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Supplemental information

Exploiting metabolic vulnerabilities after anti-VEGF antibody therapy in ovarian cancer

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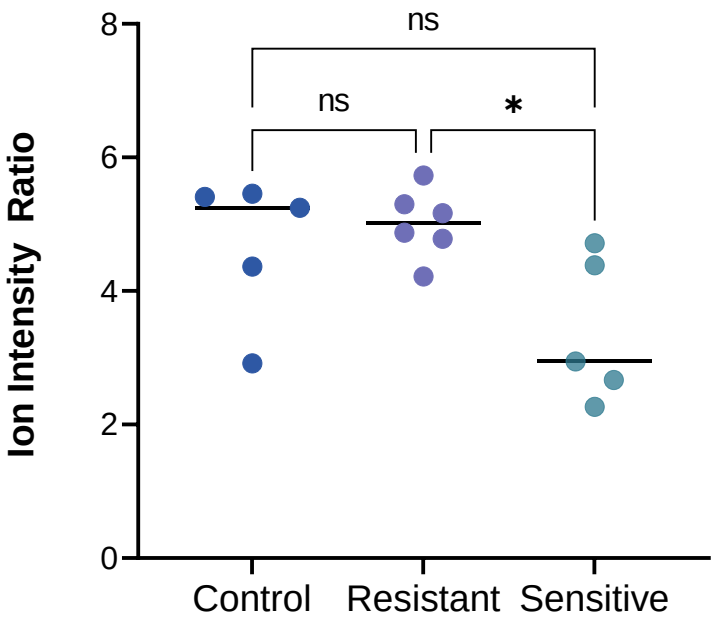

Figure S1. Normalized ion abundance comparing the ion intensity ratio of glutamate to glutamine within tumor tissues from an SKOV3ip1 ovarian cancer mouse model receiving no treatment (control) vs those that were either resistant or sensitive to AVA (bevacizumab) therapy, as monitored by in vitro imaging analysis. Related to Figure 2D. Statistical significance was determined by Tukey's HSD post hoc test, * $p < 0.05$.

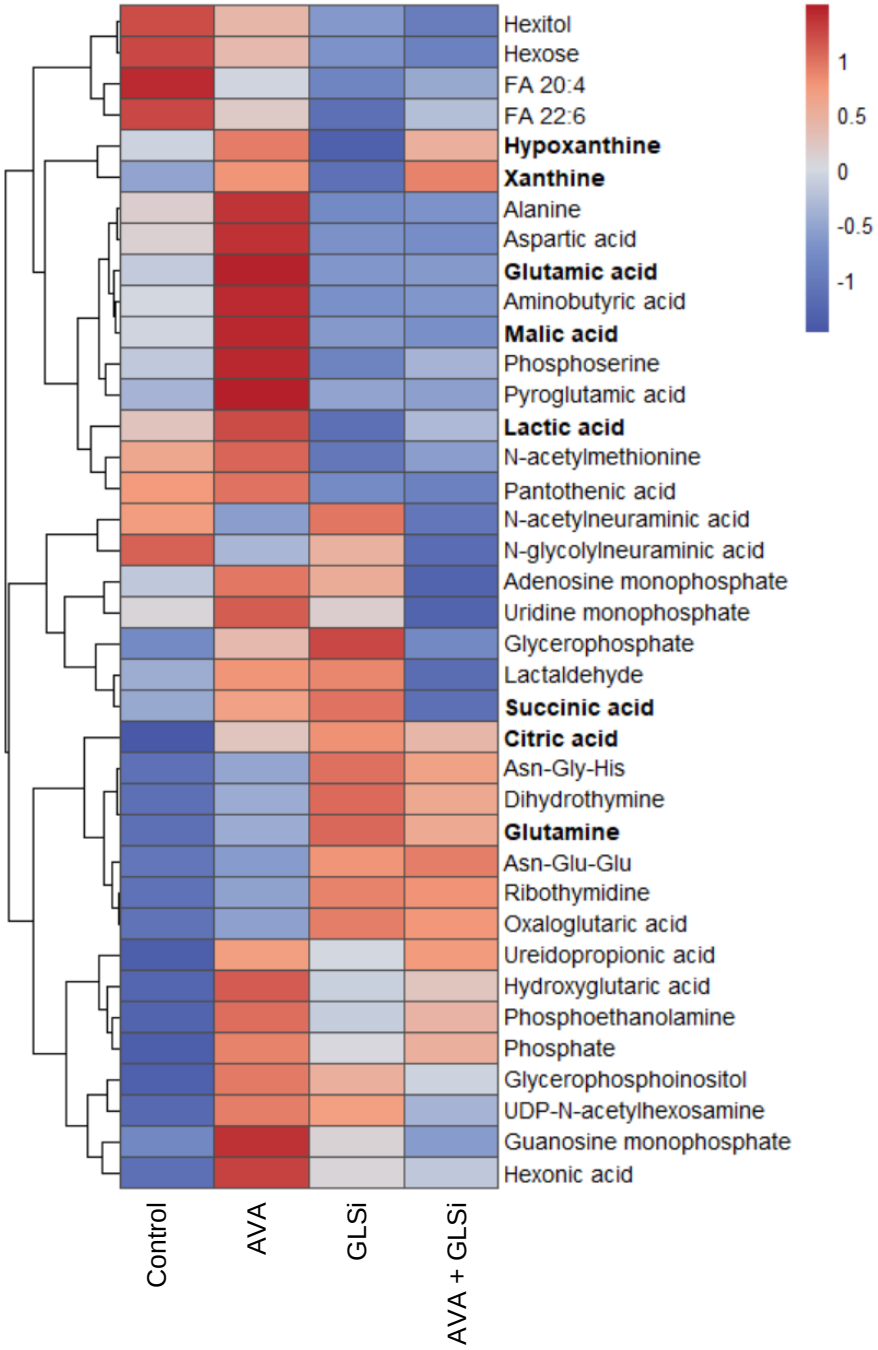

Figure S2. Intensity heat map for metabolites selected by SAM as significantly altered among SKOV3ip1 tumors treated with a vehicle control, AVA, GLSi, or AVA+GLSi (FDR < 5%) identified by DESI-MS imaging. Related to Figure 6. Features were clustered using a Euclidean-distance formula according to the average signal intensity of the corresponding *m/z* value measured from tumor-specific regions. The color scale reflects z-score standard deviations from the mean relative abundance measured for each ion. For fatty acid (FA) species, X:Y indicates the number of carbons and double bonds, respectively.

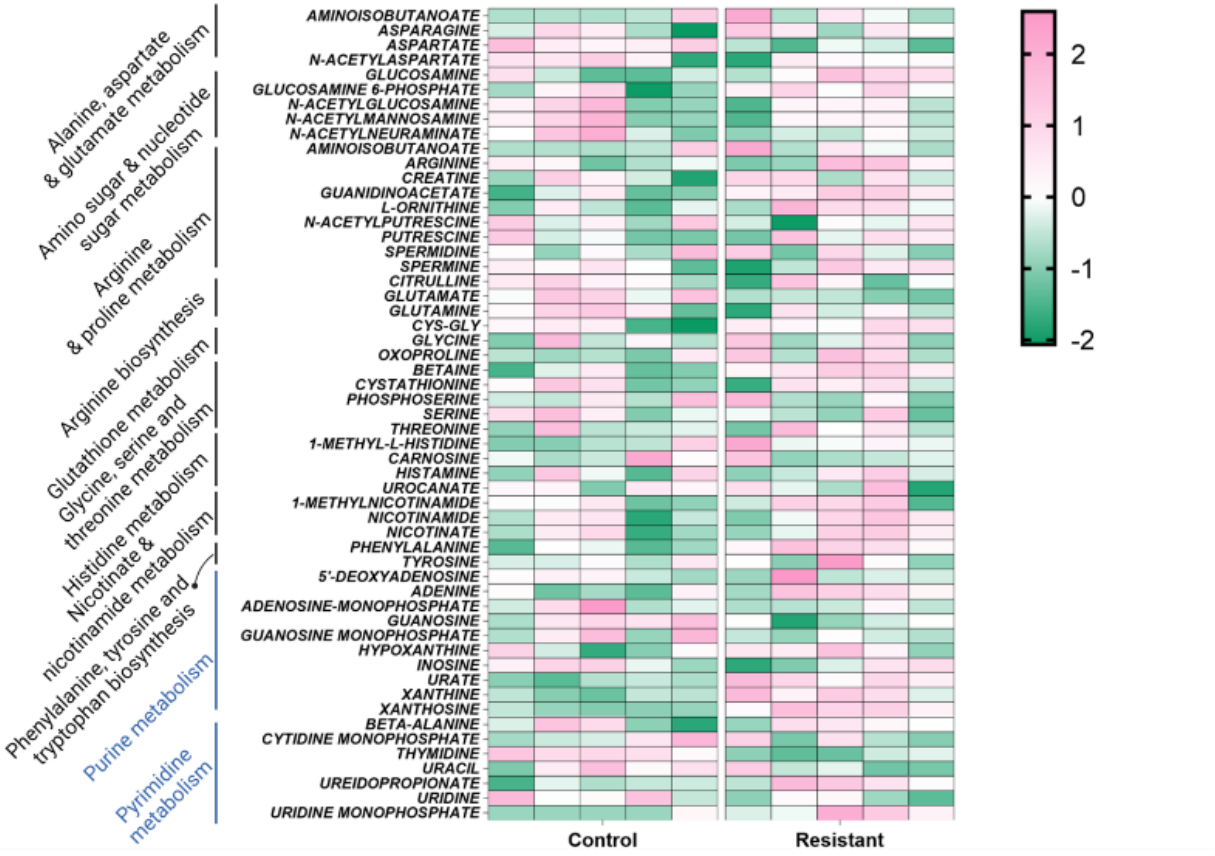

Figure S3. Heat map of altered metabolic pathways in control vs bevacizumab resistant tumor tissues. Related to Figure 2B. Purine and pyrimidine metabolism highlighted in blue on y axis. FDR < 0.05 with scale bar indicated log fold change |-2 to +2|.

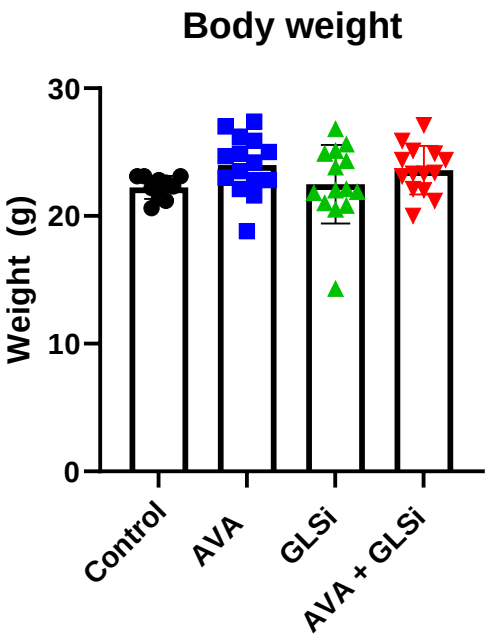

Figure S4. Body weights of SKOV3ip1 mouse model after randomization and treatment into one of four groups: control, bevacizumab (AVA), GLSi, or combination of bevacizumab plus GLSi therapy (AVA + GLSi); P = 0.16. Related to Figure 5.

Supplementary Table 1: Metabolite species selected by SAM as significantly altered among SKOV3 tumors treated with a vehicle control, Bevacuzamib, GLSi, or a combination of Bevacuzamib and GLSi (FDR < 5%) identified using DESI-MS imaging. Tentative attributions were assigned for each *m/z* value based on high mass accuracy and MS/MS measurements. For lipid species, X:Y refers to the fatty acid carbon chain length and number of double bonds, respectively.

Measured *m/z*	Tentative Attribution	Adduct	Proposed Formula	Exact *m/z*	Mass Error (ppm)	SAM Score (d)	q-value
Small Metabolites							
73.028	Lactaldehyde	[M-H]-	$C_3H_5O_2$	73.0295	-17.8	0.70	0.0186
88.039	Alanine	[M-H]-	$C_3H_6NO_2$	88.0404	-15.9	1.29	0.0000
89.023	Lactic acid	[M-H]-	$C_3H_5O_3$	89.0244	-15.5	0.77	0.0115
96.968	Phosphoric acid	[M-H]-	H_2O_4P	96.9696	-14.1	0.65	0.0390
102.055	Aminobutyric acid	[M-H]-	$C_4H_8NO_2$	102.0561	-13.8	1.65	0.0000
117.018	Succinic acid	[M-H]-	$C_4H_5O_4$	117.0193	-10.7	0.81	0.0000
127.050	Dihydrothymine	[M-H]-	$C_5H_7N_2O_2$	127.0513	-9.6	1.77	0.0000
128.034	Pyroglutamic acid	[M-H]-	$C_5H_6NO_3$	128.0353	-9.4	0.79	0.0000
131.045	Asparagine Ureidopropionic acid	[M-H]-	$C_4H_7N_2O_3$	131.0462	-9.1	0.74	0.0186
132.029	Aspartic acid	[M-H]-	$C_4H_6NO_4$	132.0302	-8.8	1.08	0.0000
133.013	Malic acid	[M-H]-	$C_4H_5O_5$	133.0143	-9.4	1.14	0.0000
135.030	Hypoxanthine	[M-H]-	$C_5H_3N_4O$	135.0312	-7.4	0.61	0.0459
140.011	Phosphoethanolamine	[M-H]-	$C_2H_7NO_4P$	140.0118	-8.2	1.03	0.0000
145.061	Glutamine	[M-H]-	$C_5H_9N_2O_3$	145.0619	-7.9	1.96	0.0000
146.045	Glutamic acid	[M-H]-	$C_5H_8NO_4$	146.0459	-7.8	1.58	0.0000
147.029	Hydroxyglutaric acid	[M-H]-	$C_5H_7O_5$	147.0299	-7.6	0.61	0.0459
151.025	Xanthine	[M-H]-	$C_5H_3N_4O_2$	151.0262	-7.5	0.65	0.0390
171.006	Glycerophosphate	[M-H]-	$C_3H_8O_6P$	171.0064	-5.3	0.76	0.0115
181.071	Hexitol	[M-H]-	$C_6H_{13}O_6$	181.0718	-4.5	0.70	0.0186
184.001	Phosphoserine	[M-H]-	$C_3H_7NO_6P$	184.0017	-4.4	0.65	0.0390
190.054	N-Acetylmethionine	[M-H]-	$C_7H_{12}NO_3S$	190.0543	-3.7	0.60	0.0459
191.019	Citric acid	[M-H]-	$C_6H_7O_7$	191.0197	-3.8	0.90	0.0000
195.050	Hexonic acid	[M-H]-	$C_6H_{11}O_7$	195.051	-3.0	1.07	0.0000
203.020	Oxaloglutaric acid	[M-H]-	$C_7H_7O_7$	203.0197	1.0	1.08	0.0000
215.032	Hexose	[M+Cl]-	$C_6H_{12}O_6Cl$	215.0350	-2.3	0.63	0.0459
217.048	Hexitol	[M+Cl]-	$C_6H_{14}O_6Cl$	217.0484	-3.4	0.82	0.0000
218.103	Pantothenic acid	[M-H]-	$C_9H_{16}NO_5$	218.1034	-2.0	0.97	0.0000
219.978	Phosphoserine	[M+Cl]-	$C_3H_8NO_6PCl$	219.9783	-1.5	0.81	0.0000
257.076	Methyluridine or Ribothymidine	[M-H]-	$C_{10}H_{13}N_2O_6$	257.0779	-9.0	0.76	0.0115
302.534	UDP-N-acetylhexosamine	[M-2H]2-	$C_{17}H_{25}N_3O_{17}P_2$	605.0659	0.5	0.64	0.0390
323.029	Uridine monophosphate	[M-H]-	$C_9H_{12}N_2O_9P$	323.0286	0.7	0.72	0.0186
324.094	N-Glycolylneuraminic acid	[M-H]-	$C_{11}H_{18}NO_{10}$	324.0936	0.7	0.63	0.0459
325.126	Asn-Gly-His	[M-H]-	$C_{12}H_{17}N_6O_5$	325.1266	-3.5	1.31	0.0000

333.060	Glycerophosphoinositol	[M-H]-	$C_9H_{18}O_{11}P$	333.0592	1.4	0.65	0.0390
346.056	Adenosine monophosphate	[M-H]-	$C_{10}H_{13}N_5O_7P$	346.0558	0.5	1.04	0.0000
362.051	Guanosine monophosphate	[M-H]-	$C_{10}H_{13}N_5O_8P$	362.0507	1.0	0.61	0.0459
389.131	Asn-Glu-Glu Asp-Glu-Gln	[M-H]-	$C_{13}H_{20}N_3O_8$	389.1314	-0.4	0.80	0.0000
606.075	UDP-N-acetylhexosamine	[M-H]-	$C_{17}H_{26}N_3O_{17}P_2$	606.0743	0.5	0.85	0.0000
Fatty Acids (FA)							
363.211	FA 22:6	[M+Cl]-	$C_{22}H_{32}O_2Cl$	363.2096	4.3	0.63	0.0459
Monoacylglycerols (MG) and Diacylglycerols (DG)							
421.312	MG 20:0	[M+Cl]-	$C_{23}H_{46}O_4Cl$	421.309	5.9	0.68	0.0253
627.477	DG 34:2	[M+Cl]-	$C_{37}H_{68}O_5Cl$	627.4761	1.0	0.75	0.0115
629.493	DG 34:1	[M+Cl]-	$C_{37}H_{70}O_5Cl$	629.4917	2.1	1.19	0.0000
655.509	DG 36:2	[M+Cl]-	$C_{39}H_{72}O_5Cl$	655.5074	2.2	0.89	0.0000

Supplementary Table 2: Metabolite species selected by SAM as significantly altered among control, Bev-resistant, and Bev-sensitive tissues (FDR < 5%) identified using DESI-MS imaging. Tentative attributions were assigned for each *m/z* value based on high mass accuracy and MS/MS measurements. For lipid species, X:Y refers to the fatty acid carbon chain length and number of double bonds, respectively.

Measured *m/z*	Tentative Attribution	Adduct	Proposed Formula	Exact *m/z*	Mass Error (ppm)	SAM Score (*d*)	q-value
Small Metabolites							
88.039	Alanine	$[M-H]^-$	$C_3H_6NO_2$	88.0404	-12.5	1.32	0.0149
96.968	Phosphoric acid	$[M-H]^-$	H_2O_4P	96.9696	-11.3	1.11	0.0489
102.055	Aminobutyric acid	$[M-H]^-$	$C_4H_8NO_2$	102.0561	-10.8	1.82	0.0000
115.002	Fumaric acid	$[M-H]^-$	$C_4H_3O_4$	115.0037	-9.6	1.17	0.0391
118.050	Threonine Homoserine	$[M-H]^-$	$C_4H_8NO_3$	118.051	-9.3	1.98	0.0000
119.034	Erythrose	$[M-H]^-$	$C_4H_7O_4$	119.035	-9.2	1.19	0.0324
125.000	Ethyl phosphate	$[M-H]^-$	$C_4H_6O_4P$	125.0009	-8.8	1.72	0.0000
128.034	Pyroglutamic acid	$[M-H]^-$	$C_5H_6NO_3$	128.0353	-8.6	1.84	0.0000
130.050	Glutamate semialdehyde Hydroxyproline Propionylglycine	$[M-H]^-$	$C_5H_8NO_3$	130.051	-8.5	1.74	0.0000
132.029	Aspartic acid	$[M-H]^-$	$C_4H_6NO_4$	132.0302	-8.3	1.93	0.0000
135.030	Hypoxanthine	$[M-H]^-$	$C_5H_3N_4O$	135.0312	-8.1	1.35	0.0149
146.045	Glutamic acid	$[M-H]^-$	$C_5H_8NO_4$	146.0459	-7.5	1.40	0.0149
151.025	Xanthine	$[M-H]^-$	$C_5H_3N_4O_2$	151.0262	-7.3	1.72	0.0000
167.020	Uric acid	$[M-H]^-$	$C_5H_3N_4O_3$	167.0211	-7.2	1.51	0.0112
174.040	N-Acetylaspartic acid	$[M-H]^-$	$C_6H_8NO_5$	174.0408	-5.2	2.13	0.0000
175.024	Ascorbate	$[M-H]^-$	$C_6H_7O_6$	175.0248	-5.7	1.39	0.0149
179.055	Hexose	$[M-H]^-$	$C_6H_{11}O_6$	179.0561	-4.5	1.73	0.0000
182.058	Phosphocholine	$[M-H]^-$	$C_5H_{13}NO_4P$	182.0587	-3.3	1.55	0.0112
184.001	Phosphoserine	$[M-H]^-$	$C_3H_7NO_6P$	184.0017	-3.8	2.39	0.0000
188.060	N-Acetylhistamine	$[M+Cl]^-$	$C_7H_{11}N_3OCl$	188.0596	4.3	1.66	0.0000
190.054	N-Acetylmethionine	$[M-H]^-$	$C_7H_{12}NO_3S$	190.0543	-3.2	1.62	0.0000
214.048	Hexosamine	$[M+Cl]^-$	$C_6H_{13}NO_5Cl$	214.0488	-2.8	1.71	0.0000
218.103	Pantothenic acid	$[M-H]^-$	$C_9H_{16}NO_5$	218.1034	-3.2	1.51	0.0112
226.996	Citric acid	$[M+Cl]^-$	$C_6H_8O_7Cl$	226.9964	-2.2	1.17	0.0391
236.021	Cysteinosuccinic acid	$[M-H]^-$	$C_7H_{10}NO_6S$	236.0234	-11.9	2.16	0.0000
241.012	Homocitric acid	$[M+Cl]^-$	$C_7H_{10}O_7Cl$	241.0121	-2.1	1.10	0.0489
245.043	Glycerophosphoglycerol	$[M-H]^-$	$C_6H_{14}O_8P$	245.0432	-1.6	1.16	0.0391
256.059	N-acetylhexosamine	$[M+Cl]^-$	$C_8H_{15}NO_6Cl$	256.0593	-0.8	1.15	0.0391
268.104	Asn-His	$[M-H]^-$	$C_{10}H_{14}N_5O_4$	268.1051	-4.5	1.65	0.0000
276.085	Asn-Glu	$[M-H]^-$	$C_9H_{14}N_3O_7$	276.0837	4.7	1.51	0.0112
289.116	Arginosuccinic acid	$[M-H]^-$	$C_{10}H_{17}N_4O_6$	289.1154	0.7	1.61	0.0000
298.114	Gly-His-Ser	$[M-H]^-$	$C_{11}H_{16}N_5O_5$	298.1157	-4.7	2.79	0.0000
306.076	Glutathione	$[M-H]^-$	$C_{10}H_{16}N_3O_6S$	306.0765	-0.3	2.02	0.0000

308.099	N-Acetylneuraminic acid	$[M-H]^-$	$C_{11}H_{18}NO_9$	308.0987	-0.6	1.52	0.0112
324.094	N-Glycolylneuraminic acid	$[M-H]^-$	$C_{11}H_{18}NO_{10}$	324.0936	0.0	1.29	0.0225
325.125	Asn-Gly-His	$[M-H]^-$	$C_{12}H_{17}N_6O_5$	325.1266	-4.6	1.13	0.0391
346.127	Ala-Glu-Glu Asp-Glu-Gln	$[M-H]^-$	$C_{13}H_{20}N_3O_8$	346.1256	4.6	1.25	0.0324
362.121	Asp-Glu-Thr	$[M-H]^-$	$C_{13}H_{20}N_3O_9$	362.1205	0.0	1.67	0.0000
328.107	Gln-Phe	$[M+Cl]^-$	$C_{14}H_{19}N_3O_4Cl$	328.107	0.3	1.37	0.0149
Fatty Acids (FA)							
301.217	FA 20:5	$[M-H]^-$	$C_{20}H_{29}O_2$	301.2173	-0.3	1.15	0.0391
319.240	FA 18:0	$[M+Cl]^-$	$C_{18}H_{36}O_2Cl$	319.2409	-2.8	1.15	0.0391
339.209	FA 20:4	$[M+Cl]^-$	$C_{20}H_{32}O_2Cl$	339.2096	-1.8	1.20	0.0324
363.210	FA 22:6	$[M+Cl]^-$	$C_{22}H_{32}O_2Cl$	363.2096	1.4	1.21	0.0324
365.226	FA 22:5	$[M+Cl]^-$	$C_{22}H_{34}O_2Cl$	365.2253	1.9	1.14	0.0391
391.245	FA 24:6	$[M+Cl]^-$	$C_{24}H_{36}O_2Cl$	391.2409	11.0	1.11	0.0489
Monoacylglycerols (MG) and Diacylglycerols (DG)							
365.246	MG 16:0	$[M+Cl]^-$	$C_{19}H_{38}O_4Cl$	365.2464	-0.3	1.32	0.0225
399.312	DG 20:0	$[M-H]^-$	$C_{23}H_{43}O_5$	399.3116	-0.3	1.16	0.0391
413.246	MG 20:4	$[M+Cl]^-$	$C_{23}H_{38}O_4Cl$	413.2464	0.0	1.32	0.0149
437.246	MG 22:6	$[M+Cl]^-$	$C_{25}H_{38}O_4Cl$	437.2464	-0.5	1.23	0.0324
439.261	MG 22:5	$[M+Cl]^-$	$C_{25}H_{40}O_4Cl$	439.2621	-1.6	1.26	0.0225