Materials for LC-MS/MS and LC-HRMS

The following standards and their deuterated derivatives were obtained from Cayman Chemicals (Ann Arbor, MI, USA): arachidonic acid, arachidonic acid-d8, linoleic acid, linoleic acid-d4, eicosapentaenoic acid, eicosapentaenoic acid-d5, docosahexaenoic acid, docosahexaenoic acid-d5, hydroxydocosahexaenoic acid (14S-HDHA, 16-HDHA, 17S/R-HDHA), LTB4-d4, LTB4, 12epi-LTB4, hydroxyeicosatetraenoic acids (5(S)-HETE-d8, 5(S)-HETE, 12(S)-HETE, 12-HETE-d8, 15(S)-HETE, 15-HETE-d8, 20-HETE-d6 and 20(S)-HETE), 18-HEPE. Internal standards for the lipidomics screening were purchased from Avanti Polar Lipids: LPI 16:0, LPI 17:1, LPI 18:0, CE 18:1-d7, Cer d18:1/16:0-d7, cholesterol-d7, DG 15:0/18:1-d7, LacCer d18:1/17:0, LPC 18:1-d7, LPC O-16:0-d4, LPE 18:1-d7, LPG 17:1, LPI 17:1, PC 15:0/18:1-d7, PC O-18:0/18:1-d9, PE 15:0/18:1-d7, PE O-18:0/18:1-d9*, PG 15:0/18:1-d7, PI 15:0/18:1-d7, PS 15:0/18:1-d7, SM d18:1/18:1-d9, TG 15:0/18:1-d7/15:0.
Acetonitrile, water, methanol, isopropanol (LC-MS grade) and butylated hydroxytoluene (BHT) were purchased from Carl Roth (Karlsruhe, Germany). Acetonitrile (ULC-MS grade) was purchased from Biosolve B. V. (Valkenswaard, Netherlands). Formic acid (pro analysis) was purchased from VWR (Darmstadt, Germany). Acetic acid was obtained from fisher scientific (Schwerte, Germany) and ammonia from Merck (Darmstadt, Germany).

Determination of fatty acids and oxylipins by LC-MS/MS

Fatty acids and oxylipins were analyzed using liquid chromatography tandem-mass spectroscopy (LC-MS/MS). The LC-MS/MS system for analysis of fatty acids consisted of a 5500 QTrap mass spectrometer (Sciex, Darmstadt, Germany), operating in negative ESI mode, an Agilent 1200 HPLC system (Agilent, Waldbronn, Germany) and an HTC Pal autosampler (Chromtech, Idstein, Germany). Analysis of oxylipins was performed on a 6500+ QTrap mass spectrometer (Sciex, Darmstadt, Germany), operating in negative ESI mode, and an Agilent 1290 Infinity II UHPLC system (Agilent, Waldbronn, Germany).

Sample extraction of arachidonic, linoleic, eicosapentaenoic and docosahexaenoic acid was performed using liquid-liquid extraction: 10 µl of plasma were gently mixed with 20 µl of methanol containing 0.1 % BHT, 20 µl of internal standard solution, 190 µL PBS and extracted twice with 600 µL ethyl acetate. Samples for standard curve and quality control were prepared similarly: 200 µl PBS, 20 µl of standard solution and 20 µl internal standard solution were mixed and extracted with ethyl acetate.

The organic phase was removed at 45 °C under a gentle stream of nitrogen. The residues were reconstituted in 50 µl of methanol:water:BHT (50:50:10^{-4}, v/v/v) prior to injection into the LC-MS/MS system. Chromatographic separation was achieved using a Gemini NX C18 column (150 mm × 2 mm ID, 5 µm, Phenomenex, Aschaffenburg, Germany) with a precolumn of the same material. A linear gradient was employed at a flow rate of 0.5 ml/min. Mobile phases were A water:ammonia (100:0.05, v/v) and B acetonitrile:ammonia (100:0.05, v/v). The gradient started at 85 % A switched to 60% A in 10 min, decreased to 10% A in 2 min, held for 1 min, reverted to 85 % A in 0.5 min following 3 min of equilibration.

Tissue samples were homogenized using a Mixer Mill MM400 (Retsch, Düsseldorf, Germany) with 4-5 zirconium dioxide balls at a tissue concentration of 0.01 mg/µL in ethanol:water (1:3, v/v) at 30 Hz for 4 min. The analysis and extraction was performed as described above using 50 µL of tissue homogenate.

Oxylipins were extracted by solid-phase-extraction (SPE) with C18 cartridges (Evolute Express ABN, Biotage, Uppsala, Sweden) preceded by a precipitation step as follows: To 200 µl of plasma, 20 µl internal standards, 100 µl of ice-cold methanol containing 0.1% BHT and 400 µl of ice-cold methanol were added and mixed. The mixture was stored at -80 °C for 30 min and afterwards centrifuged at 20.000 g, 4 °C for 5 min. The organic phase was then transferred to a new amber glass vial and dried at 45 °C under a nitrogen stream to a volume of approximately 200µl. Subsequently 400 µl of Na_2HPO_4 buffer (pH 6) was added, mixed and loaded onto the SPE cartridges. Previously, the cartridges were conditioned and equilibrated with 1 ml methanol and 1 mL water. After the sample loading, the cartridges were washed using 1 mL of a methanol:water 1:1 (v/v) with 2% formic acid solution, followed by 1 ml water. Oxylipins were eluted with 1 ml methanol containing 2 % ammonium hydroxide and then transferred to an amber glass vial. The samples were evaporated to dryness at 45 °C under a gentle stream of nitrogen. The residues were redissolved in 50 µL methanol:water 7:3 (v/v) with 0.0001 % BHT. The chromatographic separation was achieved using an Acquity UPLC BEH C18 2,1 x 100 mm, 1,7 µm column (Waters, Eschborn, Germany). The mobile phases were water and acetonitrile with 0.0025 % formic acid respectively. A 12 min linear gradient was used with a flow rate of 0.4 ml/min.

Tissue samples were homogenized by adding ethanol:water (1:3, v/v) to a tissue concentration of 0.01 mg/µl using a precellys homogenizer with a cooling module. Afterwards 250 µl homogenate were processed as described above.

All data were acquired using Analyst software v1.6.2 and quantitation was performed by MultiQuant software v3.02 (both Sciex, Darmstadt, Germany) using the internal standard method (isotope-dilution mass spectrometry). Calibration curves were calculated by linear regression with 1/x or $1/x^2$ weighting and acceptance criteria were applied as described previously [https://doi.org/10.1016/j.talanta.2019.06.004].

Semi-targeted lipid screening by LC-HRMS

Sample preparation and LC-QTOFMS analysis was performed as previously published [https://doi.org/10.1016/j.talanta.2019.120593]. Briefly, lipids were extracted from 20 µl of plasma or 20 µl serum using 150 µl of internal standard solution in methanol, 500 µl MTBE and 125 µl 50 mM ammonium formate. The upper organic phase was transferred and the aqueous phase re-extracted with 200 µl of a mixture of MTBE: methanol: water (10:3:2.5, v/v/v, upper phase). The combined organic phases were then split in to two 290 µl aliquots and dried under a nitrogen stream at 45 °C. Prior analysis, the residues were reconstituted in 120 µL of methanol for measurement in positive ion mode and 120 µl methanol:water 9:1 (v/v) containing 0.1 % formic acid and 10 mM ammonium formate for measurement in negative ion mode.
LC-MS analysis was conducted on Nexera X2 system (Shimadzu Corporation, Kyoto, Japan) coupled to a TripleTOF 6600 (Sciex, Darmstadt, Germany) using electrospray ionization. Chromatographic separation was achieved using a Zorbax RRHD Eclipse Plus C8 1.8 µm 50x2.1 mm ID column (Agilent, Waldbronn, Germany) and a 17 min linear gradient with a flow rate of 0.3 mL/min. The mobile phases were A 10 mM ammonium formate and 0.1 % formic acid in water for positive and 1 mM ammonium formate and 0.1 % formic acid in water for negative ionization mode as well as B 0.1% formic acid in acetonitrile: isopropanol (2:3, v/v) for both ionization modes. The TOF-MS Scan covered a mass range from 100 to 1000 m/z. Additionally, six data dependent spectra per cycle with a mass range from 50-1000 m/z and a CE of +/- 40 V with a 20 V collision energy spread were acquired. For quantification of the LPI parallel-reaction-monitoring was applied. QTOF-MS spectra were acquired using Analyst TF v1.7.1 and further processed using Multiquant 3.02 software (both Sciex, Darmstadt, Germany).

Multi Epitope Ligand Cartography (MELC)

MELC technology is an automated immunohistological imaging method and can be used to visualize very high numbers of antibodies on the same sample as described before (16–18). Briefly, tissues were embedded in tissue freezing medium (Tissue-Tek O.C.T. Compound,

#4583, Sakura Finetek B.V.), cryosections of 10 µm thickness were applied on silane-coated coverslips, fixed in 4 % paraformaldehyde in PBS for 15 min, permeabilized with 0.1 % Triton X100 in PBS for 15 min and blocked with 3% BSA in PBS for 1 h. The sample was placed on the stage of a Leica DM IRE2 and a picture was taken. Then, in an automated procedure, the sample was incubated for 15 min with bleachable fluorescence-labelled antibodies and rinsed with PBS. Afterward, the phase contrast and fluorescence signals were imaged by a cooled charge-coupled device camera (Apogee KX4, Apogee Instruments). A bleaching step was performed to delete fluorescence signals, and the post-bleaching image was recorded. Then the next antibody was applied and the process repeated. For data analysis, fluorescence images produced by each antibody were aligned pixel-wise and were corrected for illumination faults using flat-field correction. The post-bleaching images were subtracted from their following fluorescence image. All antibodies are listed in supplementary table 3.