## ***Supporting Information* for:**

# *In vitro* Liquid Extraction Surface Analysis Mass Spectrometry (ivLESA MS) for Direct Metabolic Analysis of Adherent Cells in Culture

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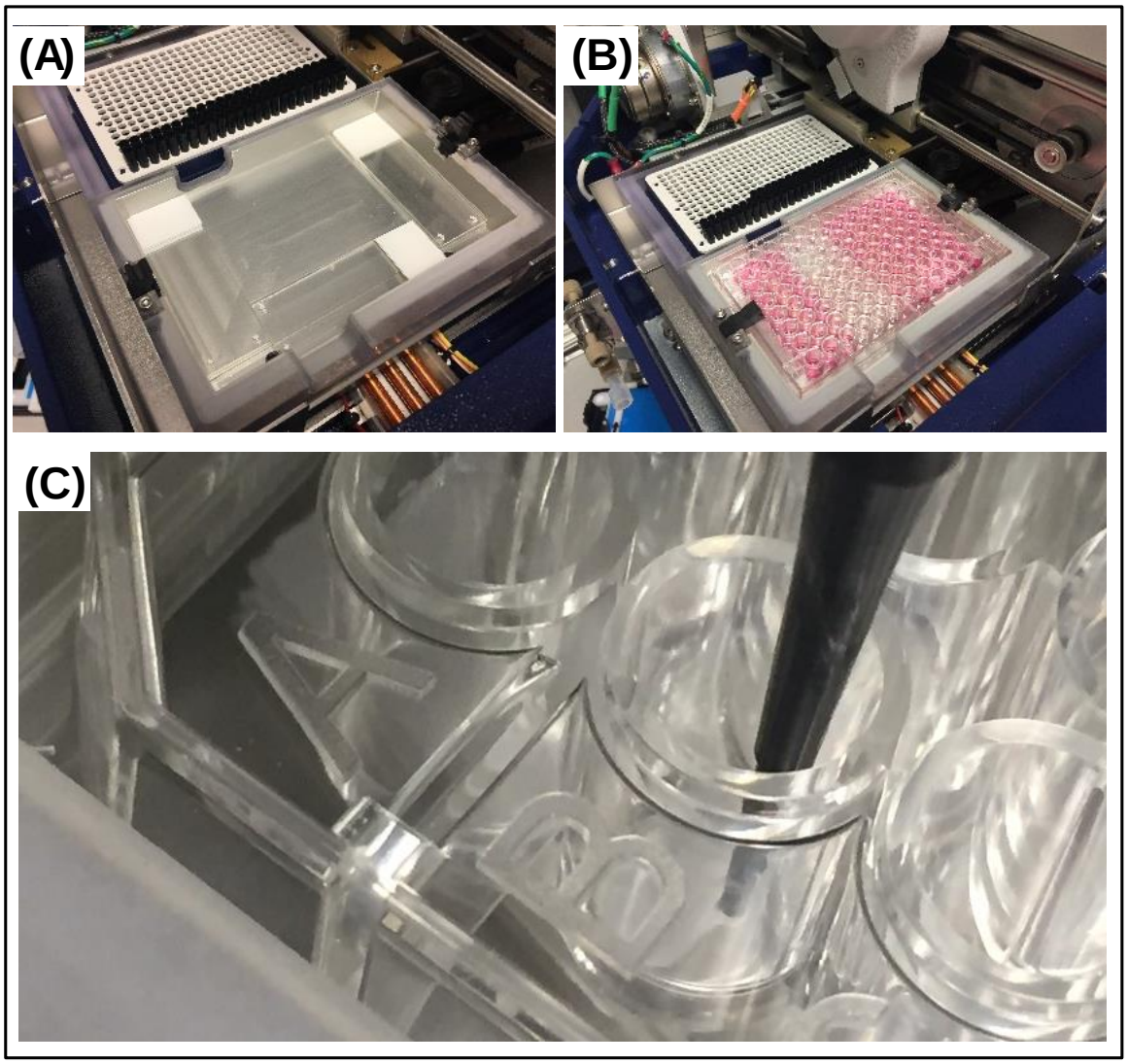
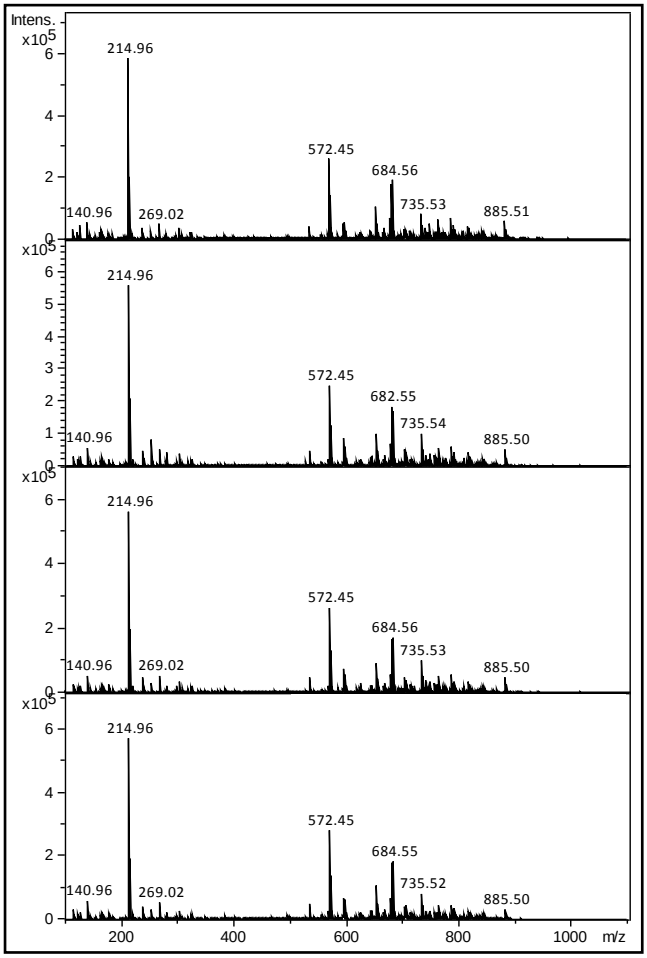



**Supplemental Figure 1.** *In vitro* liquid extraction surface analysis (ivLESA) set up for direct analysis of cells in culture. (A) Three stacked glass slides serve as a spacer used to elevate cell culture plate. (B) Photo showing 96-well cell culture plate in LESA tray holder. (C) Close up image of pipet tip aspirating from cell surface.

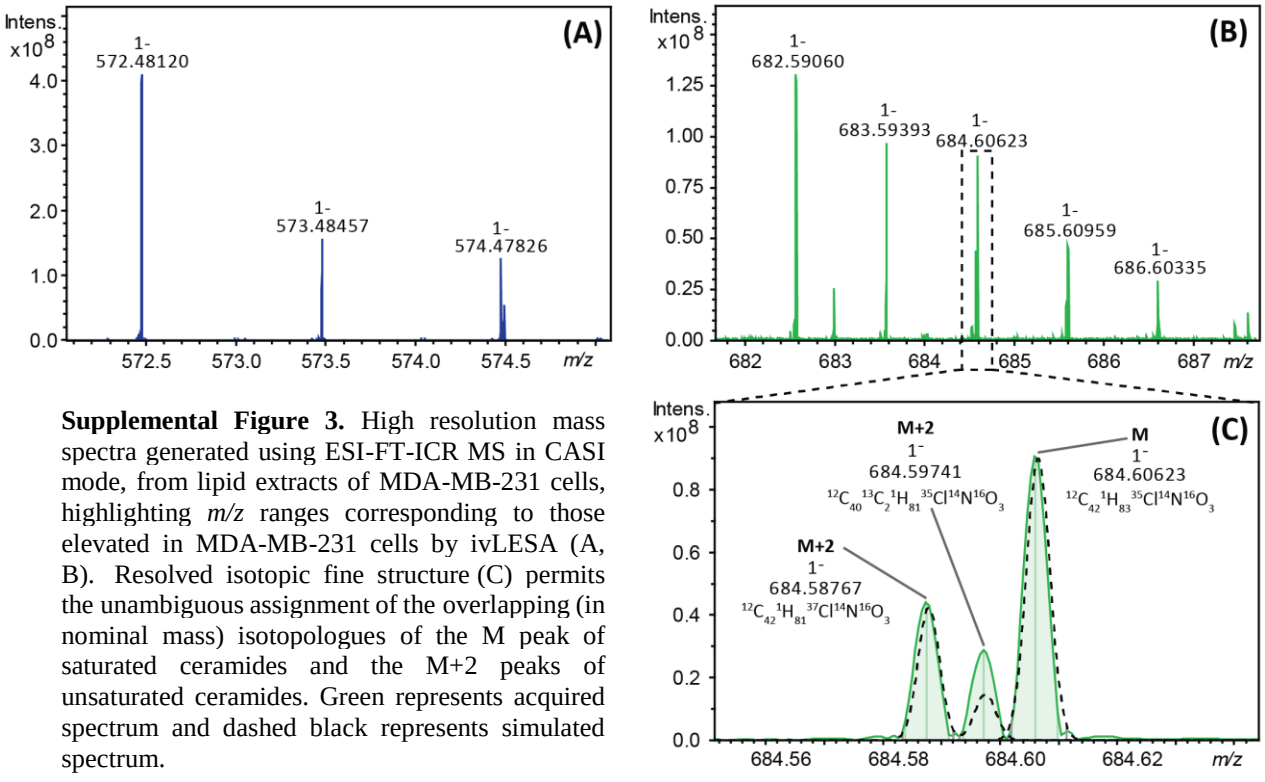



**Supplemental Figure 2.** ivLESA mass spectra generated from MDA-MB-231 cells in negative electrospray mode, taken from four different wells demonstrating reproducibility of method.

## Ceramide confirmation using high resolution mass spectrometry (HRMS)

***Lipid extraction.*** Lipids extracts from MDA-MB-231 cells were generated using a modified Folch extraction method[1]. Briefly, cells from 6-well cell culture plates were washed 2X with PBS, harvested by scraping, pelleted (1000*g*, 2 min), resuspended in 0.3 mL MeOH and vortexed with 0.6 mL $CHCl_3$ for 10 min. Phase separation was achieved using 0.25 mL $H_2O$, followed by vortexing, incubation at RT for 10 min and centrifugation (1000*g*, 2 min). Following centrifugation, the organic phase was carefully collected and diluted ten-fold in 2:1 $CHCl_3$:MeOH.

***ESI-FT-ICR MS analysis.*** Lipid extracts were introduced into the mass spectrometer at an infusion rate of 2 µL/min, and ionized at atmospheric pressure using electrospray ionization (ESI) in negative ion mode. Mass spectra were acquired using a 9.4 Tesla SolariX XR FT-ICR (Bruker Daltonics, Billerica, MA), using ramped RF-excitation and a 4 MW dataset. After confirming an MS lipid profile similar to that seen by ivLESA MS, HRMS measurements were performed in continuous accumulation of selected ions (CASI) mode at 572.5 m/z and 683.0 m/z with a 20 m/z window to obtain high mass accuracy of ions contained within the mass ranges of interest.




**Supplemental Figure 3.** High resolution mass spectra generated using ESI-FT-ICR MS in CASI mode, from lipid extracts of MDA-MB-231 cells, highlighting *m/z* ranges corresponding to those elevated in MDA-MB-231 cells by ivLESA (A, B). Resolved isotopic fine structure (C) permits the unambiguous assignment of the overlapping (in nominal mass) isotopologues of the M peak of saturated ceramides and the M+2 peaks of unsaturated ceramides. Green represents acquired spectrum and dashed black represents simulated spectrum.

| $m/z_{meas}$ | $m/z_{calc}$ | Molecular Formula | Adduct | Molecular assignment | Delta (ppm) |
|---|---|---|---|---|---|
| 572.48120 | 572.48150 | $C_{34}H_{67}NO_3$ | $M+Cl^-$ | Ceramide (d18:1/16:0) | 0.5 |
| 682.59060 | 682.59105 | $C_{42}H_{81}NO_3$ | $M+Cl^-$ | Cer(d18:1/24:1(15z)) | 0.7 |
| 684.60623 | 684.60670 | $C_{42}H_{83}NO_3$ | $M+Cl^-$ | Ceramide (d18:1/24:0) | 0.7 |
| 684.60623 | 684.60670 | $C_{42}H_{83}NO_3$ | $M+Cl^-$ | Cer(d18:0/24:1(15z)) | 0.7 |

**Supplemental Table 1.** Molecular formulas and lipid identification was performed by querying the Human Metabolome Database[2] for ions with *m/z* values of 572.4812, 682.5906 and 684.6062, with each query having a mass tolerance of ± 1 ppm. Each query returned only chloride adducts of ceramide molecules within the mass range specified (synonymous molecules have been omitted). In the case of 684.6062, two different possible ceramide species were returned, though physiologically, ceramide (d18:1/24:0) is more likely.

**References**

(1) FOLCH, J.; LEES, M.; SLOANE STANLEY, G. H. *J. Biol. Chem.* **1957**, *226* (1), 497–509.

(2) Wishart, D. S.; Tzur, D.; Knox, C.; Eisner, R.; Guo, A. C.; Young, N.; Cheng, D.; Jewell, K.; Arndt, D.; Sawhney, S.; Fung, C.; Nikolai, L.; Lewis, M.; Coutouly, M. A.; Forsythe, I.; Tang, P.; Shrivastava, S.; Jeroncic, K.; Stothard, P.; Amegbey, G.; Block, D.; Hau, D. D.; Wagner, J.; Miniaci, J.; Clements, M.; Gebremedhin, M.; Guo, N.; Zhang, Y.; Duggan, G. E.; MacInnis, G. D.; Weljie, A. M.; Dowlatabadi, R.; Bamforth, F.; Clive, D.; Greiner, R.; Li, L.; Marrie, T.; Sykes, B. D.; Vogel, H. J.; Querengesser, L. *Nucleic Acids Res.* **2007**, *35* (SUPPL. 1).