Supplementary Material

Table S1. The plasma levels of oxylipin mediators during selective modulation of COX, LOX and sEH branches in a murine model of inflammation

Table S2. The plasma levels of key oxylipin mediators in Ephx 2-null mice and their wild-type counterparts.

Table S3. The plasma levels of IL-6 during selective modulation of COX, LOX and sEH branches in a murine model of inflammation

Fig. S1. The sEHI *t*-AUCB synergized with aspirin and MK 886 in suppressing the induction of COX-2 or 5-LOX in the murine LPS-induced inflammation model but had no effect on COX-1 protein expression.

Fig. S2. Hepatic expression of the 5-LOX protein following LPS exposure is lower in *Ephx2*-null mice (gray bar) compared to wild-type (black bar).

Fig. S3. Dual inhibition of sEH and 5-LOX synergistically inhibits the production of 5-oxo-ETE.

Materials and Methods

1. Measurements of plasma cytokines

Plasma cytokine levels were analyzed using a CBA mouse inflammation kit. Briefly, thawed plasma samples (30 μL each) were mixed for 2 hours at room temperature with florescence-labeled capture beads with the PE detection reagents to measure the concentrations of interleukin-6 (IL-6), monocyte chemoattractant protien-1 (MCP-1), tumor necrosis factor-α (TNF-α) and Interferon-gamma (IFN-γ). Samples were then washed with washing buffer and analyzed on a FACScan flow cytometer (BD Immunocytometry Systems); Data were analyzed using BD Cytometric Bead Array Analysis software (BD Immunocytometry Systems).

2. Immunoblot Analysis

Western blot analysis was performed as described previously on the S-9 fraction of murine hepatic homogenates[1]. These proteins were separated by gel electrophoresis using a precast 10% SDS/PAGE gel and transferred onto PVDF membranes (Immobilon P; Millipore, Billerica, MA). The COX-1, COX-2 and 5-LOX proteins were detected with polyclonal antibodies from Cayman Chemical Company (Ann Arbor, MI) and with a horseradish peroxidase-linked IgG whole secondary antibody (Amersham Pharmacia Biosciences) at 1:5,000 dilution. The signals were visualized using a SuperSignal West Femto Substrate chemiluminescence detection system (Pierce, Rockford, IL) and detected by autoradiography. The immunodetectable bands were quantified by densitometry using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Table S1. The plasma levels of oxylipin mediators during selective modulation of COX, LOX and sEH branches in a murine model of inflammation (to be continued).

Group	LPS (mg/kg)	*t*-AUCB (mg/kg)	Aspirin (mg/kg)	MK 886 (mg/kg)	TXB_2 (nM)	6-keto-PGF1a (nM)	PGE_2 (nM)	PGD_2 (nM)	5-HETE (nM)	15-HETE (nM)	14,15-DHET (nM)	11,12-DHET (nM)
1	-	-	-	-	4.36±1.43	6.27±2.14	0.45±0.08	0.40±0.04	3.43±0.30	6.56±1.17	1.28±0.17	0.87±0.13
2	10	-	-	-	15.38±3.02*	30.90±4.53*	1.73±0.26*	3.38±0.51*	12.04±2.63*	14.26±3.84*	4.89±1.57	2.59±0.75*
3	10	0.5	-	-	5.63±2.36†	20.61±11.26	1.30±0.36†	2.84±1.16	6.51±1.26†	2.32±1.13†	1.95±0.91	0.92±0.30†
4	10	-	50	-	6.02±1.92†	7.38±3.09†	0.82±0.20†	1.35±0.64†	6.53±1.62	5.22±0.44†	2.24±0.31	1.44±0.34†
5	10	-	80	-	2.95±1.82†	5.48±1.41†	0.58±0.10†	2.20±1.04	3.14±0.47†	3.09±0.59†	1.42±0.05	0.78±0.07
6	10	0.5	50	-	2.43±0.59†,§	4.03±1.06†	0.37±0.08†,‡,§	1.14±0.01†	4.78±1.80†	3.63±1.48†	1.46±0.34	0.86±0.16
7	10	-	-	10	4.38±1.71†	4.56±1.08†	0.40±0.08†	1.48±0.53†	4.93±0.64†	3.40±0.92†	1.44±0.36	1.09±0.05
8	10	-	-	20	1.99±1.08†	4.13±0.34†	0.33±0.13†	0.72±0.64†	4.17±1.25†	2.41±0.66†	1.99±0.31	1.07±0.14
9	10	0.5	-	10	1.40±0.14†,‡	5.60±1.75†	0.56±0.04†	1.28±0.32†	2.70±0.33†,‡,¶	1.55±0.20†,¶	1.49±0.26	0.67±0.13†,¶

Data represent mean ± sd (n=4). *, significant different from the group 1; †, significantly different from the group 2; ‡, significantly different from group 3; §, significantly difference from group 4; ¶, significantly different from group 7. Significant difference ($P < 0.05$) was determined by ANOVA followed with Tukey's or Game-Howell's test.

Table S1. The plasma levels of oxylipin mediators during selective modulation of COX, LOX and sEH branches in a murine model of inflammation (continued).

Group	LPS (mg/kg)	*t*-AUCB (mg/kg)	Aspirin (mg/kg)	MK 886 (mg/kg)	8,9-DHET (nM)	5,6-DHET (nM)	14,15-EET (nM)	11,12-EET (nM)	8,9-EET (nM)	SUM(DHET) (nM)	SUM(EET) (nM)
1	-	-	-	-	3.27±0.99	0.47±0.01	1.16±0.40	2.52±1.06	2.10±0.67	5.43±1.08	5.78±2.10
2	10	-	-	-	5.53±0.98*	1.54±0.28*	0.82±0.21	1.72±0.82	1.50±0.54	13.0±2.94*	4.04±1.56
3	10	0.5	-	-	1.52±0.10†	0.39±0.07†	1.24±0.48	1.06±0.32	0.98±0.22	4.38±1.26†	3.28±1.00
4	10	-	50	-	3.99±0.68	0.99±0.68	1.32±0.13	2.00±0.42	1.46±0.61	7.68±1.28†	4.78±0.99
5	10	-	80	-	2.08±0.10†	0.51±0.06†	0.69±0.13	0.61±0.38	0.87±0.24	4.28±0.20†	2.17±0.54
6	10	0.5	50	-	2.47±0.93†	0.53±0.18†,§	1.04±0.39	1.54±0.65	1.49±0.51	4.80±1.17†,§	4.07±1.38
7	10	-	-	10	31.14±5.54†	0.62±0.18†	1.08±0.34	1.07±0.45	2.20±1.36	33.66±5.34†	4.36±1.56
8	10	-	-	20	25.77±6.14†	0.63±0.37†	0.70±0.31	1.10±0.75	1.26±0.67	28.83±6.34†	3.06±1.35
9	10	0.5	-	10	2.31±0.51†,¶	0.54±0.73†	1.52±0.29	1.24±0.24	1.40±0.25	4.46±0.62†,¶	4.16±0.40

Data represent mean ± sd (n=4). *, significant difference from the group 1; †, significantly different from the group 2; ‡, significantly different from group 3; §, significantly different from group 4; ¶, significantly different from group 7. Significant difference ($P < 0.05$) was determined by ANOVA followed with Tukey's or Game-Howell's test.

Table S2. The plasma levels of key oxylipin mediators in *Ephx*-null mice and their wild-type counterparts.

	Wildtype (n=6)				sEH knockout (n=4)			
Group	1	2	3	4	5	6	7	8
LPS (mg/kg)	-	10	10	10	-	10	10	10
Aspirin (mg/kg)	-	-	50	-	-	-	50	-
MK886 (mg/kg)	-	-	-	10	-	-	-	10
PGE_2 (nM)	0.15 ± 0.05	0.64±0.22†	0.31 ± 0.06‡	0.64±0.37†	0.13 ± 0.05	0.30±0.05*,†	0.22 ± 0.02*,‡	0.33±0.13†
PGD_2 (nM)	0.10±0.05	0.27±0.08†	0.11 ± 0.03‡	0.35±0.06†	0.13±0.08	0.28±0.08†	0.07 ± 0.01*,‡	0.20±0.05
TXB_2 (nM)	0.34±0.15	1.85±1.80	0.56±0.23	0.52±0.21	0.61±0.11	0.40±0.12	0.37±0.11	0.45±0.24
6-keto-PGF_{1a} (nM)	2.63±0.96	6.00±2.62	6.29±2.46	9.06±4.16†	3.22±1.40	3.52±0.44	6.18±0.73	3.82±0.74
5-HETE (nM)	1.03 ± 0.32	5.25 ± 1.74†	2.66 ± 0.89	1.70 ± 0.40‡	1.45 ± 0.15	3.49 ± 0.70†	2.06 ± 0.42	0.98± 0.21*,‡
15-HETE (nM)	1.41± 0.57	3.42±1.94	3.68±1.78	2.63±1.62	1.74±0.49	1.64±0.32	2.25±0.49	1.23±0.23
14,15-DHET (nM)	1.53±0.67	1.99±0.66	1.51±0.46	1.31±0.37	0.50±0.37*	0.34±0.13*	0.41±0.06*	0.22±0.09*
11,12-DHET (nM)	0.92±0.47	0.93±0.29	0.65±0.19	0.63±0.12	0.86±0.68	0.51±0.15	0.65±0.10	0.35±0.16*
8,9-DHET (nM)	0.66±0.25	1.28±0.32	0.71±0.19‡	0.69±0.11‡	0.68±0.25	0.62±0.20*	0.57±0.08	0.45±0.04*
5,6-DHET (nM)	0.31±0.12	0.68±0.20	0.28±0.11‡	0.30±0.10‡	0.25±0.09	0.44±0.23	0.28±0.03	0.18±0.04
14,15-EET (nM)	0.44±0.10	0.82±0.32	0.67±0.09	0.73±0.22	2.40±1.12*	3.21±1.02*	2.83±0.31*	1.96±0.63*
11,12-EET (nM)	0.31±0.06	0.64±0.14†	0.76±0.20	0.39±0.04‡	1.40±0.80*	1.57±0.40*	1.53±0.18*	0.91±0.16*
8,9-EET (nM)	0.24±0.04	0.53±0.14†	0.44±0.16	0.29±0.25	0.98±0.30*	1.29±0.40*	1.16±0.03*	0.72±0.15*
5,6-EET (nM)	5.55±1.57	10.93±3.42†	8.02±2.51	5.14±1.38‡	13.52±4.88*	18.22±4.16	15.89±2.89*	15.78±1.47*

Data represent mean ± sd. *, significant difference from the wild-type counterparts; †, significantly different from the control group (1 or 5) without LPS; ‡, significantly different from LPS only group (2 or 6). Significant difference ($P < 0.05$) was determined by ANOVA followed with Tukey's or Game-Howell's test.

Table S3. The plasma levels of key cytokines and chemokine during selective modulation of COX, LOX and sEH branches in a murine model of inflammation (to be continued).

Group	LPS (10 mg/kg)	*t*-AUCB (mg/kg)	Aspirin (mg/kg)	MK 886 (mg/kg)	IL-6 (pg/mL)		TNF-α (pg/mL)	
					6 h	24 h	6 h	24 h
1	-	-	-	-	45 ± 35	4 ± 2	18 ± 4	11 ± 1
2	10	-	-	-	4870 ± 3250*	124 ± 15*	225 ± 65*	38 ± 8*
3	10	0.5	-	-	5950 ± 2440*	92 ± 8*,†	275 ± 40	27 ± 1*,†
4	10	-	50	-	1370 ± 295*,†	107 ± 25*	150 ± 30*,†	31 ± 5*
5	10	0.5	50	-	6020 ± 2640*	93 ± 8*,†	205 ± 70*	37 ± 8*
6	10	-	-	10	7800 ± 2670*	243 ± 122*	250 ± 35*	33 ± 5*
7	10	0.5	-	10	9000 ± 770*	70 ± 40*,†,‡	300 ± 35*	33 ± 10*

Data represent mean ± sd (n=4). *, significant difference from the group 1; †, significantly different from the group 2; ‡, significant difference from the group 6. Significant difference ($P < 0.05$) was determined by ANOVA followed with Tukey's or Game-Howell's test.

Table S3. The plasma levels of key cytokines and chemokine during selective modulation of COX, LOX and sEH branches in a murine model of inflammation (continued).

Group	LPS (10 mg/kg)	*t*-AUCB (mg/kg)	Aspirin (mg/kg)	MK 886 (mg/kg)	MCP-1 (pg/mL)		IL-10 (pg/mL)		INF-γ (pg/mL)	
					6 h	24 h	6 h	24 h	6 h	24 h
1	-	-	-	-	105 ± 25	90 ± 15	90 ± 35	40 ± 15	8 ± 1	1.4 ± 0.1
2	10	-	-	-	8980 ± 280*	2045 ± 130*	125 ± 20	150 ± 30*	605 ± 330*	1.8 ± 0.5
3	10	0.5	-	-	10050 ± 980	1490 ± 450*,†	205 ± 95	170 ± 45*	675 ± 280*	2.1±0.9
4	10	-	50	-	6850 ± 2060*	1825 ± 580*	120 ± 30	140 ± 25*	65 ± 50*,†	1.3 ± 0.8
5	10	0.5	50	-	9300 ± 1360*	2495 ± 590*	130 ±30	155 ± 20*	370 ± 300*	1.6 ± 0.2
6	10	-	-	10	9320 ± 530*	2030 ± 200*	275 ± 40*,†	140 ± 25*	540 ± 300*	2.2 ± 1.3
7	10	0.5	-	10	8130 ± 2520*	1545 ± 110*,†,‡	165 ± 35*	115 ± 20*	625 ±230*	1.6 ± 0.3

Data represent mean ± sd (n=4). *, significant difference from the group 1; †, significantly different from the group 2; ‡, significant difference from the group 6. Significant difference (P < 0.05) was determined by ANOVA followed with Tukey's or Game-Howell's test.

Fig. S1. The sEHI *t*-AUCB synergized with aspirin and MK 886 in suppressing the induction of COX-2 or 5-LOX in the murine LPS-induced inflammation model but had no effect on COX-1 protein expression. LPS administration expectedly led to a robust increase in the levels of COX-2 and 5-LOX proteins. (***A***) *t*-AUCB significantly reduced the hepatic expression of COX-2 protein 6 h following LPS administration while aspirin and MK 886 had no effect on COX-2 expression. However the co-administration of *t*-AUCB and aspirin reduced hepatic COX-2 levels in an additive manner. (***B***) MK 886 and aspirin significantly reduced the hepatic expression of 5-LOX protein 6 h following LPS administration while *t*-AUCB had less effect on suppressing 5-LOX expression. However, co-administration of *t*-AUCB and MK 886 reduced hepatic 5-LOX levels in an additive manner. EETs have been shown to reduce the translocation of the nuclear factor NFκB and the suppression by sEHI observed here may be linked to this activity. (***C***) Aspirin, t-AUCB and MK 886 had no effect on the hepatic expression of COX-1. Data represent the relative protein levels ± SD (n=4) in murine liver after treatment as determined by Western blotting. The data are depicted as percentage of the amount of COX-2 , 5-LOX or COX-1 in control mice receiving vehicle without LPS. Different letters denoted significant difference among groups. Significantly different ($P < 0.05$) determined by ANOVA followed with Tukey's test.

Fig. S2. Hepatic expression of the 5-LOX protein following LPS exposure is lower in *Ephx2*-null mice (gray bar) compared to wild-type (black bar). In parallel to the data presented in Fig. S3, a lower level of upregulation of the 5-LOX protein was observed in *Ephx2*-null mice. The *Ephx2*-null mice, however, had lower baseline 5-LOX levels and displayed a lower level of induction upon LPS administration. The FLAP inhibitor MK886 reduced the induction of 5-LOX not only in wild-type mice but also in *Ephx2*-null mice thus in *Ephx2*-null mice the total 5-LOX levels

were about half of the wild-type mice after MK 886 administration. These data predict a synergistic interaction between chemicals which reduce arachidonic acid flow through the 5-LOX pathway and those which stabilize or mimic EETs. Data represent the average ± SD (n=4 and 6 for *Ephx2*-null and wild mice, respectively.), and are depicted as percentage of wild-type control animals receiving vehicle without LPS. * Significantly different from wild-type mice with same treatment, and † significantly different ($P < 0.05$) determined by ANOVA followed by Tukey's test.

Fig. S3. Dual inhibition of sEH and 5-LOX synergistically inhibits the production of 5-oxo-ETE. LPS administration led to significant increases in 5-oxo-ETE levels. *t*-AUCB, effective by itself, however, when co-administered with MK 886, led to a synergistic decrease in the production of 5-oxo-ETE. MK 886 expectedly decreased the production of 5-oxo-ETE. The data suggest that inhibition of sEH is beneficiary to the 5-LOX inhibition. Since animals treated with sEH inhibitors reduce the levels of 5-oxo-ETE alone or in combination with MK 886, and 5-oxo-ETE is A PPAR-γ agonist which induce sEH, this cascade should provide a negative feedback loop on sEH induction. The data represent average ± sd (n=4). * Significant different from normal control, †, significant different from LPS control, and # significant different from individual treatment. Significantly different ($P < 0.05$) determined by ANOVA followed by Tukey's or Games-Howell's posthoc comparison test.

1. Schmelzer KR, Inceoglu B, Kubala L, Kim IH, Jinks SL*, et al.* (2006) Enhancement of antinociception by coadministration of nonsteroidal anti-inflammatory drugs and soluble epoxide hydrolase inhibitors. *P Natl Acad Sci USA* 103**:** 13646-13651.

Figure S1

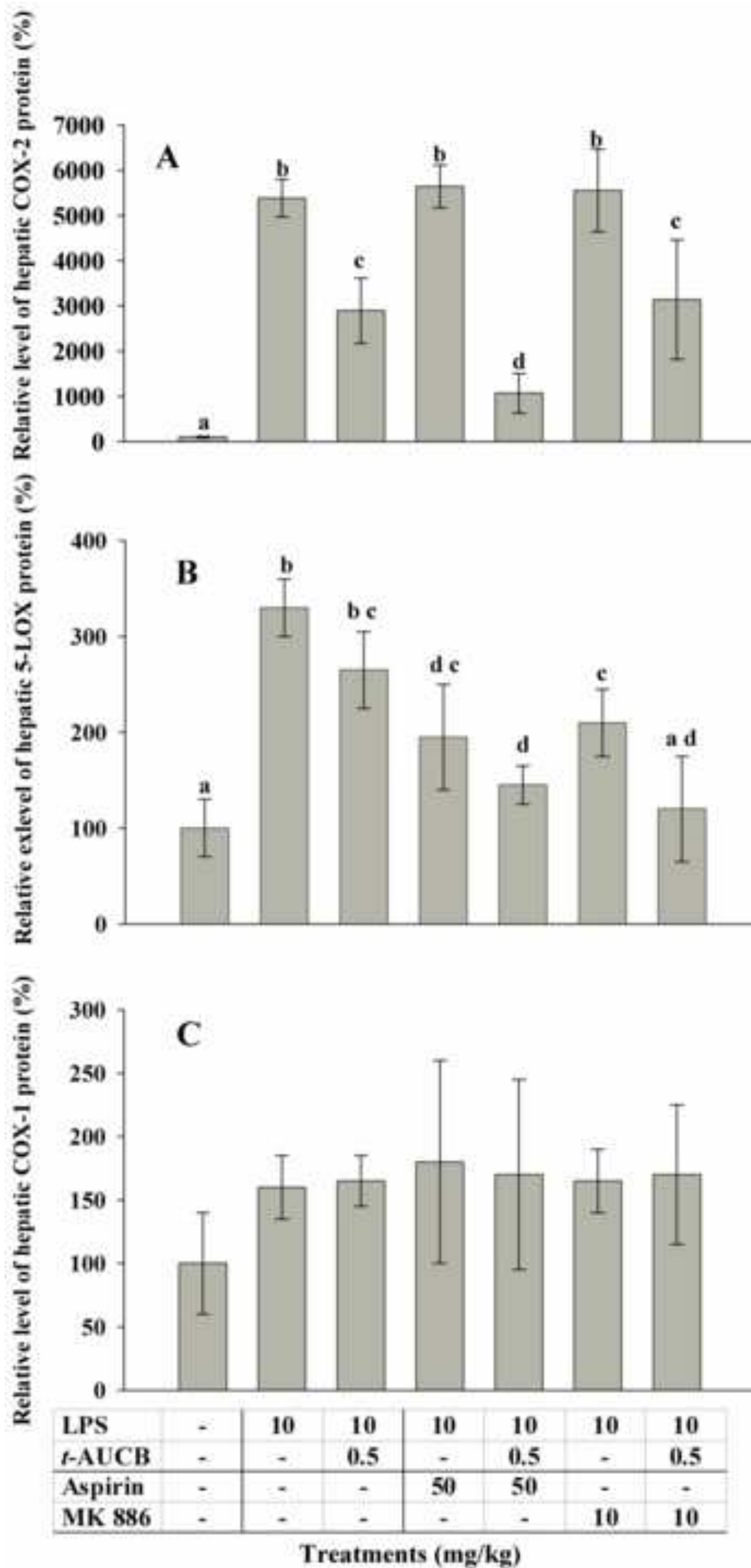

Figure S2

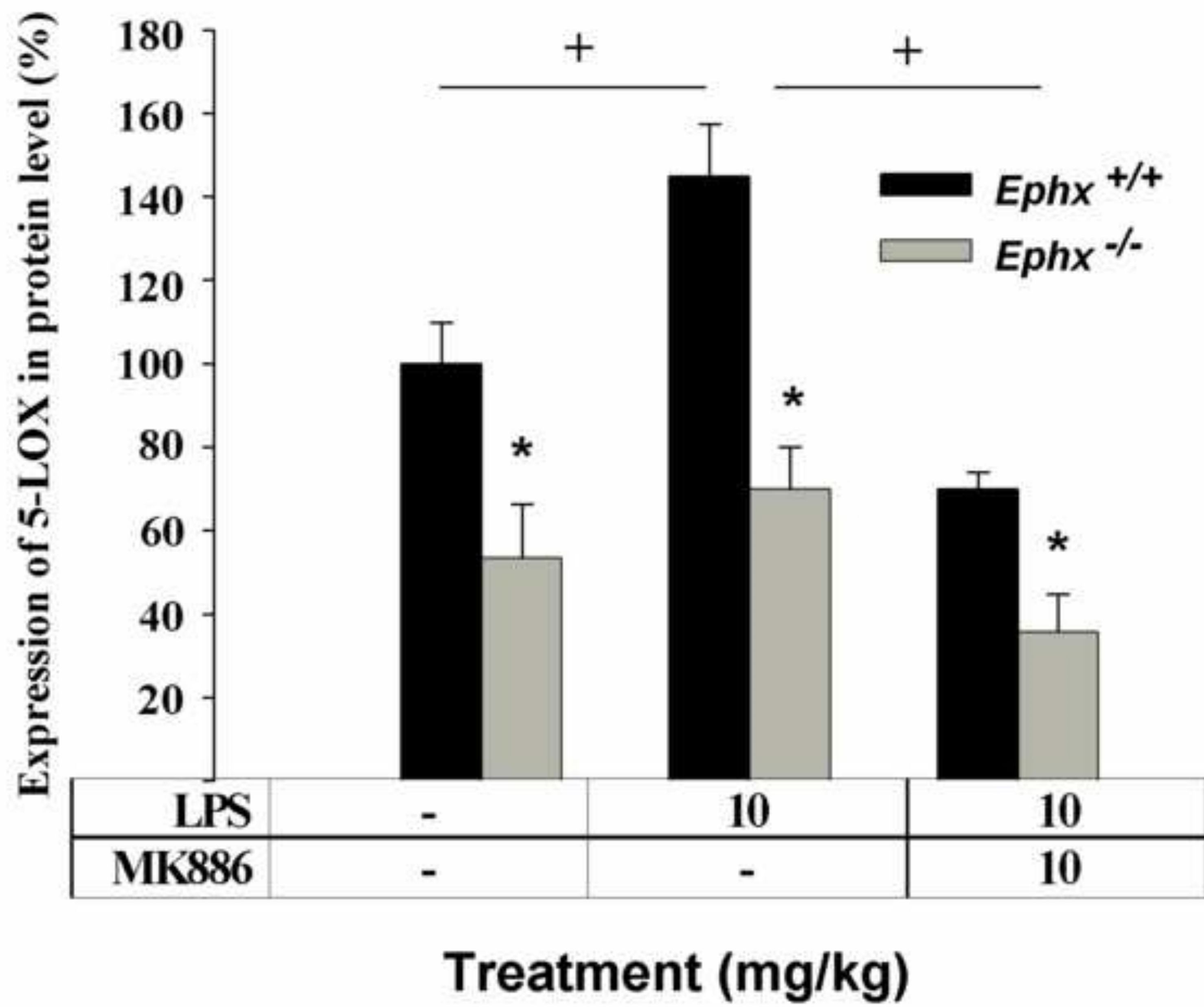

Figure S3