ONLINE METHODS

Sample Preparation for Lipid Standards

Lipid standards were purchased from Avanti Polar Lipids, Inc., and Matreya LLC and a list is included in the supplementary information section. A final concentration of 1 μM was desired for the LC-IMS-MS studies. The standards were diluted with chloroform to reach the desired concentration.

Sample Preparation for Mouse Tissue and Human Cell Lines

Mouse Tissue Collection and Extraction. Adult CD-1 mice were purchased from Charles River Laboratory (Raleigh, NC). Females were mated with fertile males of the same strain to induce pregnancy. Mice were euthanized between 0900 and 1000 h on day 8 of pregnancy (0 h on day 1 = vaginal plug). Day 8 implantation sites were microdissected to isolate the top and bottom hemispheres for subsequent lipid extraction. Due to the high sensitivity of our mass spectrometry platforms each dataset represents either a top or bottom hemisphere from a single implantation site. In total 12 samples were analyzed; top and bottom hemispheres from 6 implantation sites collected from two mice (3 implantation sites from each mouse).

Lipids were extracted from the tissue using a Folch extraction [1]. Briefly, tissue was lysed in 400 μl methanol using a tissue lyser with a 3 mm tungsten carbide bead for 3 min. Sample was transferred into a vial and 800 μl of chloroform was added. The sample was vortexed for 60 sec then shaken for 1hr at room temperature at 1000 rpm. The samples were then vortexed briefly and 300 μl of water was added to induce a bi-phase separation. The sample was gently mixed, incubated at room temperature for 10 min and then centrifuged for 5 min at 15,000 x g at 4°C. The lower organic layer was collected and transferred into another vial. The remaining

sample was washed using a blank lower organic layer to collect remaining lipids. The washed samples were vortexed for 5 sec, incubated at room temperature for 10 min and then centrifuged as stated above. The lower organic layer of the washed sample was added to the first lower organic layer and then dried *in vacuo*. 150 µl of 2:1 chloroform/methanol was added and the samples were stored at -20°C until analysis.

Human Cell Line Collection and Extraction. The cells were extracted with the same protocol as that used for the mouse tissue. The human hepatoma cell line (Huh7.5.1) is derived from Huh7 cells [2, 3] and cultured as described in [4]. Powdered extract (product no. 345066, lot no. 286061) of the seeds (achenes) of *Silybum marianum* [L.] Gaertn. was obtained from Euromed, S.A. (Barcelona, Spain), which is a part of the Madaus Group (Cologne, Germany). To eliminate stability concerns with freeze–thawing solutions of silymarin and the hygroscopic nature of DMSO, single-use aliquots of silymarin were prepared as follows. The extract was reconstituted to a concentration of 10 mM in MeOH (based on a molecular weight of 482 g/mol for the seven main flavonolignan diastereoisomers). Then, 100 µL of this solution was dispensed into 0.7 mL miniature centrifuge tubes and allowed to dry overnight; this imparts 0.482 mg of silymarin per tube. The dried aliquots were stored at −20 °C. For each experiment, aliquots of silymarin were reconstituted in 40 µL of DMSO and extensively vortexed to generate a 25 mM stock solution. For all experiments, freshly prepared silymarin stock solutions were used once and then discarded. The Huh7.5.1 cell line was treated with a non-toxic 80 µM dose of silymarin and analyzed 24 hr after treatment to understand its effect.

LC-MS/MS and LC-IMS-MS Instrumental Analyses

Analyses of all samples in this manuscript were performed using a Waters NanoAquity UPLC system interfaced with either the 6560 IMS-MS instrument (Agilent, Santa Clara, CA) [5] or the Velos-ETD Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA). Lipid extracts were dried *in vacuo*, reconstituted in 200 µL of isopropanol and analyzed by LC-MS/MS as previously described in [6] and with LC-IMS-MS. In both analyses, samples were injected (0.7 µL) onto a capillary column (26 cm x 150 µm i.d.) containing HSS T3 reversed-phase material (1.8 µm). Lipids were separated over 90-min using gradient elution (mobile phase A: acetonitrile/water (40:60) containing 10 mM ammonium acetate; mobile phase B: acetonitrile/isopropanol (10:90) containing 10 mM ammonium acetate) at a flow rate of 1 µl/min. All samples were analyzed by both LC-MS/MS and LC-IMS-MS instruments detailed below.

For the LC-MS/MS analyses, samples were analyzed in both positive and negative ionization using HCD (higher-energy collision dissociation) and CID (collision-induced dissociation). The MS data were collected from 200-2000 *m/z* at a resolution of 60,000 (automatic gain control (AGC) target: $1x10^6$) followed by data dependent ion trap MS/MS spectra (AGC target: $1x10^4$) of the 6 most abundant ions using a normalized collisional energy setting of 35 for CID and 30 for HCD. A dynamic exclusion time of 60 sec was used to discriminate against previously analyzed ions. The raw data files were imported into the in-house developed software LIQUID (Lipid Informed Quantitation and Identification) for semi-automated identification of lipid molecular species (software now commercially available through Protein Metrics, Inc as Lipify™). The identified lipids were then used to populate an Accurate Mass and Time (AMT) tag database [7] and the MS and MS/MS spectra were utilized for quantitation.

The LC-IMS-MS analyses were also performed in both positive and negative ionization and collected from 100-3200 *m/z* at a resolution of 40,000. The LC-IMS-MS data were analyzed using the in-house PNNL software for deisotoping and feature finding the multidimensional data [8]. The features were then compared to the AMT tag database generated from LC-MS/MS for identification and quantitation.

References

1. Folch, J., M. Lees, and G.H.S. Stanley, *A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues.* Journal of Biological Chemistry, 1957. **226**(1): p. 497-509.
2. Zhong, J., et al., *Robust hepatitis C virus infection in vitro.* Proc Natl Acad Sci U S A, 2005. **102**(26): p. 9294-9.
3. Li, K., et al., *Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-beta production in hepatocytes.* J Biol Chem, 2005. **280**(17): p. 16739-47.
4. Wagoner, J., et al., *Multiple effects of silymarin on the hepatitis C virus lifecycle.* Hepatology, 2010. **51**(6): p. 1912-21.
5. May, J.C., et al., *Conformational ordering of biomolecules in the gas phase: nitrogen collision cross sections measured on a prototype high resolution drift tube ion mobility-mass spectrometer.* Anal Chem, 2014. **86**(4): p. 2107-16.
6. Gao, X., et al., *A reversed-phase capillary ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) method for comprehensive top-down/bottom-up lipid profiling.* Anal Bioanal Chem, 2012. **402**(9): p. 2923-33.
7. Zimmer, J.S., et al., *Advances in proteomics data analysis and display using an accurate mass and time tag approach.* Mass Spectrom Rev, 2006. **25**(3): p. 450-82.
8. Crowell, K.L., et al., *LC-IMS-MS Feature Finder: detecting multidimensional liquid chromatography, ion mobility and mass spectrometry features in complex datasets.* Bioinformatics, 2013. **29**(21): p. 2804-5.