Supporting Information

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SI Methods

Analysis of Subcellular Redistribution of 5-LO, $cPLA_2$, CLP, and FLAP by Cell Fractionation and Immunoblotting. For fractionation by mild detergent lysis, neutrophils were suspended in ice-cold nonidet P-40-lysis buffer (10 mM Tris/HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 1 mM EDTA, 0.1% nonidet P-40, 1 mM PMSF, 60 μg/ml soybean trypsin inhibitor, and 10 μg/ml leupeptin), vortexed (3 × 5 sec), kept on ice for 10 min and centrifuged (800 × *g*/10 min/4°C). The resulting supernatants (non-nuclear fractions) were transferred to a new tube and the pellets (nuclear fractions) were resuspended in ice-cold relaxation buffer (50 mM Tris/HCl, pH 7.4, 250 mM sucrose, 25 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM PMSF, 60 μg/ml soybean trypsin inhibitor, and 10 μg/ml leupeptin). Both nuclear and non-nuclear fractions were centrifuged again (800 × *g*/10 min/4°C), for further purification. Lysis of cells and integrity of nuclei was confirmed by light microscopy with trypan blue exclusion. Nuclei in relaxation buffer were disrupted by sonication (3 × 5 sec).

For fractionation by sonication, cells were resuspended in ice-cold relaxation buffer, sonicated on ice (5 × 10 sec) and centrifuged at 100,000 × *g*/70 min/4°C. The 100,000 × *g* supernatant is referred as S100; the corresponding pellet is referred as the membrane fraction (P100).

Analysis of Proteins from Cellular Fractions by SDS/PAGE and Western Blotting. Nuclear and non-nuclear fractions and S100 and P100 fractions were immediately mixed with the same volume of SDS-b and heated for 6 min at 95°C. Aliquots (20 μl) of these samples were analyzed by SDS/PAGE using a Mini Protean system (Bio-Rad) on 8% ($cPLA_2$), 10% (5-LO), or 14% (CLP, FLAP) gels. Correct loading of the gel and transfer of proteins to the nitrocellulose membrane was confirmed by Ponceau staining. After electroblot to nitrocellulose membrane (GE Healthcare), membranes were blocked with 5% nonfat dry milk in 50 mM Tris/HCl, pH 7.4 and 100 mM NaCl (TBS) for 1 h at room temperature. Membranes were washed and then incubated with primary antibody for overnight at 4°C. 5-LO antibody (1551, affinity purified), and polyclonal anti-human CLP antiserum (raised in chicken, affinity purified) were used as 1:100 dilution. Polyclonal anti-FLAP antiserum was kindly provided by A. Hatzelmann (Konstanz, Germany) and used as 1:100 dilution. $cPLA_2$ antibody (Santa Cruz Biotechnology) was used as 1:1,000 dilution. Lamin B, a ubiquitous protein exclusively present in the nuclear membrane, was used as marker to indicate correct fractionation. The membranes were washed, incubated with 1:1,000 dilution of alkaline phosphatase-conjugated IgGs for 2 h at RT, and proteins were visualized with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (Sigma) in detection buffer (100 mM Tris/HCl, pH 9.5, 100 mM NaCl, and 5 mM $MgCl_2$).

Determination of MAPK Activation and Phosphorylation of Elk-1 by SDS/PAGE and Western Blotting. For analysis of ERK1/2, phosphorylated ERK1/2, phosphorylated p38 MAPK, and phosphorylated Elk-1, total cell lysates were separated by SDS/PAGE on a 10% gel as described above. After electroblot to Hybond ECL nitrocellulose membranes (GE Healthcare), and blocking with 2% ECL advance blocking agent (GE-Healthcare) for 1 h at RT, membranes were incubated with primary antibodies, overnight at 4°C. Antibody recognizing ERK1/2 and antibodies recognizing phosphorylated ERK1/2 (Thr-202/Tyr-204), phosphorylated p38 MAPK (Thr-180/Tyr-182), and phosphorylated Elk-1 (Ser-383) were from Cell Signaling Technology and used at 1:1,000 dilution in TBS containing 2% ECL advance blocking agent (GE-Healthcare). The membranes were then washed and incubated for 1 h at RT with ECL Plex CyDye-conjugated secondary antibodies (Cy5-conjugated anti-rabbit and Cy3-conjugated anti-mouse, (GE-Healthcare) diluted 1:2,500 in TBS. After washing, membranes were dried and then scanned in the Cy3 (Excitation Filter: 540 nm; Emission Filter: 595 nm) and in the Cy5 (Excitation Filter: 635 nm; Emission Filter: 680 nm) channels by an Ettan DIGE imaging system ((GE-Healthcare). Densitometry was performed with ImageQuant TL image analysis software ((GE-Healthcare); relative intensities were calculated as percentage of the strongest band in the corresponding membrane.

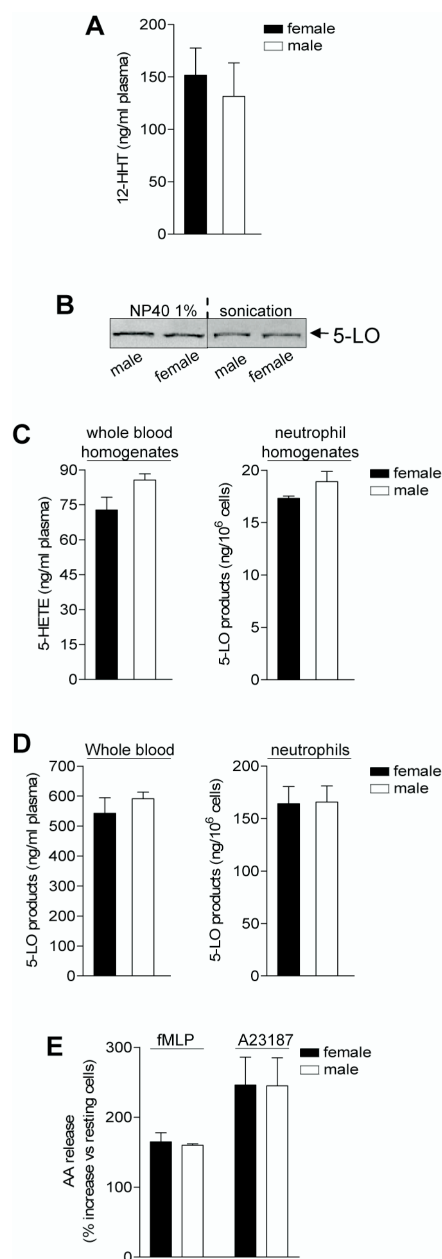

Fig. S1. (*A*) The levels of the COX-derived product 12-HHT in human whole blood stimulated with A23187 (30 μM; 10 min, 37°C) are not significantly different between females and males. Data are means + SEM; n = 6, duplicates. (*B*) 5-LO protein expression and the levels of enzymatically active 5-LO are not different between females and males. 5-LO expression in neutrophils was analyzed by immunoblotting of total cell lysates (corresponding to 3×10^5 cells) obtained by detergent (1% Nonidet P-40) lysis or by sonication (3×10 sec). Results are representative of three independent experiments. (*C*) 5-LO product formation in homogenates of whole blood and neutrophils is not significantly different between females and males. Formed 5-LO products were extracted and analyzed by HPLC. Data are means + SEM; n = 4–5, duplicates. (*D*) 5-LO product formation in human whole blood induced by 30 μM A23187 plus 100 μM AA (10 min, 37°C) (left panel), and in human neutrophils induced by 2.5 μM A23187 plus 20 μM AA (10 min, 37°C) (right panel) are not significantly different between females and males. Data are means + SEM; n = 3 (whole blood), n = 14 (neutrophils), duplicates. (*E*) AA release induced by fMLP (1 μM; 5 min, 37°C) after 30 min priming with LPS (1 μg/ml) plus Ada (0.3 U/ml), or by A23187 (2.5 μM; 10 min, 37°C) in neutrophils is not significantly different between females and males. Data are means + SEM; n = 3–4, duplicates.

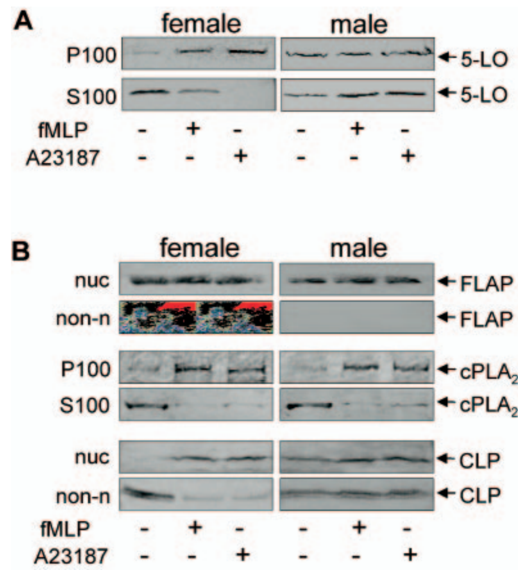

Fig. S2. (*A*) 5-LO subcellular localization and trafficking of 5-LO following activation with fMLP (1 μM; 5 min, 37°C) after 30 min priming with LPS (1 μg/ml) plus Ada (0.3 U/ml), or with A23187 (2.5 μM; 5 min, 37°C) are different in neutrophils from females and males, as analyzed by immunodetection of 5-LO in the membrane (P100) and soluble fractions (S100) of neutrophils. (*B*) The subcellular localization of CLP, but not of $cPLA_2$ and of FLAP, correlates to that of 5-LO, as analyzed by immunodetection of $cPLA_2$ in membrane (P100) and soluble fractions (S100) of neutrophils, and of FLAP and CLP in nuclear (nuc) and non-nuclear (non-n) fractions of neutrophils. Neutrophils were activated with fMLP (1 μM; 5 min, 37°C) after 30 min priming with LPS (1 μg/ml) plus Ada (0.3 U/ml), or with A23187 (2.5 μM; 5 min, 37°C). Results shown are representative of four independent experiments.

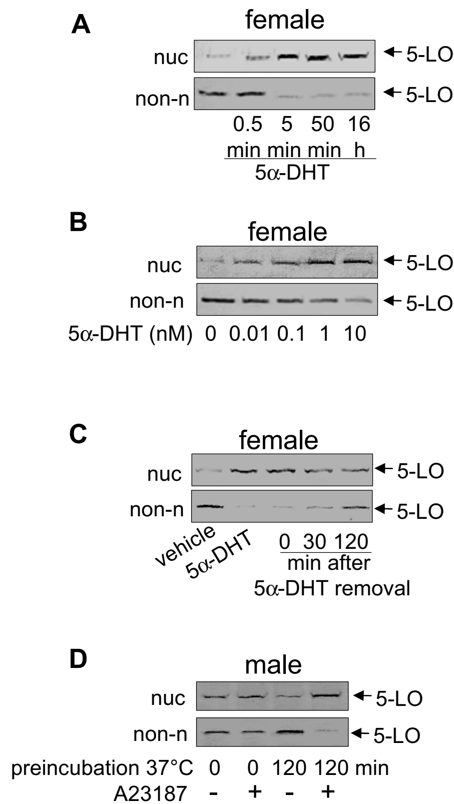

Fig. S3. 5α-DHT induces 5-LO translocation to the nuclear compartment in female neutrophils in a rapid (*A*) and concentration-dependent (*B*) manner, as analyzed by immunodetection of 5-LO in the nuclear (nuc) and non-nuclear (non-n) fractions. Cells were treated with (A) 5α-DHT (10 nM) for the times indicated, or (*B*) with the indicated concentrations of 5α-DHT for 30 min. (*C*) Female neutrophils were treated with 5α-DHT (10 nM, 30 min, 37°C), washed and kept at 37°C for 0, 30 and 120 min and then processed for immunodetection of 5-LO in the nuclear (nuc) and non-nuclear (non-n) fractions after subcellular fractionation. (*D*) Freshly isolated male neutrophils were kept at 37°C for 0 or 120 min, then A23187 (2.5 μM, 5 min, 37°C) was added and 5-LO subcellular localization was analyzed by immunodetection of 5-LO in the nuclear (nuc) and non-nuclear (non-n) fractions. The results shown are representative of $n = 3–4$ independent experiments.

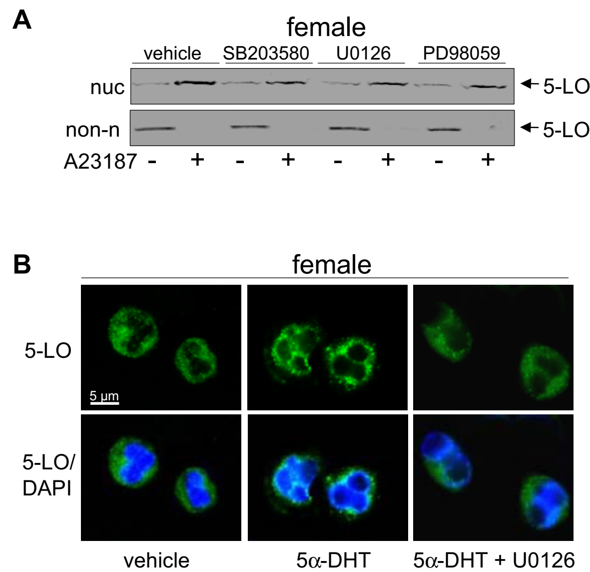

Fig. S4. (*A*) Inhibition of ERK1/2 activation by U0126 (3 μM) or PD98059 (30 μM), or of p38 MAPK by SB203580 (10 μM) does not influence 5-LO subcellular localization in resting or A23187-stimulated female neutrophils. Neutrophils were pretreated with the kinase inhibitors (or 0.3% DMSO as vehicle) for 15 min at 37°C prior stimulation with A23187 (2.5 μM, 5 min, 37°C) and 5-LO was analyzed by immunodetection in the nuclear (nuc) and non-nuclear (non-n) fractions of female cells. (*B*) Inhibition of ERK1/2 activation by 3 μM U0126 blocks nuclear localization of 5-LO induced by 5α-DHT (10 nM; 30 min, 37°C) in neutrophils from females, as analyzed by IF microscopy. Pictures with single staining for 5-LO (green) and merged with DNA-stain (DAPI, blue), are shown. The results shown are representative of three independent experiments.

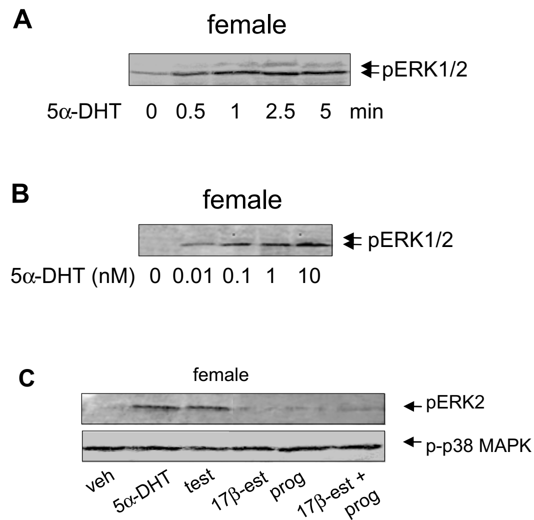

Fig. S5. 5α-DHT activates ERK1/2 in a rapid and concentration-dependent manner in neutrophils from females. (*A*) Time course for 5α-DHT (10 nM) on ERK1/2 activation and (*B*) concentration response for 5α-DHT on ERK1/2 activation (after 1.5 min) in neutrophils from females, as analyzed by immunodetection of phosphorylated ERK1/2 (pERK1/2). Cells in PGC buffer were treated with (*A*) 5α-DHT (10 nM) for the times indicated or (*B*) with the indicated concentrations of 5α-DHT for 1.5 min at 37°C. The results shown are representative of three independent experiments. (*C*) Activation of ERKs by 5α-DHT (10 nM), testosterone (10 nM), 17β-estradiol (100 nM) and/or progesterone (10 μM) after 1.5 min incubation of neutrophils from females. The results shown are representative of three independent experiments.