Supporting Information

Single Cell Mass Spectrometry Analysis of Drug-Resistant Cancer Cells: Metabolomics Studies of Synergetic Effect of Combinational Treatment

Authors: Xingxiu Chen, Mei Sun, Zhibo Yang*

AUTHOR ADDRESS

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, USA

*Address reprint requests to Dr. Zhibo Yang, Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, USA

Tel: (405) 325-1772

Email: Zhibo.Yang@ou.edu

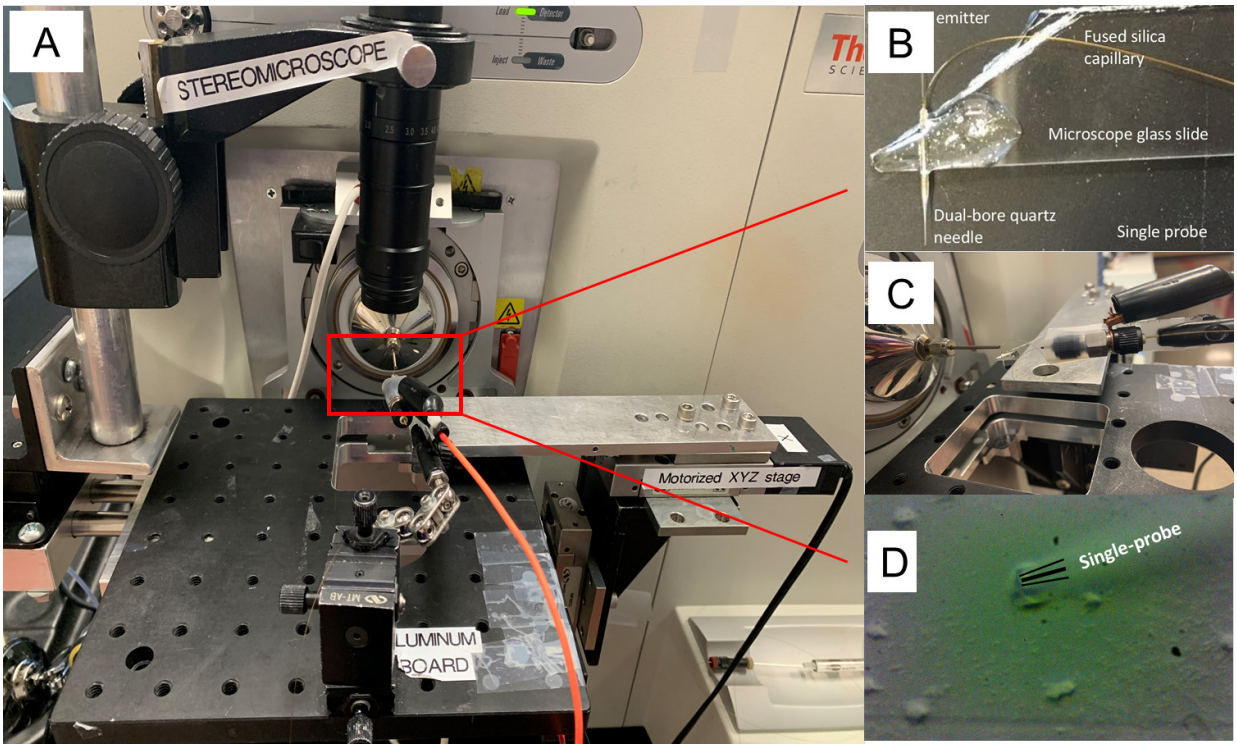


Figure S1. (A) The Single-probe SCMS setup. (B) Image of a fabricated Single-probe. A Single-probe is fabricated by inserting the fused silica capillary (OD 105 μm) and nano-ESI emitter (OD 105 μm) into the dual-bore quartz needle. A dual-bore quartz tubing (OD 500 μm; ID 127 μm) is pulled into a needle (tip size ~10 μm) using a laser micropipette puller. The OD of the bores in the dual-bore needle is gradually reduced from 500 to ~10 μm after laser pulling, and the fused silica capillary has a tight contact and seal with the inner wall of the dual-bore quartz tubing at the tapering part. This type of tight connection is secured using the UV epoxy. (C) A sideview of the Single-probe coupled with the Thermo Orbitrap XL mass spectrometer. The Single-probe is connected with the solvent-providing capillary through a conductive union. The sampling solvent is continuously delivered by a syringe pump with controlled flowrates. The ionization voltage is applied on the conductive union and transmitted to the nanoESI emitter via liquid inside the capillaries. (D) A typical microscopy image of single cells analyzed using the Single-probe device. Single cells are selected and analyzed by precisely moving the motorized XYZ-stage system, and the entire process is monitored using a digital stereomicroscope. The ion signals of the cell will be continuously collected and monitored till the signals completely disappear (usually ion signals from a cell can last for 20-25 s). The Z-stage will be lowered down, and the solvent will be continuously used to flush the inner channel of the probe (e.g., for 30 s) to ensure the ion signals from the cell completely disappear prior to the analysis of the next cell.

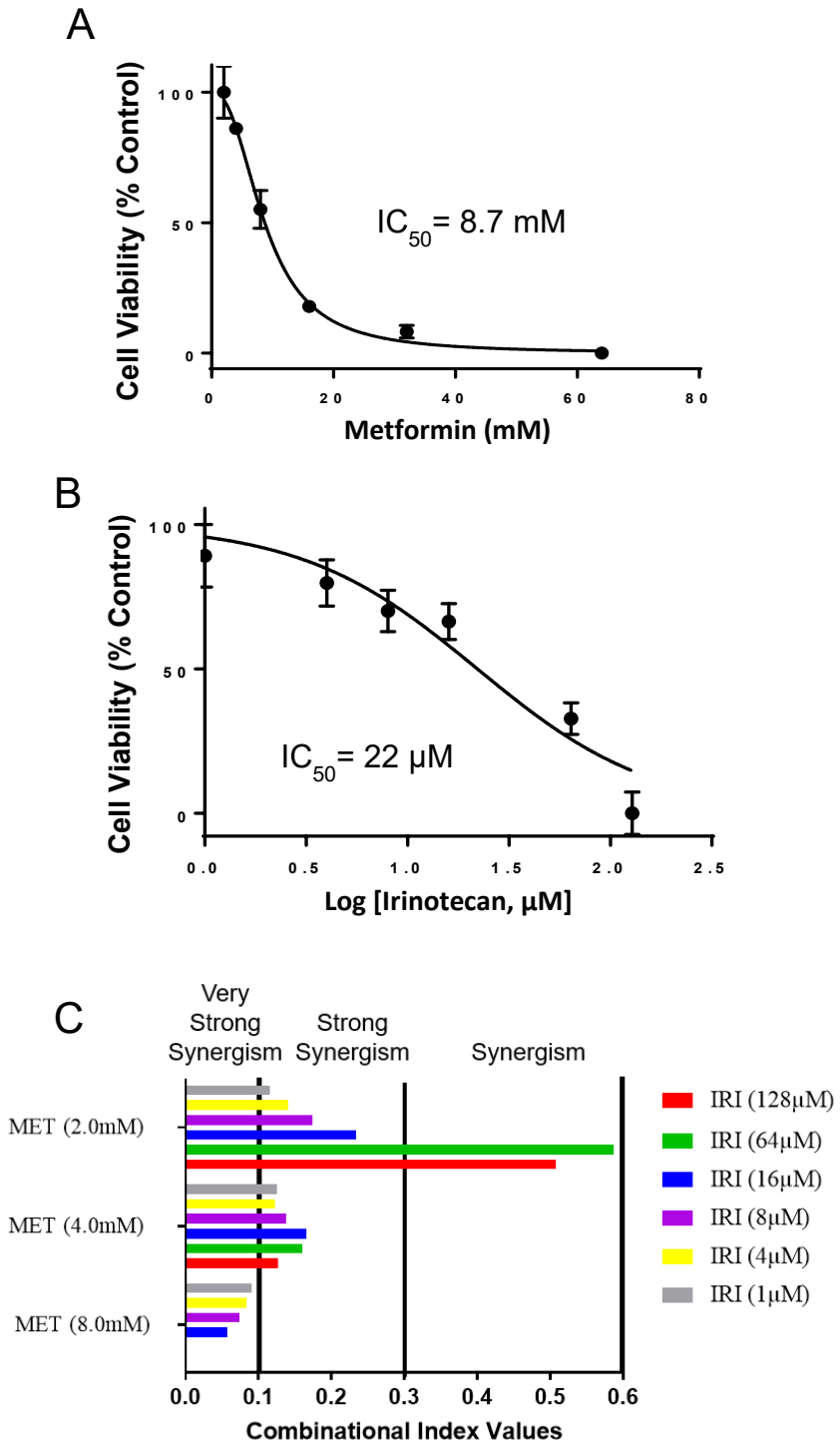
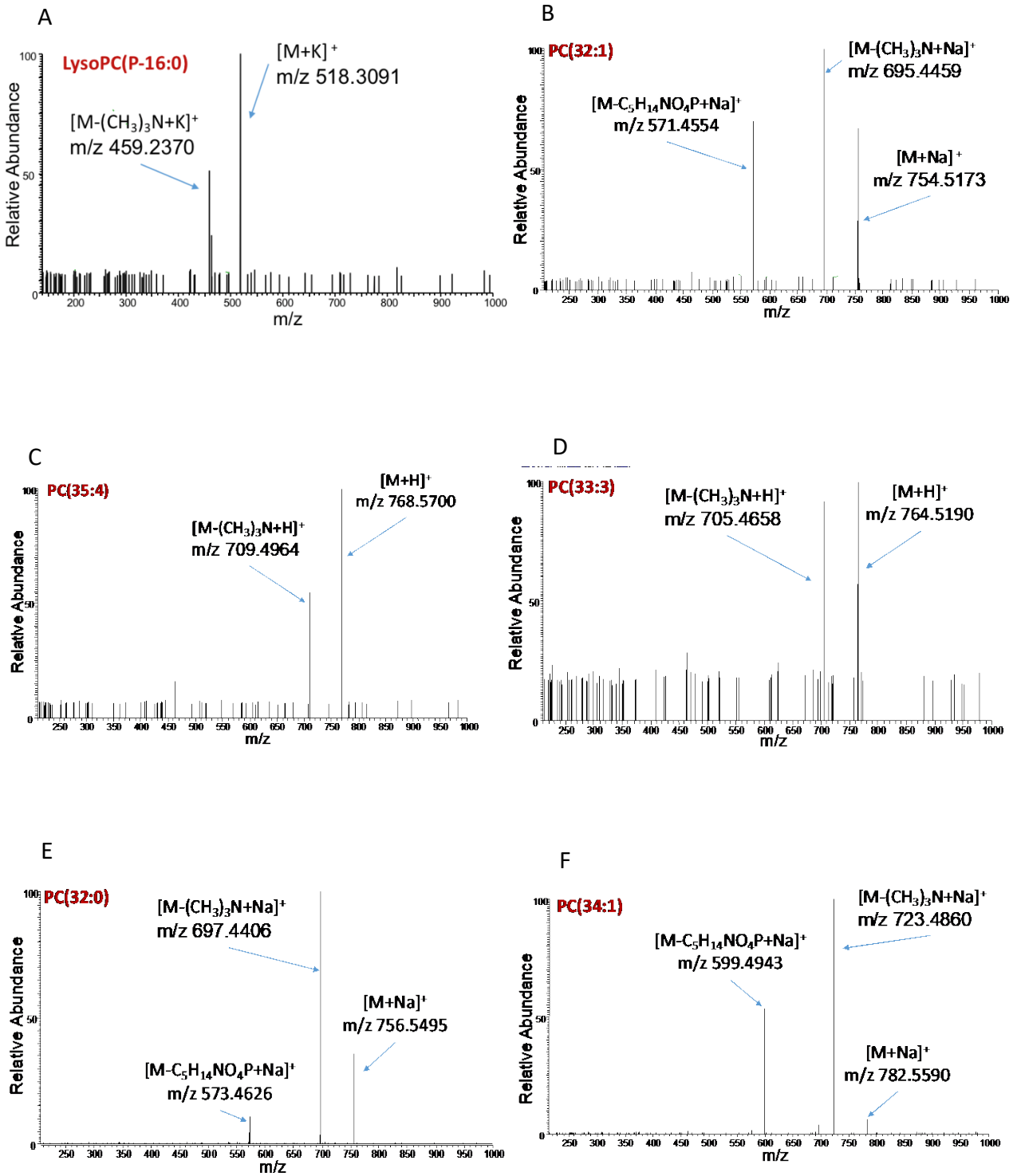
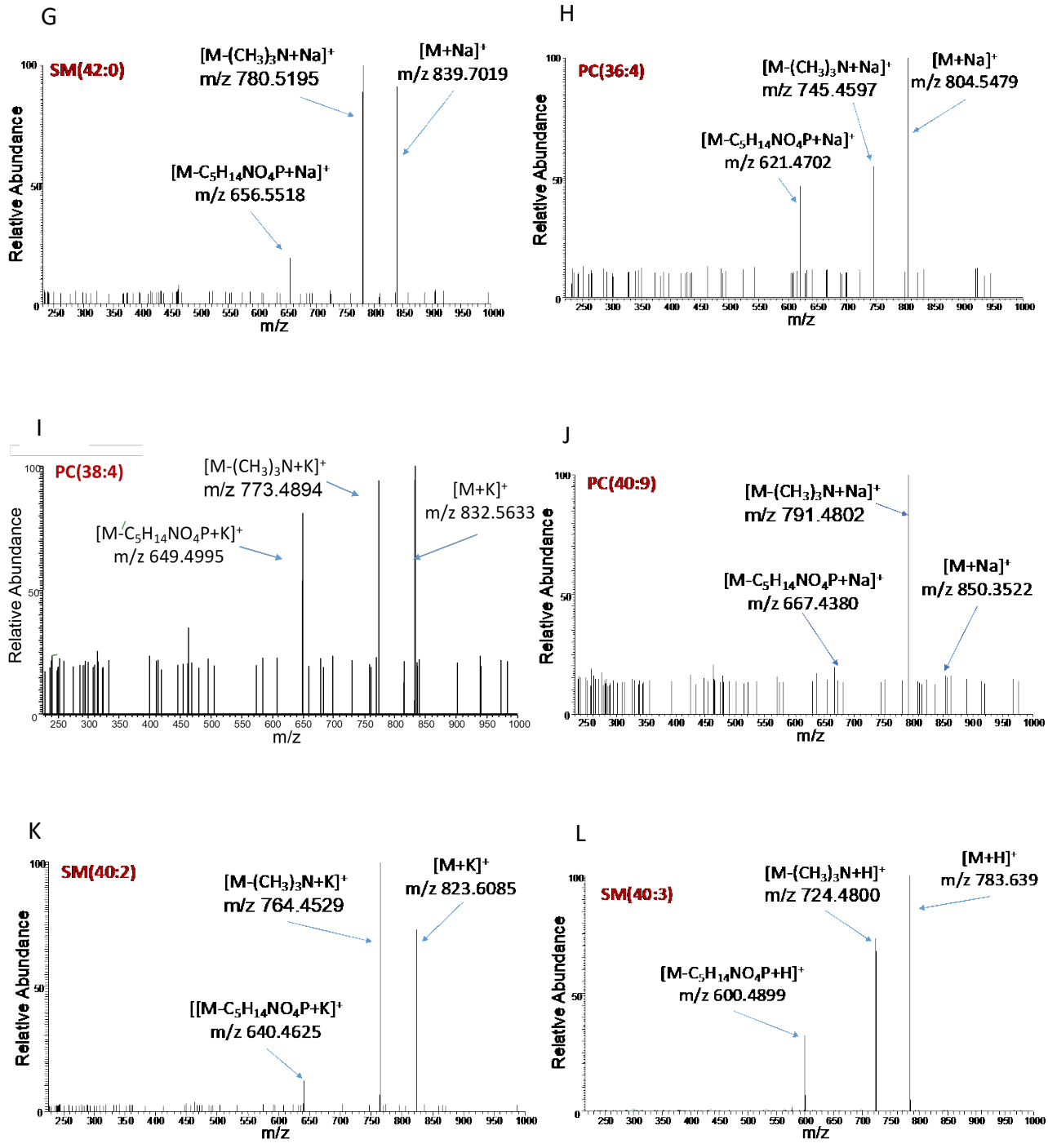
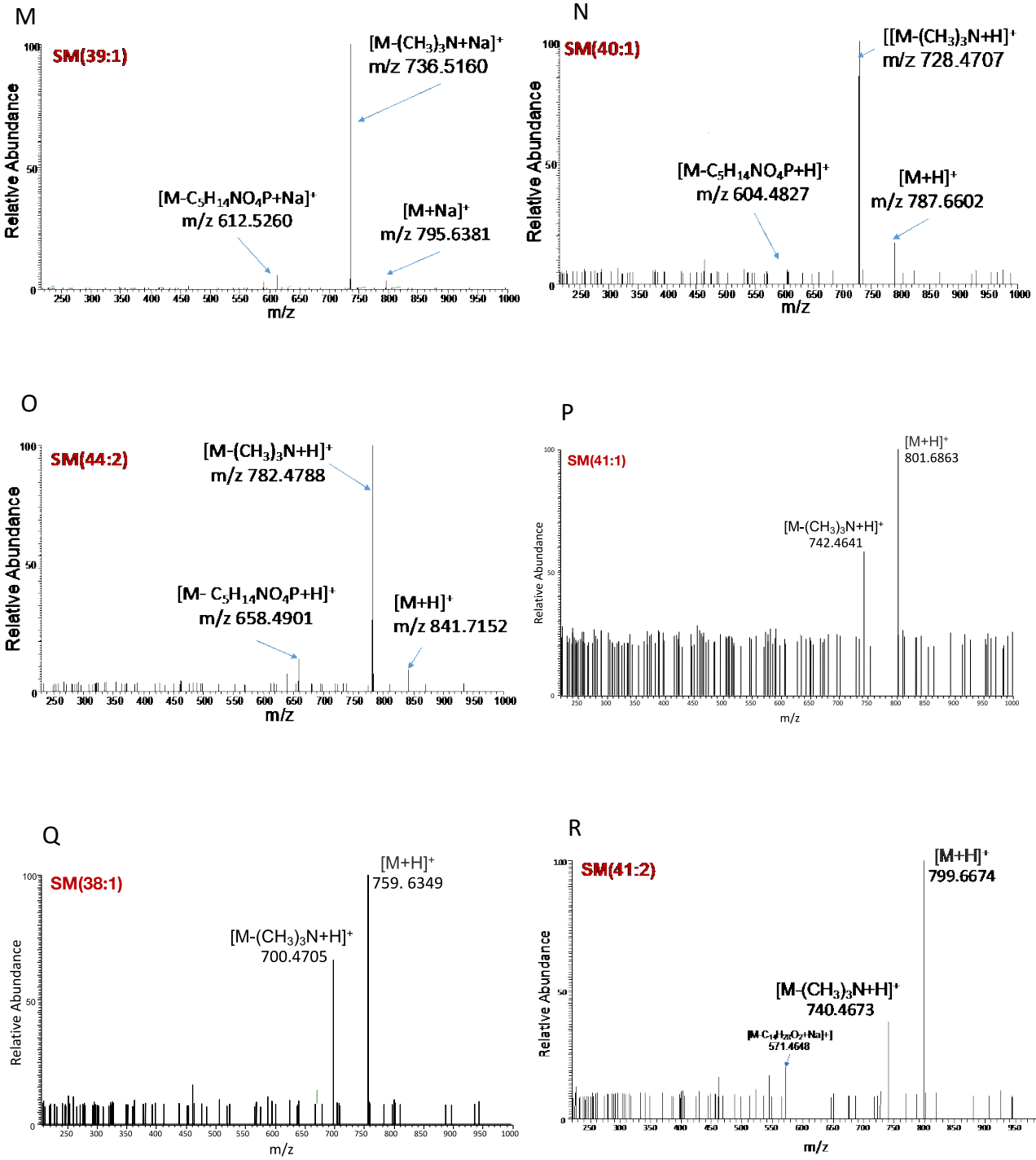


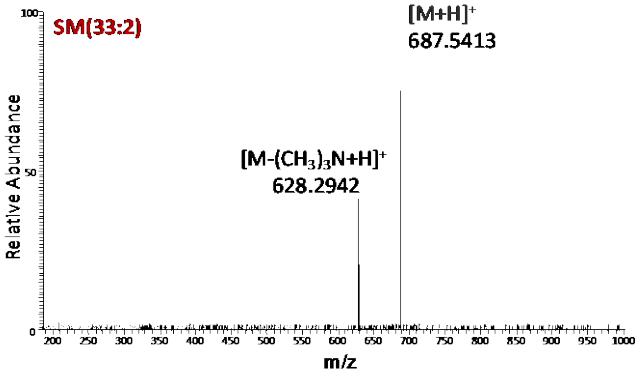
Figure S2. (A) Cell viability measurement of irinotecan (IRI)-resistant cells treated by metformin (MET). (B) Cell viability measurement of IRI-resistant cells treated by IRI. (C) Histogram of combinational index values assessing the degree of synergism: very strong (CI < 0.1), strong (0.1 < CI < 0.3), and synergism (0.3 < CI <0.7).


A
LysoPC(P-16:0)
[M+K]+
m/z 518.3091
[M-(CH3)3N+K]+
m/z 459.2370
Relative Abundance
m/z
B
PC(32:1)
[M-(CH3)3N+Na]+
m/z 695.4459
[M-C5H14NO4P+Na]+
m/z 571.4554
[M+Na]+
m/z 754.5173
Relative Abundance
m/z
C
PC(35:4)
[M+H]+
m/z 768.5700
[M-(CH3)3N+H]+
m/z 709.4964
Relative Abundance
m/z
D
PC(33:3)
[M-(CH3)3N+H]+
m/z 705.4658
[M+H]+
m/z 764.5190
Relative Abundance
m/z
E
PC(32:0)
[M-(CH3)3N+Na]+
m/z 697.4406
[M+Na]+
m/z 756.5495
[M-C5H14NO4P+Na]+
m/z 573.4626
Relative Abundance
m/z
F
PC(34:1)
[M-(CH3)3N+Na]+
m/z 723.4860
[M-C5H14NO4P+Na]+
m/z 599.4943
[M+Na]+
m/z 782.5590
Relative Abundance
m/z

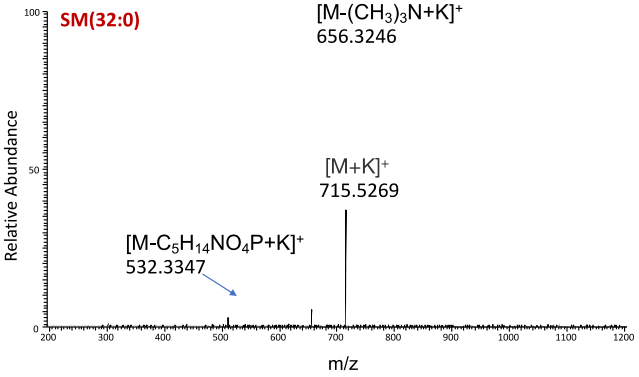

G
SM(42:0)
[M-(CH3)3N+Na]+
m/z 780.5195
[M+Na]+
m/z 839.7019
[M-C5H14NO4P+Na]+
m/z 656.5518
H
PC(36:4)
[M-(CH3)3N+Na]+
m/z 745.4597
[M+Na]+
m/z 804.5479
[M-C5H14NO4P+Na]+
m/z 621.4702
I
PC(38:4)
[M-(CH3)3N+K]+
m/z 773.4894
[M+K]+
m/z 832.5633
[M-C5H14NO4P+K]+
m/z 649.4995
J
PC(40:9)
[M-(CH3)3N+Na]+
m/z 791.4802
[M+Na]+
m/z 850.3522
[M-C5H14NO4P+Na]+
m/z 667.4380
K
SM(40:2)
[M-(CH3)3N+K]+
m/z 764.4529
[M+K]+
m/z 823.6085
[[M-C5H14NO4P+K]+
m/z 640.4625
L
SM(40:3)
[M-(CH3)3N+H]+
m/z 724.4800
[M+H]+
m/z 783.639
[M-C5H14NO4P+H]+
m/z 600.4899
Relative Abundance
m/z


M
SM(39:1)
[M-(CH3)3N+Na]+
m/z 736.5160
[M-C5H14NO4P+Na]+
m/z 612.5260
[M+Na]+
m/z 795.6381
Relative Abundance
m/z
N
SM(40:1)
[[M-(CH3)3N+H]+
m/z 728.4707
[M-C5H14NO4P+H]+
m/z 604.4827
[M+H]+
m/z 787.6602
Relative Abundance
m/z
O
SM(44:2)
[M-(CH3)3N+H]+
m/z 782.4788
[M- C5H14NO4P+H]+
m/z 658.4901
[M+H]+
m/z 841.7152
Relative Abundance
m/z
P
SM(41:1)
[M+H]+
801.6863
[M-(CH3)3N+H]+
742.4641
Relative Abundance
m/z
Q
SM(38:1)
[M+H]+
759. 6349
[M-(CH3)3N+H]+
700.4705
Relative Abundance
m/z
R
SM(41:2)
[M+H]+
799.6674
[M-(CH3)3N+H]+
740.4673
[M-C14H28O2+Na]+
571.4648
Relative Abundance
m/z

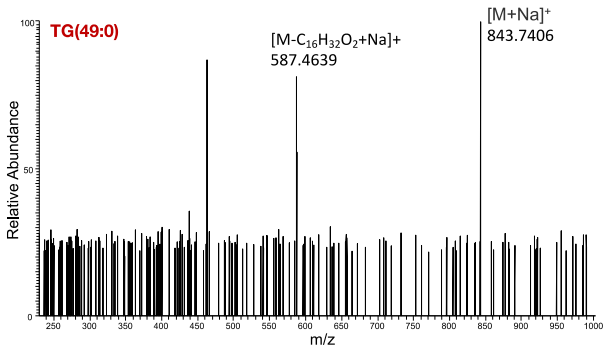
S


SM(33:2)
[M+H]+
687.5413
[M-(CH3)3N+H]+
628.2942
Relative Abundance
m/z

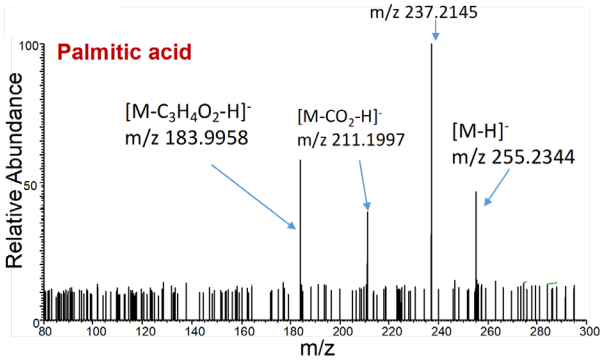
T


SM(32:0)
[M-(CH3)3N+K]+
656.3246
[M+K]+
715.5269
[M-C5H14NO4P+K]+
532.3347
Relative Abundance
m/z

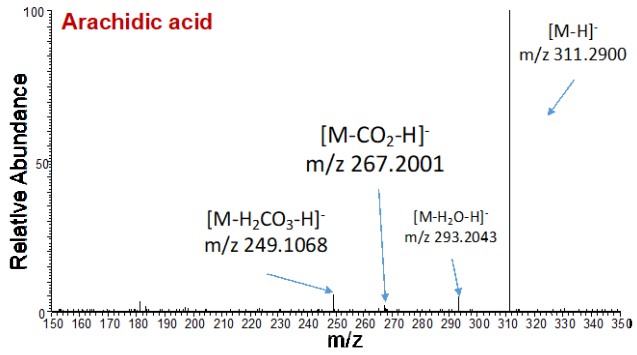
U


TG(49:0)
[M-C16H32O2+Na]+
587.4639
[M+Na]+
843.7406
Relative Abundance
m/z

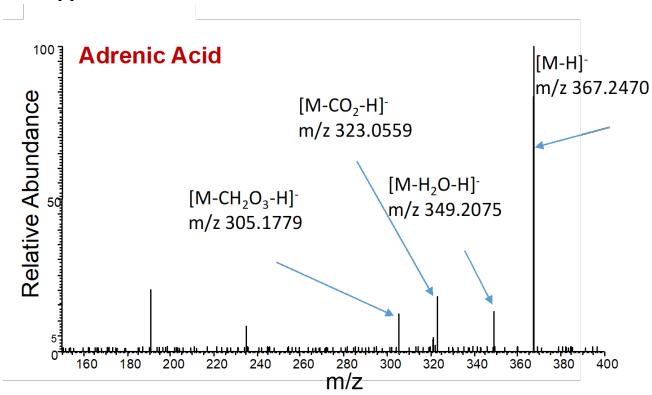
V


Palmitic acid
[M-H2O-H]-
m/z 237.2145
[M-C3H4O2-H]-
m/z 183.9958
[M-CO2-H]-
m/z 211.1997
[M-H]-
m/z 255.2344
Relative Abundance
m/z

W


Arachidic acid
[M-H]-
m/z 311.2900
[M-CO2-H]-
m/z 267.2001
[M-H2CO3-H]-
m/z 249.1068
[M-H2O-H]-
m/z 293.2043
Relative Abundance
m/z

X


Adrenic Acid
[M-H]-
m/z 367.2470
[M-CO2-H]-
m/z 323.0559
[M-CH2O3-H]-
m/z 305.1779
[M-H2O-H]-
m/z 349.2075
Relative Abundance
m/z

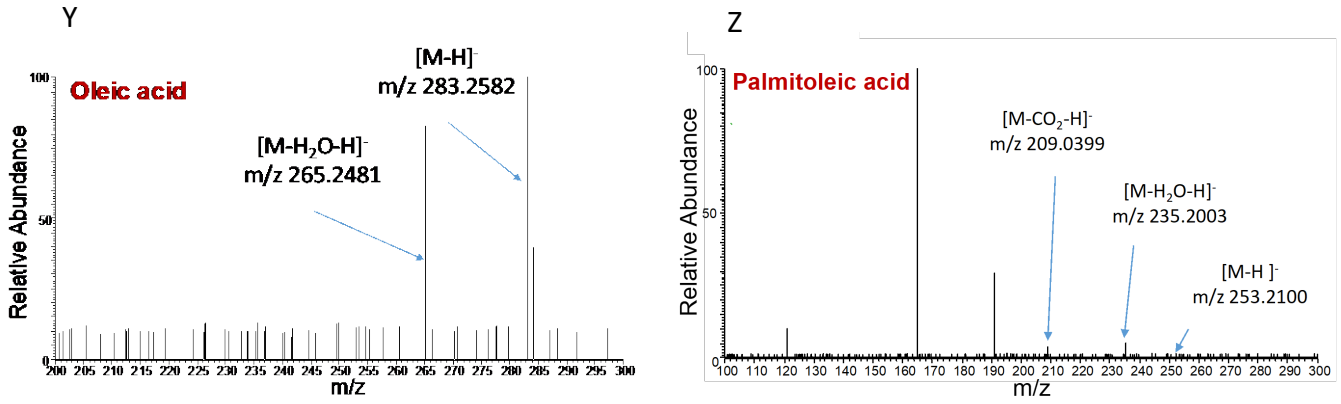
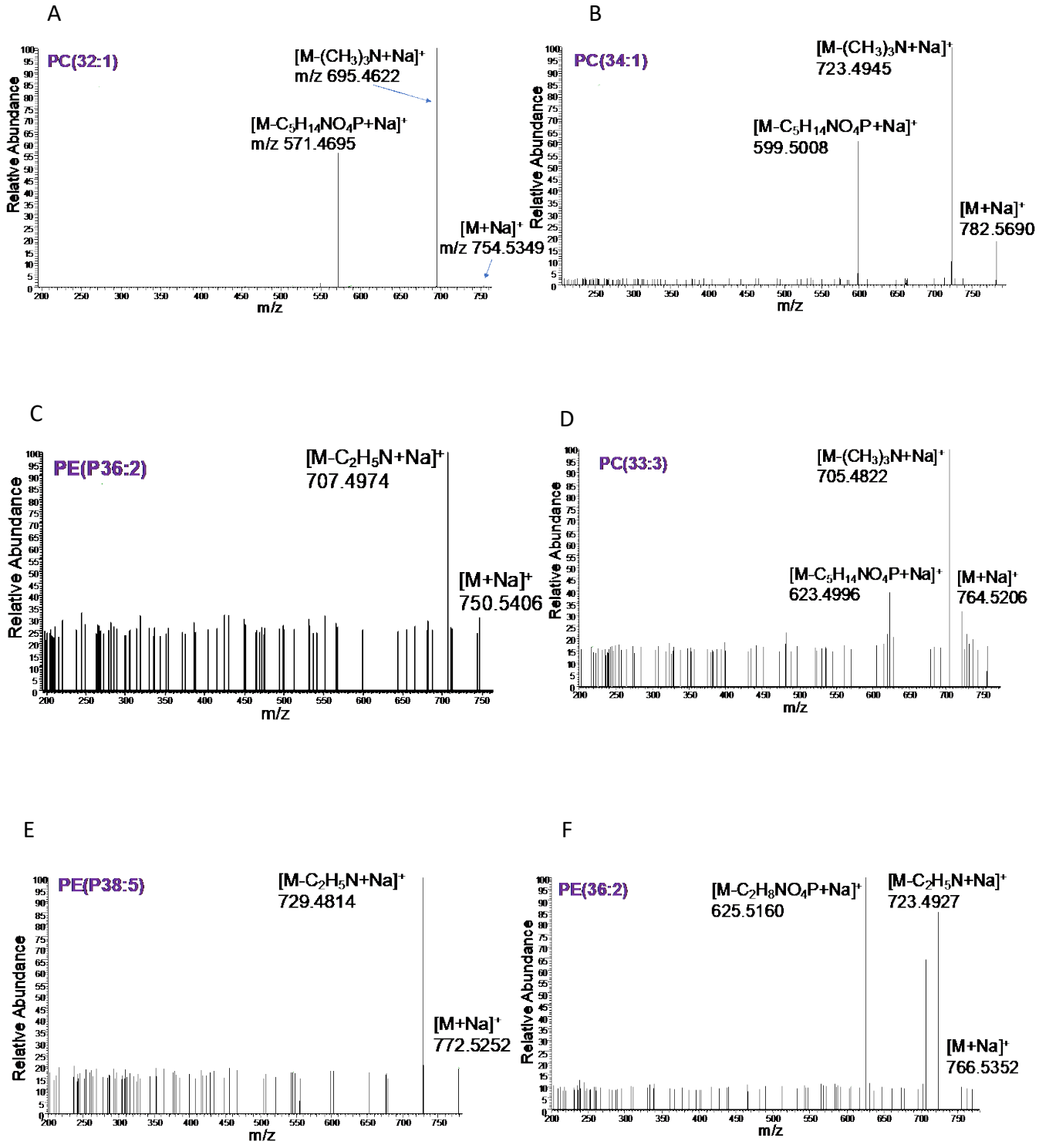
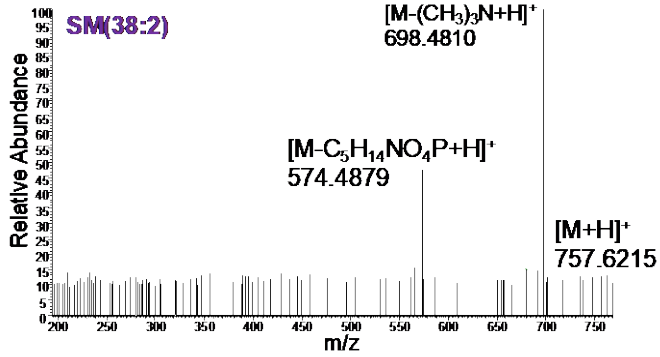


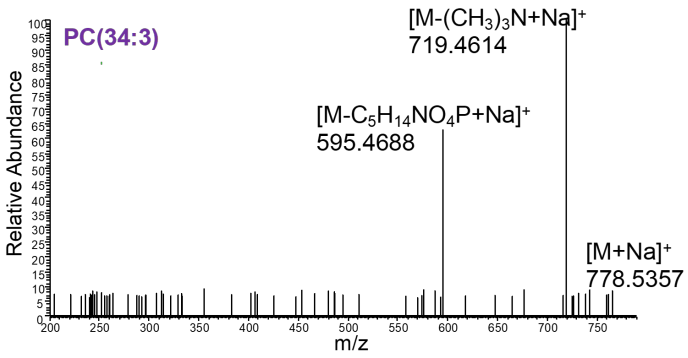
Figure S3. Online MS^2 identification of metabolites in single IRI-resistant cells using the Single-probe SCMS technique. (A) LysoPC(P-16:0), (B) PC(31:1), (C) PC(35:4), (D) PC(33:3), (E) PC(32:0), (F) PC(34:1), (G) SM(42:0), (H) PC(36:4), (I) PC(38:4), (J) PC(40:9), (K) SM(40:2), (L) SM(40:3), (M) SM(39:1), (N) SM(40:1), (O) SM(44:2), (P) SM(41:1) (Q) SM(38:1) (R) SM(41:2) (S) SM(33:2) (T) SM(32:0) (U) TG(49:0) (V) Palmitic acid, (W) Arachidic acid, (X) Adrenic acid, (Y) Oleic acid, and (Z) Palmitoleic acid. (LysoPC: lysophosphatidylcholine; PC: phosphatidylcholine; SM: sphingomyelin; TG: triglyceride).


A
PC(32:1)
[M-(CH3)3N+Na]+
m/z 695.4622
[M-C5H14NO4P+Na]+
m/z 571.4695
[M+Na]+
m/z 754.5349
Relative Abundance
m/z
B
PC(34:1)
[M-(CH3)3N+Na]+
723.4945
[M-C5H14NO4P+Na]+
599.5008
[M+Na]+
782.5690
Relative Abundance
m/z
C
PE(P36:2)
[M-C2H5N+Na]+
707.4974
[M+Na]+
750.5406
Relative Abundance
m/z
D
PC(33:3)
[M-(CH3)3N+Na]+
705.4822
[M-C5H14NO4P+Na]+
623.4996
[M+Na]+
764.5206
Relative Abundance
m/z
E
PE(P38:5)
[M-C2H5N+Na]+
729.4814
[M+Na]+
772.5252
Relative Abundance
m/z
F
PE(36:2)
[M-C2H8NO4P+Na]+
625.5160
[M-C2H5N+Na]+
723.4927
[M+Na]+
766.5352
Relative Abundance
m/z

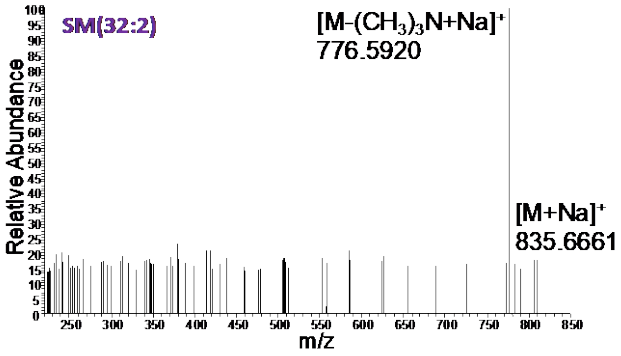
G


SM(38:2)
[M-(CH3)3N+H]+
698.4810
[M-C5H14NO4P+H]+
574.4879
[M+H]+
757.6215
Relative Abundance
m/z

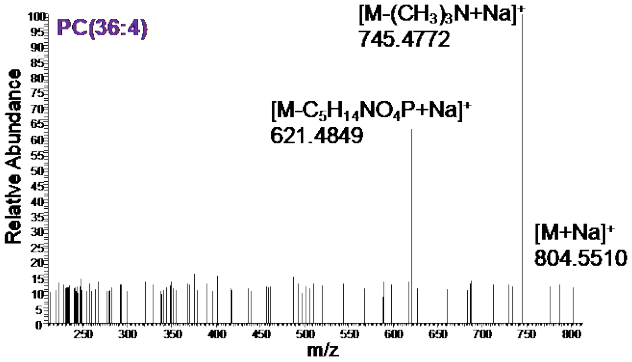
H


PC(34:3)
[M-(CH3)3N+Na]+
719.4614
[M-C5H14NO4P+Na]+
595.4688
[M+Na]+
778.5357
Relative Abundance
m/z

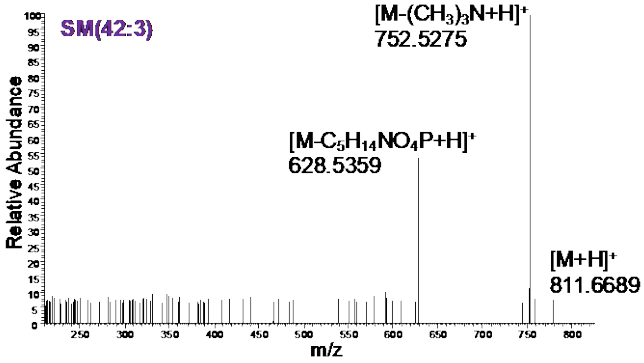
I


SM(32:2)
[M-(CH3)3N+Na]+
776.5920
[M+Na]+
835.6661
Relative Abundance
m/z

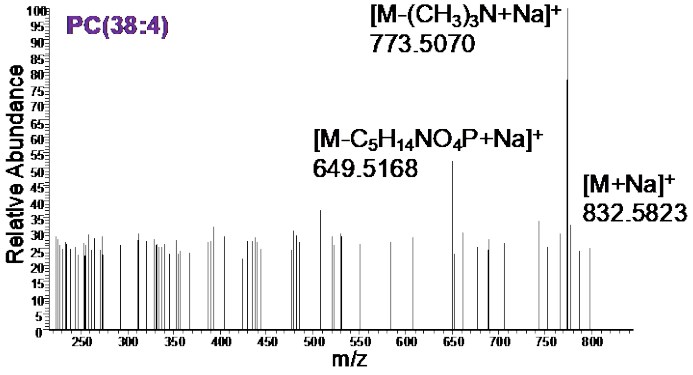
J


PC(36:4)
[M-(CH3)3N+Na]+
745.4772
[M-C5H14NO4P+Na]+
621.4849
[M+Na]+
804.5510
Relative Abundance
m/z

K


SM(42:3)
[M-(CH3)3N+H]+
752.5275
[M-C5H14NO4P+H]+
628.5359
[M+H]+
811.6689
Relative Abundance
m/z

L


PC(38:4)
[M-(CH3)3N+Na]+
773.5070
[M-C5H14NO4P+Na]+
649.5168
[M+Na]+
832.5823
Relative Abundance
m/z

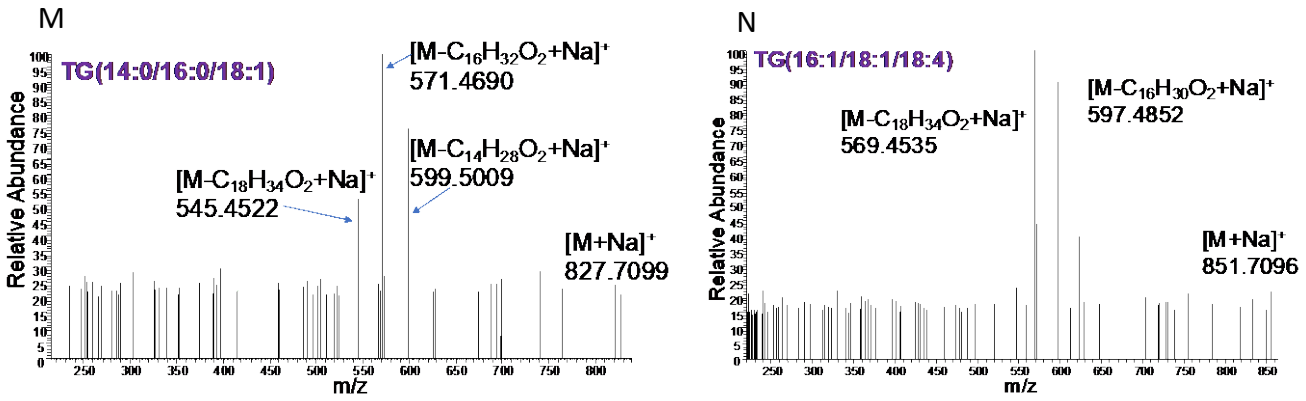


Figure S4. MS^2 identification of ions of interest from cell lysate using HPLC-MS/MS. These ions include (A) PC(32:1), (B) PC(34:1), (C) PE(P36:2), (D) PC(33:3), (E) PE(P38:5), (F) PE(36:2), (G) SM(38:2), (H) PC(34:3), (I) SM(32:2), (J) PC(36:4), (K) SM(42:3), (L) PC(38:4), (M) TG(14:0/16:0/18:1), and (N) TG(16:1/18:1/18:4). (PC: phosphatidylcholine; PE: Phosphatidylethanolamine; SM: sphingomyelin; TG: triglyceride).

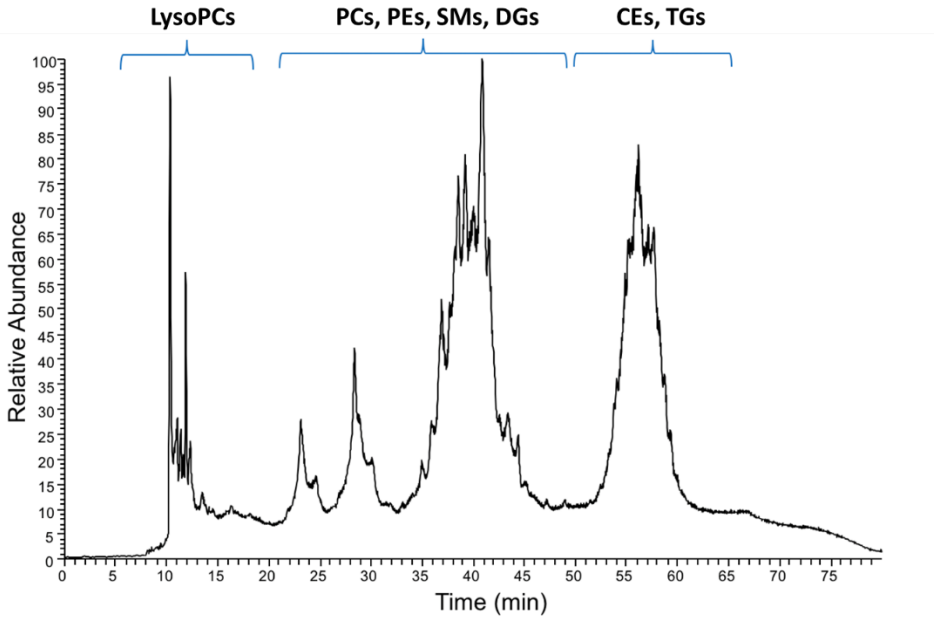


Figure S5. Chromatogram of cell lysate in HPLC-MS analysis. (LysoPC: lysophosphatidylcholine; PC: phosphatidylcholine; PE: Phosphatidylethanolamine; SM: sphingomyelin; CE: cholesterol ester; DG: diglyceride; TG: triglyceride).