Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Rapid Determination of Tumour Stroma Ratio in Squamous Cell Carcinomas with Desorption Electrospray Ionization Mass Spectrometry (DESI-MS): A Proof-Of-Concept Demonstration

Michael Woolman[a,b], Alessandra Tata[a], Delaram Dara[a], Jalna Meens[b], Elisa D'Arcangelo[c], Consuelo J. Perez[d], Shamina Saiyara Prova[d], Emma Bluemke[a], Howard J. Ginsberg[a,c,e,f], Demian Ifa[d], Alison McGuigan[c,e] Laurie Ailles[b,g] and Arash Zarrine-Afsar[a,b,f,h,*]

[a] Techna Institute for the Advancement of Technology for Health, University Health Network, 100 College Street, Toronto, ON, M5G 1P5, Canada
[b] Department of Medical Biophysics, University of Toronto, 101 College Street, Toronto, ON, M5G 1L7, Canada
[c] Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, Toronto, ON, M5S 3G9, Canada
[d] Department of Chemistry, York University, 4700 Keele Street, Toronto, ON, M3J 1P3, Canada
[e] Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College St. Toronto, ON, M5S 3E5, Canada
[f] Department of Surgery, University of Toronto, 149 College Street, Toronto, ON, M5T 1P5, Canada
[g] Princess Margaret Cancer Centre, University Health Network, 101 College St, Toronto, ON, M5G 1L7, Canada
[h] Keenan Research Center for Biomedical Science & the Li Ka Shing Knowledge Institute, St. Michael's Hospital, 30 Bond Street, Toronto, ON, M5B 1W8, Canada

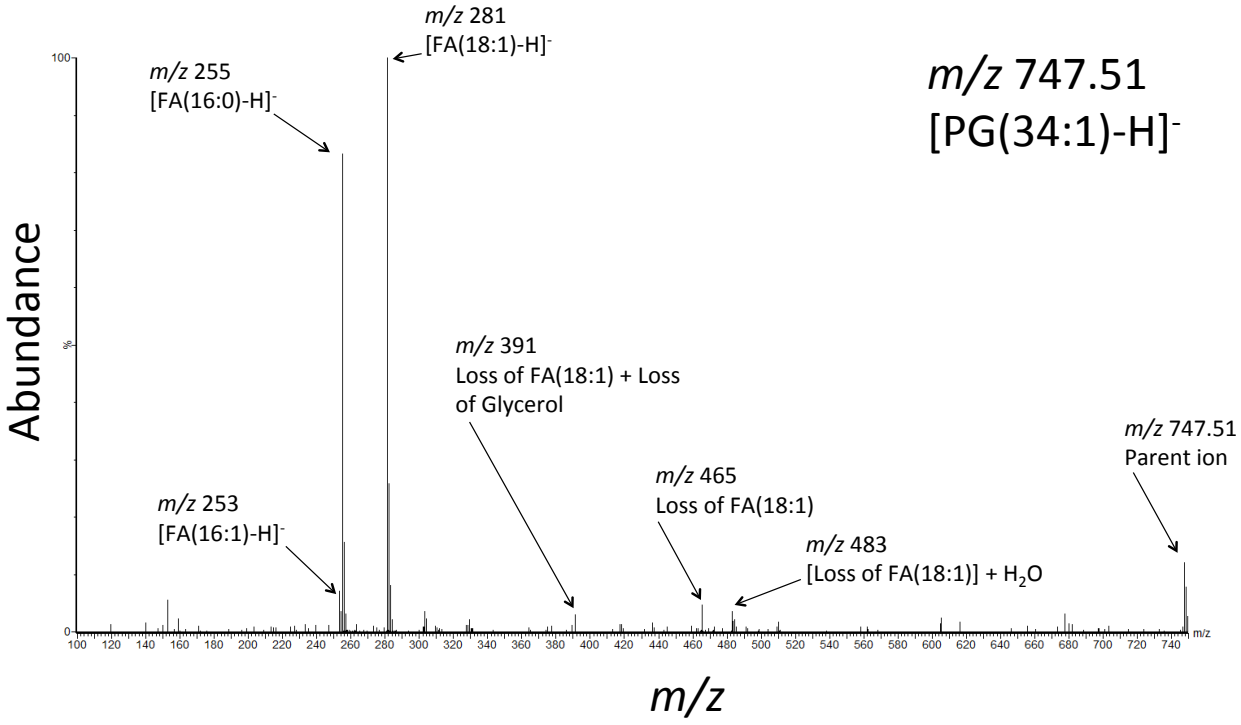
*Corresponding author: arash.zarrine.afsar@utoronto.ca

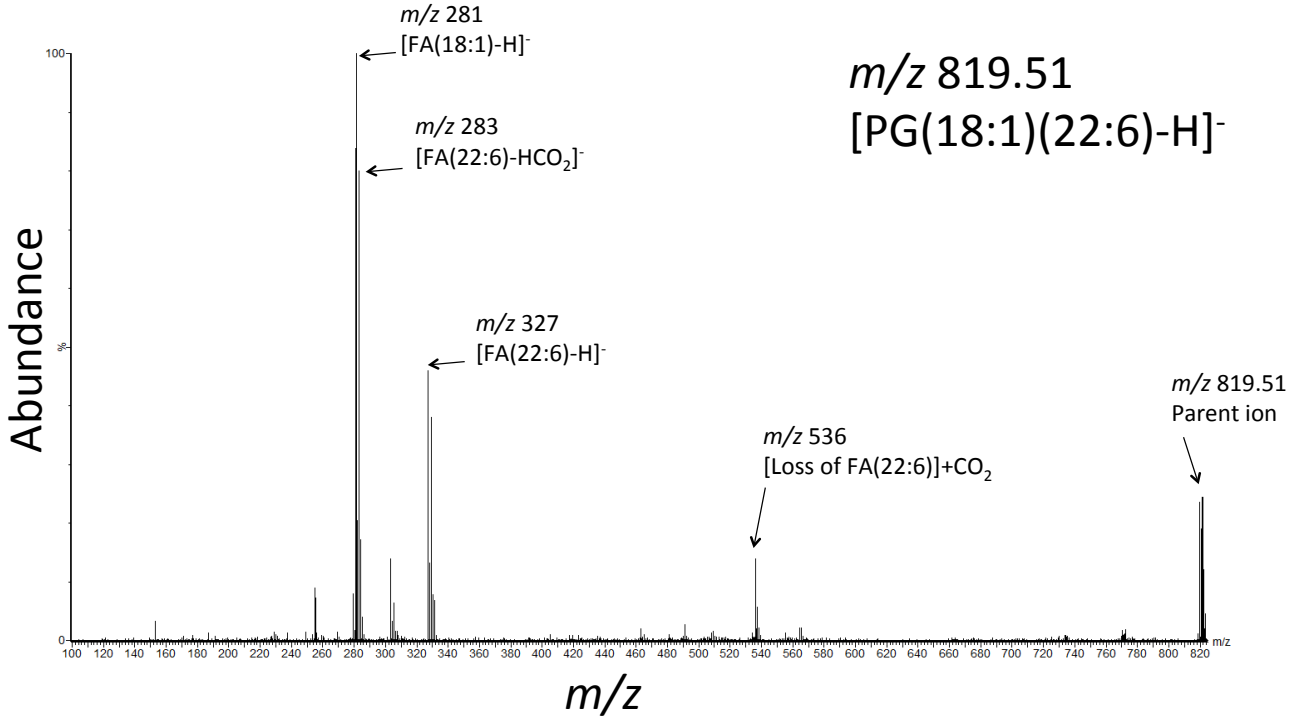
Table of Contents

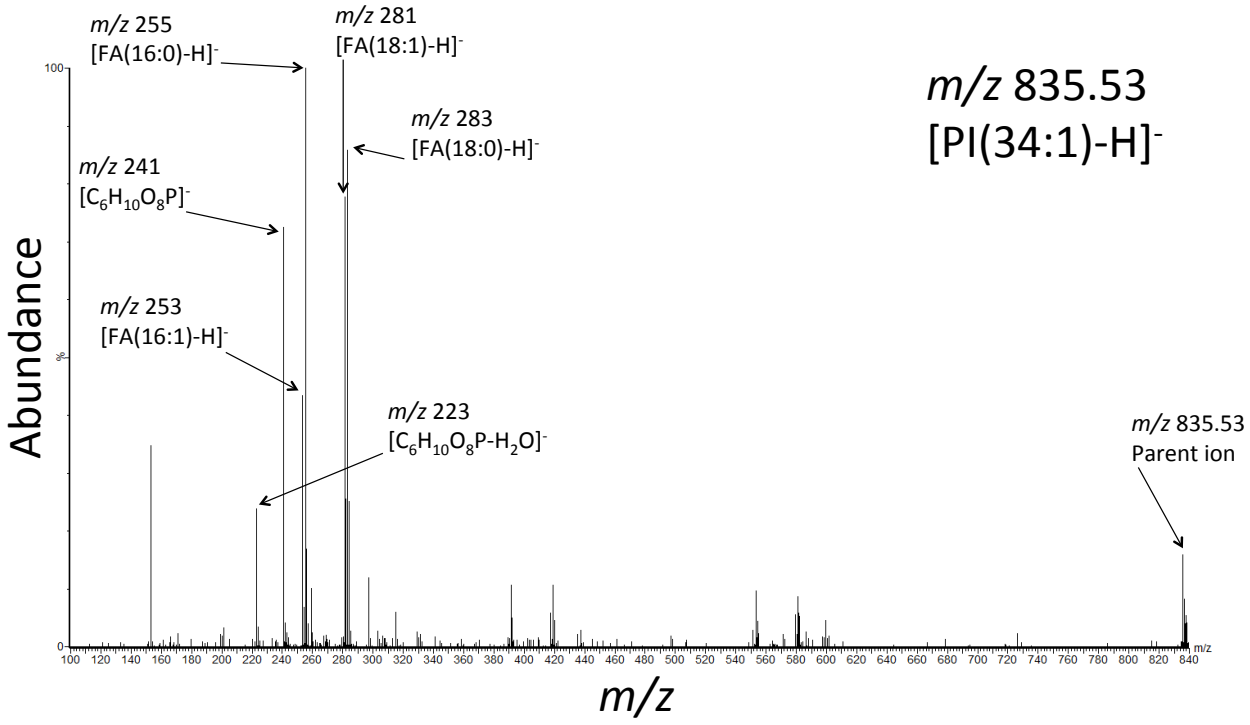
m/z	Intensity FaDu Extract	Intensity 1:1 FaDu CAF Mixture	
773.53	7.32×10^5	2.04×10^5	
835.53	2.69×10^5	9.44×10^4	
863.56	2.89×10^5	8.84×10^4	
TIC	2.25×10^7	9.49×10^6	
***m/z* Ratios**	**FaDu Extract**	**1:1 FaDu CAF Mixture**	**% Deviation in Ratios**
863.56 / 835.53	1.07	0.94	13
773.53 / 863.56	2.54	2.31	10

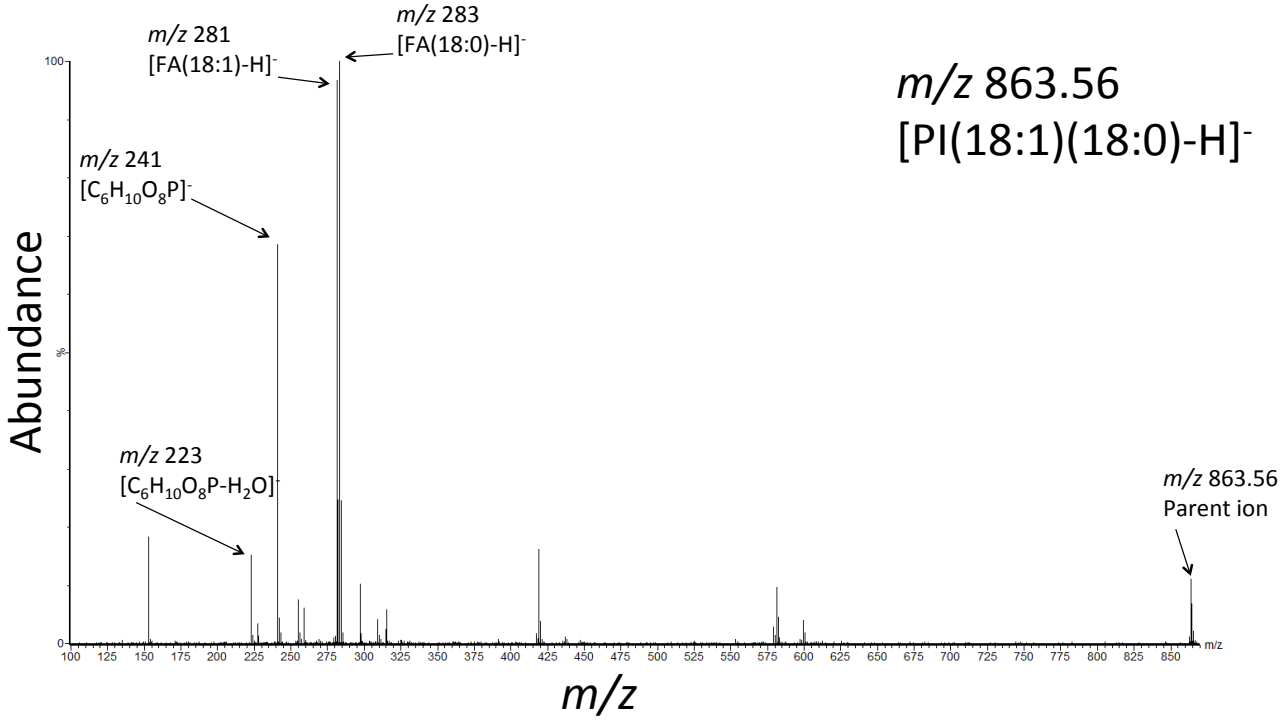
Table S1. The abundance of FaDu biomarker ions in pure cancer cell lipid extract, and in 1:1 cancer stroma mixture extract. This table summarizes the intensity values obtained from representative spectrum of lipid extract show in Fig. 1. As detailed in the manuscript, minor ion suppression on the order of ~10% was seen affecting the relative abundance of cancer biomarker ions in mixed cancer, stroma sample. The total ion count (TIC) values are also given. The percent deviation in peak intensity ratios for FaDu markers between mixture and pure cancer cell extract, likely arising due to signal suppression, is also provided.

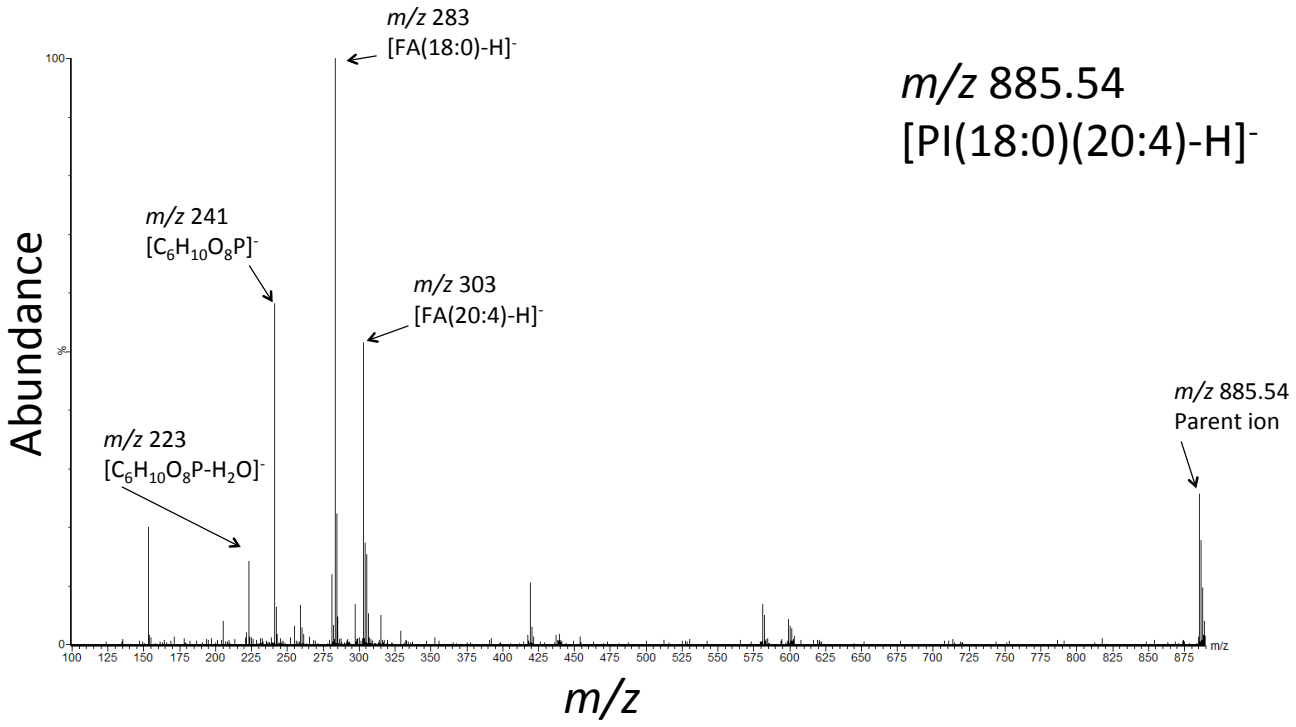
Figure S1. The MS/MS analysis of the biomarker ions described in this study. Below, we have included the ms/ms spectra that summarize the ion identity assignments presented in Table 1. The majority of ions were assigned using DESI-MS/MS analysis. The MS/MS analysis of the ion of *m/z* 773.53 $[PG(18:1)(18:1)-H]^-$ was performed on LTQ-OrbiTrap system. The other MS/MS analyses used the TOF platform utilizing a DESI-MS ion source as described in the manuscript text.

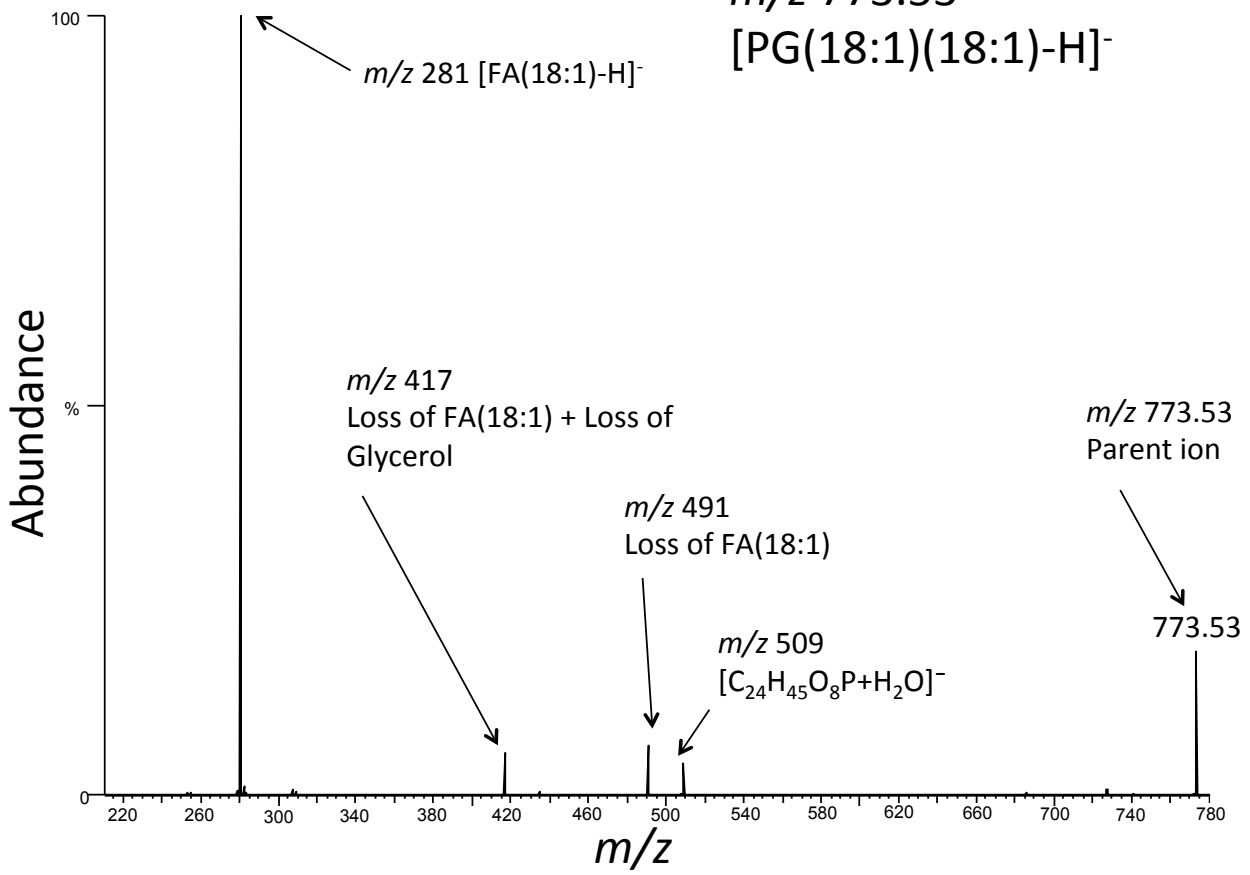

m/z 747.51
[PG(34:1)-H]-
m/z 281
[FA(18:1)-H]-
m/z 255
[FA(16:0)-H]-
m/z 391
Loss of FA(18:1) + Loss of Glycerol
m/z 747.51
Parent ion
m/z 465
Loss of FA(18:1)
m/z 253
[FA(16:1)-H]-
m/z 483
[Loss of FA(18:1)] + H2O
Abundance
m/z


m/z 819.51
[PG(18:1)(22:6)-H]-
m/z 281
[FA(18:1)-H]-
m/z 283
[FA(22:6)-HCO2]-
m/z 327
[FA(22:6)-H]-
m/z 819.51
Parent ion
m/z 536
[Loss of FA(22:6)]+CO2
Abundance
m/z


m/z 255
[FA(16:0)-H]-
m/z 281
[FA(18:1)-H]-
m/z 283
[FA(18:0)-H]-
m/z 241
[C6H10O8P]-
m/z 253
[FA(16:1)-H]-
m/z 223
[C6H10O8P-H2O]-
m/z 835.53
Parent ion
m/z 835.53
[PI(34:1)-H]-
Abundance
%
m/z


m/z 281
[FA(18:1)-H]-
m/z 283
[FA(18:0)-H]-
m/z 241
[C6H10O8P]-
m/z 223
[C6H10O8P-H2O]-
m/z 863.56
Parent ion
m/z 863.56
[PI(18:1)(18:0)-H]-
Abundance
%
m/z


m/z 283
[FA(18:0)-H]-
m/z 885.54
[PI(18:0)(20:4)-H]-
m/z 241
[C6H10O8P]-
m/z 303
[FA(20:4)-H]-
m/z 885.54
Parent ion
m/z 223
[C6H10O8P-H2O]-
Abundance
m/z


m/z 773.53
[PG(18:1)(18:1)-H]-
m/z 281 [FA(18:1)-H]-
m/z 417
Loss of FA(18:1) + Loss of
Glycerol
m/z 491
Loss of FA(18:1)
m/z 509
[C24H45O8P+H2O]-
m/z 773.53
Parent ion
773.53
Abundance
m/z

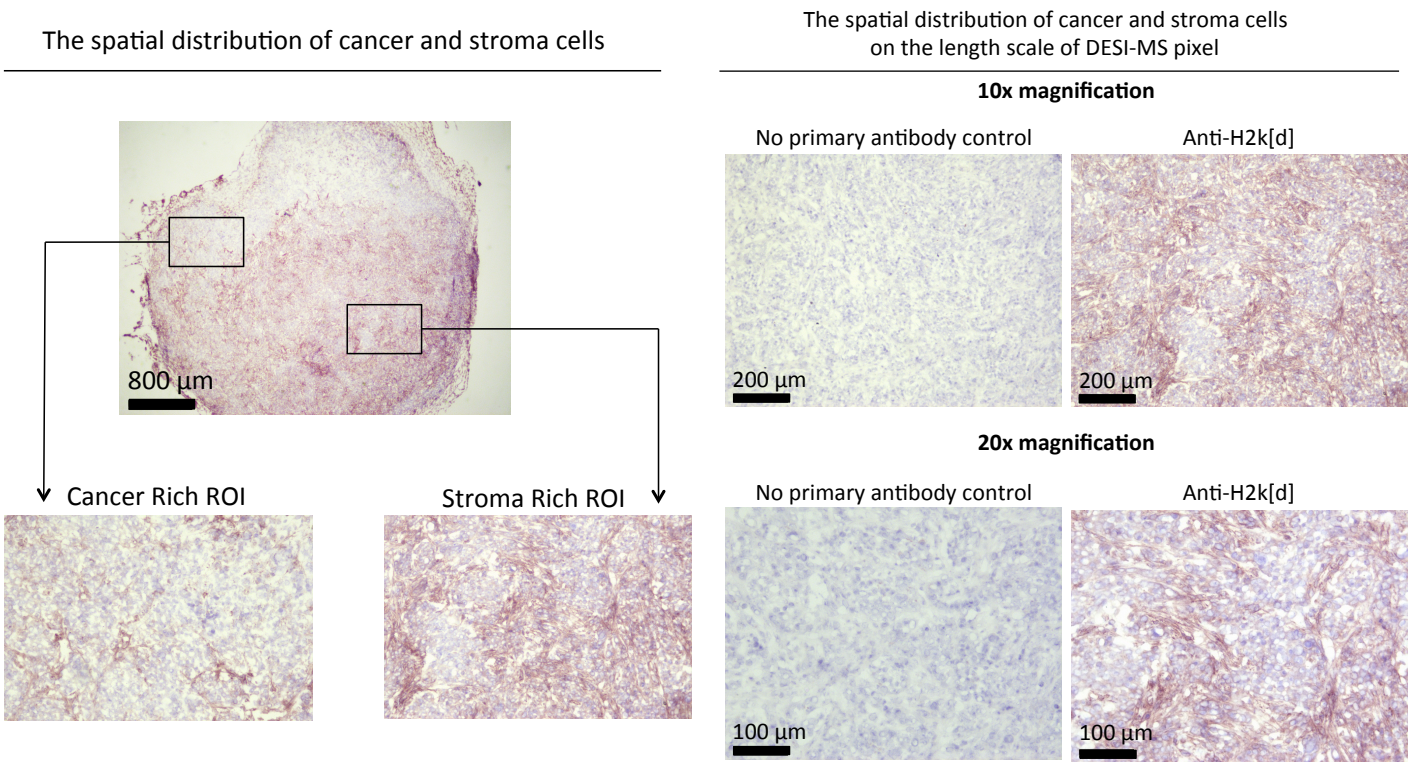


Figure S2. The Immunohistochemistry image of FaDu xenograft tumour slice. A 10 µm slice of FaDu xenograft tumour was subjected to immunostaining. This image illustrates the degree of mixing and infiltration between stroma and cancer cells in FaDu head & neck cancer model. The mixing between cancer and stroma cells is on the order of a single DESI-MS pixel. Both cancer rich and stroma rich ROIs contain cancer and stroma cells.

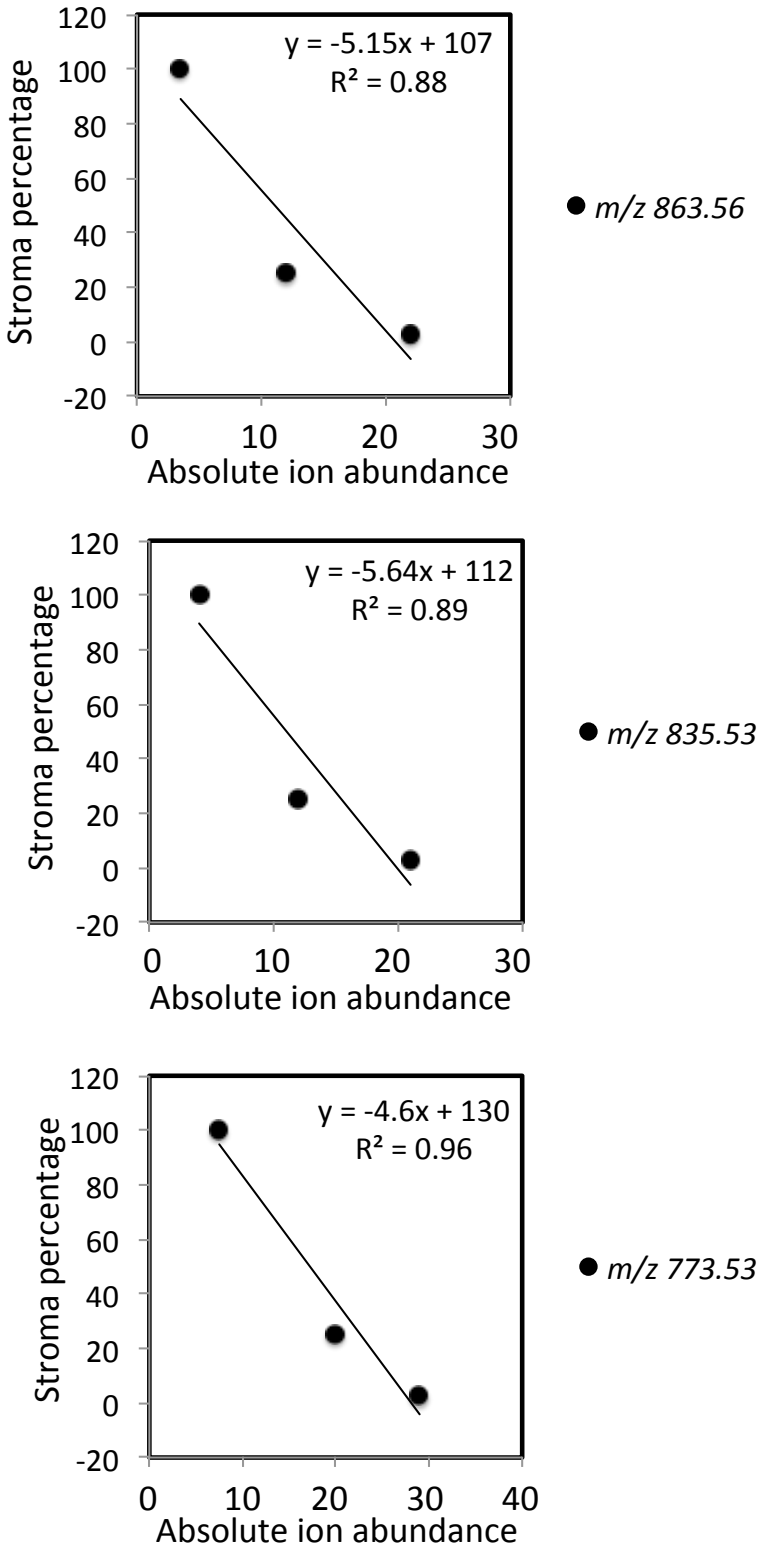


Figure S3. The quantitative concordance between stroma percentage and absolute values of FaDu biomarker ion abundance from lipid extract experiment, averaged from the repetitions shown in Fig. 5. A simple linear relationship between absolute ion abundance values and stroma percentage is assumed. The coefficient of determination R^2 values is provided for each of the FaDu markers listed in Table 1. With the caveat of limited data points, we used a liner fit model to predict stroma percentages of FaDu tumour slices from ROIs shown in Fig. 4.