Supporting information

Isolation and culture of human pulmonary macrophages

Macrophages were isolated from finely minced, macroscopically normal lung parenchyma obtained (using an adherence technique) from sites far from the tumor, as described previously. Briefly, the fluid collected from several washings of the minced peripheral lung tissues was centrifuged (2000 rpm, 10 min) and the cell pellet was resuspended in supplemented RPMI with 10% heat-inactivated foetal calf serum (FCS), 2 mM L-glutamine and antibiotics. Re-suspended viable cells (10^6 cells per mL) were then aliquoted into either a 12-well plate for transcriptional assays or a 24-well plate for cytokine assays. Following incubation for at least 1.5 hours at 37°C (in 5% CO_2 humidified atmosphere), non-adherent cells were removed by gentle washings and the remaining cells were maintained at 37°C and 5% CO_2 overnight. More than 95% of the adherent cells were macrophages. Cell viability exceeded 95% as assessed by Trypan blue dye exclusion. It has been shown that the adherence step does not significantly influence the overall transcriptional changes in alveolar macrophages, relative to flow cytometry-based isolation (Shaykhiev *et al.*, 2009).

The culture plates with adherent macrophages were washed with warm medium, and then 1 mL of fresh medium supplemented with 1% FCS was added per well. LPS (10 $ng.mL^{-1}$), IL-4 (10 $ng.mL^{-1}$) and IL-13 (50 $ng.mL^{-1}$) were then added to the wells as necessary. After 24h of stimulation, culture supernatants were harvested, centrifuged and frozen for subsequent cytokine assays. Adherent cells were harvested and frozen in TRIzol® reagent (Life Technologies, Saint Aubin, France) for RNA extraction and analysis.

Table S1: Effect of PD146176 (10 µM) on lung macrophage viability in an LDH assay

LDH (µU/mL)	Baseline	PD146176
Control	2370 ± 331	2132 ± 206
n=5		
LPS	1858 ± 50	2068 ± 156
n=5		
IL-4	2260 ± 200	2461 ± 171
n=5		
IL-13	1740 ± 221	2365 ± 222
n=5		

Table S2: Effect of LPS on production of the panel of IL-4-/IL-13-induced chemokines.

Cytokines (ng/10^6 LMs)	*Control*	*LPS*
CCL13 n=5	0.07 ± 0.01	0.12 ± 0.03
CCL18 n=5	127.45 ± 28.09	175.92 ± 35.99*
CCL22 n=5	0.42 ± 0.13	0.52 ± 0.13

*: $p<0.05$

Table S3: Effect of IL-4 and IL-13 on production of the panel of LPS-induced chemokines.

Cytokines (ng/10^6 LMs)	*Control*	*IL-4*	*Control*	*IL-13*
CCL2	1.96 ± 1.26 n=5	1.77 ± 1.16	2.48 ± 1.20 n=5	2.37 ± 1.45
CCL3	0.56 ± 0.18 n=5	0.68 ± 0.16	0.78 ± 0.26 n=5	0.75 ± 0.17
CCL4	1.50 ± 0.23 n=5	2.18 ± 0.49	1.50 ± 0.23 n=5	2.01 ± 0.56
CXCL1	0.37 ± 0.10 n=5	0.18 ± 0.08*	0.31 ± 0.09 n=5	0.17 ± 0.07*
CXCL10	0.002 ± 0.002 n=6	0.03 ± 0.02	0.009 ± 0.006 n=5	0.01 ± 0.007

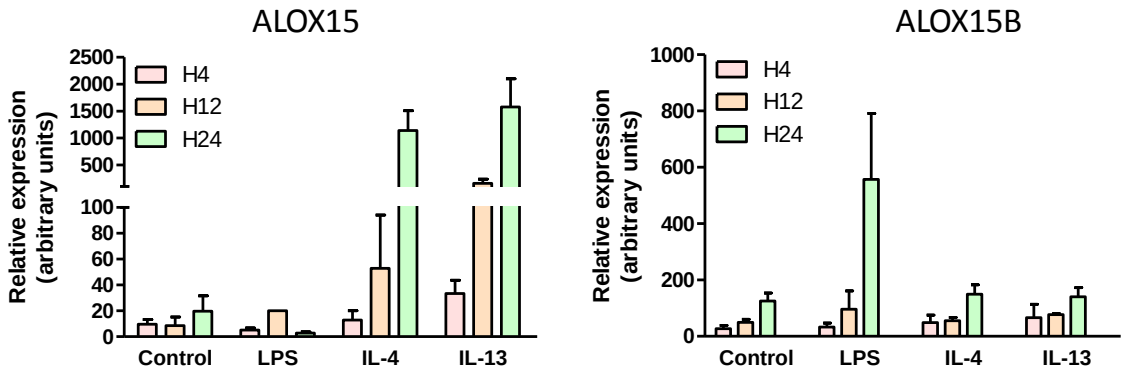
*: $p<0.05$

Table S4: Effect of LPS and Th2 cytokines on mRNA expression of M1- and M2a-related markers of polarization in human lung macrophages.

Relative cytokine expression ($2^{-\Delta Ct}$ x 1000)	*Baseline*	*LPS*	*IL-4*	*IL-13*
CCL2	121 *[99-132]*	3190 *[918-8896]*	29 *[26-30]*	28 *[21-36]*
CCL3	1844 *[1232-8694]*	113772 *[35261-430539]*	1778 *[1705-7210]*	889 *[813-9190]*
CCL4	298 *[186-540]*	22589 *[21466-34611]*	762 *[224-1431]*	447 *[167-1076]*
CXCL1	133 *[100-190]*	43148 *[20730-92331]*	11 *[6-70]*	11 *[10-13]*
CXCL8	1686 *[1532-3329]*	175067 *[119473-177875]*	706 *[387-1606]*	694 *[608-934]*
CXCL10	2 *[1-17]*	16 *[10-43]*	3 *[2-5]*	2 *[1-5]*
TNF	170 *[98-176]*	1295 *[673-1372]*	118 *[115-122]*	121 *[85-145]*
CCL13	1 *[1-2]*	5 *[3-7]*	87 *[7-190]*	44 *[25-88]*
CCL22	265 *[47-278]*	815 *[263-1515]*	3016 *[453-3050]*	1203 *[675-1726]*

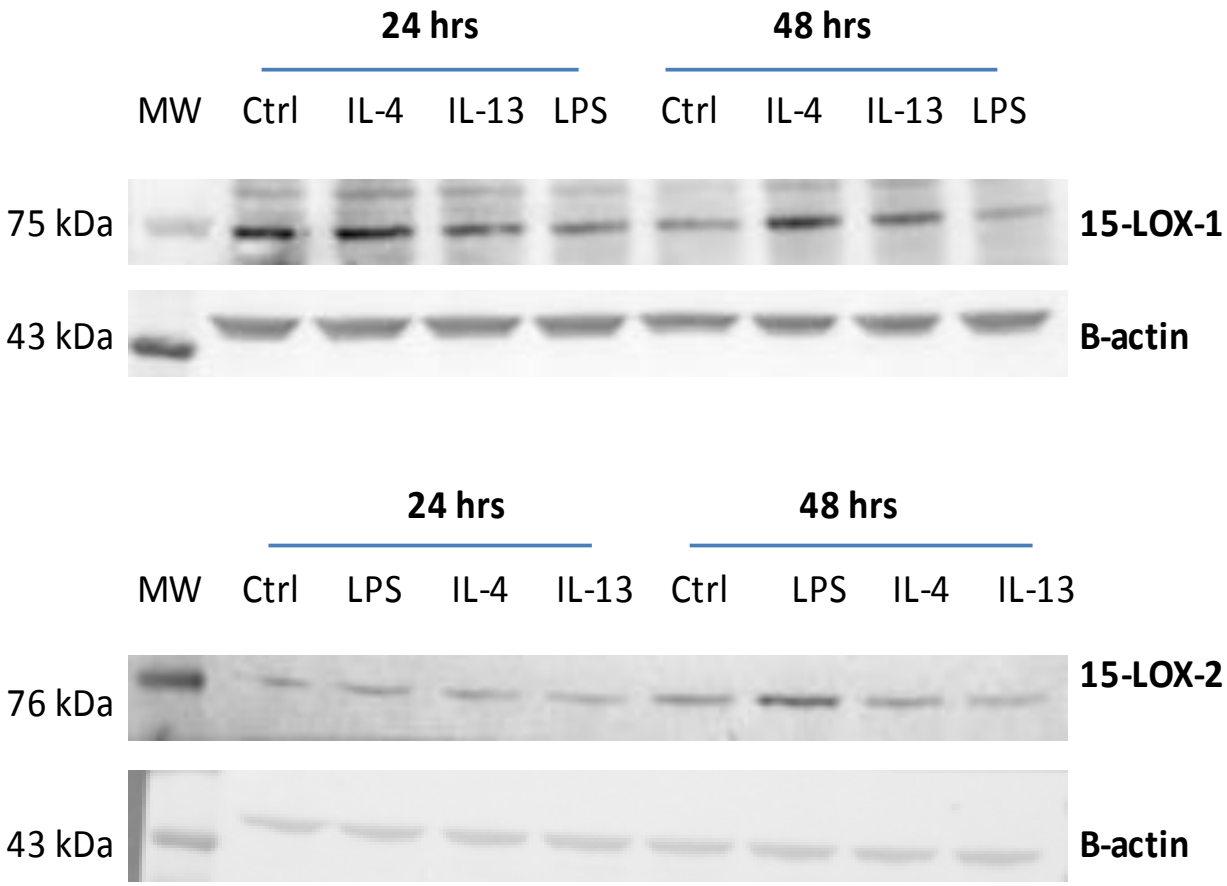
Lung macrophages were incubated with LPS (10 ng.mL^{-1}), IL-4 (10 ng.mL^{-1}) or IL-13 (50 ng.mL^{-1}) for 24 hrs. Levels of gene expression were determined by RT-qPCR.
The values correspond to the median [range] from three different donors.

Figure S1: Time course of ALOX15 and ALOX15B mRNA expression in polarized human lung macrophages.



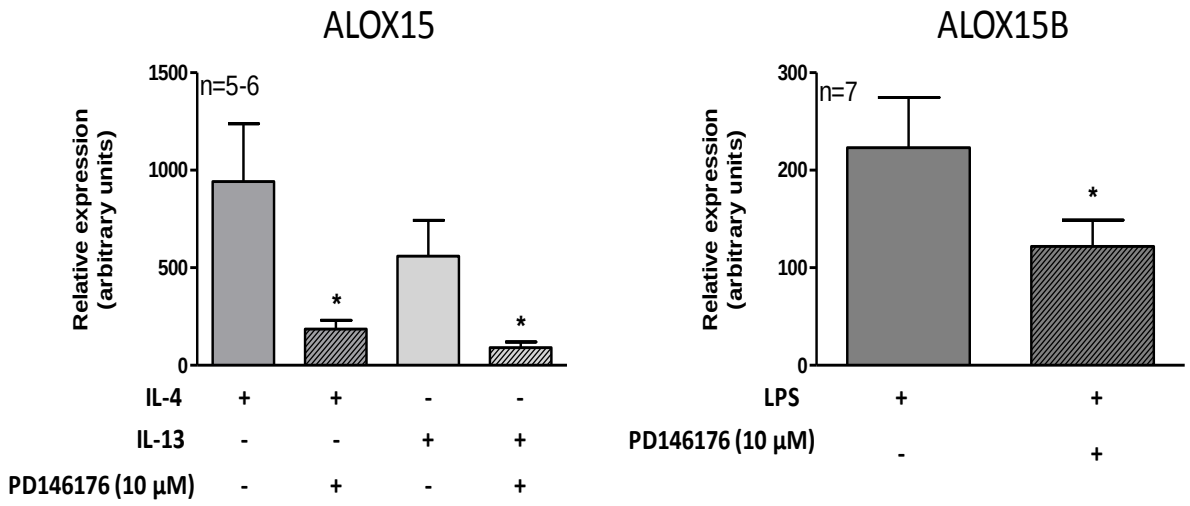
Lung macrophages were incubated with LPS (10 ng.mL^{-1}), IL-4 (10 ng.mL^{-1}) or IL-13 (50 ng.mL^{-1}) for 4, 12 and 24 hrs. Levels of ALOX15 and ALOX15B mRNA expression were determined by RT-qPCR. The data correspond to the mean ± SEM of three independent experiments.

Figure S2: Western blot analysis of 15-LOX-1 and 15-LOX-2 protein expression in human lung macrophages stimulated by LPS, IL-4 or IL-13 for 24 and 48 hrs



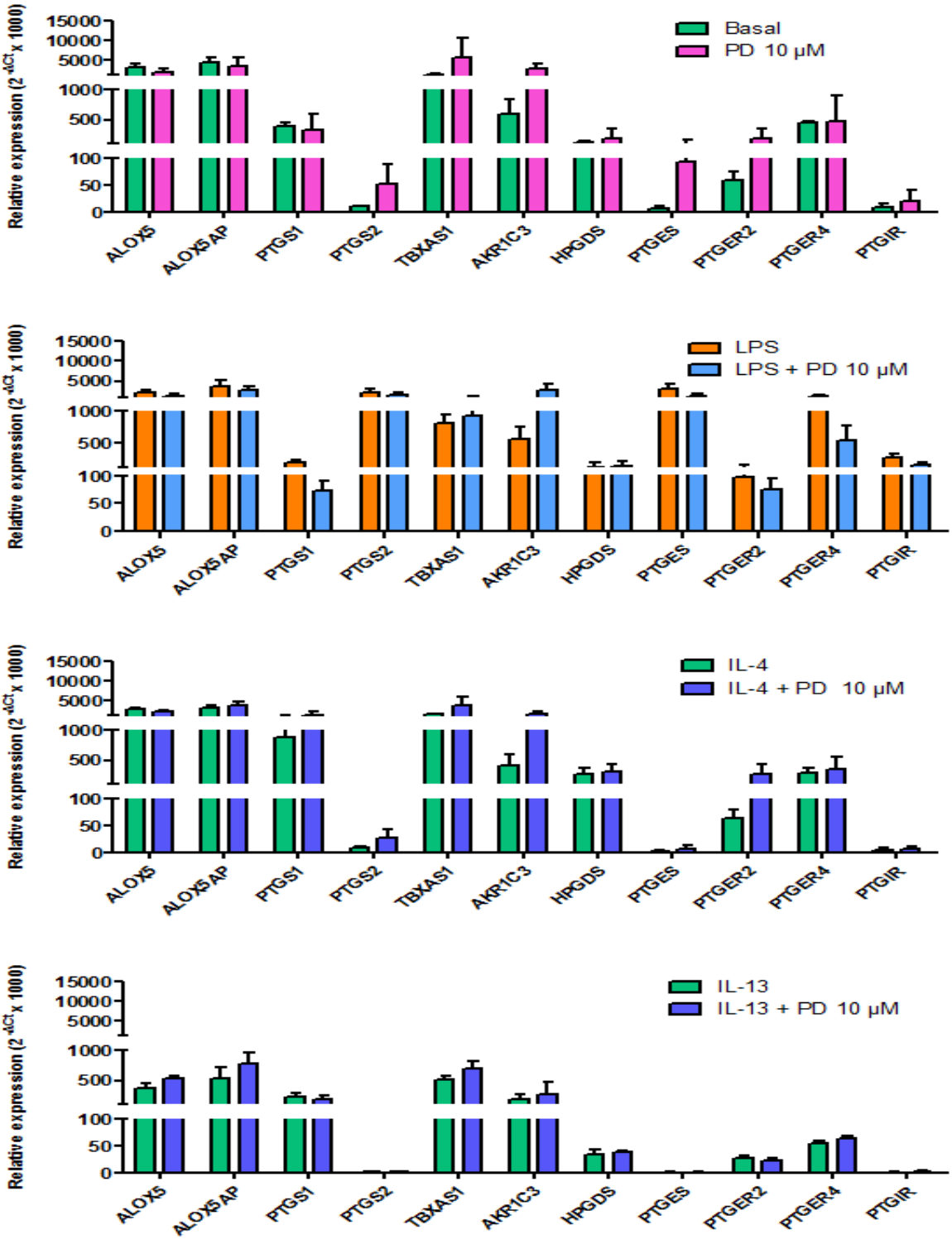
Lung macrophages were incubated with LPS, IL-4, IL-13 or vehicle for 24s or 48 hrs. The cells were then washed with PBS and lysed with lysis buffer (Novagen, San Diego, CA, USA) containing 1% protease inhibitor cocktail and phosphatase inhibitor cocktail (Roche, Mannheim, Germany) for 30 min on ice. Equal amounts of cell lysate (40 µg) were separated on a NuPAGE® 4-10% SDS-PAGE gel (Life Technologies) and transferred into a nitrocellulose membrane, which was further incubated for 2 hrs at room temperature with 5% (w/v) non-fat powdered milk in TBS containing 0.1% Tween 20 and then overnight at 4°C with an anti-15-LOX-1 antibody (Cayman Chemical Europe, Tallinn, Estonia, 1/1000), an anti-15-LOX-2 antibody (Abcam®, Cambridge, United-Kingdom, 1/200) and anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, California, 1/200). After washing, the membranes were incubated for 2 hrs with horseradish-peroxidase-conjugated anti-mouse/rabbit/donkey antibody (1/10000), as appropriate. Blots were then incubated with an enhanced chemiluminescence solution for 1 min and developed with the Molecular Imager ChemiDoc XRS System (Bio-Rad, Marnes-la-Coquette, France).

Figure S3: Effect of PD146176 on ALOX15 and ALOX15B expression in human lung macrophages



Lung macrophages were incubated with LPS (10 ng.mL^{-1}), IL-4 (10 ng.mL^{-1}) or IL-13 (50 ng.mL^{-1}) for 24 hrs in the presence or absence of PD146176. Levels of ALOX15 and ALOX15B mRNA expression were determined by RT-qPCR. Data correspond to the mean ± SEM of three independent experiments.

Figure S4: Effect of PD146176 on mRNA expression of genes involved in the eicosanoid pathways in human lung macrophages



Macrophages were treated for 1 hrs with PD146176 10 µM before being stimulated by LPS (10 ng.mL^{-1}) IL-4 (10 ng.mL^{-1}) or IL-13 (50 ng.mL^{-1}) for 24 hrs. Levels of gene expression were determined by RT-qPCR. Data are reported as the mean ± SEM of 3 to 5 independent experiments.