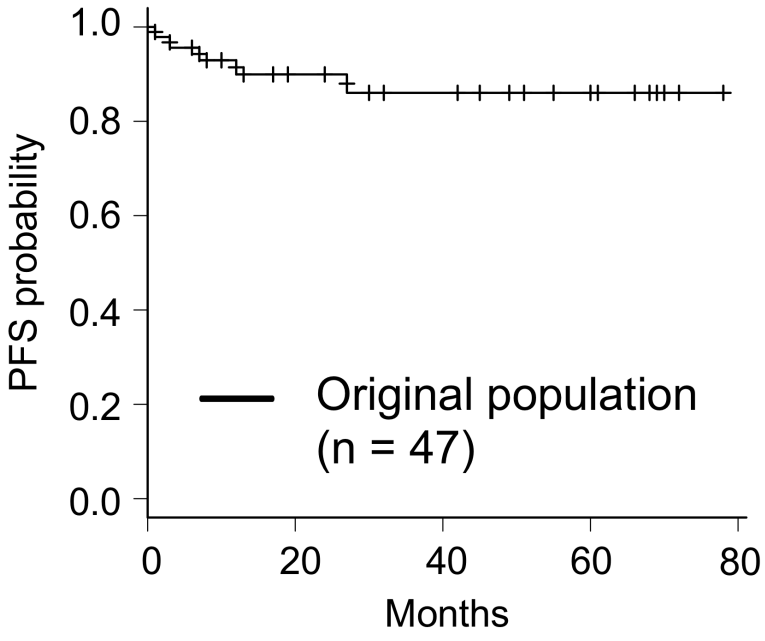
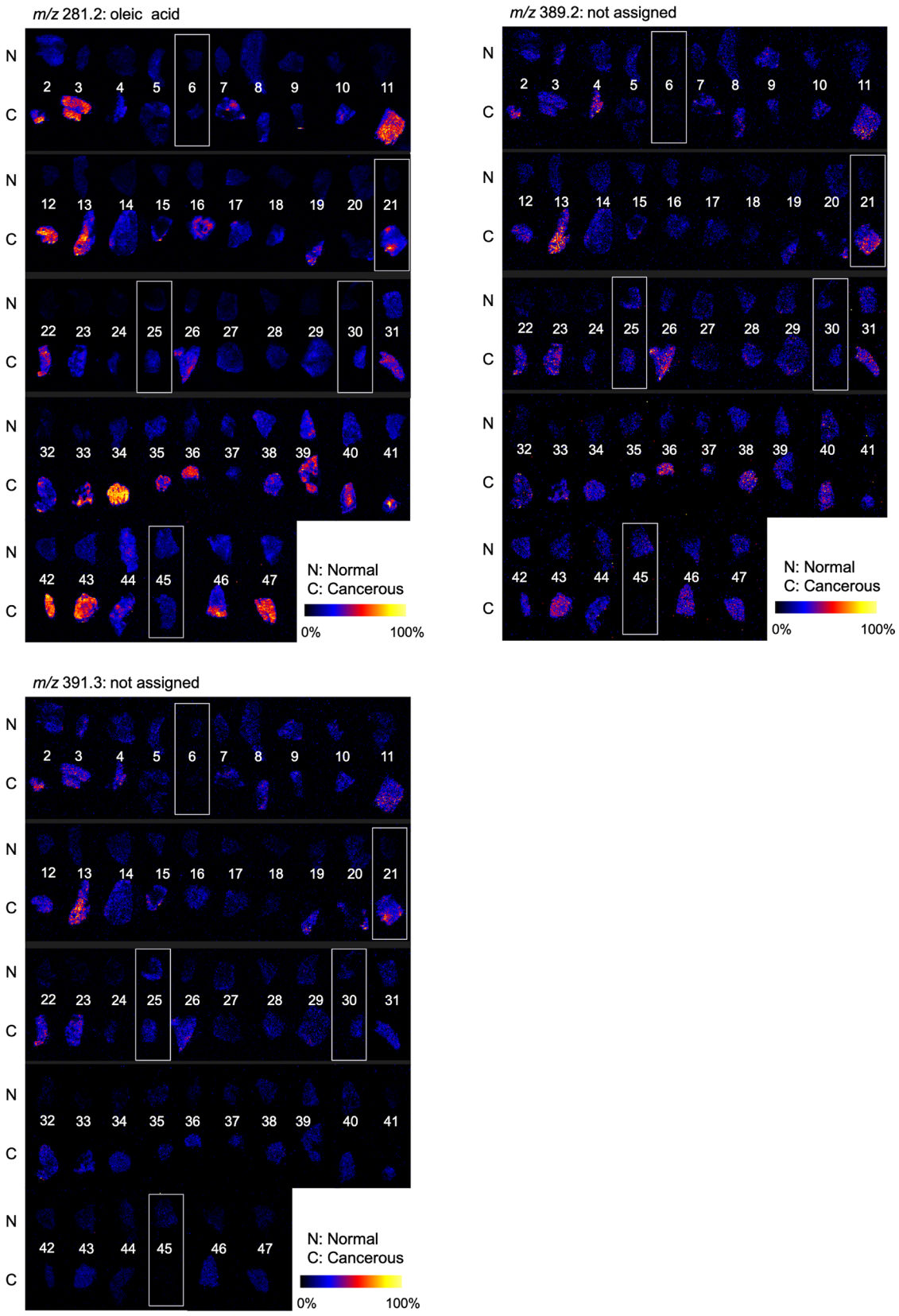
Discovery of lipid biomarkers correlated with disease progression in clear cell renal cell carcinoma using desorption electrospray ionization imaging mass spectrometry

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Kaplan–Meier survival curve of PFS for original population. This survival curve shows observed period (months) (x-axis) and progression-free survival probability (y-axis) in all patients.



Supplementary Figure 2: Images showing ions of *m/z* 281.2 (oleic acid), 389.2 (not assigned), and 391.3 (not assigned) identified as biomarkers using DESI-IMS data from all samples except No. 1. Images for sample No. 1 by DESI-IMS were shown as representative images in the main article. High to low ion intensity was shown on a scale from white to black, respectively. Most cancerous areas were colored in red or blue as compared to normal areas. The samples surrounded with a square box were obtained from patients who showed disease progression.

Supplementary Table 1: LC-MS/MS data used for identification of glycerophospholipids

Observed mass	Theoretical mass	Delta (Da)	Fragment ions	Ion assignments	Elemental Formula
773.5341	773.5338	0.0009	152.00, 281.25	PG(18:1/18:1)	$C_{42}H_{78}O_{10}P^-$
885.5493	885.5499	0.0009	152.00, 241.01, 259.02, 259.24, 259.24, 283.26	PI(18:0/20:4)	$C_{47}H_{82}O_{13}P^-$

Two important two glycerophospholipids in this study were identified by LC-MS/MS. PG = glycerophosphoglycerol, PI = glycerophosphoinositol.

Supplementary Table 2: Searching a computationally-generated database of lipid classes

Input mass	Matched mass	Delta (Da)	Name	Elemental formula
253.2174	253.2173	0.0001	FA(16:1)	$C_{16}H_{29}O_2^-$
255.2330	255.2330	0.0000	FA(16:0)	$C_{16}H_{31}O_2^-$
279.2330	279.2330	0.0000	FA(18:2)	$C_{18}H_{31}O_2^-$
281.2486	281.2486	0.0000	FA(18:1)	$C_{18}H_{33}O_2^-$
303.2329	303.2330	0.0001	FA(20:4)	$C_{20}H_{31}O_2^-$
309.2799	309.2799	0.0000	FA(20:1)	$C_{20}H_{37}O_2^-$
327.2333	327.2330	0.0003	FA(22:6)	$C_{22}H_{31}O_2^-$
329.2476	329.2486	0.0010	FA(22:5)	$C_{22}H_{33}O_2^-$
389.2462	389.2545	0.0083	FA(20:0(OH4,Ep))	$C_{27}H_{37}O_7^-$
	389.2545	0.0083	FA(20:0(OH4,Ke))	$C_{27}H_{37}O_7^-$
391.2586	391.2490	0.0096	FA(23:2(Ke2,Ep))	$C_{23}H_{35}O_5^-$
	391.2490	0.0096	FA(23:2(Ke,Ep2))	$C_{23}H_{35}O_5^-$
	391.2490	0.0096	FA(23:3(OH,Ep2))	$C_{23}H_{35}O_5^-$
	391.2490	0.0096	FA(23:3(OH,Ke2))	$C_{23}H_{35}O_5^-$
	391.2490	0.0096	FA(23:3(OH,Ke,Ep))	$C_{23}H_{35}O_5^-$
	391.2490	0.0096	FA(23:4(OH2,Ep))	$C_{23}H_{35}O_5^-$
	391.2490	0.0096	FA(23:4(OH2,Ke))	$C_{23}H_{35}O_5^-$
	391.2490	0.0096	FA(23:5(OH3))	$C_{23}H_{35}O_5^-$

Each input mass is measured mass from mass lock correction described in the main text. The ion of *m/z* 187.0976 did not have any hit on this database search. FA = fatty acid. FA(16:1): palmitoleic acid, FA(16:0): palmitic acid, FA(18:2): linoleic acid, FA(18:1): oleic acid, FA(20:4): arachidonic acid, FA(22:6): docosahexaenoic acid (DHA), FA(22:5): docosapentaenoic acid (DPA).

Supplementary Table 3: Searching LIPID MAPS structure database (LMSD)

Input mass	Matched mass	Delta (Da)	Name	Elemental formula
187.0976	187.0976	0.0000	Azelaic acid	$C_9H_{15}O_4^-$
187.0976	187.0976	0.0000	cis- and trans-Ethyl 2,4-dimethyl-1,3-dioxolane-2-acetate	$C_9H_{15}O_4^-$
187.0976	187.0976	0.0000	(+/–)-Ethyl 3-acetoxy-2-methylbutyrate	$C_9H_{15}O_4^-$
187.0976	187.0976	0.0000	(+/–)-Methyl 5-acetoxyhexanoate	$C_9H_{15}O_4^-$
187.0976	187.0977	0.0001	3-Methylsubericacid	$C_9H_{15}O_4^-$
391.2586	391.2643	0.0057	5-((3Z,6Z,9Z,12Z,15Z,18Z)-henicosa-3,6,9,12,15,18-hexaen-1-yl)resorcinol	$C_{27}H_{35}O_2^-$

This search focused on *m/z* 187.0976, 389.2462, and 391.2586, which could not be identified using a computationally-generated database of lipid classes. The ion of *m/z* 389.2462 did not have any hit on LMSD search.