

Shotgun lipidomics. Livers were isolated from PBS-perfused mice, and 10 μ g was homogenized in ddH₂O using a Precellys homogenizer (PeeqLab Biotechnology). For lipid extraction, 50 μ l of the homogenate was added to 500 μ l of extraction mix (CHCl₃/MeOH 1/5 containing internal standards: 210 pmol PE(31:1), 396 pmol PC(31:1), 98 pmol PS(31:1), 84 pmol PI(34:0), 56 pmol PA(31:1), 51 pmol PG (28:0), 28 pmol CL(56:0), 39 pmol LPA (17:0), 35 pmol LPC(17:1), 38 pmol LPE (17:0), 32 pmol Cer(17:0), 99 pmol SM(17:0), 55 pmol GlcCer(12:0), 14 pmol GM3 (18:0-D3), 359 pmol TG(47:1), 111 pmol CE(17:1), 64 pmol DG(31:1), 103 pmol MG(17:1), 724 pmol Chol(d6), 45 pmol Car(15:0)) were added. The sample was sonicated for 2 min and centrifuged at 20 000 g for 2 min. The supernatant was collected into a new tube, and 200 μ l chloroform and 800 μ l 1% AcOH water were added. The sample was shaken and centrifuged for 2 min at 20 000 g. The upper aqueous phase was removed, and the lower phase was transferred into a new tube and evaporated in a speed vac (45°C, 10 min). Spray buffer (500 μ l of 8/5/1 2-propanol/MeOH/water, 10 mM ammonium acetate) was added to the sample and sonicated for 5 min, infused at 10 μ l/min into a Thermo Q Exactive Plus spectrometer equipped with the HESI II ion source for shotgun lipidomics. MS1 spectra (resolution 280 000) were recorded in 100 m/z windows from 250 to 1200 m/z (pos.) and 200 - 1700 m/z (neg.) followed by recording MS/MS spectra (res. 70 000) by data-independent acquisition in 1 m/z windows from 200 to 1200 (pos.) and 200 to 1700 (neg.) m/z. Raw files were converted to .mzml files and imported into and analyzed by LipidXplorer software using custom mfql files to identify sample lipids and internal standards. For further data processing, absolute amounts were calculated using the internal standard intensities followed by the calculated mol% of the identified lipids.