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Supplementary information

Albumin orchestrates a natural host defence mechanism against mucormycosis

In the format provided by the authors and unedited

MATERIALS AND METHODS FFA and oxilypins analysis

1. Reagents and solutions

LC-MS grade methanol (MeOH) and acetonitrile (ACN) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Butylated hydroxytoluene (BHT) was obtained from Fluka Honeywell Research Chemicals (Seelze, Germany). Glacial acetic acid was sourced from VWR Chemicals (Pennsylvania, USA), and ethyl acetate from Sigma-Aldrich (Steinheim, Germany). Disodium ethylenediaminetetraacetic acid (Na_2EDTA) was purchased from SERVA Electrophoresis GmbH (Heidelberg, Germany). Ultrapure MilliQ H_2O was obtained using a Milli-Qplus185 system (18 MΩ·cm, Millipore, Bedford, MA, USA). Deuterated oxylipin standards, including 20(*S*)-HETE-d_6, (±)14,15-DiHETrE-d_{11}, and (±)11(12)-EpETrE-d_{11}, were acquired from Cayman Chemical (Ann Arbor, MI, USA), while palmitic acid-d_{31} was purchased from Sigma-Aldrich (Steinheim, Germany).

An internal standard solution containing 2 µM of each deuterated oxylipin was prepared in MeOH. A premix solution consisted of 0.002% BHT, 250 µM Na_2EDTA, and 0.01% CH_3COOH in MeOH/H_2O (1:4, v/v). The Solid Phase Extraction (SPE) buffer was prepared with MilliQ H_2O containing 5% MeOH and 0.1% CH_3COOH.

2. Extraction of Free Oxylipins and Fatty Acids from Serum Samples

A 200 µL aliquot of vortexed serum was added to 600 µL of premix solution and 10 µL of deuterated oxylipin mix in 1.5 mL centrifuge tubes. Samples were vortexed and centrifuged at 16,100 *x*g for 10 min at 4 ºC. The supernatant (~800 µL) was collected for SPE extraction. SPE was performed using 60 mg Oasis HLB cartridges,

pre-washed with 1 column volume (~3 mL) of ethyl acetate, 2 volumes of MeOH, and pre-conditioned with 2 volumes of SPE buffer. The supernatant was loaded onto the cartridges, adjusted to 3 mL with SPE buffer, and eluted by gravity. The column was washed twice with 2 column volumes of SPE buffer and dried under vacuum (15 psi) for 20 min. Oxylipins were eluted with 0.5 mL MeOH and 1.5 mL ethyl acetate into 2 mL centrifuge tubes. The extracts were dried under vacuum using a HyperVAC centrifugal concentrator (Gyrozen, Seoul, South Korea) at 30 ºC for approximately 40 min at 2,000 × g and 10 bar. Samples were reconstituted in 100 µL LC-MS grade MeOH and transferred into amber LC-MS vials with glass inserts for analysis.

3. Quality Assurance (QA) and Quality Control (QC) Analysis

Quality management (QM) was maintained throughout the entire analysis process –before, during, and after– to ensure robust data integrity. Effective QM was achieved by adhering to the mQACC (Metabolomics QA and QC Consortium) guidelines, as detailed by Kirwan et al[1]. QC was ensured using intra-study QC samples, prepared by pooling equal volumes of serum or BALF from each sample prior to extraction. A total of ten injections of a single QC sample were made continuously at the beginning of the analysis until system equilibration was achieved. Subsequently, QC samples were injected at regular intervals –every 4 or 5 randomized samples– to assess the stability, reproducibility, and performance of the system. This was done to correct any signal deviations within the analytical sequence. Blank samples were prepared by adding 200 µL of MilliQ H_2O. QC samples and blank samples followed the same preparation steps as experimental samples. Two non-injected blanks (mobile phases) were analyzed before the

sequence, and two blanks were injected at both the beginning and end of the sequence to subtract background signals and identify any potential sources of contamination.

Chromatographic peaks of the measured oxylipins were compared against 68 commercial standards. Free fatty acids were compared against 34 fatty acids characterized in a plasma reference material. All measured lipids were evaluated for retention time (RT) matching, elution order, peak symmetry, and peak area. Data treatment parameters are summarized in Tables S1 and S2.

Agilent MassHunter Qualitative Analysis Navigator Software B.10.00 (Agilent Technologies, Waldbronn, Germany) was used to generate quality control charts, overlays of equipment pressure, and Extracted Ion Chromatograms (EIC) for internal standards in both samples and QC samples. This allowed for the verification of system mass accuracy, data quality, and reproducibility.

4. RP-UHPLC-ESI(–)-QTOF-MS data acquisition

Reversed-phase Ultra-High Performance Liquid Chromatography (RP-UHPLC) coupled with Quadrupole Time-of-Flight Mass Spectrometry (QTOF-MS) equipped with a dual Agilent Jet Stream (AJS) electrospray ionization (ESI) source was used for data acquisition, following the method described by Camunas-Alberca et al.[2]

The mobile phases for liquid chromatography were as follows: (A) 0.1% CH_3COOH in MilliQ H_2O and (B) 0.1% CH_3COOH in a mixture of ACN/MeOH (80:15, v/v). The chromatography gradient was initiated at 35% B (0–2 min), followed by 85% B (12–17 min), 100% B (27–29 min), and returning to 35% B (29.10–30 min). The flow rate

started at 0.25 mL/min until 17 min, increased to 0.40 mL/min at 27 min, and then returned to 0.30 mL/min at 30 min. Total run time was 31 min. A multiwash strategy was used to prepare starting conditions with a mixture of A/B (65:35, v/v) and MeOH/H_2O (50:50, v/v). An Agilent 1290 Infinity II Multisampler system was employed to inject 2 µL of serum samples and 10 µL of BALF samples, maintaining the multisampler temperature at 4 °C to preserve compound integrity. An Agilent ZORBAX Eclipse Plus C18 (2.1 mm x 150 mm x 1.8 µm) reverse phase column and its UHPLC guard column ZORBAX Eclipse Plus C18 (2.1 mm x 5 mm x 1.8 µm) (Agilent Technologies, Santa Clara, CA, USA) were used and maintained at 45 °C.

Agilent 6546 QTOF ion source was set with the following parameters: 100 V fragmentor, 65 V skimmer, 4,000 V capillary voltage, 1,000 V nozzle voltage, 750 V octopole radio frequency voltage, 10 L/min nebulizer gas flow, 300°C gas temperature, 35 psi nebulizer gas pressure, 11 L/min sheath gas flow, and 350°C sheath gas temperature. A reference mass solution was continuously infused into the system via an Agilent 1260 Iso Pump at a flow rate of 1 mL/min with a 1:100 split ratio for mass correction. The reference masses used were *m/z* 112.9856, *m/z* 980.01634, and *m/z* 1,033.9881. MS data were collected in Full Scan mode from *m/z* 100 to 1,200 with a scan rate of 1 spectra/s.

Data acquisition was performed using Agilent MassHunter Workstation Software LC/MS Data Acquisition B.09.00, while Agilent MassHunter Qualitative Analysis Navigator B.10.00 Software was used for data visualization (Agilent Technologies, Waldbronn, Germany).

5. Data treatment

Peak integration for oxylipins and fatty acids was performed using Agilent MassHunter Quantitative Analysis Software B.08.00 (Agilent Technologies, Waldbronn, Germany). Peak areas were processed using SIMCA v 17.0.2 (Umetrics, Umea, Sweden), and various scaling methods were tested to optimize QC grouping. Principal Component Analysis (PCA-X) models were used to identify analytical variability. For serum samples, QC-SVRC (quality control support vector regression) normalization was applied to correct variability, while no normalization was applied to BALF samples. MATLAB v R2023b (The MathWorks, Maticks, MA, USA) was used for data normalization.

Semiquantification of the compounds was achieved using deuterated internal standards, including deuterated oxylipins and palmitic acid-d_{31}, which were matched based on structural similarity. Concentrations from blank samples were subtracted from study samples, and compounds with a coefficient of variation (CV) greater than 30% in QC samples were excluded from further analysis. Free oxylipins were reported as nM, and free fatty acids as µM.

6. Lipid nomenclature

The lipid nomenclature followed the classification system established by LIPID MAPS (https://www.lipidmaps.org/resources/tools/nomenclature) and the shorthand nomenclature outlined in their guidelines (https://lipidmaps.org/lipid_nomenclature)[3]

Table S1. LC-MS Parameters for free oxylipins from commercial standards used in compound identification in samples. Precursor polyunsaturated fatty acids for oxylipins include LA, linoleic acid (18:2n-6); ALA, alpha-linolenic acid (18:3n-3); DGLA, dihomo-gamma-linolenic acid (20:3n-6); AA, arachidonic acid (20:4n-6); EPA, eicosapentaenoic acid (20:5n-3); DHA, docosahexaenoic acid (22:6n-3).

Oxylipin Common Name	**Shorthand Notation**	**Molecular Formula**	**Precursor Fatty Acid**	**Exact mass $[M-H]^-$ *m/z***	**Retention Time (min)**
Commercial oxylipin standards					
20-COOH-LTB4	FA 20:5;O4	C20H30O6	AA	365.1970	5.28
6-keto-PGF1α	FA 20:3;O4	C20H34O6	AA	369.2283	5.38
20-OH-LTB4	FA 20:4;O3	C20H32O5	AA	351.2177	5.58
TXB2	FA 20:3;O4	C20H34O6	AA	369.2283	6.60
PGF2α	FA 20:3;O3	C20H34O5	AA	353.2333	7.30
PGE2	FA 20:4;O3	C20H32O5	AA	351.2177	7.52
PGD2	FA 20:4;O3	C20H32O5	AA	351.2177	7.88
LTD4	FA 25:7;O4	C25H40N2O6S	AA	495.2534	8.27
LXA4	FA 20:4;O3	C20H32O5	AA	351.2177	8.31
LTE4	FA 23:6;O3	C23H37NO5S	AA	438.2320	9.33
PGJ2	FA 20:5;O2	C20H30O4	AA	333.2071	9.36

PGB2	FA 20:5;O2	C20H30O4	AA	333.2071	9.58
8,15-DiHETE	FA 20:4;O2	C20H32O4	AA	335.2228	10.08
6-trans-LTB4	FA 20:4;O2	C20H32O4	AA	335.2228	10.28
5,15-DiHETE	FA 20:4;O2	C20H32O4	AA	335.2228	10.56
LTB4	FA 20:4;O2	C20H32O4	AA	335.2228	10.58
14,15-DiHETrE	FA 20:3;O2	C20H34O4	AA	337.2384	11.52
11,12-DiHETrE	FA 20:3;O2	C20H34O4	AA	337.2384	11.87
8,9-DiHETrE	FA 20:3;O2	C20H34O4	AA	337.2384	12.13
15-deoxy-PGJ2	FA 20:6;O	C20H28O3	AA	315.1966	12.32
20-HETE	FA 20:4;O	C20H32O3	AA	319.2279	12.42
5,6-DiHETrE	FA 20:3;O2	C20H34O4	AA	337.2384	12.53
15-HETE	FA 20:4;O	C20H32O3	AA	319.2279	13.12
11-HETE	FA 20:4;O	C20H32O3	AA	319.2279	13.32
15-oxo-ETE	FA 20:5;O	C20H30O3	AA	317.2122	13.39
12-HETE	FA 20:4;O	C20H32O3	AA	319.2279	13.49
8-HETE	FA 20:4;O	C20H32O3	AA	319.2279	13.49
9-HETE	FA 20:4;O	C20H32O3	AA	319.2279	13.64
5-HETE	FA 20:4;O	C20H32O3	AA	319.2279	13.75
14(15)-EpETrE	FA 20:4;O	C20H32O3	AA	319.2279	14.17

12-oxo-ETE	FA 20:5;O	C20H30O3	AA	317.2122	14.37
5-oxo-ETE	FA 20:5;O	C20H30O3	AA	317.2122	14.37
11(12)-EpETrE	FA 20:4;O	C20H32O3	AA	319.2279	14.51
8(9)-EpETrE	FA 20:4;O	C20H32O3	AA	319.2279	14.64
5(6)-EpETrE	FA 20:4;O	C20H32O3	AA	319.2279	14.81
9-HOTrE	FA 18:3;O	C18H30O3	ALA	293.2122	11.93
9-HpOTrE	FA 18:3;O2	C18H30O4	ALA	309.2071	12.07
13-HOTrE	FA 18:3;O	C18H30O3	ALA	293.2122	12.10
PGE1	FA 20:3;O3	C20H34O5	DGLA	353.2333	7.72
PGD1	FA 20:3;O3	C20H34O5	DGLA	353.2333	7.82
LTB3	FA 20:3;O2	C20H34O4	DGLA	337.2384	11.52
15-HETrE	FA 20:3;O	C20H34O3	DGLA	321.2435	13.64
17-HDoHE	FA 22:6;O	C22H32O3	DHA	343.2279	13.15
19(20)-EpDPE	FA 22:6;O	C22H32O3	DHA	343.2279	13.97
13(14)-EpDPE	FA 22:6;O	C22H32O3	DHA	343.2279	14.22
16(17)-EpDPE	FA 22:6;O	C22H32O3	DHA	343.2279	14.22
10(11)-EpDPE	FA 22:6;O	C22H32O3	DHA	343.2279	14.34
7(8)-EpDPE	FA 22:6;O	C22H32O3	DHA	343.2279	14.49

PGE3	FA 20:5;O3	C20H30O5	EPA	349.2020	6.63
17,18-DiHETE	FA 20:4;O2	C20H32O4	EPA	335.2228	10.48
14,15-DiHETE	FA 20:4;O2	C20H32O4	EPA	335.2228	10.76
15-HEPE	FA 20:5;O	C20H30O3	EPA	317.2122	12.38
8-HEPE	FA 20:5;O	C20H30O3	EPA	317.2122	12.50
12-HEPE	FA 20:5;O	C20H30O3	EPA	317.2122	12.57
5-HEPE	FA 20:5;O	C20H30O3	EPA	317.2122	12.77
17(18)-EpETE	FA 20:5;O	C20H30O3	EPA	317.2122	13.20
11(12)-EpETE	FA 20:5;O	C20H30O3	EPA	317.2122	13.44
14(15)-EpETE	FA 20:5;O	C20H30O3	EPA	317.2122	13.44
8(9)-EpETE	FA 20:5;O	C20H30O3	EPA	317.2122	13.50
9,12,13-TriHOME	FA 18:1;O3	C18H34O5	LA	329.2333	7.14
9,10,13-TriHOME	FA 18:1;O3	C18H34O5	LA	329.2333	7.25
12,13-DiHOME	FA 18:1;O2	C18H34O4	LA	313.2384	11.00

9,10-DiHOME	FA 18:1;O2	C18H34O4	LA	313.2384	11.21
13-HODE	FA 18:2;O	C18H32O3	LA	295.2279	12.90
13-oxo-ODE	FA 18:3;O	C18H30O3	LA	293.2122	13.22
9-oxo-ODE	FA 18:3;O	C18H30O3	LA	293.2122	13.44
12(13)-EpOME	FA 18:2;O	C18H32O3	LA	295.2279	14.14
9(10)-EpOME	FA 18:2;O	C18H32O3	LA	295.2279	14.25
Commercial deuterated internal standards					
11(12)-EpETrE-d_{11}	-	C20H21D11O3	AA	330.2970	14.05
14,15-DiHETrE-d_{11}	-	C20H23D11O4	AA	348.3076	11.00
20-HETE-d_6	-	C20H26D6O3	AA	325.2656	12.00

Table S2. LC-MS Parameters for free fatty acids from commercial standards used in compound identification in samples. MCFA, Medium-chain Fatty Acids; LCFA, Long-chain Fatty Acids.

Fatty Acid Systematic Name – Common Name	**Shorthand Notation**	**Molecular Formula**	**Exact mass $[M-H]^-$ *m/z***	**Retention Time (min)**
MCFA				

Hexanoic acid Caproic acid	FA 6:0	C6H12O2	115.0765	5.10
Octanoic acid Caprylic acid	FA 8:0	C8H16O2	143.1078	8.70
Decanoic acid Capric acid	FA 10:0	C10H20O2	171.1391	11.50
Dodecanoic acid Lauric acid	FA 12:0	C12H24O2	199.1704	13.70
LCFA				
Tetradecanoic acid Myristic acid	FA 14:0	C14H28O2	277.2016	15.78
Tetradecenoic acid	FA 14:1	C14H26O2	225.1860	14.28
Pentadecanoic acid	FA 15:0	C15H30O2	241.2173	17.99
Hexadecanoic acid Palmitic acid	FA 16:0	C16H32O2	255.2329	19.99
Hexadecenoic acid	FA 16:1	C16H30O2	253.2173	16.87
Hexadecanoic acid	FA 16:2	C16H28O2	251.2016	15.05
Hexadecatrienoic acid	FA 16:3	C16H26O2	249.1860	14.23
Heptadecanoic acid Margaric acid	FA 17:0	C17H34O2	269.2486	21.80
Heptadecenoic acid	FA 17:1	C17H32O2	267.2329	18.78
Octadecanoic acid Stearic acid	FA 18:0	C18H36O2	283.2642	23.41

Octadecenoic acid Oleic acid	FA 18:1	C18H34O2	281.2486	20.68
Octadecadienoic acid Linolenic acid (LA)	FA 18:2	C18H32O2	279.2329	17.79
Octadecatrienoic acid α-linolenic acid (ALA)	FA 18:3	C18H30O2	277.2173	15.79
Nonadecanoic acid	FA 19:0	C19H38O2	297.2799	24.82
Nonadecenoic acid	FA 19:1	C19H36O2	295.2642	22.32
Eicosanoic acid Arachidic acid	FA 20:0	C20H40O2	311.2955	26.11
Eicosenoic acid	FA 20:1	C20H38O2	309.2799	23.76
Eicosadienoic acid	FA 20:2	C20H36O2	307.2642	21.41
Eicosatrienoic acid Dihomo-α-linolenic acid (DGLA)	FA 20:3	C20H34O2	305.2486	19.91
Eicosatetraenoic acid Arachidonic acid (AA)	FA 20:4	C20H32O2	303.2329	17.32
Eicosapentaenoic acid (EPA)	FA 20:5	C20H30O2	301.2173	15.50
Docosanoic acid	FA 22:0	C22H44O2	339.3268	28.35
Docosenoic acid	FA 22:1	C22H42O2	337.3112	26.26
Docosadienoic acid	FA 22:2	C22H40O2	335.2955	24.29
Docosatrienoic acid	FA 22:3	C22H38O2	333.2799	22.55

Docosatetraenoic acid	FA 22:4	C22H36O2	331.2642	20.27
Docosapentaenoic acid	FA 22:5	C22H34O2	329.2486	18.91
Docosahexaenoic acid (DHA)	FA 22:6	C22H32O2	329.2486	16.69
Tricosanoic acid	FA 23:0	C23H46O2	327.2329	28.55
Tetracosenoic acid	FA 24:1	C24H46O2	365.3425	28.39
Commercial deuterated internal standard				
Palmitic acid-d_{31}	-	C16HD31O2	286.4275	19.50

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