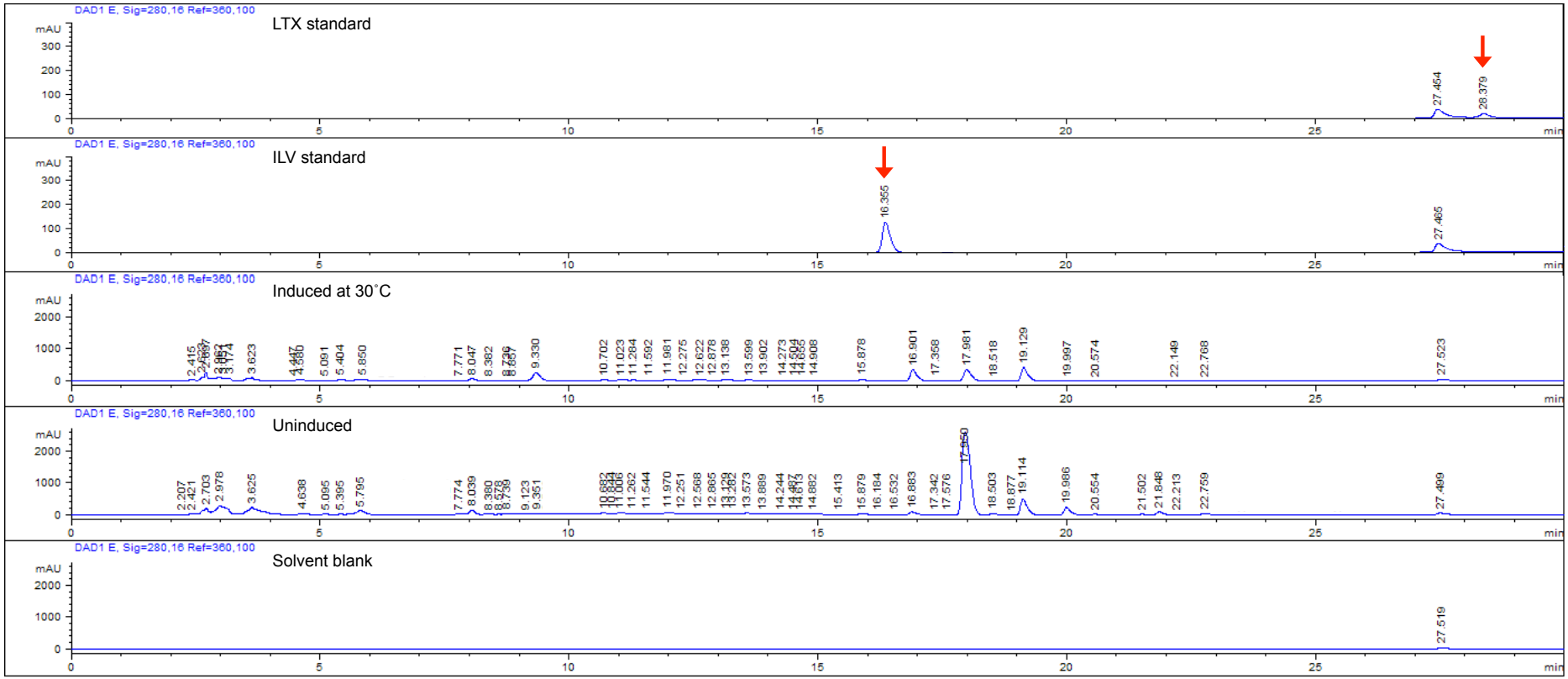
NMR spectra were obtained using the Bruker Avance III 300 system in MeOH-d_4 (CD_3OD), and the solvent peak was used as an internal standard (δ_H 3.31).

Supplementary Table 2: ^{1}H (600 MHz) NMR data for LTX, **1**

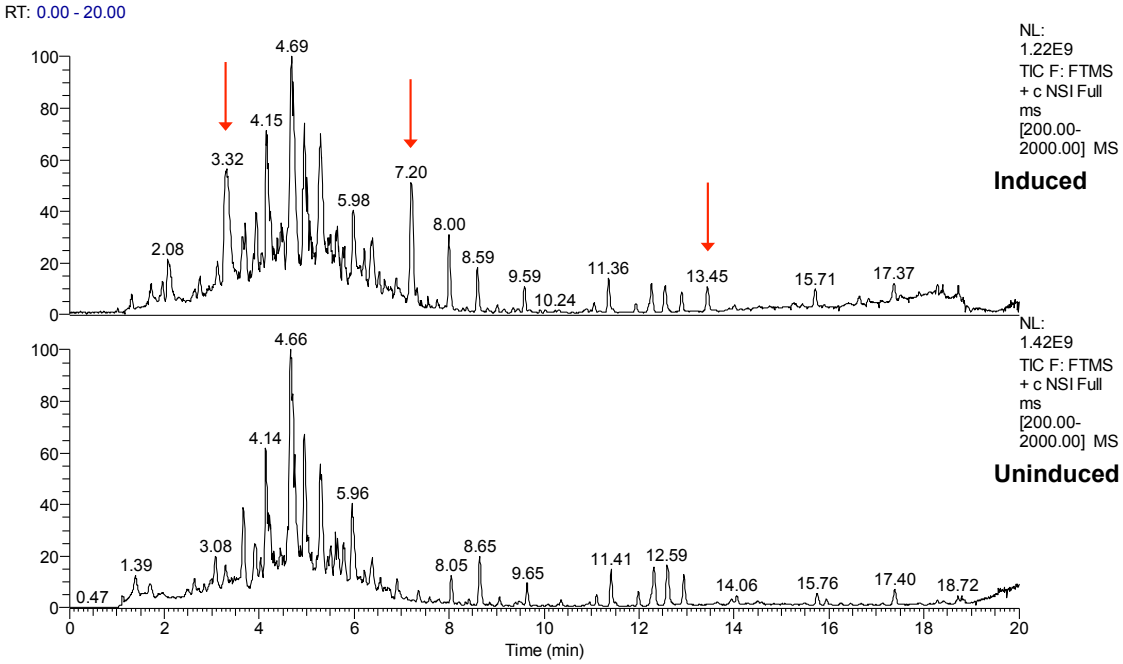
No.	δ_H (multiplicity, J=Hz)
1	not observed
2	6.91 (s)
5	6.47 (d, 8.0)
6	6.93 (d, 8.0)
8	3.10 (dd, 17.4, 2.3)
	2.05 (dd, 17.4, 3.7)
9	4.29 (s)
10	not observed
12	4.42 (d, 10)
14	3.65 (dd, 11.0, 4.7)
	3.6 (dd, 11.0, 4.7)
15	2.54 (m)
16	0.88 (d)
17	0.63 (d)
18	2.84 (s)
20	1.46s)
21	6.13 (ddd, 17.6, 10.7, 4.8)
22	5.13 (dd, 10.7)
	5.07 (dd, 17.6)
23	1.81 (td, 13.2, 13.2, 4.9)
	1.74 (ddd, 13.2, 11.8, 4.9)
24	2.02 (br m)
	1.61 (m*)
25	5.01 (br m)
27	1.47 (s)
28	1.58 (br s)

*Signal was obscured by an adjacent peak.

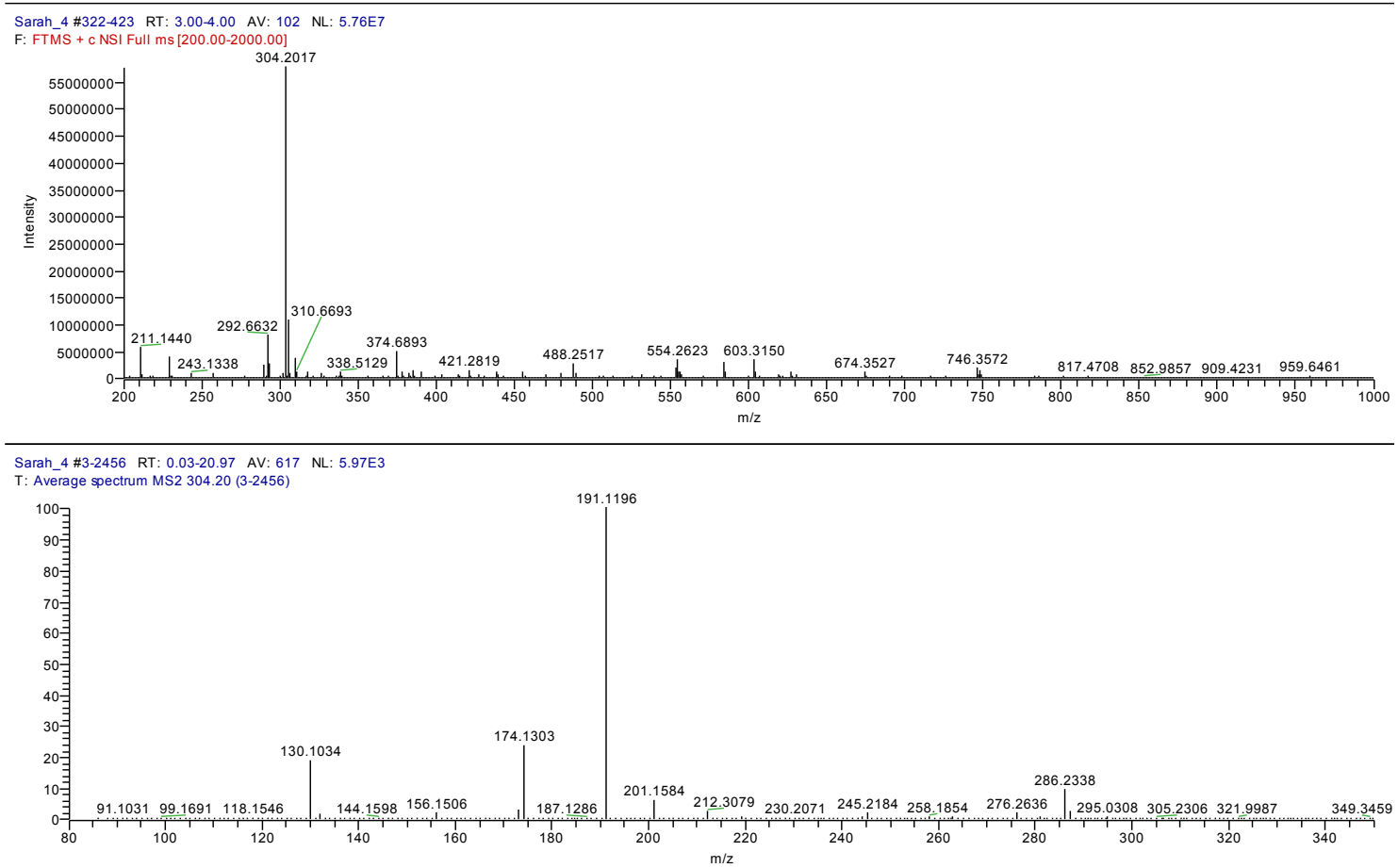
NMR spectra were obtained using the Bruker Avance III 600 system in MeOH-d_4 (CD_3OD), and the solvent peak was used as an internal standard (δ_H 3.31).



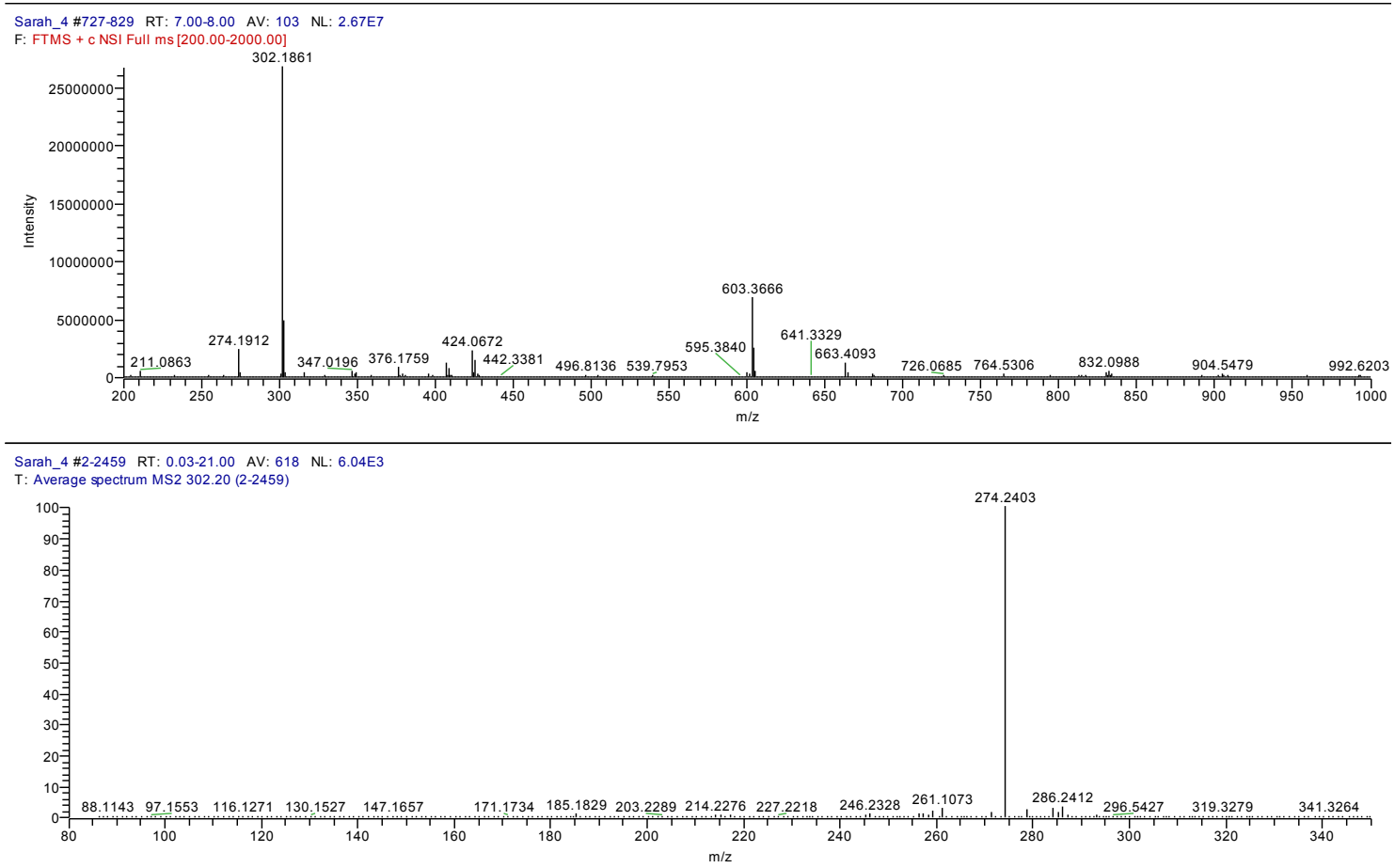
Supplementary Figure 1. HPLC analysis of crude *E. coli* extracts grown at 30˚C. Solvent gradient 5-95% acetonitrile over 30 min. No production was observed at 30˚C. Ten micrograms each of LTX and ILV standards (indicated with red arrows) were injected.



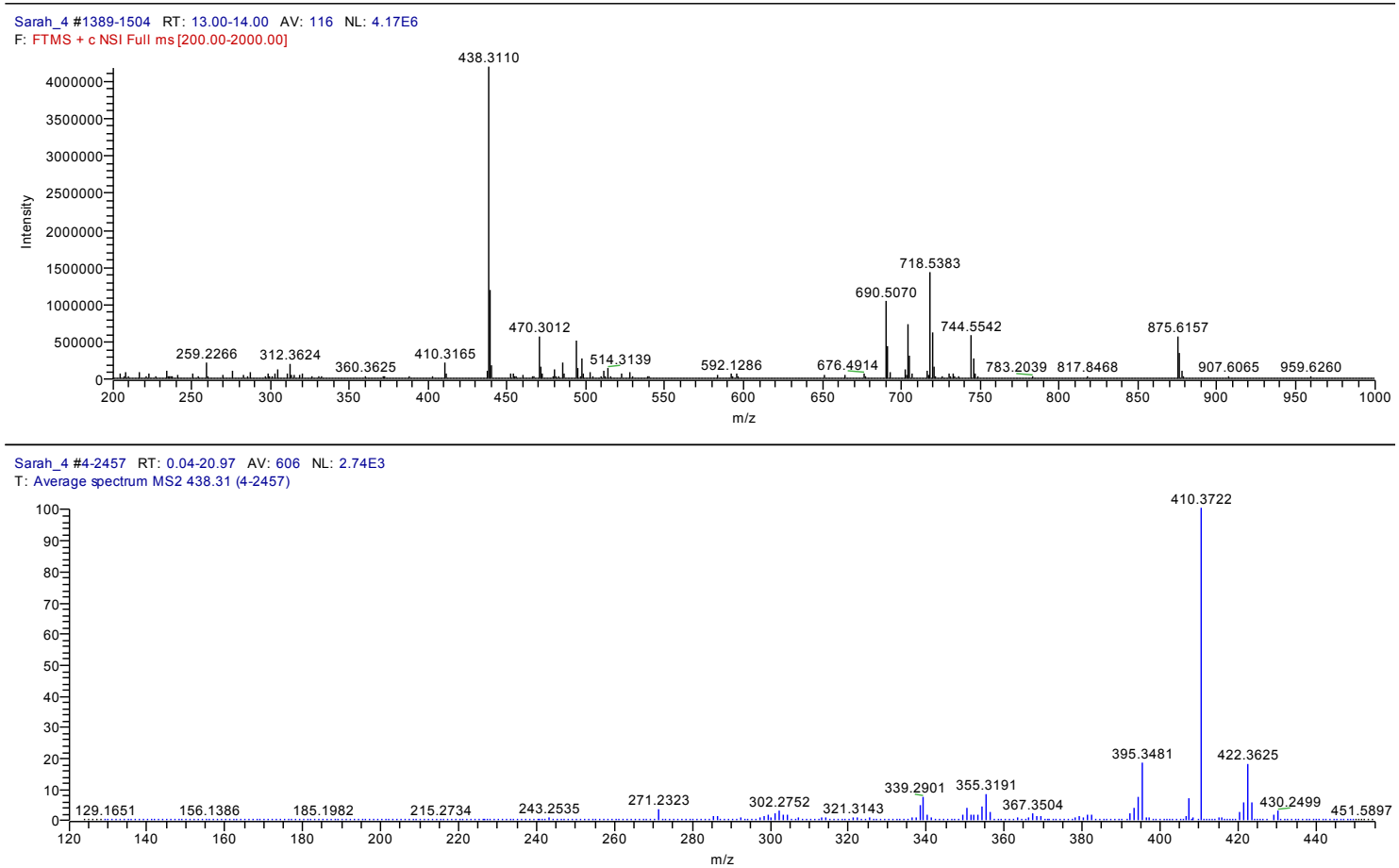
Supplementary Figure 2. LC-MS total ion count chromatogram of tetracycline-induced *E. coli* pCC-Ptet-ltx fermentation crude methanol extracts. Clear differences between the induced and uninduced cultures are indicated with red arrows.



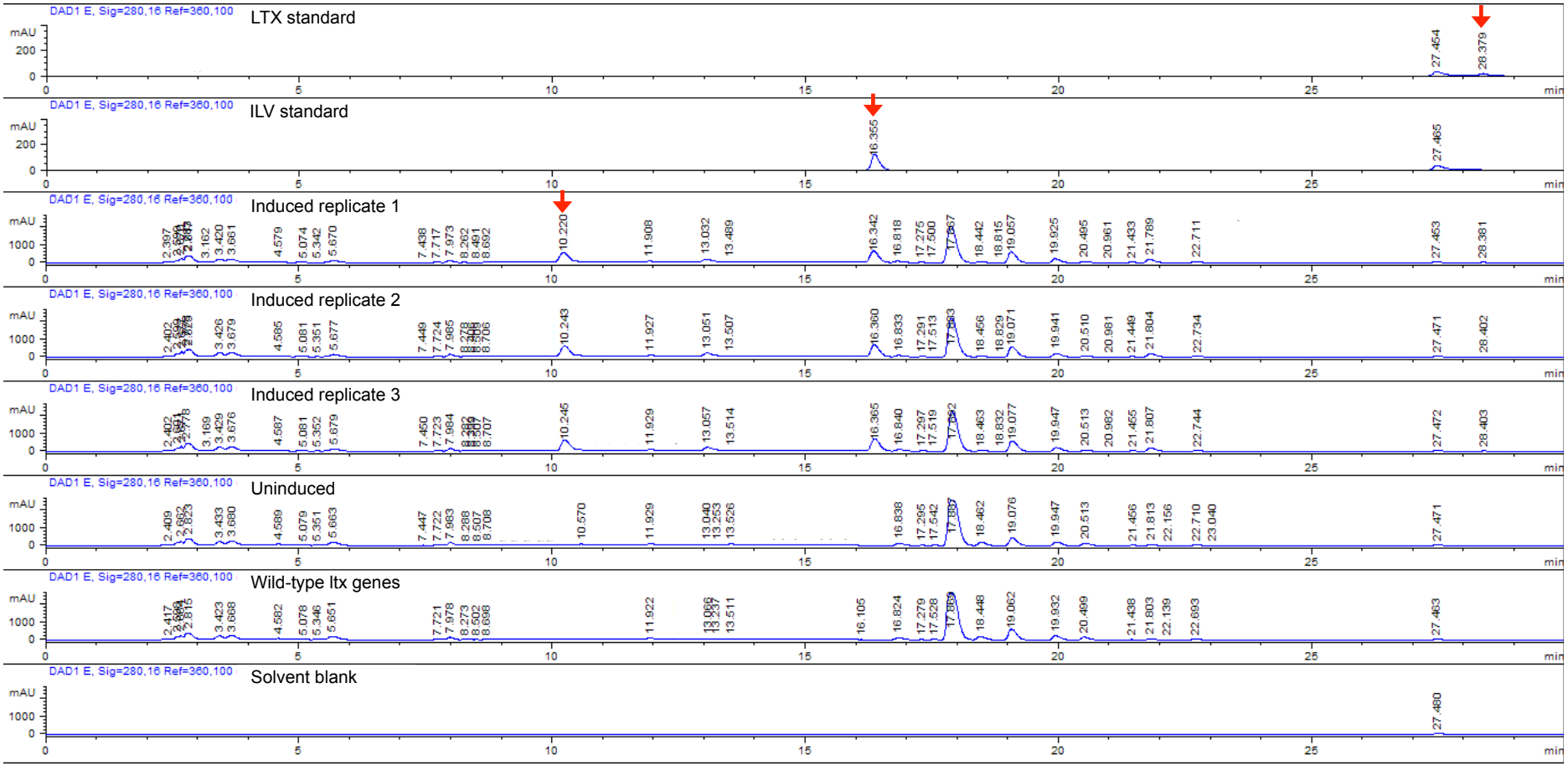
Supplementary Figure 3a. LC-MS/MS of tetracycline-induced *E. coli* pCC-Ptet-ltx fermentation crude methanol extract. Upper window, full spectra (0-1000 m/z) at retention time 3-4 min; Lower window, secondary ionization of 304.2 ± 0.5 *m/z*.



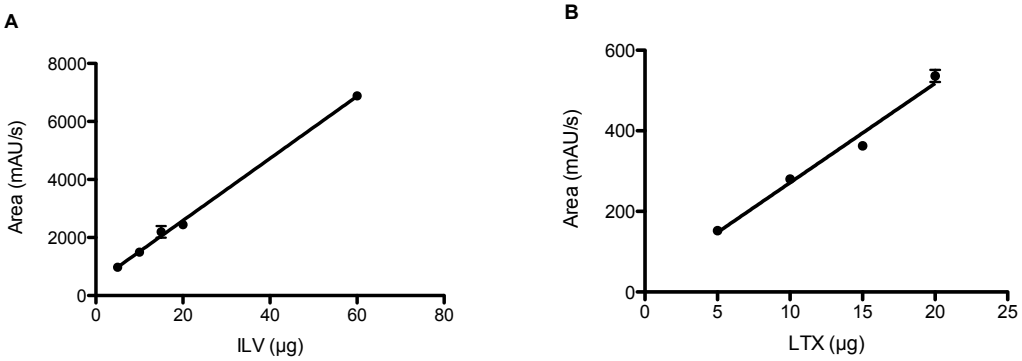
Supplementary Figure 3b. LC-MS/MS of tetracycline-induced *E. coli* pCC-Ptet-ltx fermentation crude methanol extract. Upper window, full spectra (0-1000 m/z) at retention time 7-8 min; Lower window, secondary ionization of 302.2 ± 0.5 *m/z*.



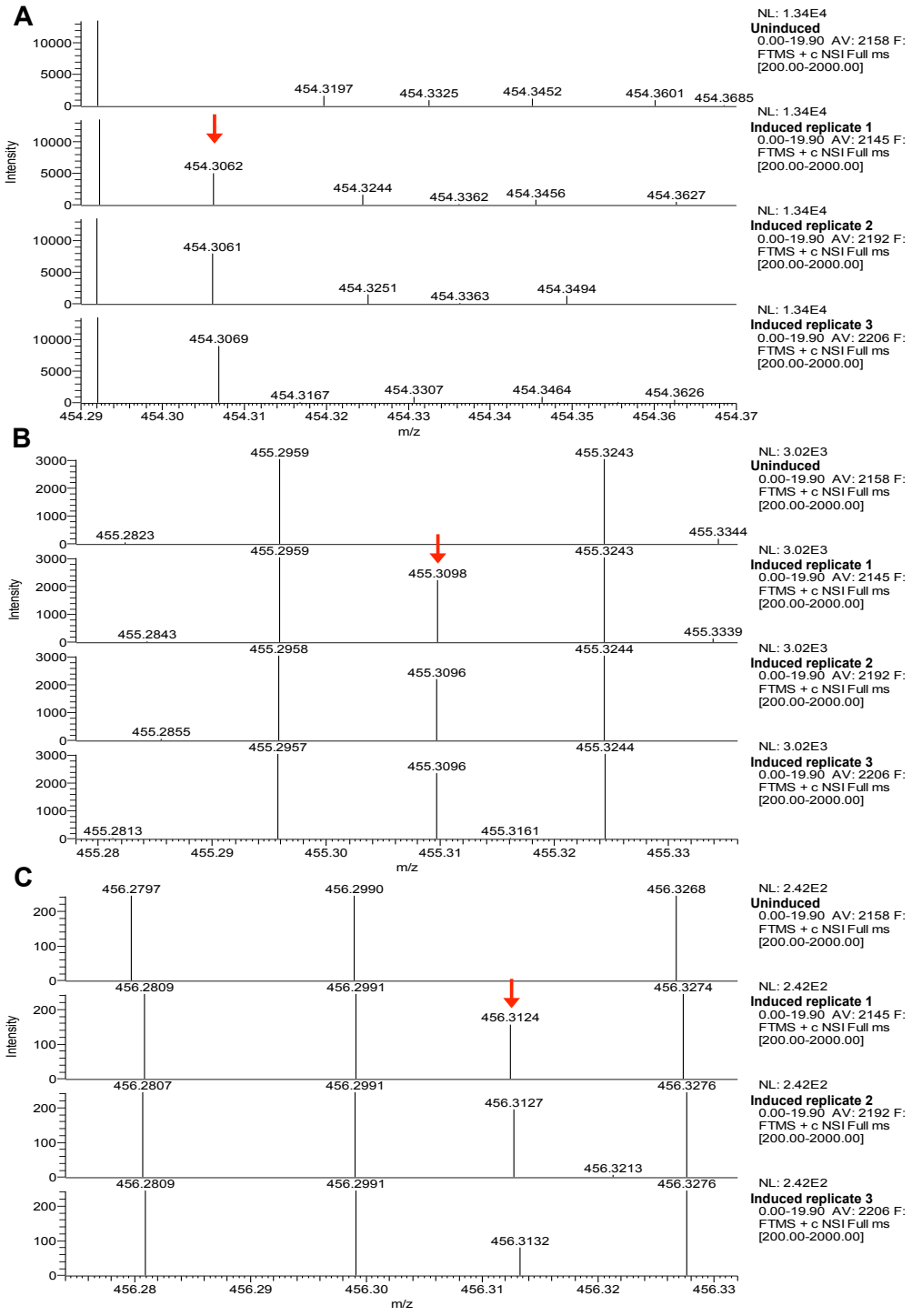
Supplementary Figure 3c. LC-MS/MS of tetracycline-induced *E. coli* pCC-Ptet-ltx fermentation crude methanol extract. Upper window, full spectra (0-1000 m/z) at retention time 13-14 min; Lower window, secondary ionization of 438.31 ± 0.5 *m/z*.



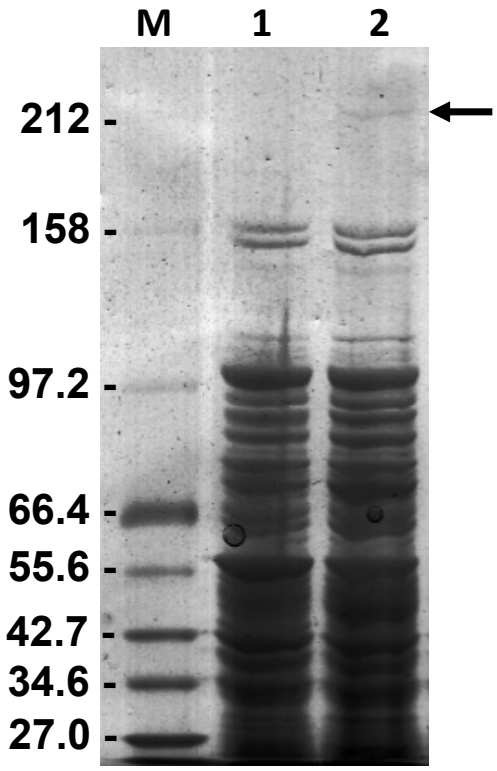
Supplementary Figure 4. HPLC analysis of crude *E. coli* extracts of tetracycline induced pCC-Ptet-ltx (3 replicates), uninduced pCC-Ptet-ltx and wild-type ltx gene cluster cultures cultivated at 18˚C. Solvent gradient 5-95% acetonitrile over 30 min. Ten micrograms each of LTX and ILV standards (indicated with red arrows) and 2% of the crude extracts were injected. The peak corresponding to NVMT is indicated with a red arrow in the induced samples.



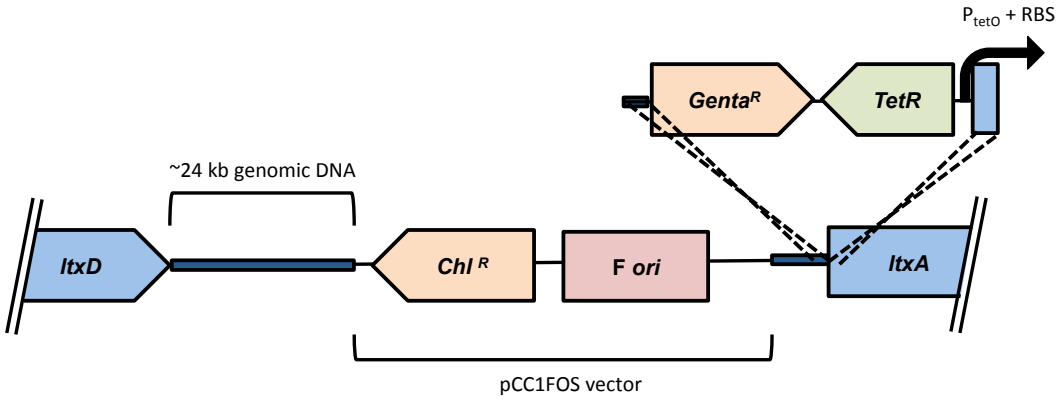
Supplementary Figure 5. Standard curves for quantification of (A) ILV ($R^2 = 0.998$) and (B) LTX ($R^2 = 0.981$) by HPLC. Each data point represents the average of at least two experiments.



Supplementary Figure 6. Zoomed HR-ESI-MS spectra showing presence of ions indicative of the presence of lyngbyatoxin B or C isoforms. The experimental lyngbyatoxin B/C molecular ion isotope distribution for peaks corresponding to the expected is shown, (A) 454.31 $[M+H]^+$, (B) 455.31 $[M+H]^+$, (C) 456.31 $[M+H]^+$. In all instances the top pane is uninduced, followed by triplicate induced cultures.



Supplementary Figure 7. SDS-PAGE of *E. coli* soluble protein fractions. M, broad range protein marker (2-212 kDa) (New England Biolabs); 1, uninduced GB05-MtaA pCC-Ptet-ltx; 2, tetracycline-induced GB05-MtaA pCC-Ptet-ltx on an Invitrogen NuPAGE Novex 3-8% Tris-Acetate 1.0 mm gel. Arrows indicate the band corresponding to LtxA. Protein identity was confirmed by LC-MS/MS analysis of the excised and tryptic digested protein band, with assignment as LtxA by MASCOT using the NCBI nr protein database.



Supplementary Figure 8. Strategy for insertion of the tetracycline-inducible promoter (P_{tetO}) cassette $Genta^R$-TetR-P_{tetO}, consisting of gentamicin resistance (*aacC1*), tetracycline repressor (*tetR*) and P_{tetO} by recombineering using 40 nt homology arms to upstream of *ltxA* and to *ltxA* coding sequence (including the start codon).