METHODS

Mice. Wild type C57BL/6J and *Alox15*$^{-/-}$ mice on a C57BL/6J background were purchased from Jackson Laboratories (USA). Mice were fed a normal chow global diet 2918 containing 18.5% protein and 5.5% fat (Harlan Mucedola, Italy) and housed in individually ventilated cages (IVC) under specific pathogen-free conditions in full compliance to FELASA recommendations at the Animal House Facility of the Biomedical Research Foundation of the Academy of Athens. All procedures had received prior approval from the Institutions and Regional Ethical Review Boards and were in accordance with the US National Institutes of Health Statement of Compliance (Assurance) with Standards for Humane Care and Use of Laboratory Animals (#A5736–01) and with the European Union Directive 86/609/EEC for animal research.

Pre-clinical model of ovalbumin (OVA)-induced AAI. Induction of AAI in C57BL/6 mice required two systemic sensitization injections with 7.5 μg OVA (Albumin, from chicken egg white, Grade V, Sigma-Aldrich) emulsified in aluminum salts ($AlOH_3$) and subsequent exposure after 7 days, to 5 % w/v aerosolized OVA (35 min challenge for 3 sequential days). Mice that were sensitized and challenged with PBS were used as non-diseased controls. Untreated (non-sensitized, non-challenged) mice were used as naïve controls. All mice were 8-10 weeks of age at the time of experimentation.

Bronchoalveolar lavage & Differential cell counts. BAL of the whole lung was performed twice with 0.5 mL saline. 100,000 cells were subjected to cytospin

centrifugation at 600 rpm for 3 min. Cytospins were stained with May-Grumwald-Giemsa and analysed by differential cell counting.

Edema assessment. Tissues were weighed before and after drying at 60 °C for 2 days. The weight difference reflected fluid accumulation in the tissue.

Lymph node isolation & culture. Mediastinal lymph nodes (MLN) were isolated from mice. Single cell suspensions were generated with the use of a 70 μm pore size cell strainer. 0.5 million cells/well were plated in triplicates in U-shaped 96-well plates. Cells were *in vitro* re-stimulated with 100 μg/mL OVA. Culture supernatants were collected after 48 hours and stored at -20 °C.

T-cell responses. The concentrations of T_H2 cytokines were determined by ELISA (Ready-SET- Go! Kits eBioscience, California, USA). The immunoassay was performed on MLN culture supernatants.

Dexamethasone dosing. DXM was systemically administered intraperitoneally (IP) in the form of water-soluble dexamethasone-21-phosphate disodium salt (Sigma-Aldrich) at 1mg/kg (mpk) dose per day, for 3 sequential days.

Chemical reagents. PGE2-d4 (9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic-3,3,4,4-d4 acid), PDX (10(S),17(S)-dihydroxy-4Z,7Z,11E,13Z,15E,19Z-docosahexaenoic acid) were purchased from Cayman Chem. All solvents and eluent additives used were

of LC-MS grade. Acetonitrile (ACN) (Riedel-de Haen), water (Sigma), ammonium acetate (Fluka), formic acid (Fluka) were purchased from Sigma-Aldrich.

Calibration curve. Lung tissue and serum was isolated from naïve C57Bl/6 mice. Lungs were homogenized in 4 mL ice-cold 70% methanol/ 1 gram of wet tissue. Lung and serum calibration curves ranged from 2.5 ng/g to 50 ng/g for PDX and from 1 ng/mL to 10 ng/mL for RvD1, RvE1, LXA4, PDX, PGD2, PGE2, EXC4, LTB4, 17-HDHA and 7-HDHA respectively.

Sample preparation. After homogenization, 200 μL of lung homogenate was spiked with internal standard (IS: PGE2-d4). Samples were cleaned-up by centrifugation and supernatants were collected. Solid precipitates were washed twice with 100 μL ice-cold 50% MeOH (v/v). Mouse serum was collected at designated time-points. For the liquid chromatography-tandem mass spectrometry with multiple reaction monitoring (LC-MRM) analyses 200 μL of mouse serum was spiked with 10 μL 1 μg/mL IS.

Solid Phase Extraction (SPE). The resulting samples were acidified with 1 mL aqueous solution of 0.1% formic acid (FA) (v/v) and were immediately subjected to SPE (Oasis HLB Extraction plate, C8, 30 μm, 10 mg of sorbent, Waters Corporation). 96-well SPE cartridges were preconditioned with 500 μL MeOH and equilibrated with 500 μL H_2O. The cartridges were then washed sequentially with 500 μL H_2O and 500 μL 15% MeOH (v/v). Finally, the analytes were eluted with 200 μL 90% ACN (v/v). Samples were evaporated to dryness in a SpeedVac (ThermoSavant SPD1010, NY, USA) at room

temperature, under vacuum. The dry residue was dissolved in 100 µL of 50:50 mobile phase A:B, followed by vortex and sonication.

HPLC-MS/MS analysis. HPLC was performed with an Ultimate 3000 Series system (Dionex Corporation, USA). The mobile phase consisted of solvents A: 10 % ACN, 90 % water, 2 mM ammonium acetate, 0.1 % FA and B: 90 % ACN, 10 % water, 2 mM ammonium acetate, 0.1 % FA. A multi-linear gradient was performed with a flow rate of 300 µL/min. A Waters Symmetry C8 column (2.1 x 50 mm, 3.5 µm) was used at stable 25 °C. The injection volume was 10 µL. Mass spectrometry was performed on an API 4000 QTRAP MS/MS system fitted with a TurboIonSpray source and a hybrid triple quadruple linear ion trap (QqLIT) mass spectrometer (Applied Biosystems, Concord, Ontario, Canada). The instrument was operated in negative ion mode.

Absolute & Relative quantification of LM in biological matrices. Quantification was performed in multiple reaction monitoring (MRM) mode of operation. Values of DHA, 17-HDHA and 7-HDHA presented in Fig.1E, Fig. 2B and Fig.4B are expressed as fold changes relative to the levels of the respective analytes in naïve mice. In the case of PD1 and PDX, where levels in naïve mice were below the limit of quantification (LOQ)~~BLQ~~, fold changes are expressed relative to half the ~~L~~LoQ.

Statistical analysis. Statistical significance of differences was assessed by a *two-tailed Student t test* for parametric data and a *Mann-Whitney U test* for nonparametric data. *P* values of less than 0.05 were considered statistically significant.