SUPPLEMENTARY INFORMATION

Application of Desorption Electrospray Ionization Mass Spectrometry Imaging in Breast Cancer Margin Analysis

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DESI Mass Spectrometry Imaging

The stage holding the glass slides mounted with tissue sections moved horizontally at the speed of 200 µm/s and vertically by 200 µm step to generate 2D image. The stage movement was controlled by OminiSpray 2D (Prosolia Inc., IN). The nondestructive solvent containing acetonitrile (ACN)/Dimethylformamide (DMF) (50:50 v/v) was used. (1, 2) Flow rate of 1 µL/min was selected for the solvent spray. The spectra were acquired within the mass range *m/z* 50 - 1,100 with Bruker software Hystar (Bruker Daltonics, Billerica, USA). To validate day-to-day reproducibility, mouse brain sections were tested in exactly the same conditions at the beginning of each day, before acquiring data. To display 2D images, FireFly (Prosolia Inc., IN) was used to convert the data to be compatible with Biomap. All images obtained from Biomap were displayed with the same intensity scale in each figure. The ion intensities were normalized before the subtraction. All the spectra were re-plotted using Excel.

An additional image analysis has been done using SCiLS lab 2014a software (SCiLS GmbH, Bremen, Germany). DESI-MSI raw data was first converted to be able to be opened in fleximaging 4.0 (Bruker Daltonics, Billerica, USA). The data were then imported into SCiLS Lab 2014a software and converted to the SCiLS H5 format. The standard preprocessing pipeline was used for data analysis (3). This pipeline includes baseline removal, and total ion count normalization.

Lipid Extraction from Tumor Center Sample of Research Subject #9

For lipid extraction, the tumor sample was homogenized in 150 µL of MeOH/$CHCl_3$ (2:1 v/v) with a french press. 50 µL of KCl 0.88 % were then added to the solution. After homogenization, sample was then centrifuged 50 seconds (3500 *g* at RT) and the upper phase was removed. The lower phase was washed with 30 µL of H_2O/MeOH (2:1 v/v) and briefly vortexed. The final biphasic mixture was centrifuged (10 min, 2000 *g*). The lower phase was evaporated under vacuum and dissolved in 100 µL of a mixture of MeOH/$CHCl_3$ /formic acid (49:49:2 v/v/v) with 5 mM ammonium acetate for MS analyses.

ESI-FT-ICR MS Analyses

Mass spectra were acquired using a SolariX XR FT-ICR (12 T) (Bruker Daltonics, Billerica, USA). Positively charged ions were generated at atmospheric pressure by ESI, transferred into the mass spectrometer via a heater glass capillary and collected in a collision cell external to the ICR cell. Ions are then ejected, entering an RF-only ion guide, and transferred into a dynamically harmonized ICR cell (paracell). Mass spectra were acquired from *m/z* 74 - 2,500, using a ramped RF-excitation. The dataset

size was set to 4MW, yielding a 0.839 s transient. Depending upon the precursor ion's intensity, a single spectrum was acquired by signal averaging 20-100 individual scans.

The lipid samples were first analyzed in MS only mode to determine possible precursor ions for MSMS analysis. For collision induced dissociation (qCID) experiments, precursors are isolated in the quadrupole using a 3 amu window and are dissociated inside the collision cell (18-25 eV). Accumulation times varied from 1-2 s depending on the precursor ion's intensity. All experiments were calibrated externally. The instrument's resolving power exceeded the theoretically estimation for all ions observed.

Lipid molecular formulas were determined from the MS mass spectra. Monoisotopic masses were labeled using DataAnalysis 4.2 software (Bruker Daltonics, Billerica, USA) with the FTMS peak-picking algorithm according to default parameters. Molecular formulas were determined manually using the SmartFormula algorithm in DataAnalysis 4.2. Tolerance was set to 1 ppm. Identification was then done by searches against "LIPID Metabolites And Pathways Strategy" (Lipid Maps) database (website: http://www.lipidmaps.org/). Identifications were performed by "Text-based searches" using the experimental monoisotopic mass of each lipid. Mass tolerance was set to ±0.01 Da. The identification of the phospholipids was confirmed by the detection of major product ions typically yield from the CID of PI-, PS-, and PE-derived $[M-H]^-$ ions during the MSMS analyses. For the other fatty acids (*m/z* 74 to 500), identifications were confirmed by calculating the possible molecular formulas of each MS precursor ion with a tolerance of 1 ppm from MS and MSMS data using SmartFormula3D algorithm in DataAnalysis 4.2. MetFrag tool (website: http://msbi.ipb-halle.de/MetFrag/) was also used to identify product ions from MSMS data and determine the structure of each fatty acid. Tolerance was also set to 1 ppm.

References

1. Eberlin LS*, et al.* (2011) Desorption electrospray ionization then MALDI mass spectrometry imaging of lipid and protein distributions in single tissue sections. *Analytical chemistry* 83(22):8366-8371.
2. Eberlin LS*, et al.* (2011) Nondestructive, histologically compatible tissue imaging by desorption electrospray ionization mass spectrometry. *Chembiochem : a European journal of chemical biology* 12(14):2129-2132.
3. Trede D*, et al.* (2012) On the importance of mathematical methods for analysis of MALDI-imaging mass spectrometry data. *Journal of integrative bioinformatics* 9(1):189.

m/z_{IT} [a]	$m/z_{FT\text{-}ICR}$ [b]	m/z_{th}	Error | ppm |	Assignment	Elemental Formula [c]	References
885.7	885.54966	885.54985	0.22	PI(38:4)	$C_{47}H_{82}O_{13}P^-$	59, 63-65
863.7	863.56535	863.56550	0.17	PI(36:1)	$C_{45}H_{84}O_{13}P^-$	65, 66
844.7	844.60717	844.60731	0.16	PS(40:1)	$C_{46}H_{87}NO_{10}P^-$	-
835.7	835.53398	835.53420	0.27	PI(34:1)	$C_{43}H_{80}O_{13}P^-$	65, 66
794.7	794.57023	794.57053	0.38	PE(40:4)	$C_{45}H_{81}NO_8P^-$	64
788.7	788.54451	788.54471	0.25	PS(36:1)	$C_{42}H_{79}NO_{10}P^-$	59, 63, 64
768.7	768.55474	768.55488	0.18	PE(38:3)	$C_{43}H_{79}NO_8P^-$	64
750.7	750.54424	750.54431	0.10	Plasm-PE(38:4)	$C_{43}H_{77}NO_7P^-$	64
655.6	655.47067	655.47081	0.20	PA(O-16:0/18:3)/PA(P-16:0/18:2)	$C_{37}H_{68}O_7P^-$	-
445.4	445.40411	445.40401	0.23	FA(30:3)	$C_{30}H_{53}O_2^-$	-
415.4	415.35809	415.35815	0.16	FA(28:4)	$C_{28}H_{47}O_2^-$	-
391.4	391.35811	391.35815	0.12	-	$C_{26}H_{47}O_2^-$	-
365.4	365.34244	365.34250	0.11	FA(24:1) (nervonic acid)	$C_{24}H_{45}O_2^-$	-
303.2	303.23292	303.23295	0.10	FA(20:4) (arachidonic acid)	$C_{20}H_{31}O_2^-$	59, 64, 66
281.2	281.24858	281.24860	0.08	FA(18:1) (oleic acid)	$C_{18}H_{33}O_2^-$	64, 66
255.2	255.23292	255.23295	0.12	FA(16:0) (palmitic acid)	$C_{16}H_{31}O_2^-$	63, 64

Table S1. List of the lipids detected during the analyses of the tissue sections of the 14 research subjects. [a] *m/z* values detected during the DESI-MSI analyses. [b] *m/z* values detected during the ESI-FT-ICR analyses. [c] Elemental formula of the anionic ion species ($[M-H]^-$).

$m/z_{FT\text{-}ICR}$	Assignment	Characteristic Fragments
885.54966	PI(38:4)[a]	223.00145, 241.01202, 259.02264, 297.03827, 315.04890
863.56535	PI(36:1)[a]	223.00147, 241.01202, 297.03824
844.60717	PS(40:1)[b]	757.57552 (NL 87)
835.53398	PI(34:1)[a]	241.01215
788.54451	PS(36:1)[b]	701.51303 (NL 87)
768.55474	PE(38:3)[a]	196.03822

Table S2. Ion fragments detected during the ESI-FT-ICR MSMS analyses of the PL species listed in Table S1. [a] *m/z* values of fragments characteristic of the polar headgroups of each PL. [b] *m/z* values of the ion fragments produced through a neutral headgroup loss ('NL') of 87.

LM-ID	Common names	Formula	Category
LMFA01020357	25:2(5Z,9Z)(24Me)	C26H48O2	Fatty Acyls [FA]
LMFA01020358	25:2(5Z,9Z)(23Me)	C26H48O2	Fatty Acyls [FA]
LMFA01030133	-	C26H48O2	Fatty Acyls [FA]
LMFA01030424	-	C26H48O2	Fatty Acyls [FA]
LMFA01030425	-	C26H48O2	Fatty Acyls [FA]
LMFA01030876	26:2(9Z,19Z)	C26H48O2	Fatty Acyls [FA]
LMFA07010656	4-Methyl-3-heptyl linoleate	C26H48O2	Fatty Acyls [FA]

Table S3: Lipid species identified from *m/z* 391.35811 by database searching in lipidmaps.

LM_ID	COMMON_NAME	FORMULA	CATEGORY
LMGP10020079	PA(O-16:0/18:3(9Z,12Z,15Z))	C37H69O7P	Glycerophospholipids [GP]
LMGP10020080	PA(O-16:0/18:3(6Z,9Z,12Z))	C37H69O7P	Glycerophospholipids [GP]
LMGP10030088	PA(P-16:0/18:2(9Z,12Z))	C37H69O7P	Glycerophospholipids [GP]

Table S4: Lipid species identified from *m/z* 655.47067 by database searching in lipidmaps.

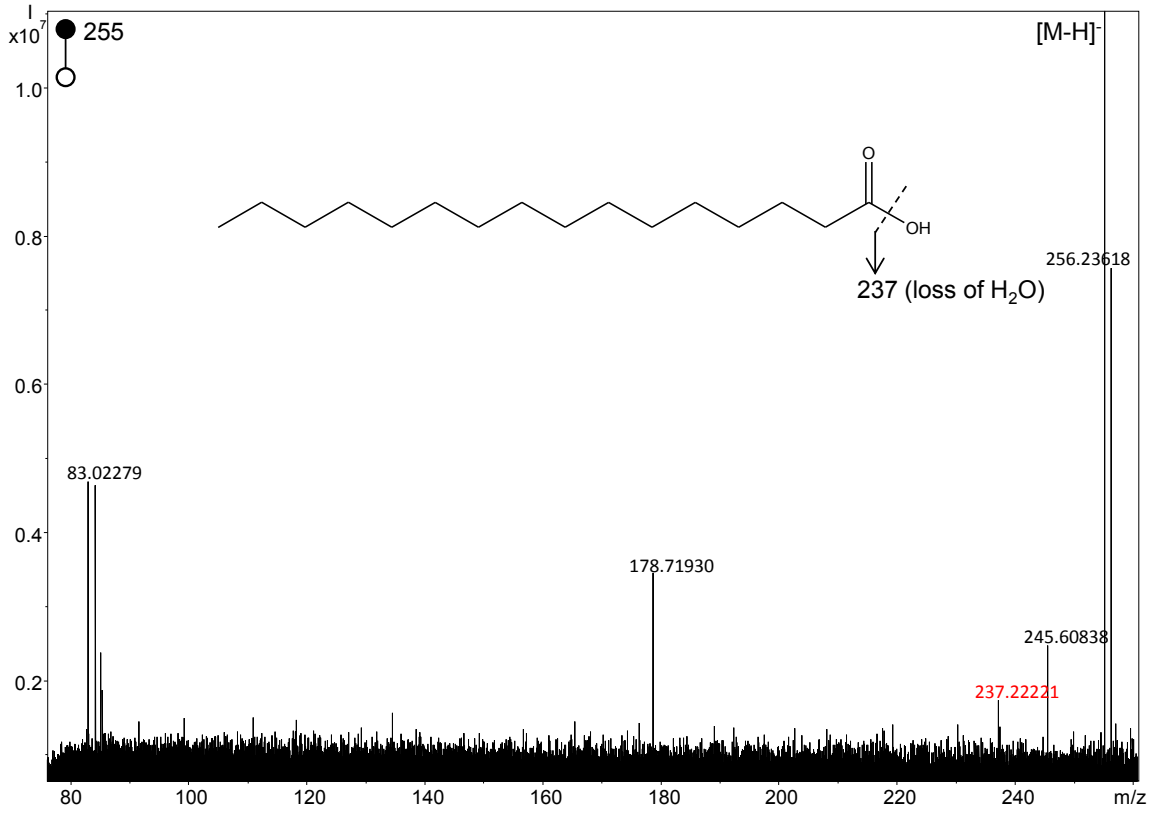

Fig. S1. ESI-FTICR MSMS analysis of a lipid at *m/z* 255.23292 extracted from tumor center tissue sections of research subject #9.

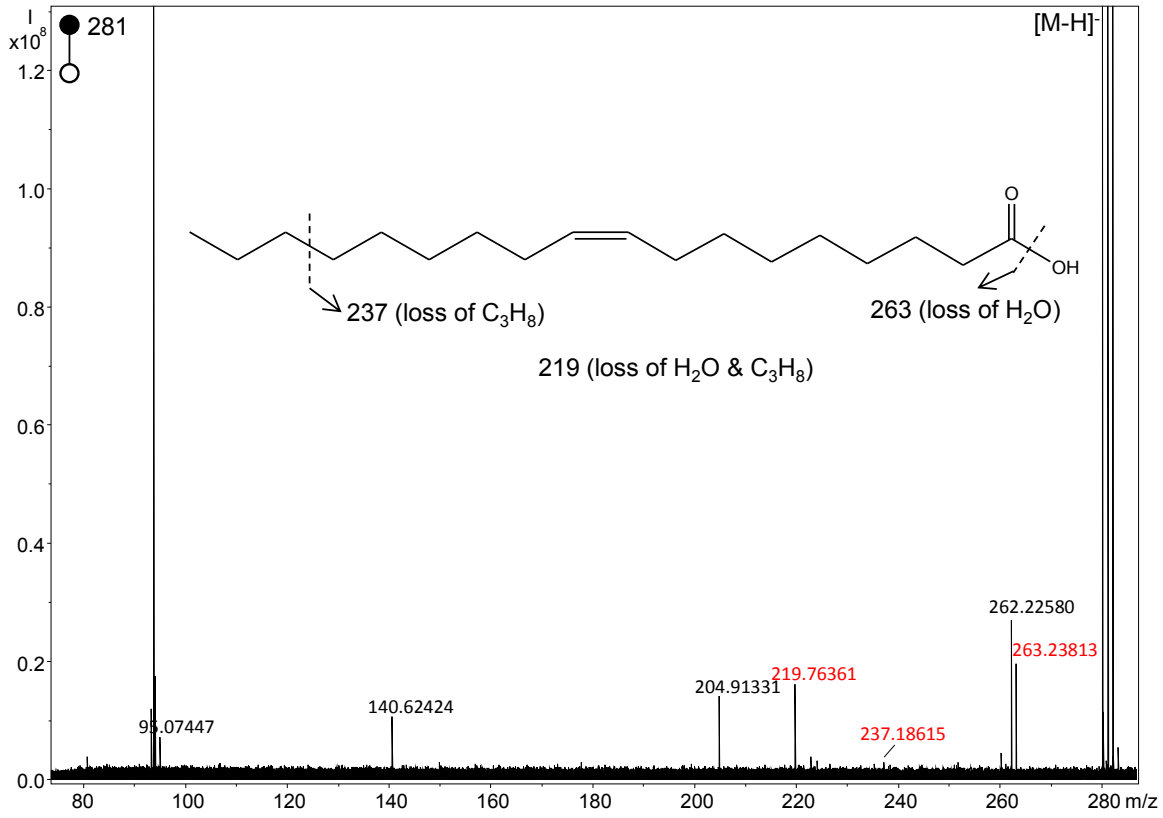

Fig. S2. ESI-FTICR MSMS analysis of a lipid at *m/z* 281.24858 extracted from tumor center tissue sections of research subject #9.

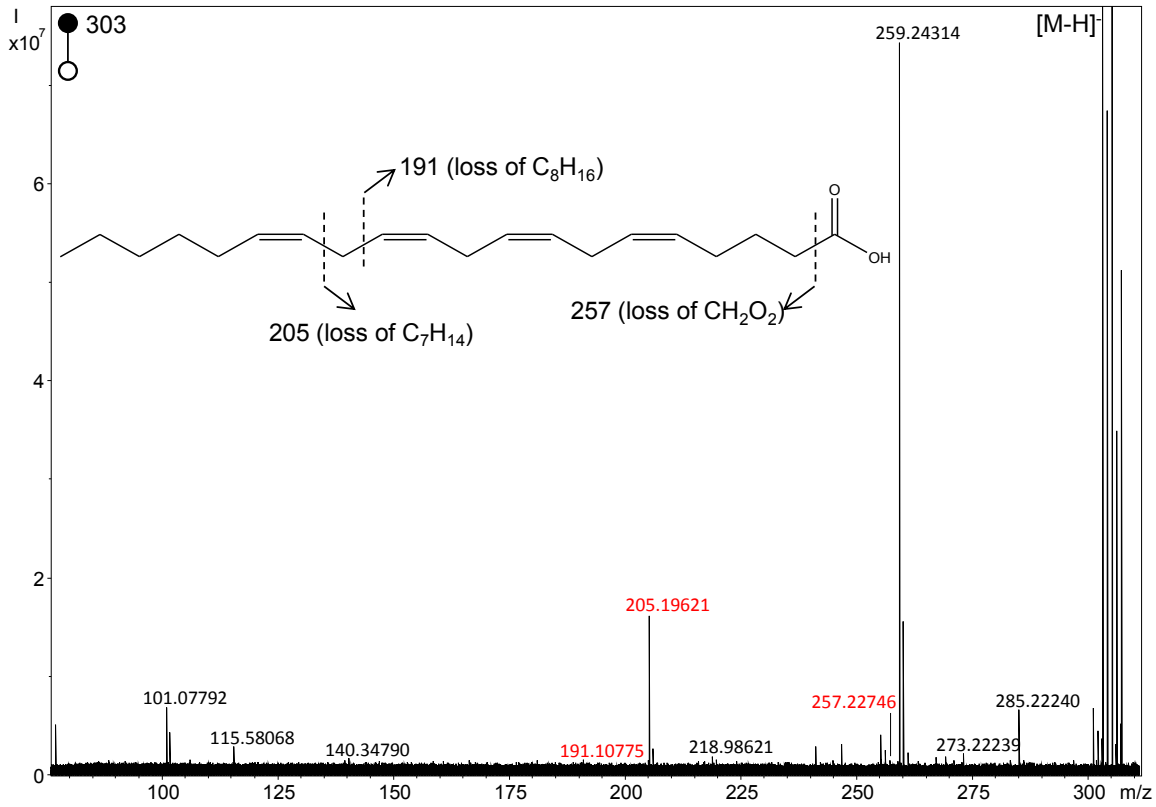

Fig. S3. ESI-FTICR MSMS analysis of a lipid at *m/z* 303.23292 extracted from tumor center tissue sections of research subject #9.

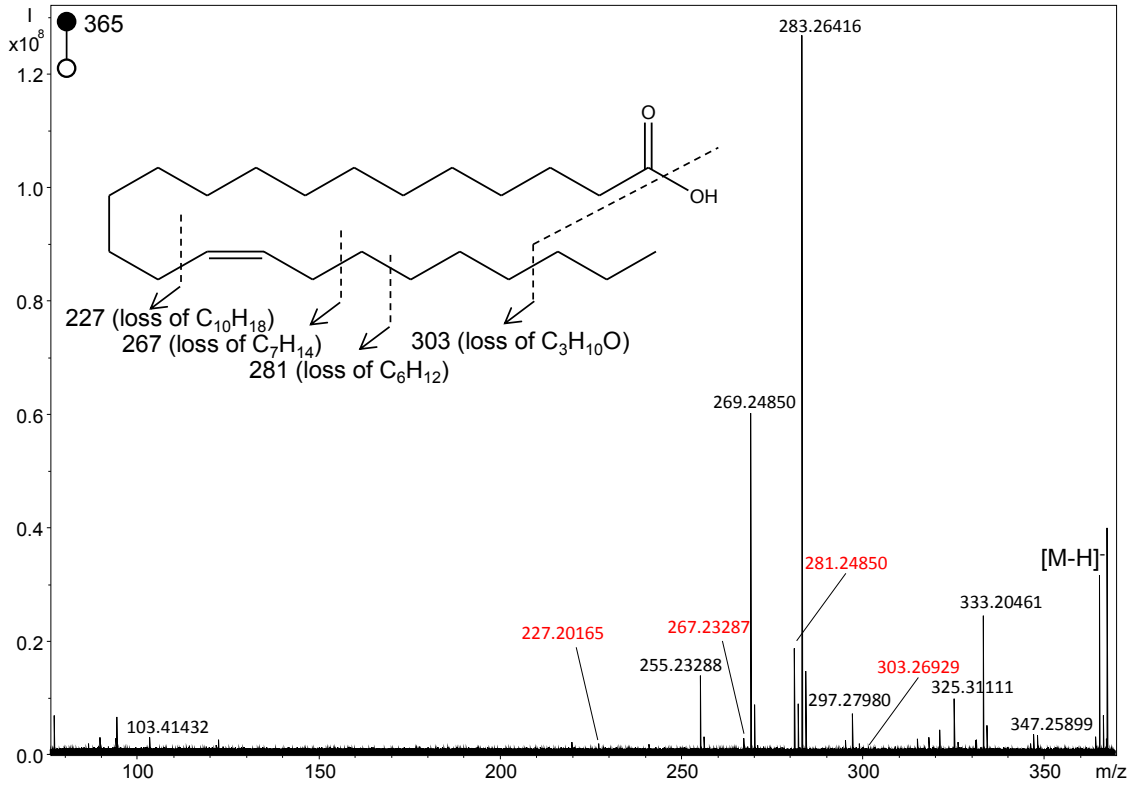

Fig. S4. ESI-FTICR MSMS analysis of a lipid at *m/z* 365.34250 extracted from tumor center tissue sections of research subject #9.

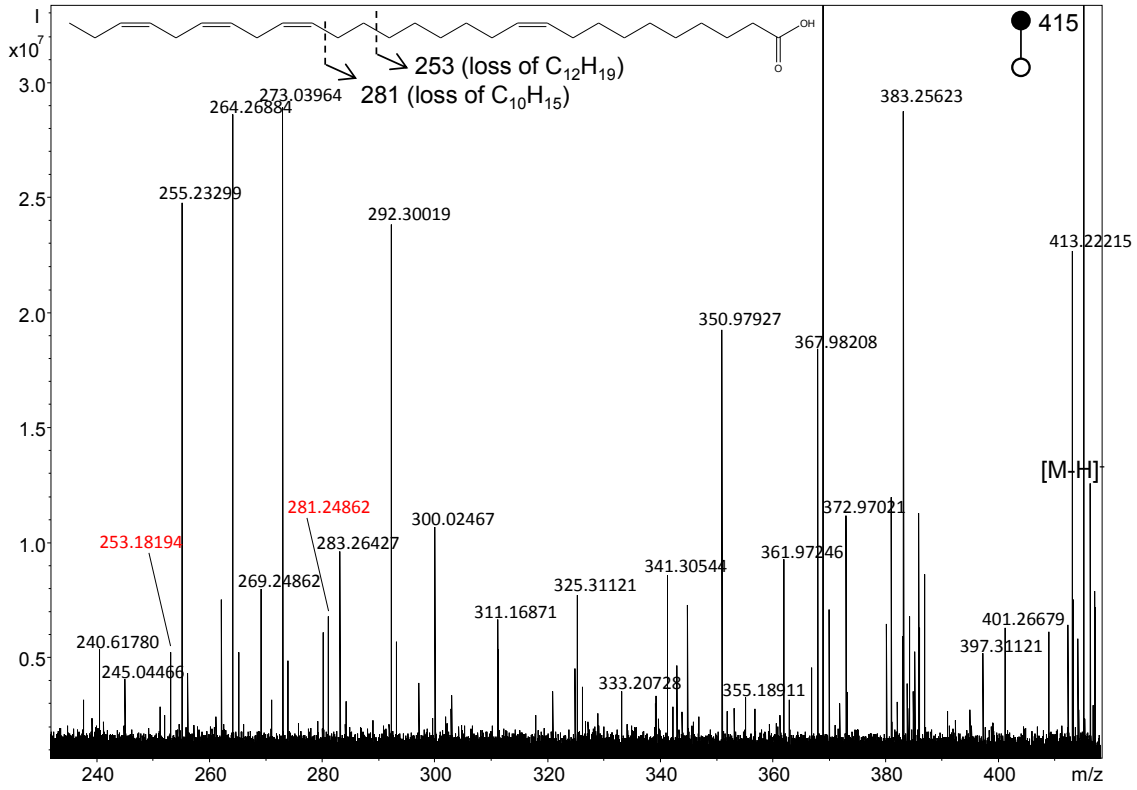

Fig. S5. ESI-FTICR MSMS analysis of a lipid at *m/z* 415.35809 extracted from tumor center tissue sections of research subject #9.

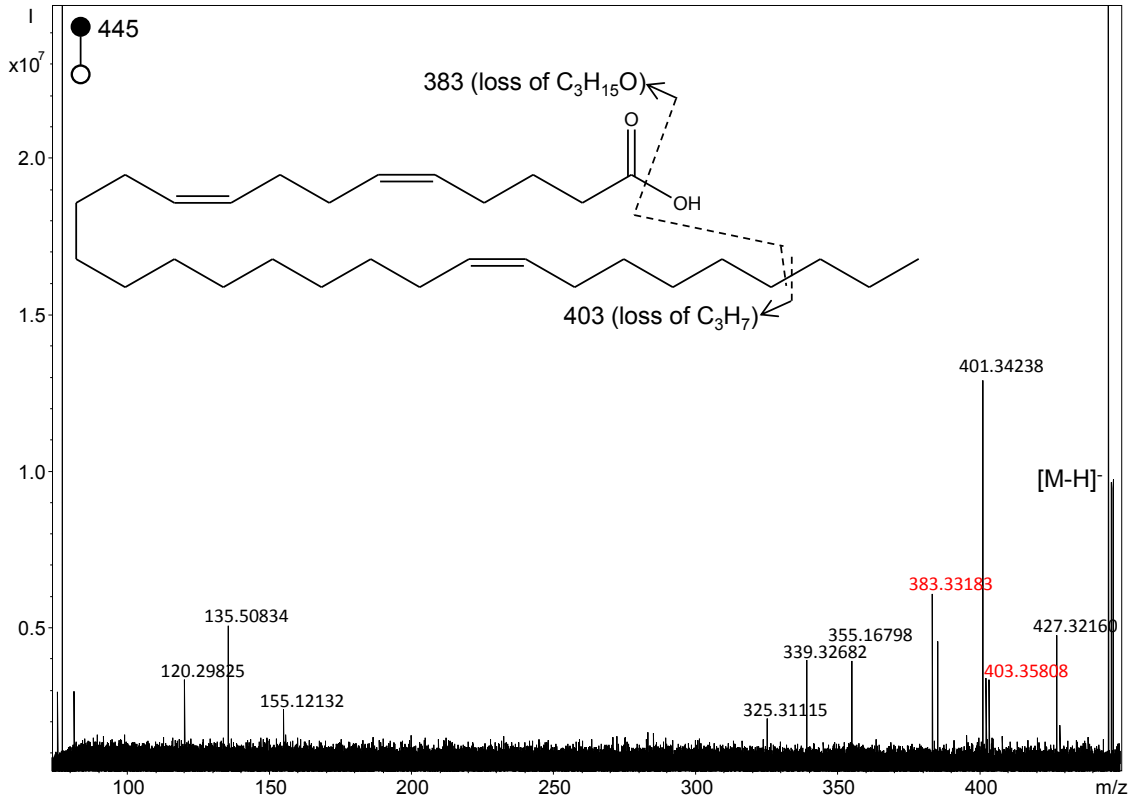

Fig. S6. ESI-FTICR MSMS analysis of a lipid at *m/z* 445.40411 extracted from tumor center tissue sections of research subject #9.

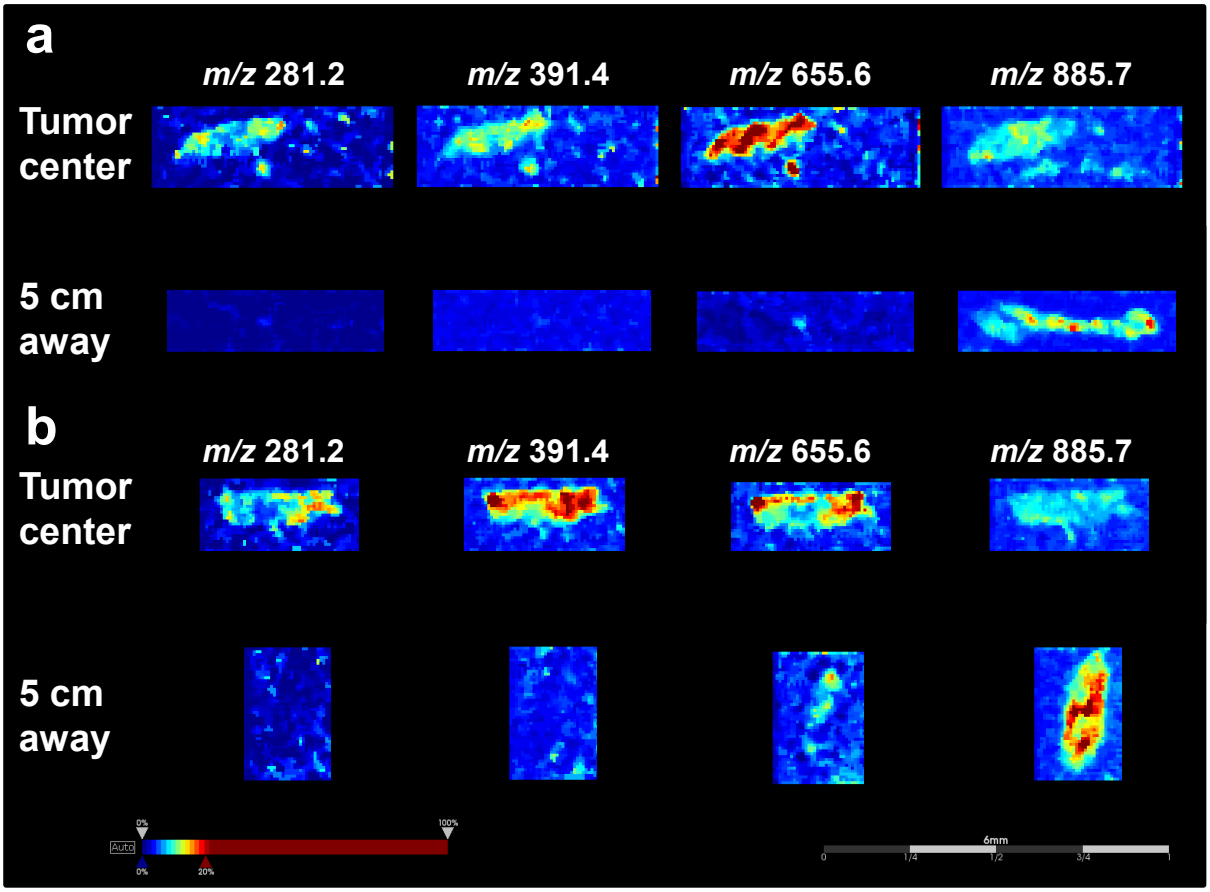

Fig. S7. Ion map comparison of tumor center and 5 cm away from tumor tissue sections from research subjects #9 (**a**) and #14 (**b**) in SCiLS software. Ion maps display the distribution of ions at *m/z* 281.2, *m/z* 391.4, *m/z* 655.6, and *m/z* 885.7 (**d**).

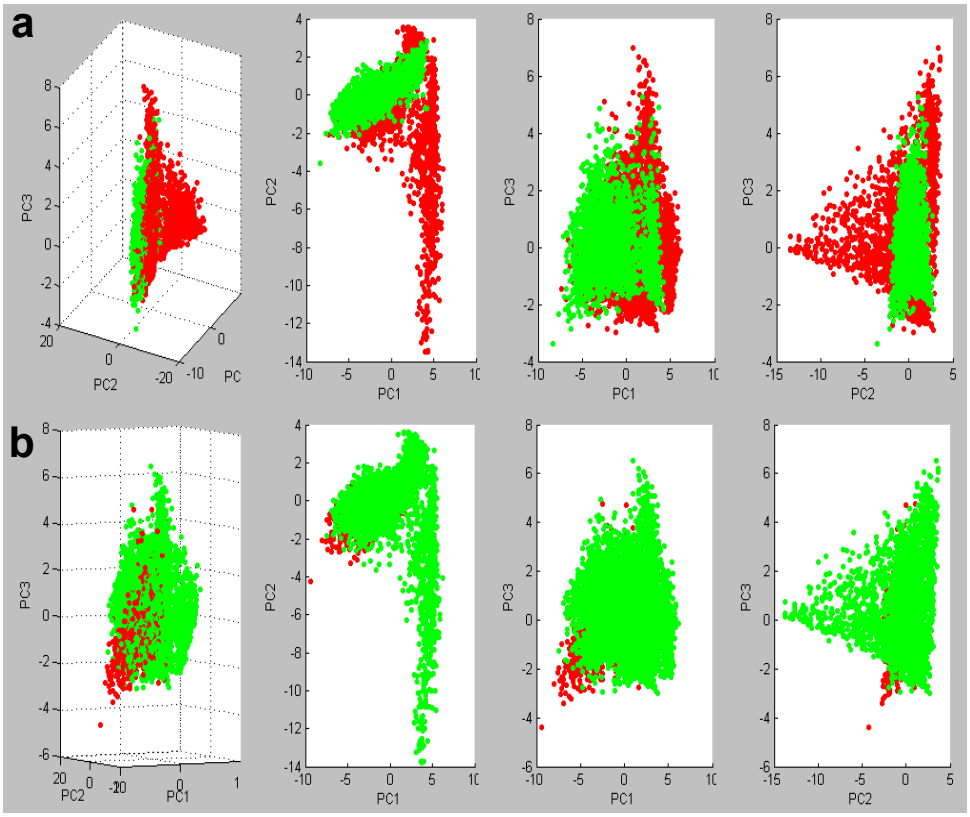

Fig. S8. PCA analysis of DESI-MSI data from ER and PR tumor center (**a**) and Her2 tumor center (**b**) specimens. Score plots of the three first principal components display mass spectra from tumor center specimens ER+/PR+ (red dots) and ER-/PR- (green dots).