SUPPLEMENTAL FIGURES AND TABLES

Cardiolipins are biomarkers of mitochondria-rich thyroid oncocytic tumors

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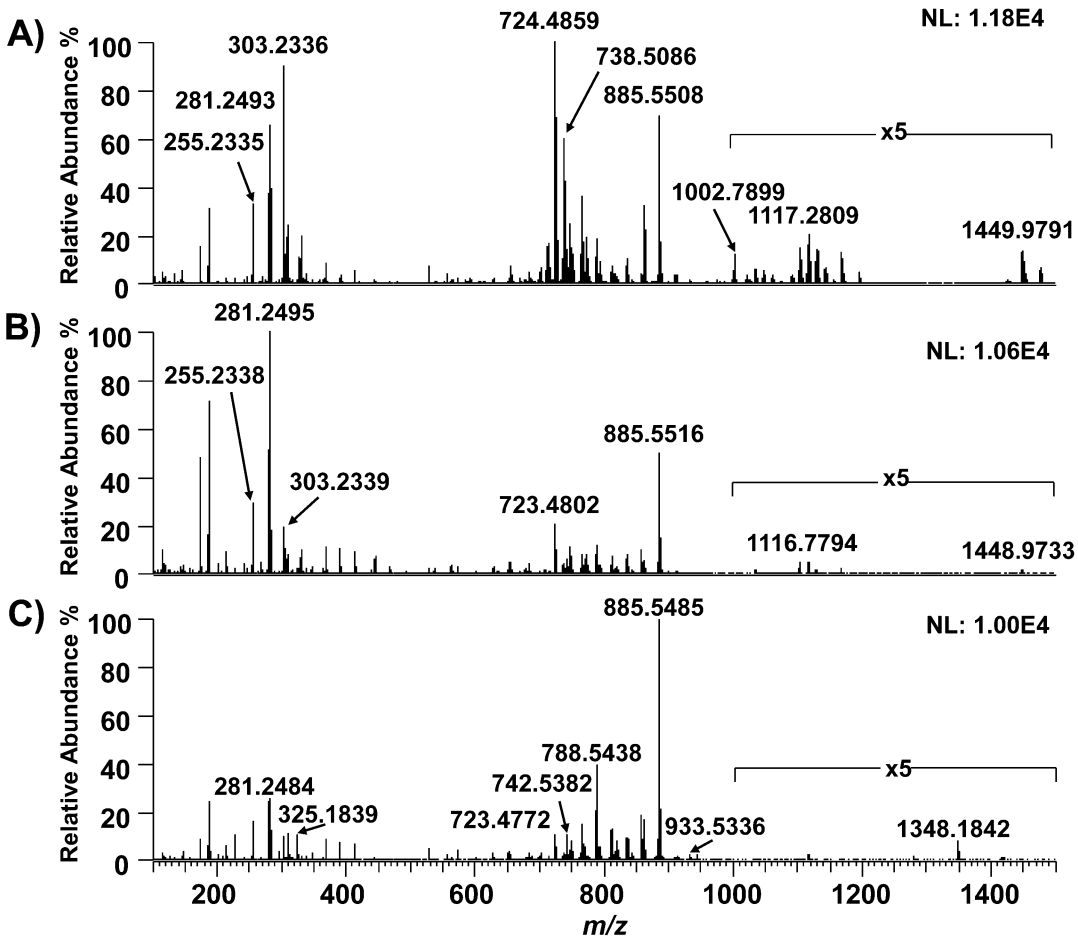
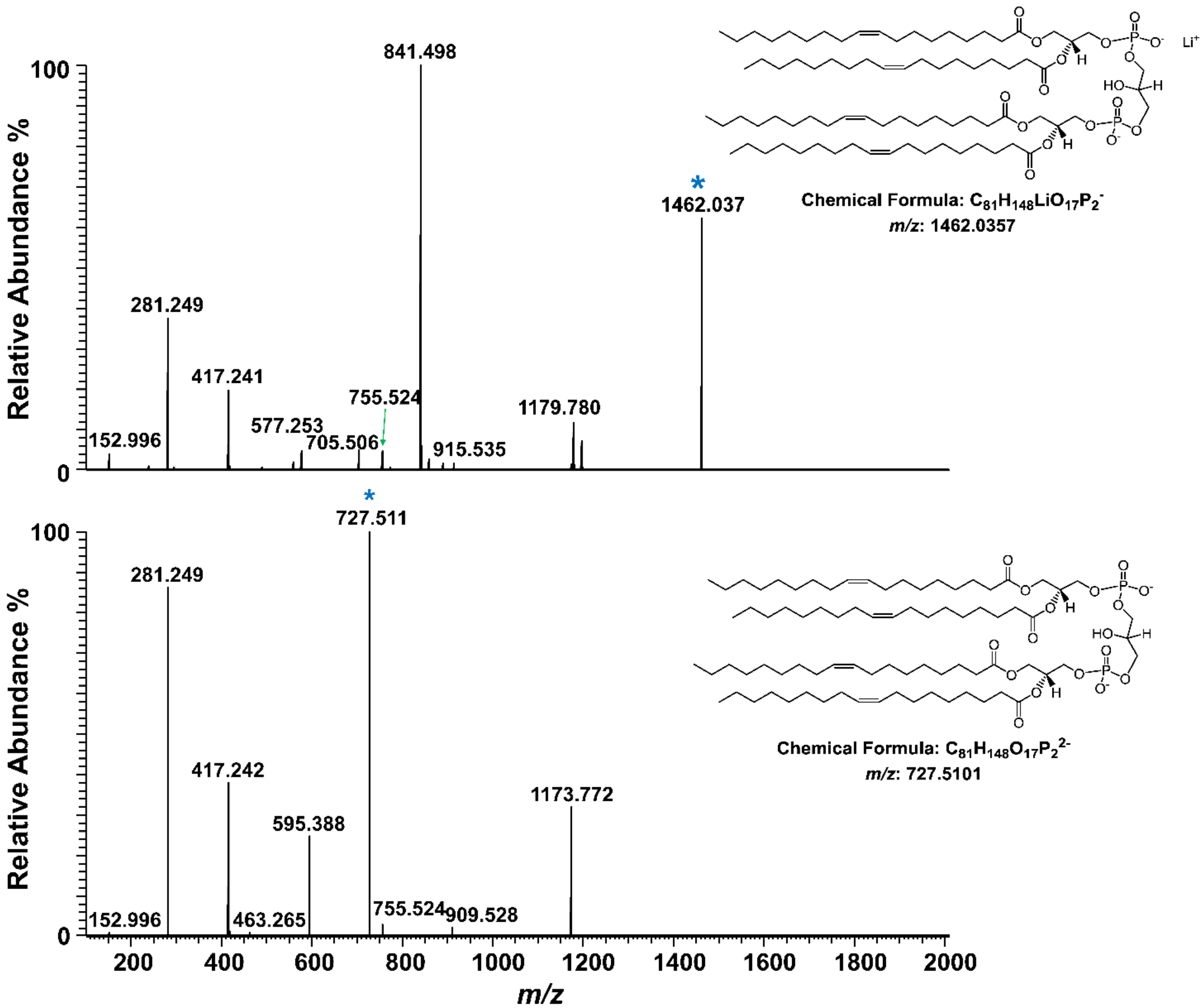
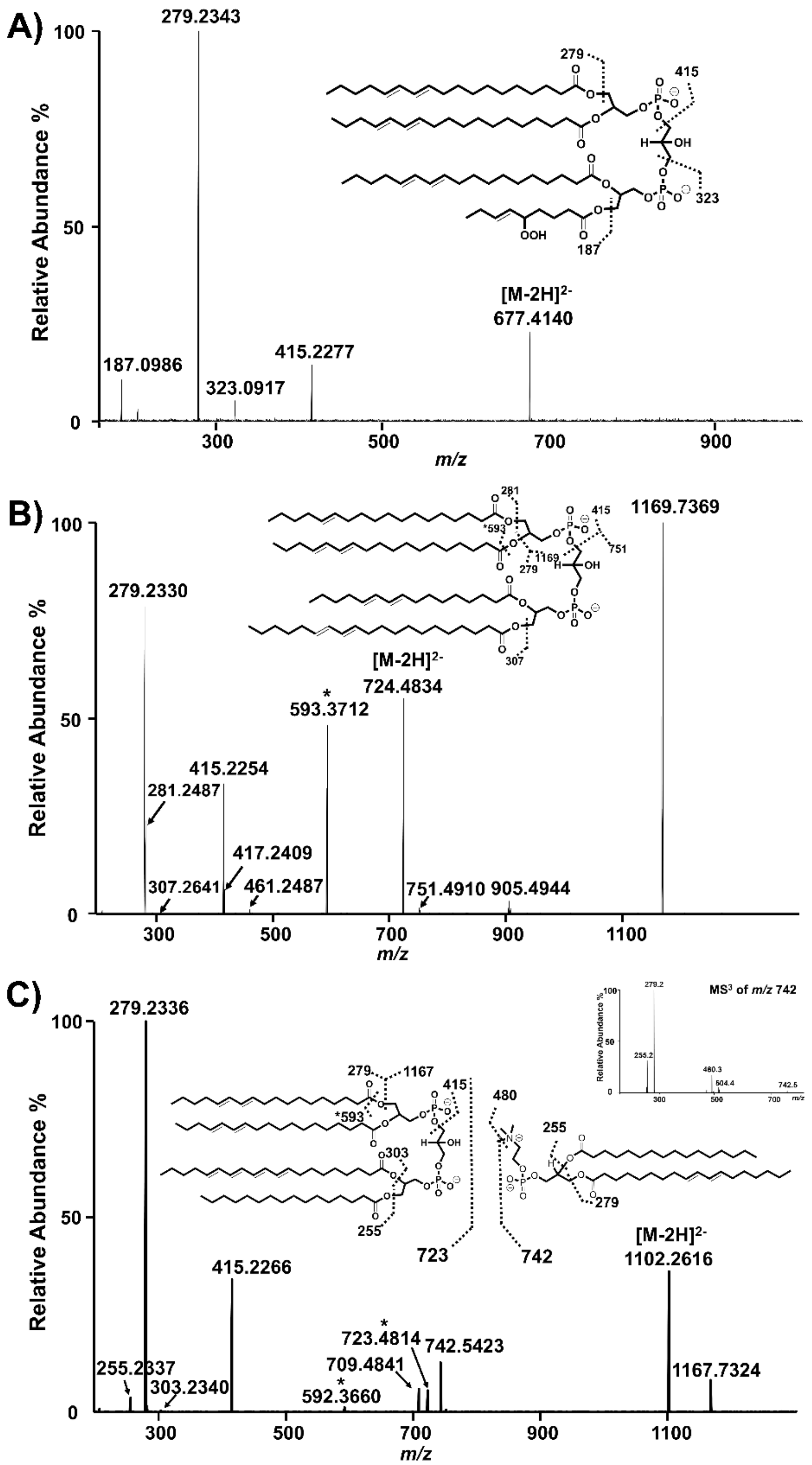
This supplemental file contains data that supports the claims and results described in the main manuscript. Figures include full mass spectra data acquired for the representative thyroid samples, examples of fragmentation patterns obtained for all the main molecular classes identified, mass spectra results obtained for confirmatory experiments using lipid standards, examples of 2D DESI-MS ion images and mass spectra for several additional samples, optical images of immunohistochemistry results, mass spectra results for isolated mitochondria experiments, and a table with detailed identification results for all molecules described in the main manuscript.



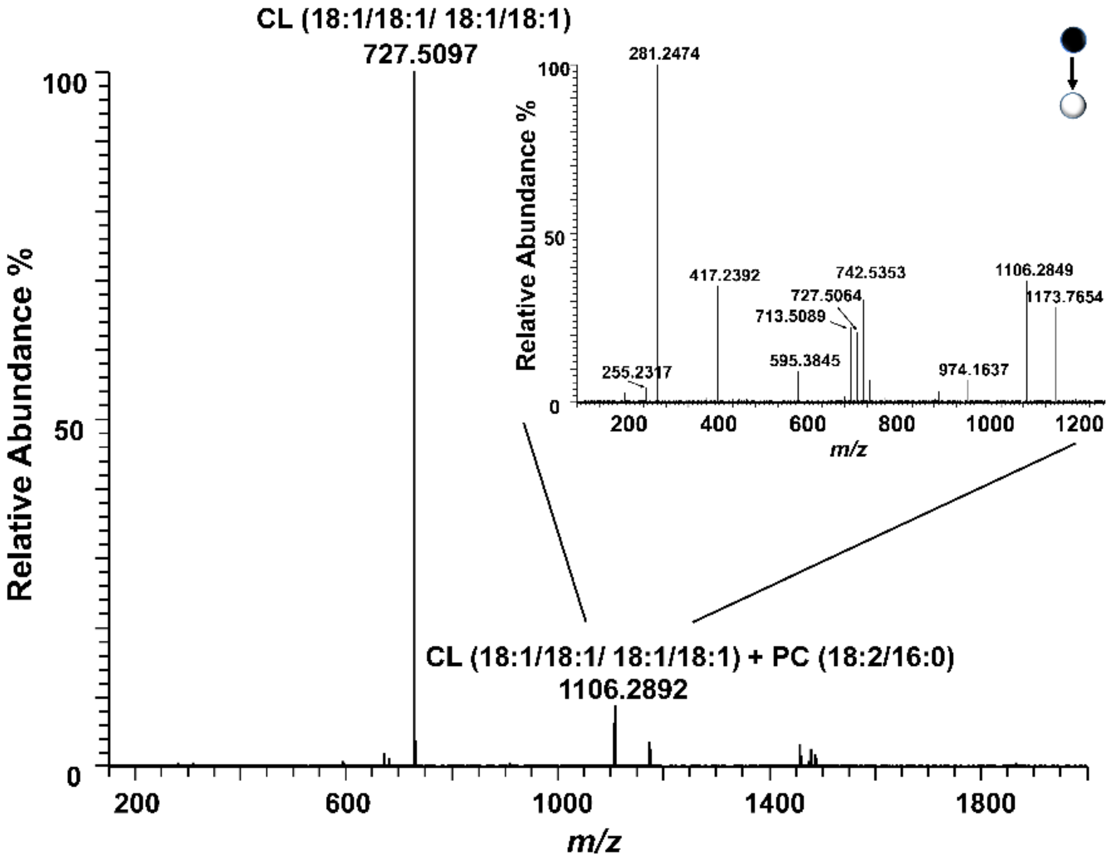
Supporting Figure 1. Comparison of DESI-MS results for oncocytic thyroid tumor, non-oncocytic thyroid tumor, and normal thyroid tissues from *m/z* 100-1500. Representative negative ion mode DESI mass spectra of A) oncocytic tumor, B) non-oncocytic tumor, and D) normal thyroid tissue.



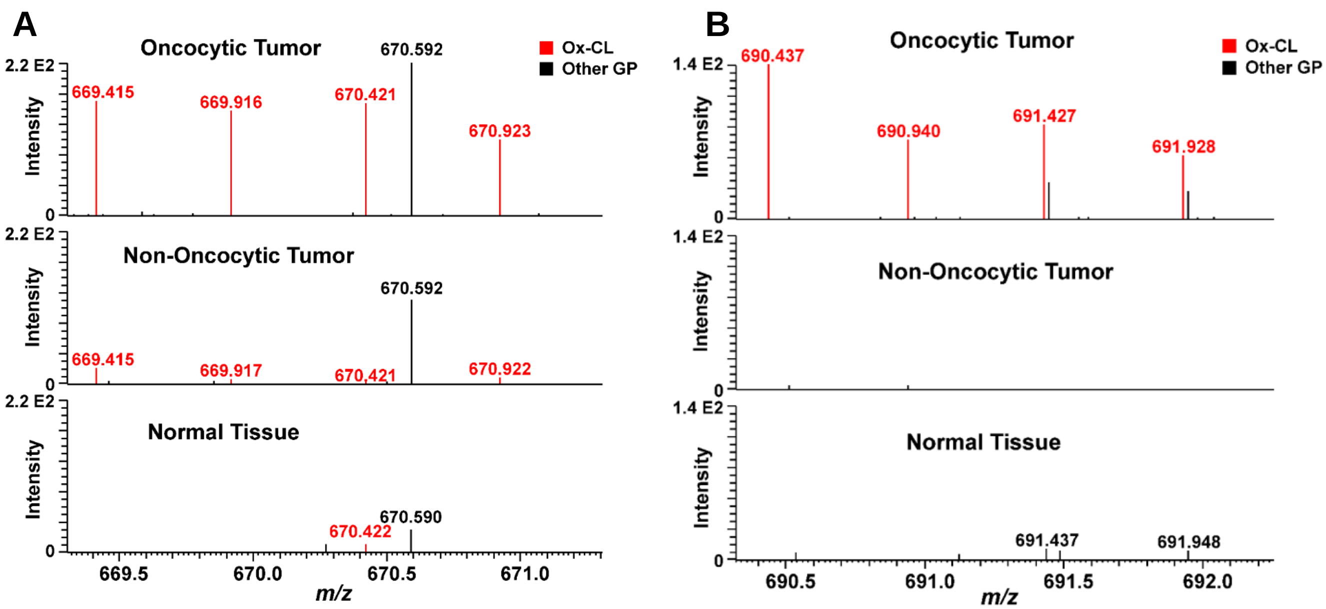
Supporting Figure 2. Comparison of HCD fragmentation patterns of the standard CL(18:1/18:1/18:1/18:1) obtained from its lithium adduct $[CL\text{-}2H+Li]^-$ at *m/z* 1462.037 and the doubly-charged $[CL\text{-}2H]^{2-}$ at *m/z* 727.5101 in the negative ion mode. Lithium adducts were obtained by adding LiCl to the DESI spray.



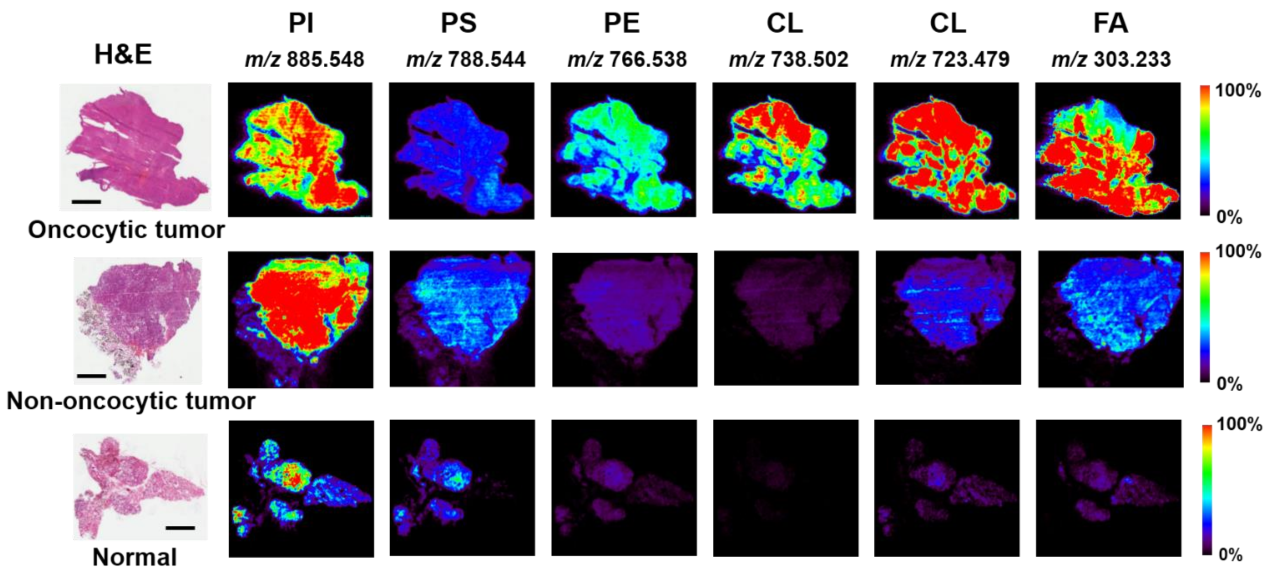
Supporting Figure 3. Tandem MS of three cardiolipins, **A)** ox-CL(18:2/18:2/18:2/9:1(OOH)), **B)** CL(20:2/18:2/18:1/16:2 or 18:2/18:2/18:2/18:1), and **C)** CL+PC (106:12).



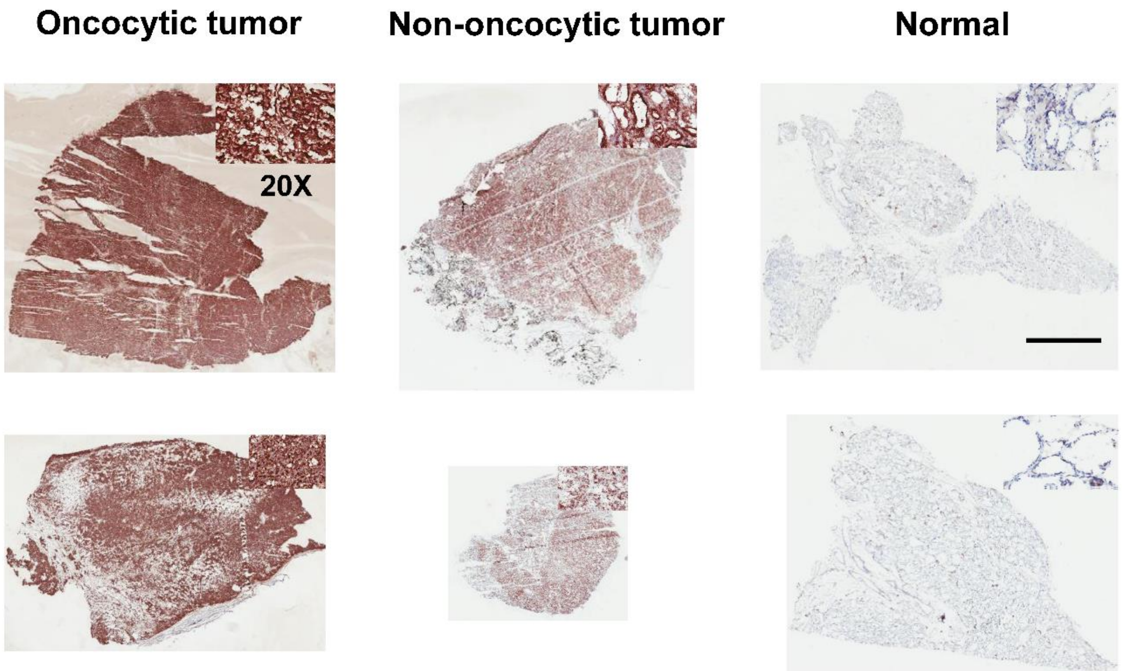
Supporting Figure 4. Analysis of mixture of CL (18:1/18:1/18:1:18:1) and PC (18:2/16:0) standards using DESI-MS. The inset shows the MS/MS of the ion at *m/z* 1106.2892 which was formed after mixing CL and PC together.



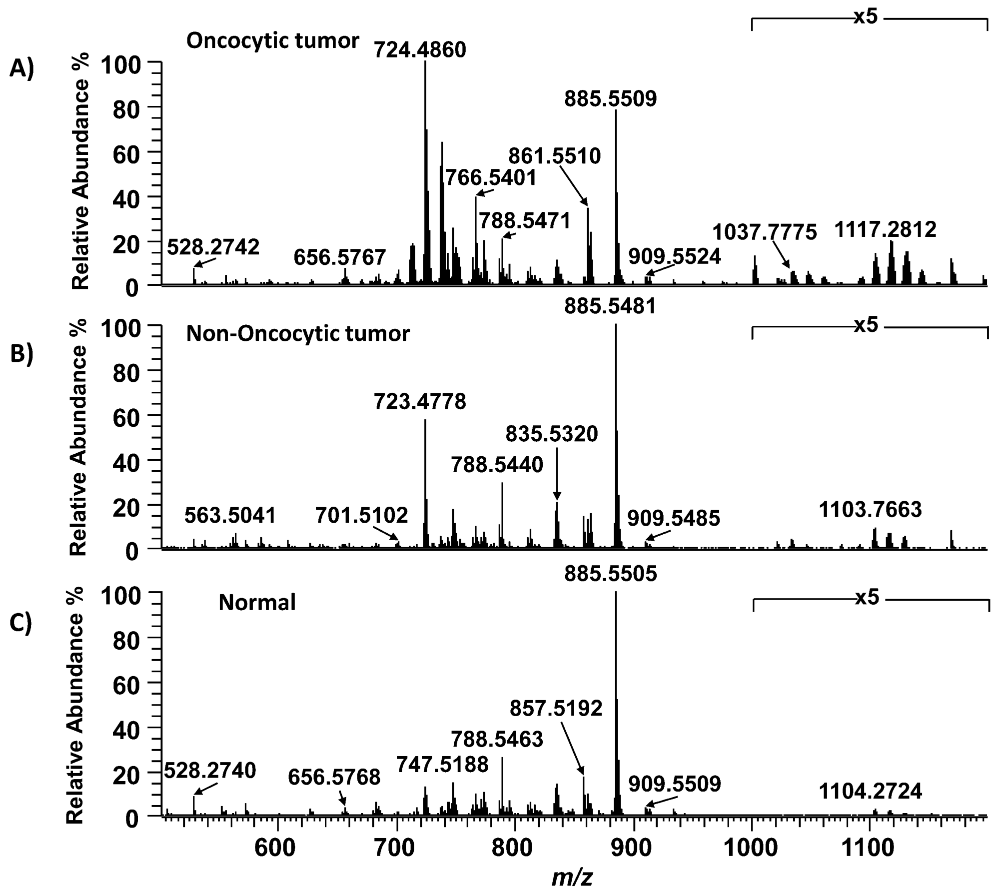
Supporting Figure 5. Two distinct mass spectra region in which ox-CL are observed, **A)** *m/z* 669 – 671, and **B)** *m/z* 690 to 692. Peaks corresponding to ox-CL were labeled in red while other peaks corresponding to GP or noise are labeled in black. Note that while the total ion abundance of the mass spectra is the same for oncocytic, non-oncocytic, and normal tissues, the relative abundance of ox-CL is drastically high in oncocytic tumors.



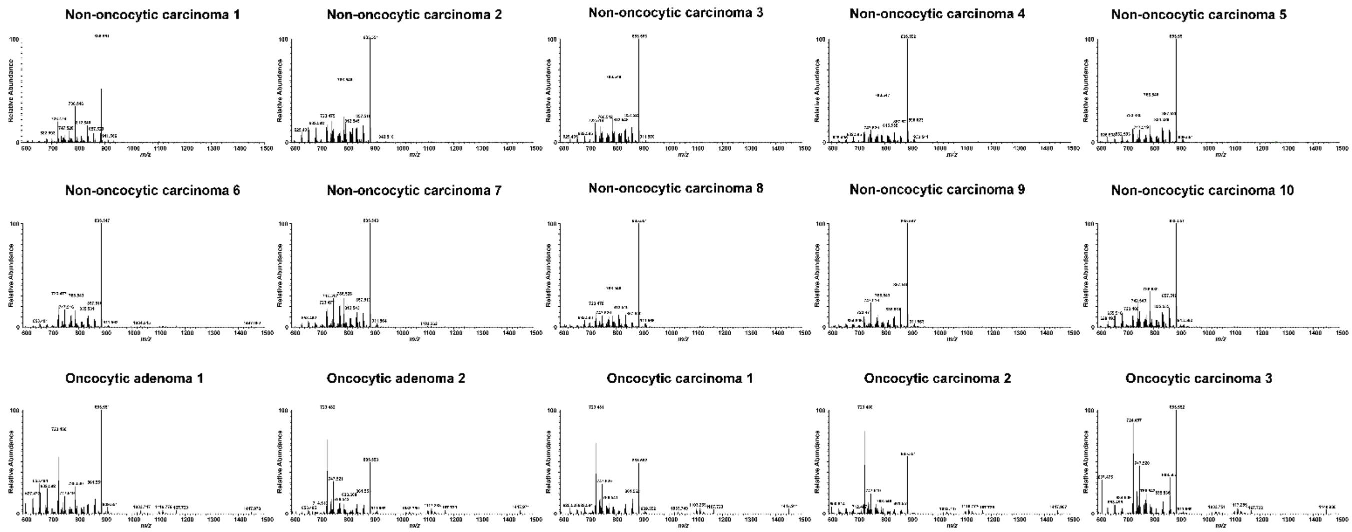
Supporting Figure 6. DESI-MSI analysis of an oncocytic tumor, non-oncocytic tumor and normal thyroid tissues. The images on the left are from H&E stained tissues which were analyzed by non-destructive DESI-MSI. Scale bar=4 mm. Six representative images from different lipid ions, including PI (20:4/18:0) (*m/z* 885.548), PS (18:1/18:0) (*m/z* 788.544), PE (20:4/18:0) (*m/z* 766.538), CL (20:4/20:2/18:1/16:0 or 20:3/18:2/18:1/18:1 or 20:2/18:2/18:2/18:1) (*m/z* 738.502), CL (18:2/18:2/18:2/18:2 or 20:4/18:2/18:2/16:0) (*m/z* 723.479) and FA (20:4) (*m/z* 303.233) are presented.



Supporting Figure 7. IHC staining images of Oncocytic tumor, Non-oncocytic tumor, and Normal thyroid tissues. Scale bar=4 mm.



Supporting Figure 8. DESI-MS analysis of isolated mitochondria from A) oncocytic tumor, B) non-oncocytic tumor, and C) normal thyroid tissue.



Supporting Figure 9. DESI mass spectra obtained from a second, independent set of samples (n=15) including 10 non-oncocytic thyroid tumors (5 papillary and 5 follicular) and 5 oncocytic thyroid tumors (3 hurthle cell carcinomas and 2 hurthle cell adenomas).

Supporting Table 1. Full list of MLCL, ox-CL, CL, CL+DG and CL+PC species identified using high mass resolution/high mass accuracy and tandem mass spectrometry analyses.

Measured *m/z*	Lipid Class[a]	Tentative Attribution	Exact *m/z*	Mass Error (ppm)[c]	Proposed Formula
592.3641	**MLCL**	CL(54:5)	592.3640	0.2	$C_{63}H_{112}O_{16}P_2$
593.3722	**MLCL**	CL(54:4)	593.3718	0.7	$C_{63}H_{114}O_{16}P_2$
669.4135	**ox-CL[b]**	20:4/18:2/16:0/9:1(OH)	669.4137	-0.2	$C_{72}H_{126}O_{18}P_2$
670.4215	**ox-CL**	18:2/18:2/18:1/9:1(OH)	670.4215	-0.1	$C_{72}H_{128}O_{18}P_2$
677.4108	**ox-CL**	18:2/18:2/18:2/9:1(OOH)	677.4112	-0.1	$C_{72}H_{126}O_{19}P_2$
678.4187	**ox-CL**	18:2/18:2/18:1/9:1(OOH)	678.4190	-0.3	$C_{72}H_{128}O_{19}P_2$
689.4292	**ox-CL**	18:2/18:2/18:2/12:2(OH)	689.4293	-0.2	$C_{75}H_{130}O_{18}P_2$
690.4352	**ox-CL**	18:2/18:2/18:1/12:2(OH) 20:4/18:1/16:0/12:2(OH)	690.4372	-2.8	$C_{75}H_{132}O_{18}P_2$
691.4261	**ox-CL**	20:2/18:2/16:0/12:2(OOH)	691.4268	-1.0	$C_{74}H_{130}O_{19}P_2$
697.4279	**ox-CL**	CL(OO-65:8)	697.4268	1.1	$C_{75}H_{130}O_{19}P_2$
697.4635	**CL**	18:2/18:2/18:2/14:0 20:2/18:2/16:2/14:0	697.4632	0.5	$C_{77}H_{138}O_{17}P_2$
698.4355	**ox-CL**	CL(OO-65:7)	698.4346	0.9	$C_{75}H_{132}O_{19}P_2$
698.4709	**CL**	18:2/18:2/18:1/14:0	698.4710	-0.2	$C_{77}H_{140}O_{17}P_2$
699.4437	**ox-CL**	CL(OO-65:6)	699.4425	1.2	$C_{75}H_{134}O_{19}P_2$
699.4774	**CL**	18:2/18:2/18:0/14:0	699.4788	-2.0	$C_{77}H_{142}O_{17}P_2$
700.4866	**CL**	18:1/18:1/18:1/14:0	700.4867	-0.1	$C_{77}H_{144}O_{17}P_2$
701.4929	**CL**	18:1/18:1/18:0/14:0	701.4945	-0.5	$C_{77}H_{146}O_{17}P_2$
706.4869	**CL**	18:2/18:1/18:1/15:0	706.4867	0.3	$C_{78}H_{144}O_{17}P_2$
710.4709	**CL**	18:2/18:2/18:2/16:1	710.4710	0.1	$C_{79}H_{140}O_{17}P_2$
711.4767	**CL**	18:2/18:2/18:1/16:1 18:2/18:2/18:2/16:0	711.4788	0.4	$C_{79}H_{142}O_{17}P_2$
712.4849	**CL**	18:2/18:2/18:1/16:0 18:2/18:1/18:1/16:1	712.4867	0.5	$C_{79}H_{144}O_{17}P_2$
713.4927	**CL**	18:2/18:1/18:1/16:0	713.4945	0.6	$C_{79}H_{146}O_{17}P_2$
714.5012	**CL**	18:1/18:1/18:1/16:0	714.5023	0.4	$C_{79}H_{148}O_{17}P_2$
722.4711	**CL**	20:4/18:3/18:1/16:1	722.4710	0.1	$C_{81}H_{140}O_{17}P_2$
723.4789	**CL**	18:2/18:2/18:2/18:2 20:4/18:2/18:2/16:0	723.4788	0.1	$C_{81}H_{142}O_{17}P_2$
724.4851	**CL**	18:2/18:2/18:2/18:1 20:2/18:2/18:1/16:2	724.4867	-1.7	$C_{81}H_{144}O_{17}P_2$
725.4936	**CL**	20:3/18:2/18:1/16:0 20:2/18:2/18:1/16:1	725.4945	-0.9	$C_{81}H_{146}O_{17}P_2$
726.5015	**CL**	20:2/18:2/18:1/16:0	726.5023	-0.8	$C_{81}H_{148}O_{17}P_2$
727.5097	**CL**	20:2/18:2/18:0/16:0 20:2/18:1/18:1/16:0	727.5101	-0.5	$C_{81}H_{150}O_{17}P_2$
730.4684	**ox-CL**	CL(O72:9)	730.4685	-0.2	$C_{81}H_{138}O_{18}P_2$
731.4768	**ox-CL**	CL(O72:8)	730.4763	0.8	$C_{81}H_{140}O_{18}P_2$
732.4821	**ox-CL**	18:2/18:1/19:1/17:3(OH) 18:4(OH)/18:2/18:1/16:0	730.4841	-2.8	$C_{81}H_{142}O_{18}P_2$
735.4783	**CL**	20:4/18:2/18:2/18:2	735.4788	-0.7	$C_{83}H_{142}O_{17}P_2$

736.4866	**CL**	20:4/18:2/18:2/18:1 20:3/18:2/18:2/18:2	736.4867	-0.1	$C_{83}H_{144}O_{17}P_2$
737.4944	**CL**	20:4/18:2/18:1/18:1 20:3/18:2/18:2/18:1 20:2/18:2/18:2/18:2	737.4945	-0.1	$C_{83}H_{146}O_{17}P_2$
738.5022	**CL**	20:4/20:2/18:1/16:0 20:3/18:2/18:1/18:1 20:2/18:2/18:2/18:1	738.5023	-0.2	$C_{83}H_{148}O_{17}P_2$
739.4740	**ox-CL**	CL(OO72:8)	739.4738	0.2	$C_{81}H_{142}O_{19}P_2$
740.4803	**ox-CL**	CL(OO72:7)	740.4810	-1.3	$C_{81}H_{144}O_{19}P_2$
745.4914	**ox-CL**	CL(O74:8)	745.4910	-0.7	$C_{83}H_{144}O_{18}P_2$
746.4982	**ox-CL**	CL(O74:7)	746.4998	-2.1	$C_{83}H_{146}O_{18}P_2$
747.4780	**CL**	22:6/20:4/18:2/16:0	747.4788	-0.8	$C_{85}H_{142}O_{17}P_2$
748.4836	**CL**	22:6/20:4/18:1/16:0	748.4867	0.1	$C_{85}H_{144}O_{17}P_2$
749.4942	**CL**	22:5/20:4/18:1/16:0	749.4945	-0.4	$C_{85}H_{146}O_{17}P_2$
750.5024	**CL**	22:4/20:4/18:1/16:0	750.5023	-1.1	$C_{85}H_{148}O_{17}P_2$
751.5101	**CL**	22:4/20:4/18:0/16:0	751.5101	0.1	$C_{85}H_{150}O_{17}P_2$
752.5172	**CL**	22:4/20:4/18:0/16:0	752.5180	-0.9	$C_{85}H_{152}O_{17}P_2$
753.5263	**CL**	22:4/20:3/18:0/16:0	752.5258	0.7	$C_{85}H_{154}O_{17}P_2$
1019.7316	**CL+DG**	CL+DG(106:10)	1019.7322	-0.6	$C_{118}H_{210}O_{22}P_2$
1020.7387	**CL+DG**	CL+DG(106:9)	1020.7400	-1.3	$C_{118}H_{212}O_{22}P_2$
1021.7440	**CL+DG**	CL+DG(106:8)	1021.7478	-3.7	$C_{118}H_{214}O_{22}P_2$
1022.7525	**CL+DG**	CL+DG(106:7)	1022.7556	-3.0	$C_{118}H_{216}O_{22}P_2$
1031.7322	**CL+DG**	CL+DG(108:12)	1031.7322	<0.1	$C_{120}H_{210}O_{22}P_2$
1032.7390	**CL+DG**	CL+DG(108:11)	1032.7410	-1.9	$C_{120}H_{212}O_{22}P_2$
1033.7461	**CL+DG**	CL+DG(108:10)	1033.7478	-1.6	$C_{120}H_{214}O_{22}P_2$
1034.7526	**CL+DG**	CL+DG(108:9)	1034.7556	-2.9	$C_{120}H_{216}O_{22}P_2$
1035.7604	**CL+DG**	CL+DG(108:8)	1035.7635	-3.0	$C_{120}H_{218}O_{22}P_2$
1036.7675	**CL+DG**	CL+DG(108:7)	1036.7713	-3.7	$C_{120}H_{220}O_{22}P_2$
1044.7405	**CL+DG**	CL+DG(110:13)	1044.7465	-5.7	$C_{122}H_{214}O_{22}P_2$
1045.7477	**CL+DG**	CL+DG(110:12)	1045.7478	-0.1	$C_{122}H_{216}O_{22}P_2$
1046.7529	**CL+DG**	CL+DG(110:11)	1046.7556	-2.6	$C_{122}H_{218}O_{22}P_2$
1047.7614	**CL+DG**	CL+DG(110:10)	1047.7635	-2.0	$C_{122}H_{220}O_{22}P_2$
1048.7702	**CL+DG**	CL+DG(110:9)	1048.7713	-1.0	$C_{122}H_{222}O_{22}P_2$
1049.7748	**CL+DG**	CL+DG(110:8)	1049.7791	-4.1	$C_{122}H_{224}O_{22}P_2$
1057.7463	**CL+DG**	CL+DG(112:14)	1057.7478	-1.4	$C_{124}H_{214}O_{22}P_2$
1058.7523	**CL+DG**	CL+DG(112:13)	1058.7556	-3.1	$C_{124}H_{216}O_{22}P_2$
1059.7627	**CL+DG**	CL+DG(112:12)	1059.7635	-0.8	$C_{124}H_{218}O_{22}P_2$
1060.7675	**CL+DG**	CL+DG(112:11)	1060.7713	-3.6	$C_{124}H_{220}O_{22}P_2$
1061.7766	**CL+DG**	CL+DG(112:10)	1061.7791	-2.4	$C_{124}H_{222}O_{22}P_2$
1062.7833	**CL+DG**	CL+DG(112:9)	1062.7869	-3.4	$C_{124}H_{224}O_{22}P_2$
1071.7613	**CL+DG**	CL+DG(114:14)	1071.7635	-2.1	$C_{126}H_{218}O_{22}P_2$
1072.7684	**CL+DG**	CL+DG(114:13)	1072.7713	-2.7	$C_{126}H_{220}O_{22}P_2$
1073.7771	**CL+DG**	CL+DG(114:12)	1073.7863	-8.6	$C_{126}H_{222}O_{22}P_2$
1074.7843	**CL+DG**	CL+DG(114:11)	1074.7869	-2.4	$C_{126}H_{224}O_{22}P_2$
1075.7928	**CL+DG**	CL+DG(114:10)	1075.7948	-1.9	$C_{126}H_{226}O_{22}P_2$
1089.2522	**CL+PC**	CL+PC(104:11)	1089.2521	0.1	$C_{121}H_{220}O_{25}NP_3$
1090.2583	**CL+PC**	CL+PC(104:10)	1090.2599	-1.5	$C_{121}H_{222}O_{25}NP_3$
1091.2647	**CL+PC**	CL+PC(104:9)	1091.2677	-2.7	$C_{121}H_{224}O_{25}NP_3$

1092.2732	**CL+PC**	CL+PC(104:8)	1092.2756	-2.2	$C_{121}H_{226}O_{25}NP_3$
1093.2802	**CL+PC**	CL+PC(104:7)	1093.2834	-2.9	$C_{121}H_{228}O_{25}NP_3$
1094.2873	**CL+PC**	CL+PC(104:8)	1094.2912	-3.6	$C_{121}H_{230}O_{25}NP_3$
1102.2593	**CL+PC**	CL+PC(106:12)	1102.2599	-0.5	$C_{123}H_{222}O_{25}NP_3$
1103.2670	**CL+PC**	CL+PC(106:11)	1103.2677	-0.6	$C_{125}H_{224}O_{25}NP_3$
1104.2745	**CL+PC**	CL+PC(106:10)	1104.2756	-1.0	$C_{125}H_{224}O_{25}NP_3$
1105.2811	**CL+PC**	CL+PC(106:9)	1105.2834	-2.1	$C_{125}H_{224}O_{25}NP_3$
1115.2657	**CL+PC**	CL+PC(108:11)	1115.2677	-1.8	$C_{125}H_{224}O_{25}NP_3$
1116.2740	**CL+PC**	CL+PC(108:10)	1116.2756	-1.4	$C_{125}H_{226}O_{25}NP_3$
1117.2816	**CL+PC**	CL+PC(108:9)	1117.2834	-1.6	$C_{125}H_{228}O_{25}NP_3$
1118.2878	**CL+PC**	CL+PC(108:8)	1118.2912	-3.0	$C_{125}H_{230}O_{25}NP_3$
1119.2960	**CL+PC**	CL+PC(108:7)	1119.2990	-2.7	$C_{125}H_{232}O_{25}NP_3$
1128.2744	**CL+PC**	CL+PC(110:14)	1128.2756	-1.1	$C_{127}H_{226}O_{25}NP_3$
1129.2818	**CL+PC**	CL+PC(110:13)	1129.2834	-1.4	$C_{127}H_{228}O_{25}NP_3$
1130.2880	**CL+PC**	CL+PC(110:12)	1130.2912	-2.8	$C_{127}H_{230}O_{25}NP_3$
1131.2939	**CL+PC**	CL+PC(110:11)	1131.2990	-4.5	$C_{127}H_{232}O_{25}NP_3$
1132.3024	**CL+PC**	CL+PC(110:10)	1132.3069	-4.0	$C_{127}H_{234}O_{25}NP_3$
1133.3098	**CL+PC**	CL+PC(110:9)	1133.3147	-4.3	$C_{127}H_{236}O_{25}NP_3$
1141.2814	**CL+PC**	CL+PC(112:13)	1141.2834	-1.8	$C_{129}H_{228}O_{25}NP_3$
1142.2887	**CL+PC**	CL+PC(112:12)	1142.2912	-2.2	$C_{129}H_{230}O_{25}NP_3$
1143.2960	**CL+PC**	CL+PC(112:11)	1143.2990	-2.6	$C_{129}H_{232}O_{25}NP_3$
1144.3048	**CL+PC**	CL+PC(112:10)	1144.3069	-1.8	$C_{129}H_{234}O_{25}NP_3$
1145.3104	**CL+PC**	CL+PC(112:9)	1145.3147	-3.8	$C_{129}H_{236}O_{25}NP_3$
1146.3199	**CL+PC**	CL+PC(112:8)	1146.3225	-2.3	$C_{129}H_{238}O_{25}NP_3$

[a] CL = cardiolipin (X:Y) denotes the total number of carbons and double bonds in the fatty acid chains.

[b] ox-CL =oxidized cardiolipin

[c] Mass errors were calculated based on the exact monoisotopic *m/z* of the deprotonated form of the assigned molecules.