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SUPPLEMENTARY INFORMATION

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Structural and mechanistic insights into 5-lipoxygenase inhibition by natural products

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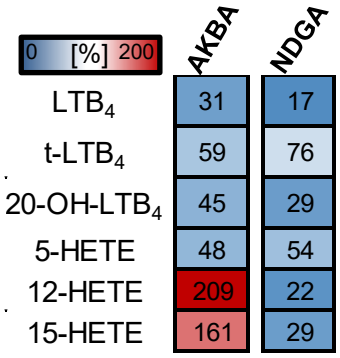
	Stable-5-LOX with NDGA (6N2W)	Stable-5-LOX with AKBA (6NCF)
Data collection		
Space group	P 1 2_1 1	P 1 2_1 1
Cell dimensions		
a, *b*, *c* (Å)	76.7, 204.4, 48.2	76.9, 203.7, 110.4
α, β, γ (°)	90, 99.9, 90	90, 109.7, 90
Resolution (Å)	51.09- 2.71 (2.81 - 2.71)	101.9-3.0 (3.11-3.0)
R_{sym} or R_{merge}	0.25 (1.45)	0.22 (0.85)
I / σI	6.58 (0.86)	6.01 (1.80)
Completeness (%)	98.58 (89.09)	97.98 (98.27)
Redundancy	3.7 (3.4)	3.8 (3.9)
Refinement		
Resolution (Å)	2.71 (2.74–2.71)	30.4 - 3.00 (3.05-3.00)
No. reflections	38857 (3389)	62566 (6248)
R_{work} / R_{free}	0.213 / 0.263	0.266 / 0.291
No. atoms	10035	21902
Protein	9850	21830
Ligand/ion	24	41
Water	171	31
B-factors	49.54	33.66
Protein	49.57	33.72
Ligand/ion	65.92	17.49
Water	45.35	10.03
R.m.s. deviations		
Bond lengths (Å)	0.007	0.004
Bond angles (°)	0.82	0.74

Supplementary Table 1. **Data collections and refinement statistics.** Statistics for the highest resolution shell are shown in parentheses.

	pg/1x10^6 cells	vehicle	AKBA	[%]	NDGA	[%]
5-LOX	LTB_4	1600 ± 195	953 ± 267	60	1.6 ± 0.6	0.1
	t-LTB_4	571 ± 130	262 ± 55	46	1.1 ± 0.6	0.2
	5-HETE	5040 ± 791	4140 ± 1597	82	298 ± 95	6
	12-HETE	60 ± 13	551 ± 142	918	76 ± 10	127
	15-HETE	414 ± 75	566 ± 283	137	530 ± 175	128
5-LOX/FLAP	LTB_4	8608 ± 1465	1598 ± 472	19	51 ± 20	0.5
	t-LTB_4	4931 ± 1175	427 ± 106	9	5 ± 1.2	0.1
	5-HETE	19,834 ± 4301	8453 ± 2548	43	228 ± 45	1
	12-HETE	105 ± 21	1188 ± 435	1131	50 ± 22	48
	15-HETE	296 ± 73	439 ± 100	148	325 ± 58	110
12-LOX	LTB_4	133 ± 13	103 ± 21	77	23 ± 7	17
	t-LTB_4	51 ± 12	26 ± 3	51	14 ± 5	27
	5-HETE	n.d.	n.d.	n.d.	n.d.	n.d.
	12-HETE	36,977 ± 2968	29,763 ± 2492	80	31,362 ± 5194	85
	15-HETE	n.d.	n.d.	n.d.	n.d.	n.d.
15-LOX-1	LTB_4	5 ± 0.2	6 ± 1.3	120	0.2 ± 0.5	4
	t-LTB_4	1.7 ± 1.0	1.9 ± 0.6	100	0.2 ± 0.6	0
	5-HETE	229 ± 49	244 ± 87	107	181 ± 50	79
	12-HETE	219 ± 113	265 ± 155	121	73 ± 39	33
	15-HETE	2360 ± 752	1956 ± 827	83	425 ± 96	18
15-LOX-2	LTB_4	6.4 ± 2.4	30 ± 5	460	n.d.	n.d.
	t-LTB_4	2.4 ± 0.9	8.5 ± 2.6	100	n.d.	n.d.
	5-HETE	138 ± 46	230 ± 53	167	193 ± 49	140
	12-HETE	50 ± 23	59 ± 22	118	42 ± 13	84
	15-HETE	24,221 ± 9176	33,458 ± 9061	138	3966 ± 1193	16

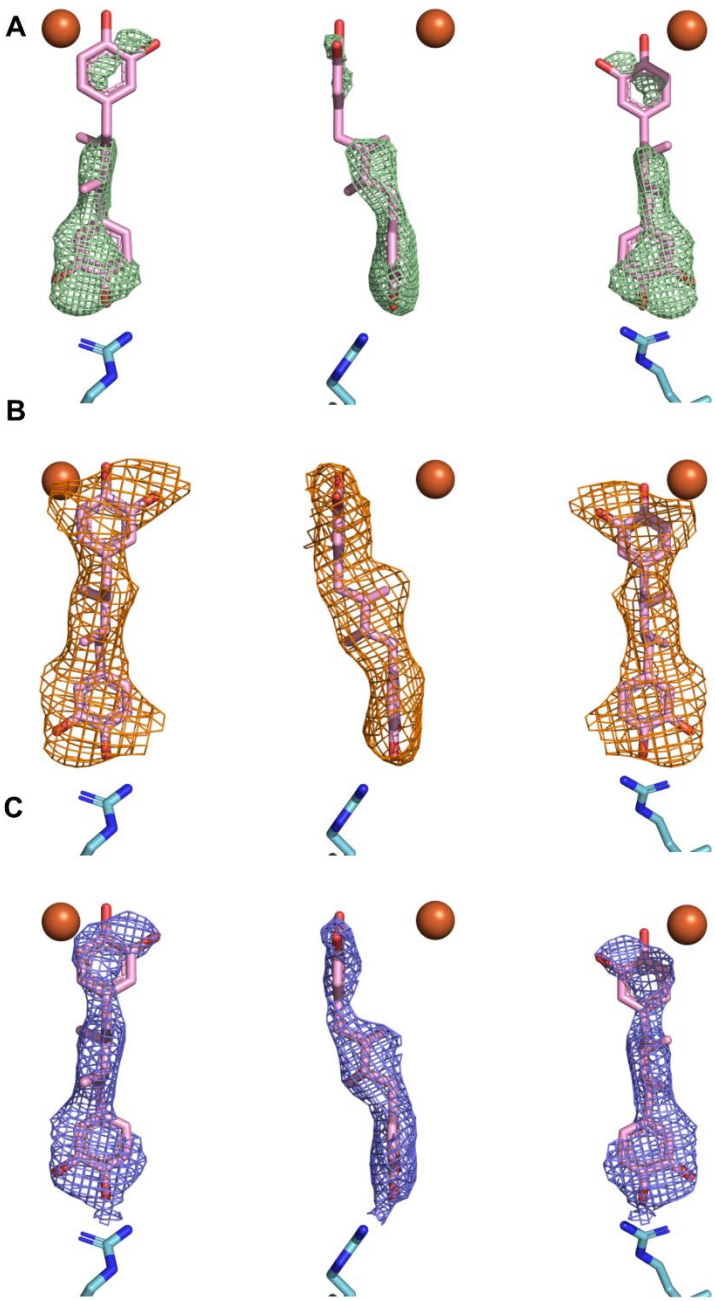
Supplementary Table 2. Modulation of the LM profile by AKBA and NDGA in stably transfected HEK293 cells. Data are presented as absolute values in pg/10^6 cells (mean ± S.E.M., n = 3) and as percentage of control (100%, white) in a heat map.



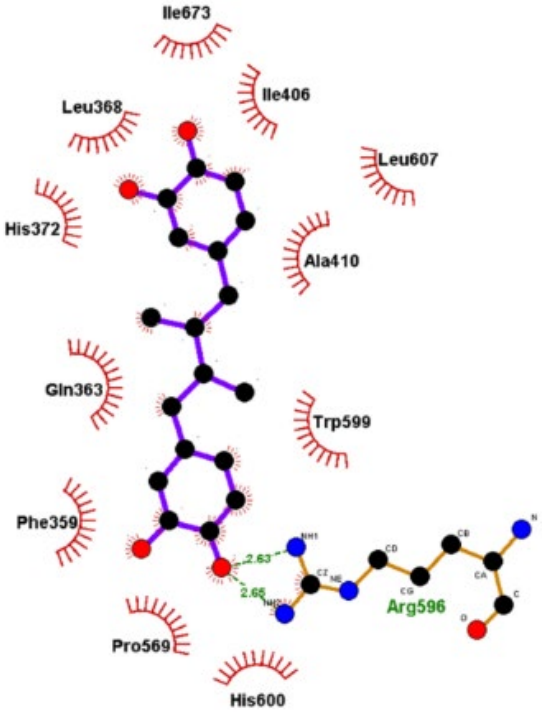
Supplementary Table 3. **Effects of AKBA or NDGA on lipid mediator formation in human neutrophils activated with *E. coli*.** Percentage inhibition is shown in a heat map (100%, white) upon 10 µM AKBA or 1 µM NDGA. Data are means of n=3 independent experiments.

5-LOX	12-LOX	15-LOX-1	15-LOX-2
H-Bond			
R101	R98	R98	Q108
H130	H127	H127	Q137
R138	R135	R135	R145
Main Chain			
V110	S107	S107	V117
Hydrophobic			
L66	F62	F62	L66
V110	S107	S107	V117
I126	M123	L123	V133
K133 Cδ	K130	E130	E140
T137	D134	E134	A144

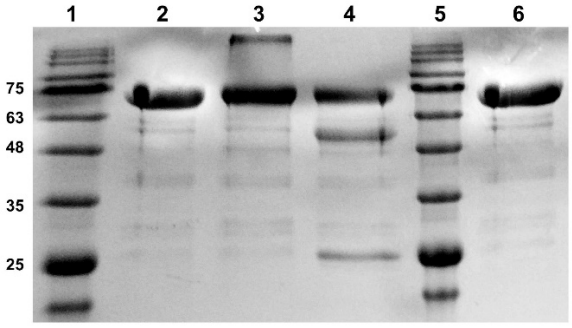
Supplementary Table 4. **Variation in the amino acids that line the interdomain crevice of lipoxygenases.** The corresponding cavities of 12-LOX, 15-LOX-1, and 15-LOX-2 differ at multiple AKBA contact points. Key differences in the binding cavities are highlighted in pink. (The hydrocarbon portions of Lys133 and Thr137 contact the ligand, hence their classification as hydrophobic in the table.)



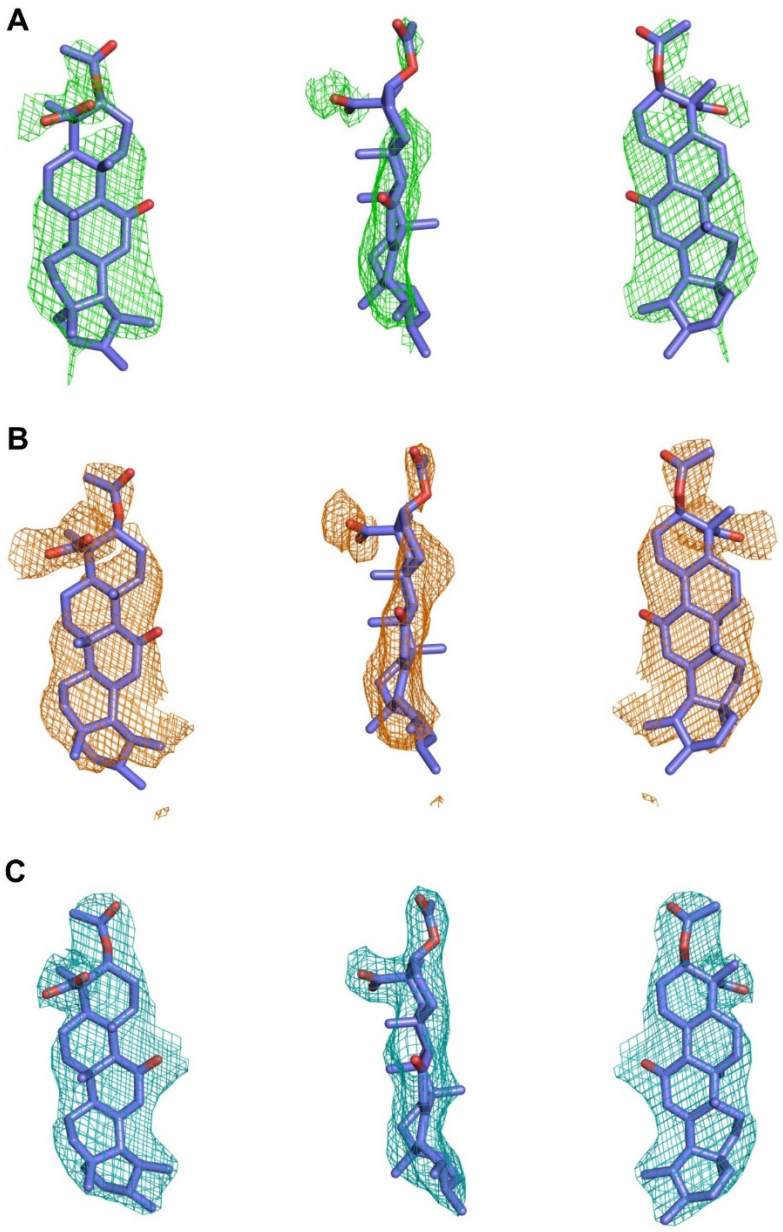
Supplementary Figure 1. Electron density for NDGA. (A) Omit map (Fo - Fc) of NDGA is shown as green mesh and contoured at 3.0 σ. NDGA is represented as sticks (C, pink; O, red) with images rotated by 90°. **(B)** Polder[1] omit map is shown as orange mesh and contoured at 3 σ. **(C)** Electron density (2Fo – Fc) is shown as cyan mesh and is contoured at 1.0 σ. All maps are carved to 1.6 Å around NDGA.



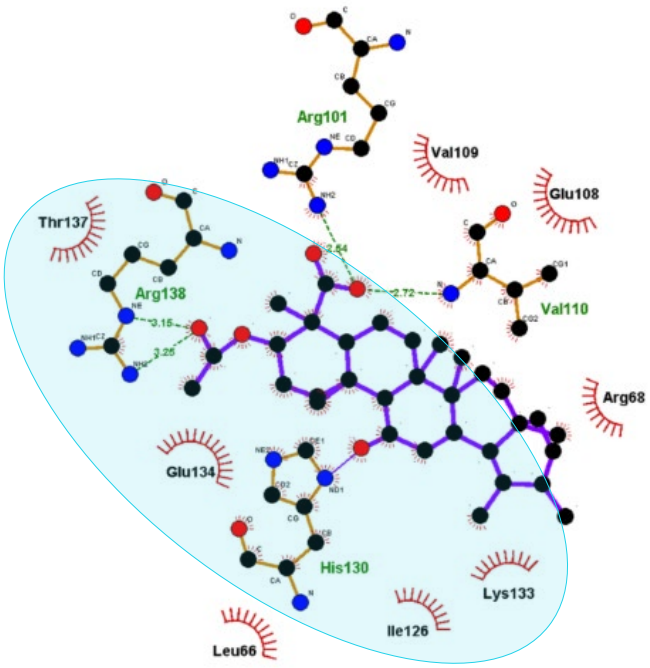
Supplementary Figure 2. Schematic of the NDGA binding site in Stable-5-LOX. Drawing generated with LigPlot v.2.1[2]. Amino acids that line the cavity and contribute van der Waal contacts are indicated with spoked arcs. Those amino acids that make polar contacts are rendered as ball and stick. Ligand atoms that make contact with the protein are indicated with red spokes.



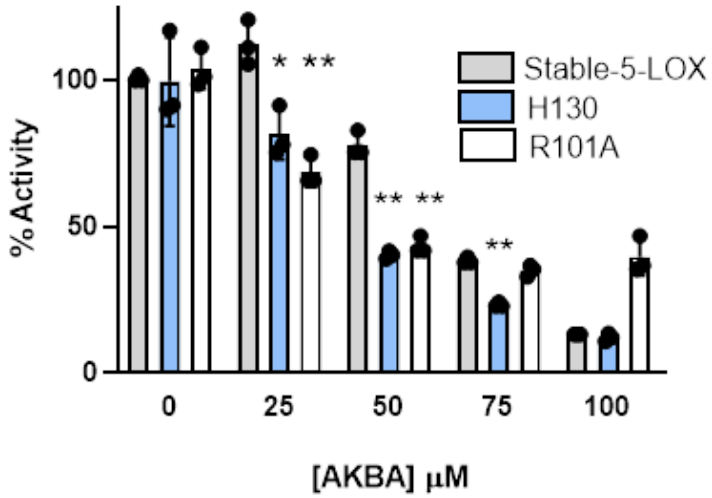
Supplementary Figure 3. Limited proteolysis of Stable-5-LOX in the presence of small molecules. SDS gel of limited proteolysis experiments as described in Materials and Methods: Lanes 1 and 5, MW markers, kD; lane 2, DMSO (carrier); lane 3, arachidonic acid (150 μM); lane 4, NDGA (150 μM), and lane 6, caffeic acid (150 μM). Only NDGA dramatically increases the 5-LOX susceptibility to proteolysis by pepsin.



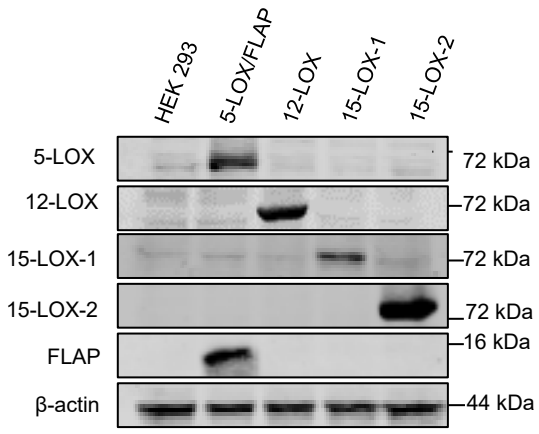
Supplementary Figure 4. Electron density for AKBA. (A) Omit map (Fo - Fc) of AKBA is shown as green mesh and contoured at 3 σ. AKBA is represented as sticks (C, blue; O, red) with images rotated by 90°. **(B)** Polder[1] omit map is shown as orange mesh and contoured at 3 σ. **(C)** Electron density (2Fo – Fc) is shown as cyan mesh and is contoured at 1.5 σ. All maps are carved to 1.6 Å of AKBA.



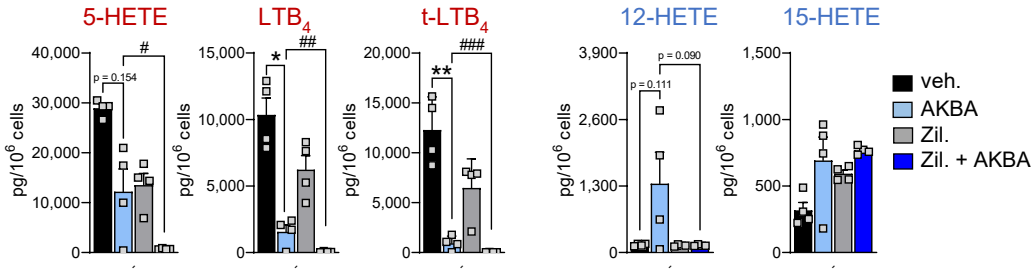
Supplementary Figure 5. Schematic of the AKBA binding site in Stable-5-LOX. Drawing generated with LigPlot v.2.1[2]. Amino acids that line the cavity and contribute van der Waal contacts are indicated with spoked arcs. Those amino acids that make polar contacts are rendered as ball and stick. Ligand atoms that make contact with the protein are indicated with red spokes. The oval encircles the amino acids of the catalytic domain that contribute to binding to AKBA.



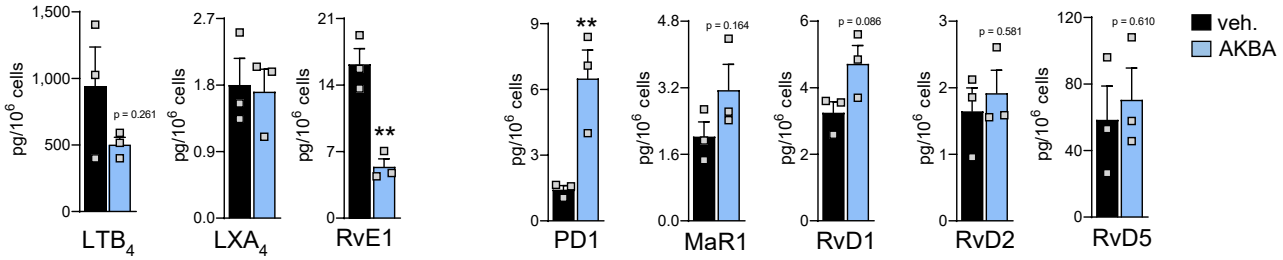
Supplementary Figure 6. Mutations to the AKBA binding site in Stable-5-LOX result in changes in AKBA sensitivity. Stable-5-LOX and the H1301A and R101A mutants were assayed in triplicate in the presence of AKBA at the indicated concentrations, as described in Material and Methods. Both mutations render the enzyme more sensitive to the presence of AKBA than wild-type Stable-5-LOX. Students paired t-test vs. Stable-5-LOX: * $p < 0.0001$, ** $p < 0.00001$.



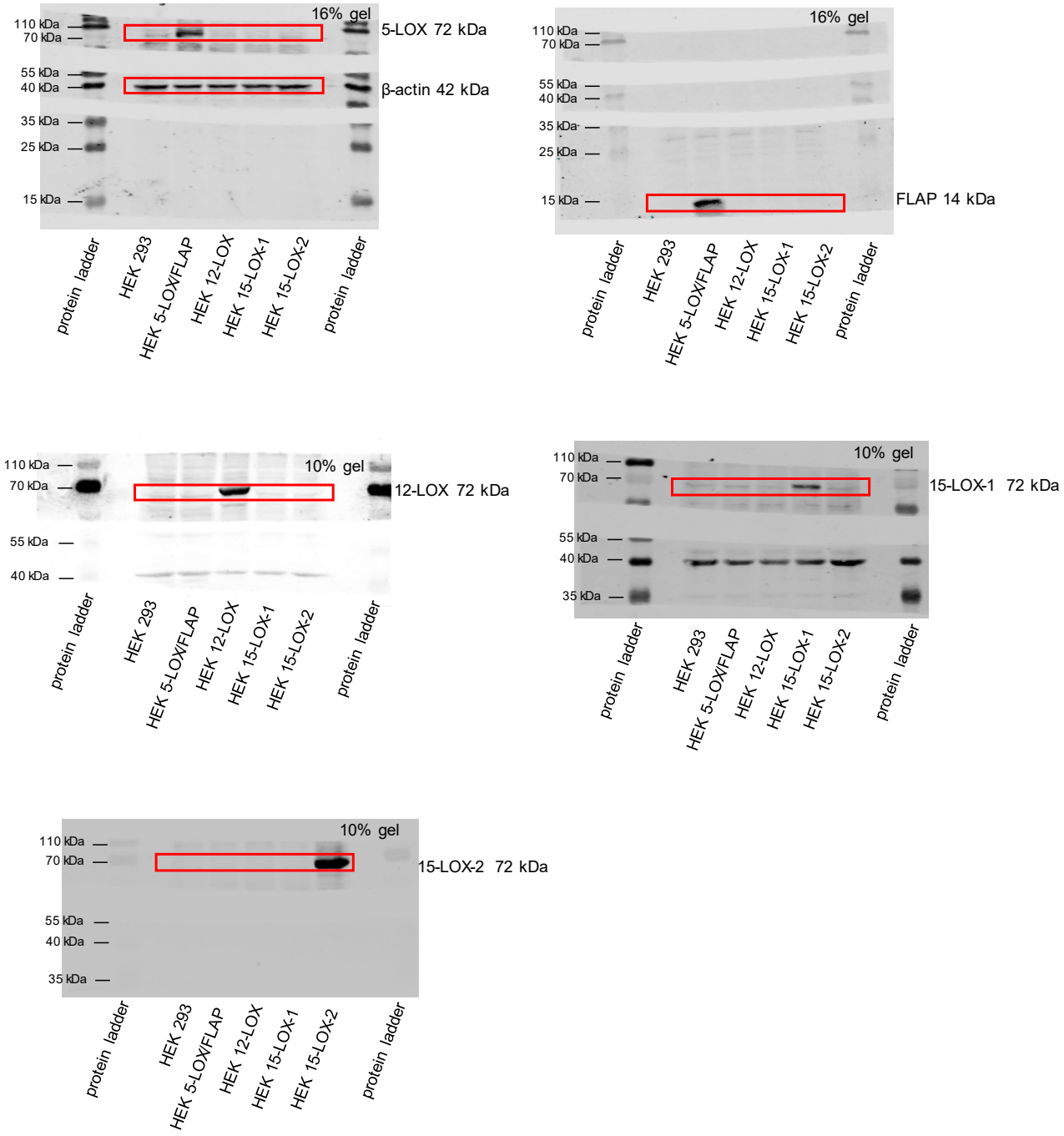
Supplementary Figure 7. Western blot analysis of LOXs and FLAP in HEK293 cells. Cell lysates of HEK293 cells corresponding to 2 × 10^6 cells were separated on 10% polyacrylamide gels for 5-LOX, 12-LOX, 15-LOX-1 and 15-LOX-2 or on a 16% polyacrylamide gels for FLAP, and blotted onto nitrocellulose membranes (Amersham™ Protran Supported 0.45 µm nitrocellulose, GE Healthcare, Freiburg, Germany). Uncropped blots are presented in **Supplementary Figure 10.**



Supplementary Figure 8. Zileuton abolishes AKBA-induced 12-HETE formation in 5-LOX/FLAP-expressing HEK293 cells. 1 x 10^6 HEK293 cells expressing 5-LOX and FLAP were pretreated with 25 µM AKBA, 3 µM zileuton (zil.) or vehicle (0.1% DMSO) for 10 min before stimulation with 2.5 µM A23187 plus 1 µM AA for another 10 min. Formed LM were analysed by UPLC-MS-MS, shown in pg/10^6 cells of n = 4 independent experiments. Data were log-transformed for statistical analysis, *p < 0.05, **p < 0.01 AKBA vs. veh. control; #p < 0.05, ##p < 0.01, ###p < 0.001 Zil. + AKBA vs. AKBA ANOVA + Dunnett`s multiple comparisons test.



Supplementary Figure 9. Effects of AKBA on SPM formation in 5-LOX/FLAP-expressing HEK293 cells. 1 x 10^6 HEK293 cells expressing 5-LOX and FLAP were incubated with 25 µM AKBA or vehicle plus 1 µM AA, 1 µM EPA and 1 µM DHA for 90 min before stimulation with 2.5 µM A23187 for another 10 min. Formed LM were isolated by SPE and analysed by UPLC-MS-MS. Data are shown as pg/10^6 cells of n = 3 independent experiments. Data were log-transformed for statistical analysis, $^{**}p < 0.01$ AKBA vs. vehicle, Student´s paired t-test.



Supplementary Figure 10. Uncropped blots and gels shown in Supplementary Figure 7.

References

1. Liebschner, D. *et al.* Polder maps: improving OMIT maps by excluding bulk solvent. *Acta crystallographica. Section D, Structural biology* 73, 148-157, doi:10.1107/S2059798316018210 (2017).
2. Laskowski, R. A. & Swindells, M. B. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J Chem Inf Model* **51**, 2778-2786, doi:10.1021/ci200227u (2011).