Preparation and LC/MS analysis of sEV lipids

Samples were collected and aliquots representing the same EV counts were dried under nitrogen, and resuspended in 2:2:1 acetonitrile:methanol:H_2O (v/v) (for polar metabolites, eg. fatty acids, phospholipids) or in 100% isopropanol (for less-polar metabolites, eg. ceramides). The samples were mixed on an orbital shaker (360 rpm) for 1 min at room temperature before the LC/MS analysis.

An Agilent 1290 Infinity II LC-system coupled to an Agilent 6545 QTOF mass spectrometer with a dual Agilent Jet Stream electrospray ionization source. Analysis of eramides and fatty acids was further verified in high resolution using an Orbitrap ID-X Tribrid mass spectrometer (Thermo Scientific). A Vanquish Horizon UHPLC system, was interfaced with the mass spectrometer via electrospray ionization in both positive and negative ion mode with a spray voltage of 3.5 and 2.8 kV, respectively. Lipids were separated on a CORTECS UPLC C18 column (2.1 x 100 mm, 1.6 um; part No. 186007095) including a Waters UPLC HSS VanGuard Pre-Column (2.1 x 5mm, 1.8 um; part No. 186007949) at a temperature of 60C and a flow rate of 250 mL/min. The mobile phases consisted of A: 60% acetonitrile, 40% water, 0.1% formic acid, 10 mM ammonium formate, 2.5 mM medronic acid, and B: 90% 2-propanol, 10% acetonitrile, 0.1% formic acid, 10 mM ammonium formate (in 1 mL water). The following linear gradient was used: 0-2 min, 30% B; 17 min, 75% B; 20 min, 85% B; 23-26 min, 100% B; 26 min, 30% B followed by a re-equilibration phase of 5 min. Lipids were detected in positive ion mode with following source parameters: gas temperature 250C, drying gas flow 11 L/min, nebulizer pressure 35 psi, sheath gas temperature 300C, sheath gas flow 12 L/min, VCap 3000 V, nozzle voltage 500 V, Fragmentor 160 V, Skimmer 65 V, Oct 1 RF Vpp 750 V,

and m/z range 50-1700. Data were acquired under continuous reference mass correction at m/z 121.0509 and 922.0890 in positive ion mode. Samples were randomized before analysis and a quality-control (QC) sample was injected to monitor instrument signal stability.