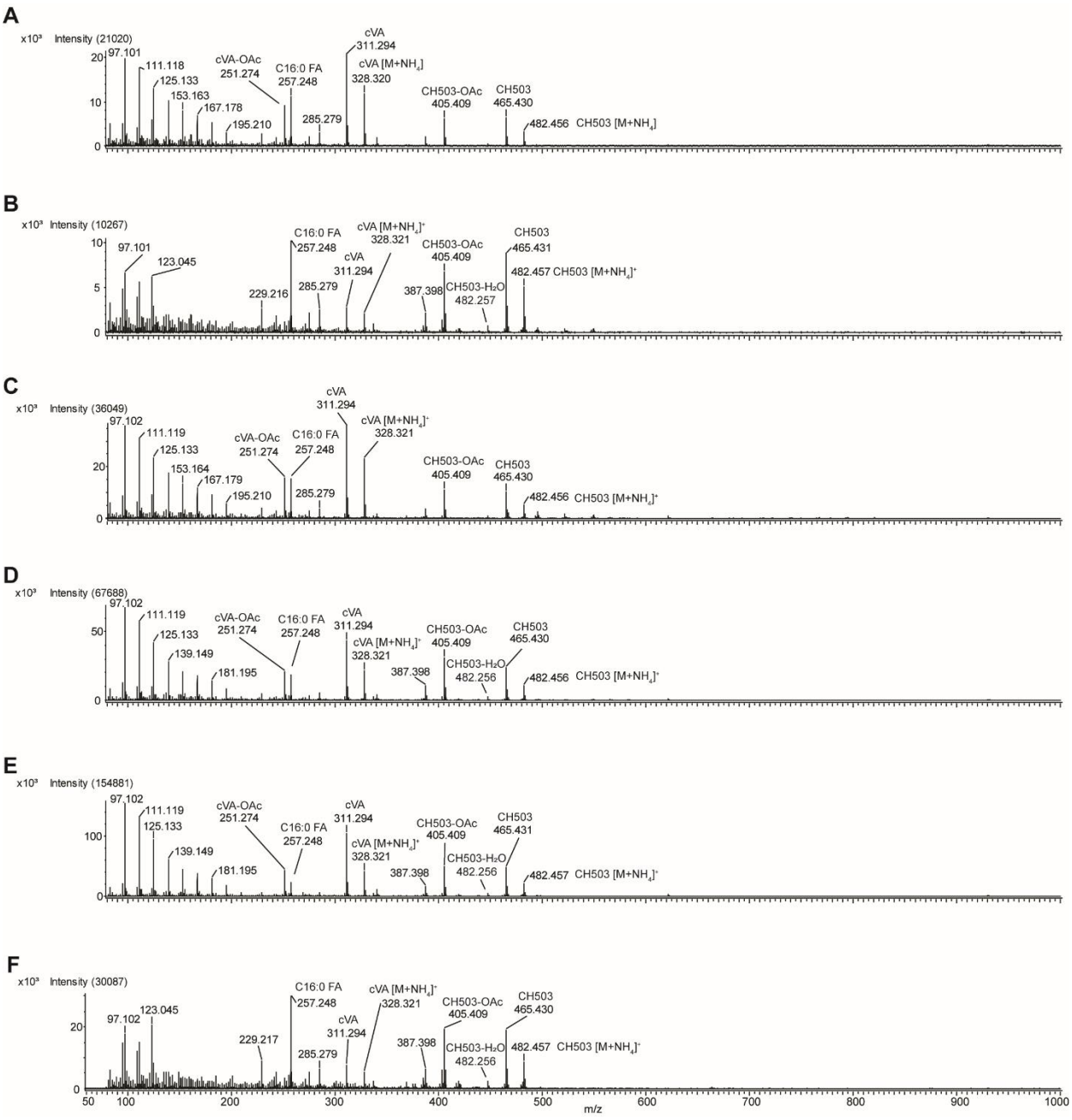
Electronic Supplementary Material (ESI) for Analyst.

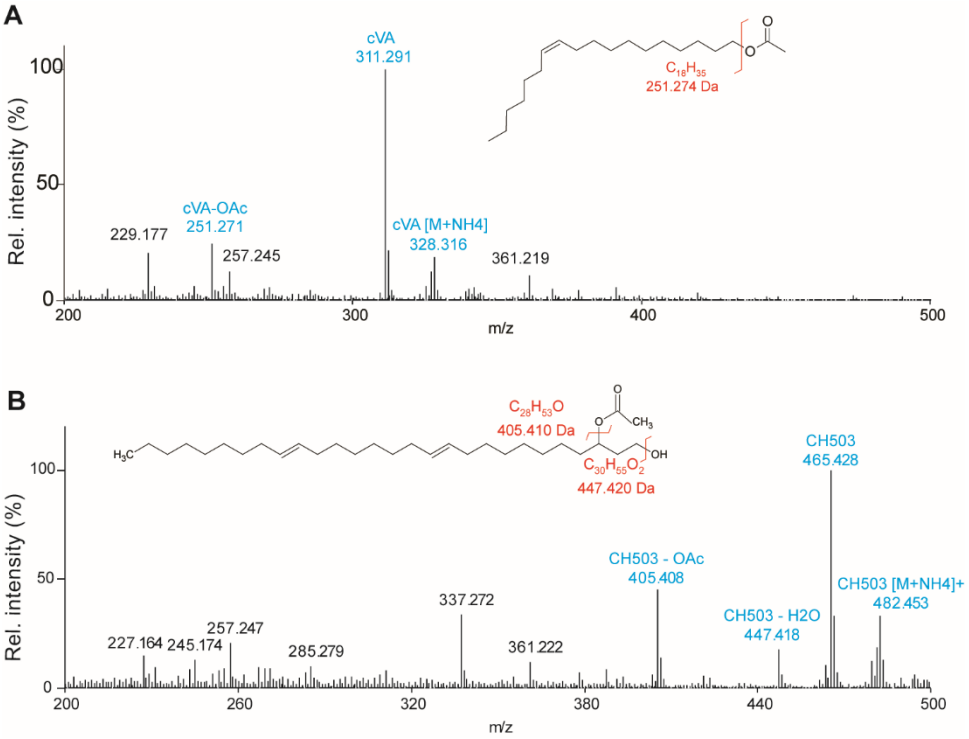
Supp. Table 1. Signals and chemical classes identified from the direct analysis of the EB using DART MS and laser desorption ionization MS.

Elemental composition[1]	Meas. *m/z*	Calc. *m/z*	Δ (mmu)[2]	Ion Form	#C: #DB[3]	Chemical class[4]	LDI MS[5] *m/z*	Calc. *m/z*	Δ (mmu)
C14 H25 O2	225.182	225.185	-0.003	$[M+H]^+$	C14:2	FA	-	-	-
C14 H27 O2	227.203	227.201	0.002	$[M+H]^+$	C14:1	FA	265.16	265.16	0.00
C14 H29 O2	229.213	229.217	-0.004	$[M+H]^+$	C14:0	FA	267.16	267.17	-0.01
C18 H34	251.274	251.274	0.000	$[M+H]^+$	-	cVA-OAc	-	-	-
C16 H31 O2	255.232	255.232	0.000	$[M+H]^+$	C16:1	FA	293.18	293.19	-0.01
C16 H33 O2	257.247	257.248	-0.001	$[M+H]^+$	C16:0	FA	295.20	295.20	-
C18 H33 O2	281.246	281.248	-0.002	$[M+H]^+$	C18:2	FA	319.20	319.20	-
C18 H35 O2	283.259	283.264	-0.005	$[M+H]^+$	C18:1	FA	321.21	321.22	-0.01
C26 H34 O1 N1	376.269	376.264	0.005	$[M+H]^+$	-	unk	-	-	-
C20 H39 O2	311.294	311.295	-0.001	$[M+H]^+$	-	cVA	349.25	349.25	0.00
C22 H41 O4	369.299	369.300	-0.001	$[M+H]^+$	-	unk	407.25	407.26	-0.01
C28 H53 O1	405.409	405.409	0.000	$[M+H]^+$	-	CH503-OAc	443.35	443.37	-0.02
C30 H57 O3	465.43	465.431	-0.001	$[M+H]^+$	-	CH503	503.37	503.39	-0.02
C33 H61 O4	521.456	521.457	-0.001	$[M+H]^+$	C30:1	DAG-H_2O	-	-	-
C33 H63 O4	523.471	523.470	0.001	$[M+H]^+$	C30:0	DAG-H_2O	561.43	561.43	0.00
C33 H63 O5	539.462	539.468	-0.006	$[M+H]^+$	C30:1	DAG	577.42	577.42	0.00
C33 H65 O5	541.488	541.483	0.005	$[M+H]^+$	C30:0	DAG	-	-	-
C35 H61 O4	545.455	545.457	-0.002	$[M+H]^+$	C32:3	DAG-H_2O	-	-	-
C35 H63 O4	547.472	547.469	0.003	$[M+H]^+$	C32:2	DAG-H_2O	-	-	-
C35 H63 O5	563.47	563.468	0.002	$[M+H]^+$	C32:3	DAG	-	-	-
C35 H65 O4	549.488	549.488	0.000	$[M+H]^+$	C32:1	DAG-H_2O	587.44	587.44	0.00
C35 H65 O5	565.483	565.483	0.000	$[M+H]^+$	C32:2	DAG	603.43	603.44	-0.01
C35 H65 O6	581.475	581.478	-0.003	$[M+H]^+$	C32:1	TAG	-	-	-
C35 H67 O5	567.498	567.499	-0.001	$[M+H]^+$	C32:0	DAG	605.44	605.45	-0.01
C37 H65 O4	573.492	573.488	0.004	$[M+H]^+$	C34:3	DAG-H_2O	-	-	-
C37 H67 O4	575.501	575.504	-0.003	$[M+H]^+$	C34:2	DAG-H_2O	-	-	-
C37 H67 O5	591.5	591.499	0.001	$[M+H]^+$	C34:3	DAG	-	-	-
C37 H69 O4	577.519	577.52	-0.001	$[M+H]^+$	C34:1	DAG-H_2O	615.47	615.48	-0.01
C37 H69 O5	593.514	593.515	-0.001	$[M+H]^+$	C34:2	DAG	-	-	-
C37 H72 O5 N1	*610.536*	*610.541*	*-0.005*	$[M+NH4]^+$	*C34:2*	*DAG*	-	-	
C37 H71 O5	595.522	595.53	-0.008	$[M+H]^+$	C34:1	DAG	633.47	633.48	-0.01
C39 H69 O4	601.52	601.52	0.000	$[M+H]^+$	C36:3	DAG-H_2O	-	-	-
C39 H71 O4	603.535	603.53	0.005	$[M+H]^+$	C36:2	DAG-H_2O	-	-	-
C39 H71 O5	619.53	619.53	0.000	$[M+H]^+$	C36:3	DAG	-	-	-
C39 H73 O4	605.551	605.551	0.000	$[M+H]^+$	C36:1	DAG-H_2O	-	-	-
C40 H77 O4	621.576	621.582	-0.006	$[M+H]^+$	C37:0	DAG-H_2O	659.52	659.53	-0.01
C41 H82 O6 N1	*684.606*	684.614	-0.008	$[M+NH4]^+$	C38:0	TAG	-	-	-
C43 H86 O6 N1	*712.641*	712.646	-0.005	$[M+NH4]^+$	C40:0	TAG	-	-	-
C43 H84 O6 N1	*710.612*	710.609	0.003	$[M+NH4]^+$	C40:1	TAG	-	-	-
C45 H87 O6	723.646	723.65	-0.004	$[M+H]^+$	C42:0	TAG	761.59	761.60	-0.01
C45 H90 O6 N1	*740.673*	*740.677*	*-0.004*	$[M+NH4]^+$	*C42:0*	*TAG*	-	-	-
C45 H85 O6	721.631	721.635	-0.004	$[M+H]^+$	C42:1	TAG	759.58	759.59	-0.01
C45 H88 O6 N1	*738.654*	*738.661*	*-0.007*	$[M+NH4]^+$	*C42:1*	*TAG*	-	-	-
C47 H94 O6 N1	768.7	768.708	-0.008	$[M+NH4]^+$	C44:0	TAG	-	-	-
C47 H89 O6	749.663	749.666	-0.003	$[M+H]^+$	C44:1	TAG	787.60	787.62	-0.02
C47 H92 O6 N1	*766.687*	*766.692*	*-0.005*	$[M+NH4]^+$	*C44:1*	*TAG*	-	-	-
C47 H87 O6	747.645	747.65	-0.005	$[M+H]^+$	C44:2	TAG	785.58	785.60	-0.02
C47 H90 O6 N1	*764.665*	*764.677*	*-0.012*	$[M+NH4]^+$	*C44:2*	*TAG*	-	-	-
C49 H93 O6	777.694	777.697	-0.003	$[M+H]^+$	C46:1	TAG	815.64	815.65	-0.01
C49 H96 O6 N1	*794.718*	*794.723*	*-0.005*	$[M+NH4]^+$	*C46:1*	*TAG*	-	-	-
C49 H91 O6	775.681	775.682	-0.001	$[M+H]^+$	C46:2	TAG	813.62	813.64	-0.02
C49 H94 O6 N1	*792.702*	*792.708*	*-0.006*	$[M+NH4]^+$	*C46:2*	*TAG*	-	-	-
C49 H89 O6	773.661	773.666	-0.005	$[M+H]^+$	C46:3	TAG	-	-	-
C49 H92 O6 N1	*790.688*	*790.692*	*-0.004*	$[M+NH4]^+$	*C46:3*	*TAG*	-	-	-
C51 H97 O6	805.724	805.729	-0.005	$[M+H]^+$	C48:1	TAG	843.66	843.68	-0.02
C51 H100 O6 N1	*822.748*	*822.755*	*-0.007*	$[M+NH4]^+$	*C48:1*	*TAG*	-	-	-
C51 H95 O6	803.71	803.713	-0.003	$[M+H]^+$	C48:2	TAG	841.68	841.67	0.01
C51 H98 O6 N1	*820.731*	*820.739*	*-0.008*	$[M+NH4]^+$	*C48:2*	*TAG*	-	-	-
C51 H93 O6	801.694	801.698	-0.004	$[M+H]^+$	C48:3	TAG	-	-	-

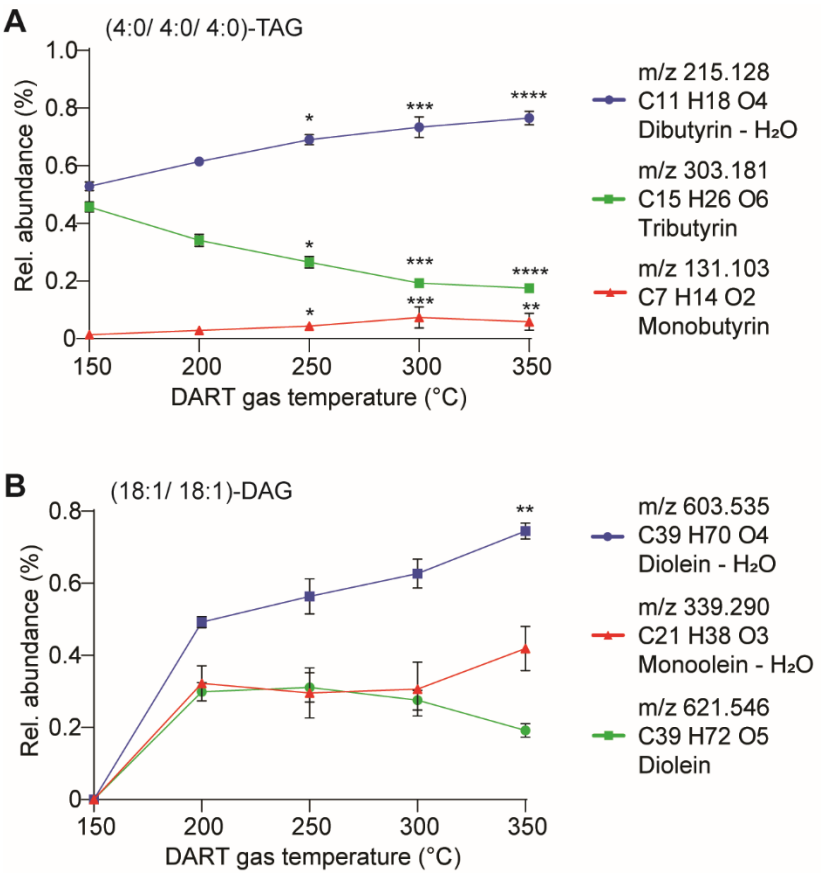
[1]Italics indicate molecules that were detected as $[M+H]^+$ and $[M+NH_4]^+$; [2]mmu: milli-mass units; [3]No. of carbons and no. of double bonds predicted in FA chains; [4]Assignment is based on exact mass measurements and degree of unsaturation; some FAs and DAGs may be decomposition products; FA: fatty acid; DAG: diacylglyceride; TAG: triacylglyceride; unk: unknown; [5]LDI: laser desorption ionization MS; signals are detected as $[M+K]^+$.



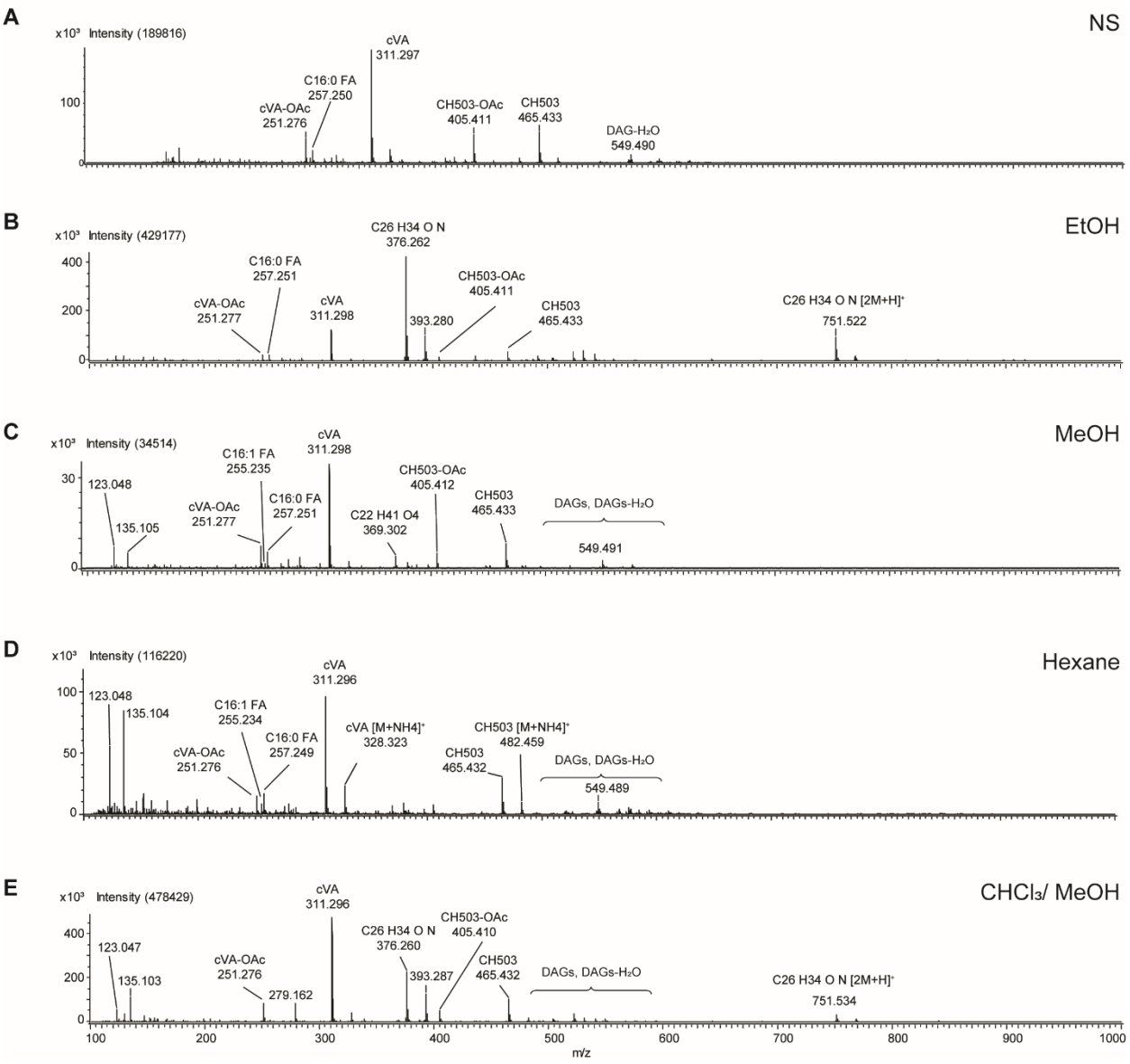
Supp. Fig. 1. (**A-F**) DART MS spectra measured from six replicate EBs analyzed on the same sample strip at 300 ˚C. The spectra are qualitatively similar and contain the major signals corresponding to cVA, deacetylated cVA, CH503, and deacetylated CH503. Some quantitative variation is evident - e.g., in **B** and **F**, the intensity of the FA signal at m/z 257.248 is comparable or higher than that of cVA and CH503. Unless otherwise indicated, ions are detected as $[M+H]^+$.



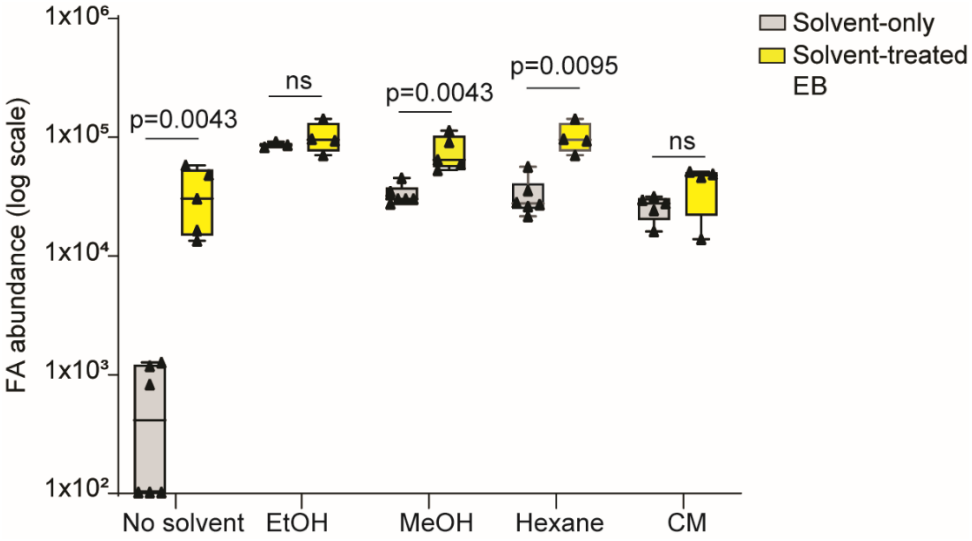
Supp. Fig. 2. DART MS analysis of cVA and CH503 synthetic standards shows a loss of acetate from both molecules and loss of water from CH503. The patterns of pheromone decomposition are consistent with those observed from direct analysis of EB tissue. Signals found in both synthetic standards and direct tissue analysis are labeled in blue. Analysis was performed at 300 ˚C.



Supp. Fig. 3. Effect of ion source temperature on synthetic tributyrin [(4:0/ 4:0/ 4:0)-TAG] and diolein [(18:1/ 18:1)-DAG]. (**A**) The loss of C4:0 acyl groups from tributyrin significantly increases with temperature; *p=0.03-0.04; **: p=0.0051; ***: p=0.0002-0.0007; ****: p<0.0001, Kruskal-Wallis. **(B)**. DAGs are detected as dehydrated ions at every temperature tested; **: p=0.0023, Kruskal-Wallis. For both graphs, each point represents the mean ± S.D, n=5-7.



Supp. Fig. 4. Representative spectra from EBs washed with various solvents; NS: no solvent. Mass signals corresponding to cVA, CH503, deacetylated fragments, fatty acids (FA), diacylglycerides (DAGs) and triacylglycerides (TAGs) are labeled.



Supp. Fig. 5. The abundance of fatty acids (FA) in solvent-only controls and EBs treated with solvents measured at 250 ˚C. The FA levels were similar between solvent-only and solvent-treated EBs in ethanol and chloroform/ MeOH (CM) conditions. Levels of FAs in untreated EBs or EBs treated with MeOH or hexane could be distinguished from baseline levels. For all graphs, bars indicate mean ± S.E.M; Mann-Whitney test, n=4-6.