Figure S1

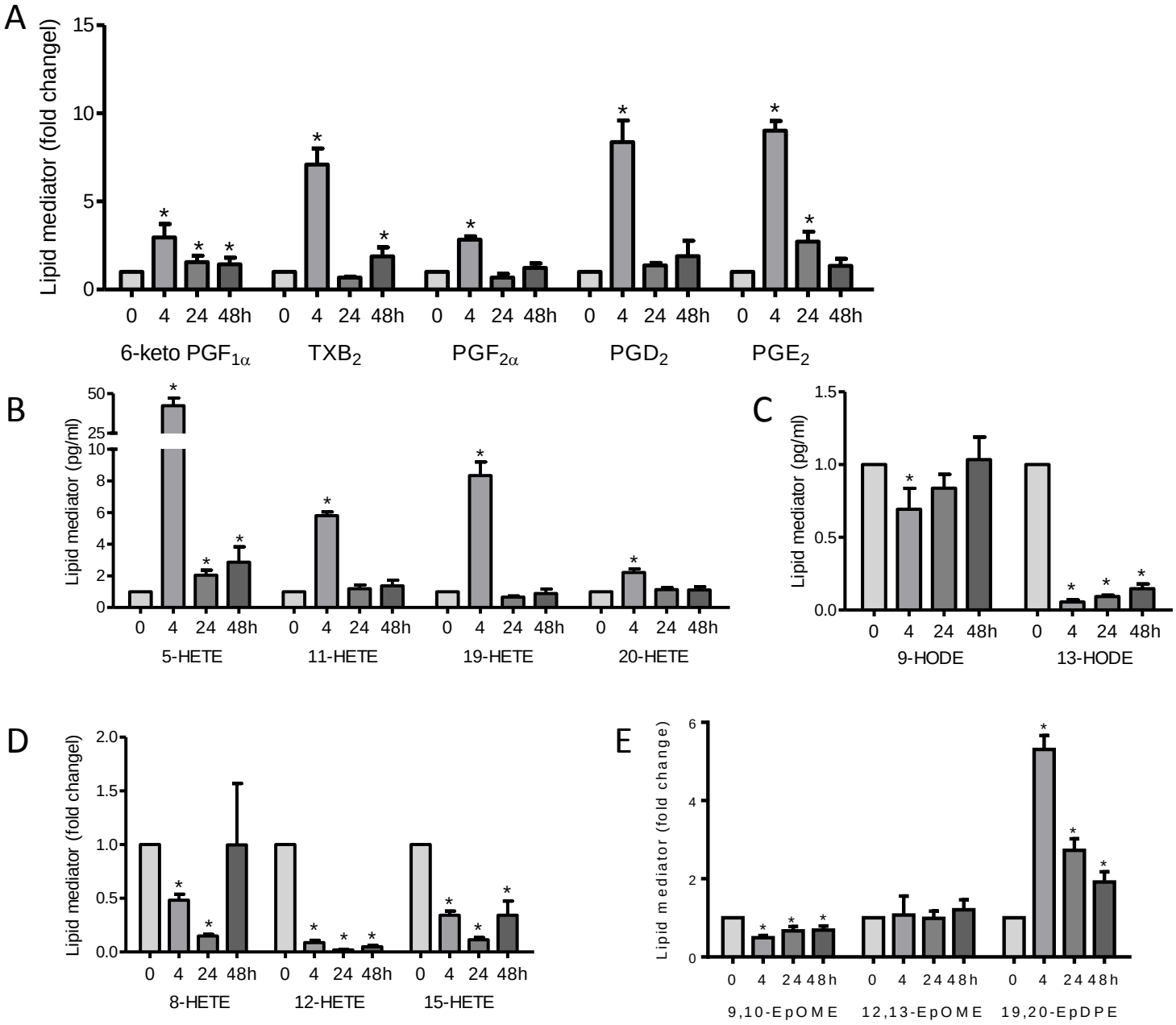

Figure S1. Oxylipin formation in acute inflammation and resolution. Mean fold change in (A) COX products: eicosanoids; (B) 5-, 11-, 19- and 20-HETE; 9- and 13-HODE; (D) 8-, 12-, and 15-HETE; and (E) 9,10- and 12,13-EpOME, and 19,20-EpDPE following zymosan A challenge (1mg i.p.) 0-48h. Each mediator was normalized to its paired levels found in the naïve cavity in each experiment; the levels found in the naïve cavity given an arbitrary value of 1. 4h represents the peak of acute inflammation (PMNs), resolution peaks at 48h. Data is mean ± s.e.m from 4-8 mice per group. *denotes $p<0.05$ by one-sample t-test from 0h.

Figure S2

Figure S2. Epoxygenase enzyme expression in acute inflammation and resolution. Examples of RT-PCR analysis of *Cyp2j5*, *Cyp2j6*, *Cyp2j9*, *Cyp2j13*, *Cyp2c44*, *Cyp2u1*, *PPAR-a* and *b-actin* in the cells from the peritoneal cavity of mice 0h, 24h, 48h and 72h post zymosan (1mg; i.p.). -RT indicates samples taken from 48h where the reverse transcriptase was omitted.

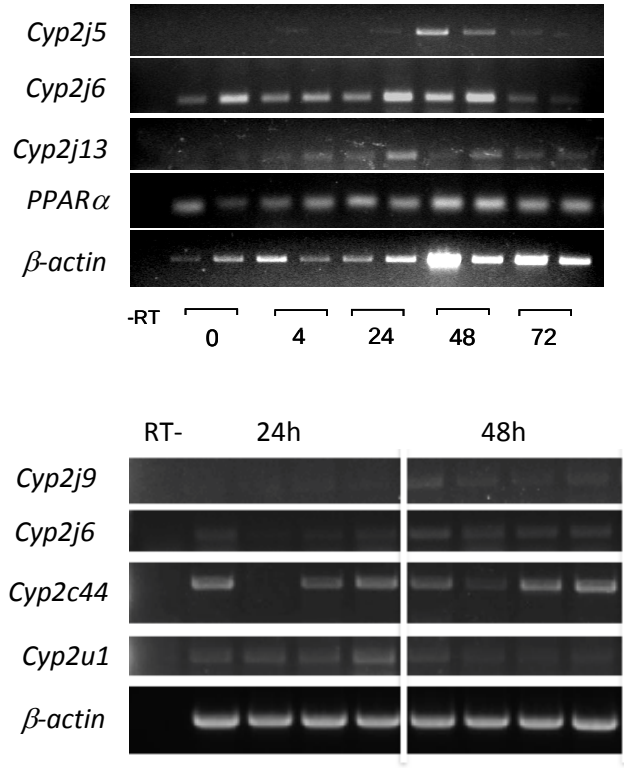

Figure S3

Figure S3. Time-course of monocyte and PMN accumulation during inflammatory resolution. Accumulation of (A) Ly6c$^+$ monocytes and (B) Ly6g$^+$ PMNs at 48h, 72h and 96h post zymosan (1mg; i.p.). Mice were treated from 24h onwards every 12h with either sterile PBS (open bars; vehicle) or epoxI (SKF525A; closed bars; 30mg/kg i.p.). Data represents the mean ± s.e.m of accumulated cells from n= 6 mice per experimental group.

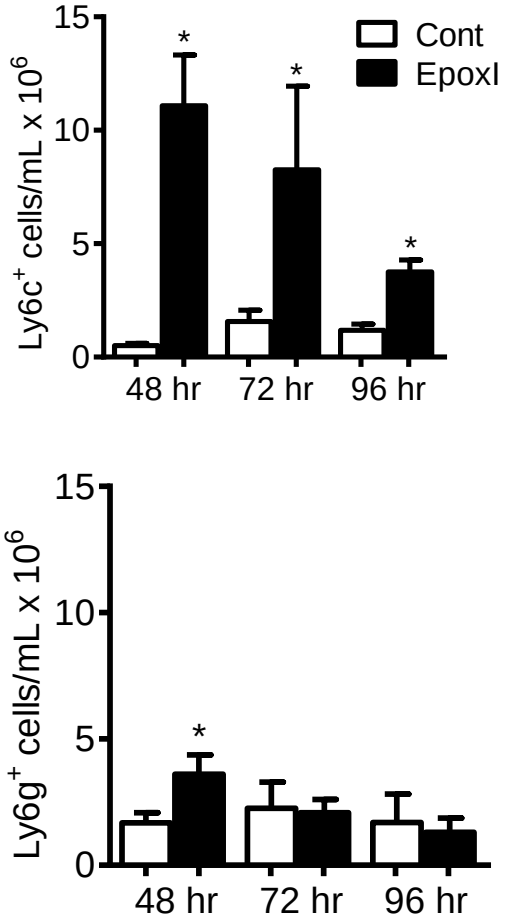

Figure S4

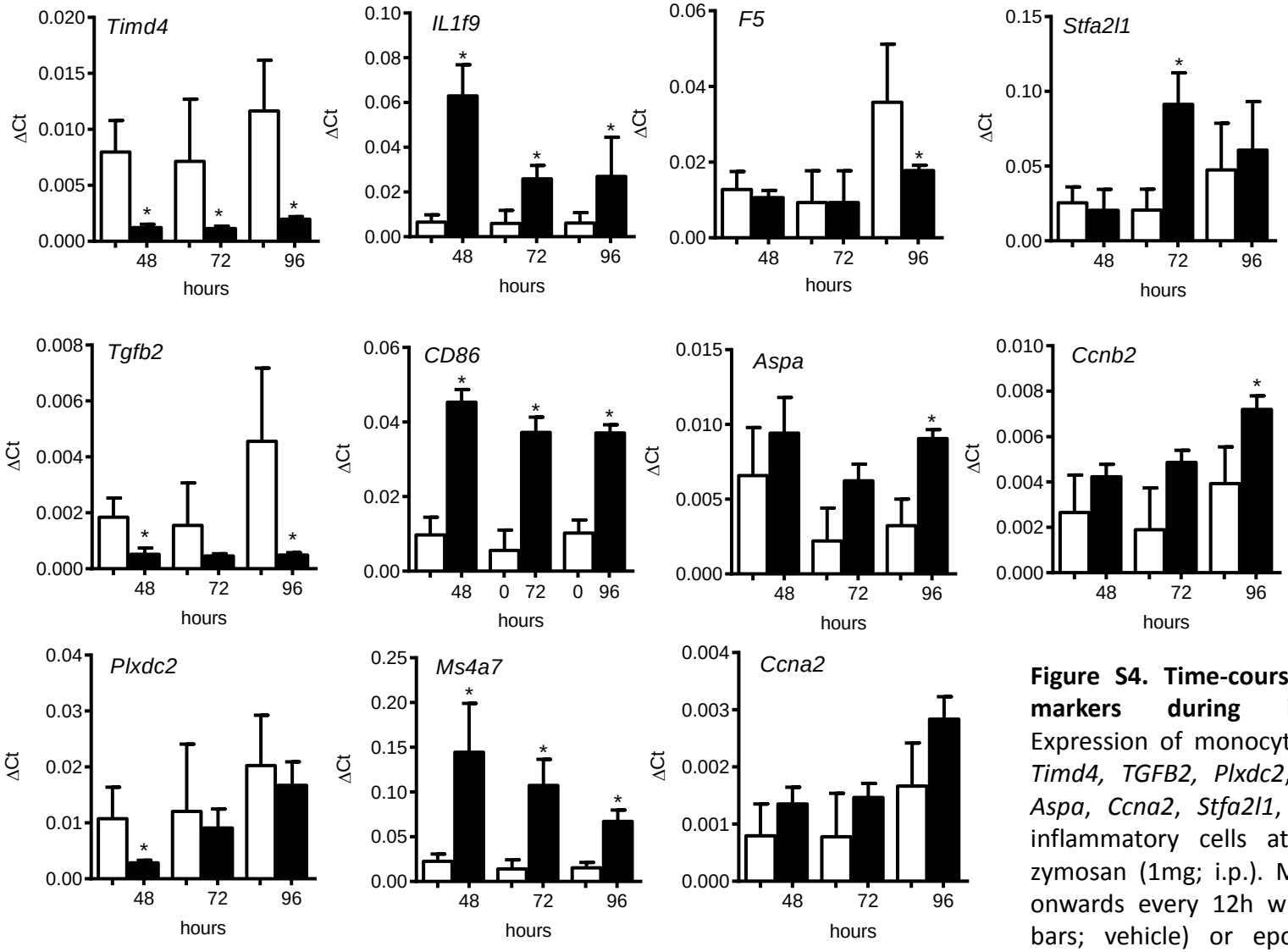

Figure S4. Time-course of resolution phenotype markers during inflammatory resolution. Expression of monocyte phenotype panel markers *Timd4, TGFB2, Plxdc2*, *IL1f19*, *CD86*, *Ms4a7*, *F5*, *Aspa*, *Ccna2*, *Stfa2l1*, and *Ccnb2* in the elicited inflammatory cells at 48h, 72h and 96h post zymosan (1mg; i.p.). Mice were treated from 24h onwards every 12h with either sterile PBS (open bars; vehicle) or epoxI (SKF525A; closed bars; 30mg/kg i.p.). Changes in mRNA levels were determined by qRT-PCR and the data represents the mean ± s.e.m in DCt from n= 4 mice per experimental group.

Figure S5

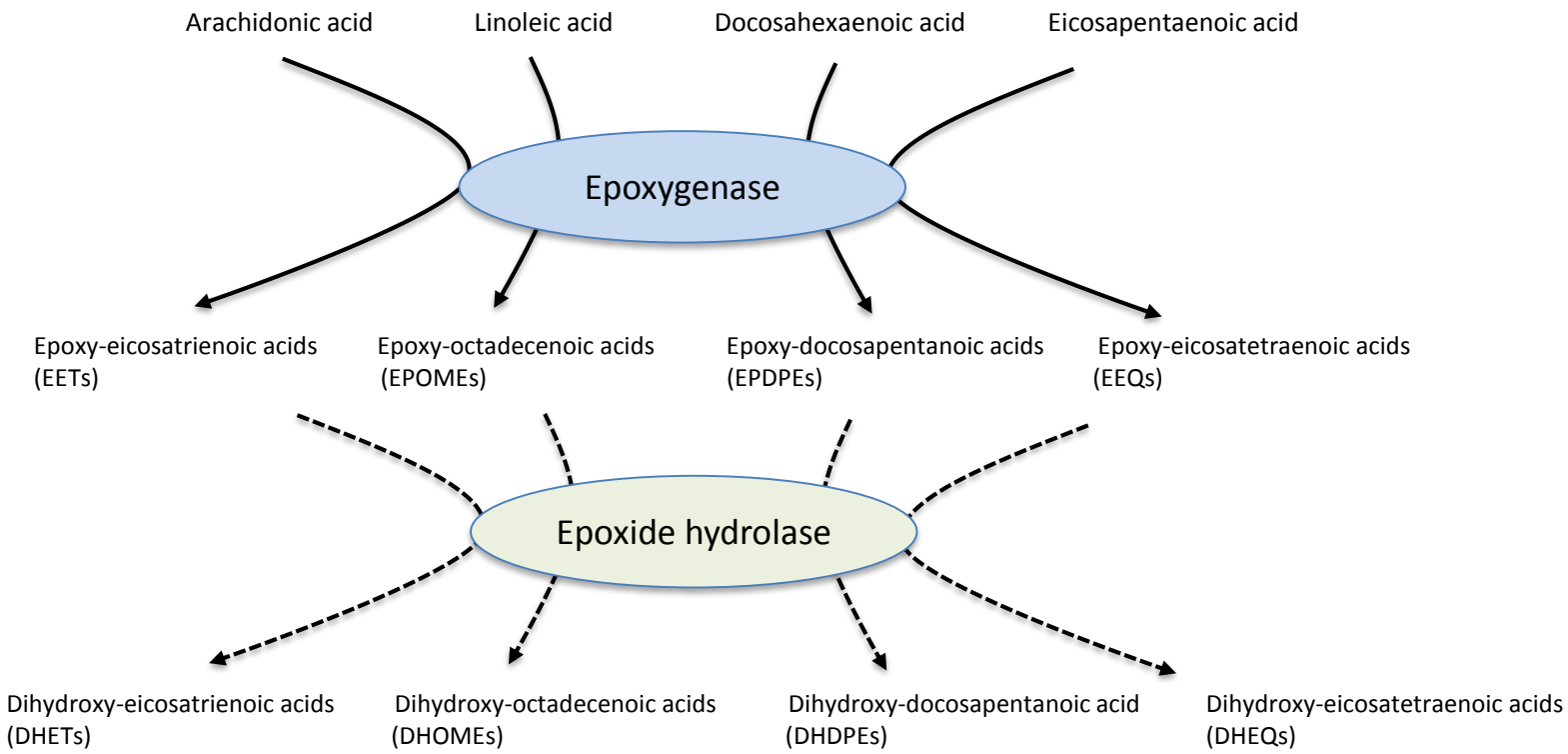

Figure S8. Epoxy-oxylipin family generation from epoxygenase enzymes. Epoxygenase enzymes utilize fatty acid substrates such as arachidonic acid, linoleic acid, docosahexaenoic acid and eicosapentaenoic acid to produce epoxy-oxylipins such as the EETs, EPOMEs, EPDPEs and EEQs respectively by insertion of molecular oxygen at the site of the double bond. These epoxy-oxylipin products are subsequently metabolized to DHETs, DHOMEs, DHDPEs and DHEQs respectively by epoxide hydrolase enzymes.

Figure S6

Figure S5. 9-EPOME induces Ccl2 mRNA in inflammatory monocytes *ex vivo.* Relative *Ccl2* mRNA expression compared to b-actin in zymosan elicited monocytes treated *ex vivo* with 9,10-EpOME (1mM) or 12,13-EpOME (1mM). Cells were elicited at 36h and treated for a further 7h. The data represents the mean ± s.e.m from n=3-5 mice per group. * denotes $p<0.05$ by one sample t-test between or either treatment.

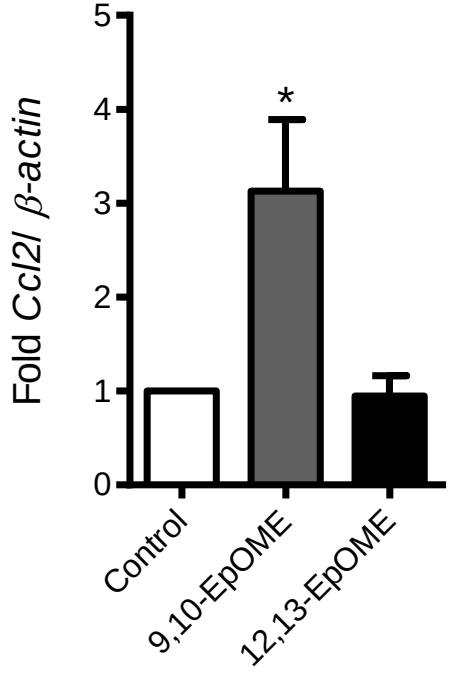

Figure S6

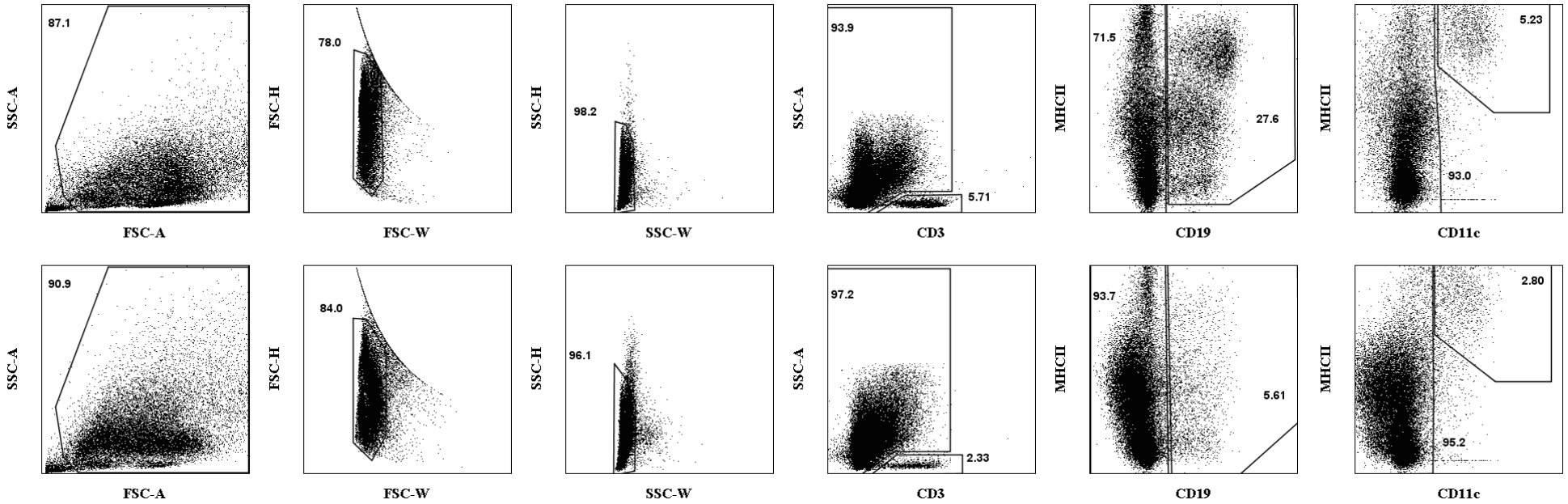

Figure S6. Supplemental Methods: flow cytometry gating strategy. Top panels show example of gated cells from vehicle control treated mice (48h), while bottom panels show comparable gating from EpoxI treated mice (30mg/kg; 48h).

Figure S7

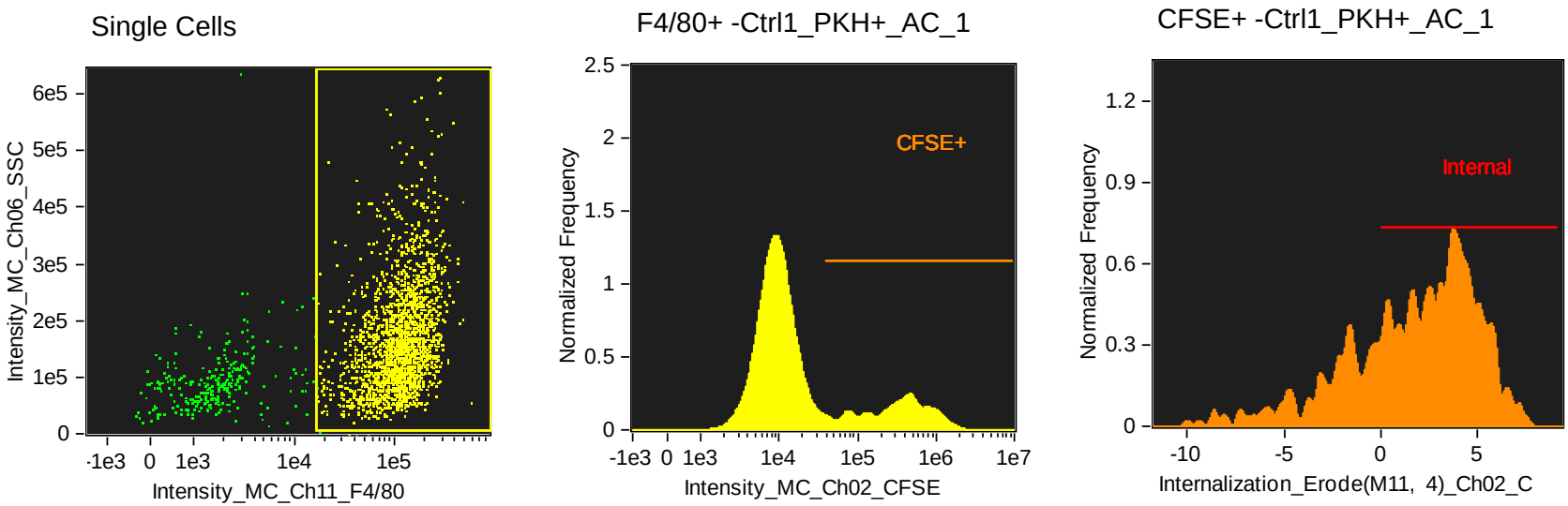

Figure S7. Supplemental Methods: ImageStream analysis of macrophage / monocyte phagocytosis of CFSE labeled apoptotic cells. Sequential images showing identification of single cells, identification of CFSE positive cells, and cell populations that have internalized CFSE positive cells.

Table S1. **Oxylipin formation in the presence of EpoxI.** Comparison of oxylipins in terms of fold (left) and total amounts (pg/ml; right) at 48h in the presence or absence of EpoxI (SKF525A; 30mg/kg) given at 24h and 36h. Inflammation was initiated by 1mg zymosan A per mouse. The data represents the mean ± s.e.m from n=3-8 mice per group.

	Control			EpoxI		
Oxylipin	fold	s.e.m	n	fold	s.e.m	n
6-keto PGF_{1a}	1.4	0.4	7	2	0.3	8
TXB_2	1.9	0.5	5	1	0.2	8
PGF_{2a}	1.2	0.2	7	1	0.1	8
PGD_2	1.9	0.9	7	1.6	0.3	8
PGE_2	1.3	0.4	7	1.3	0.2	8
5-HETE	1.4	0.2	5	0.8	0.1	8
8-HETE	0.11	0.03	5	0.12	0.03	8
11-HETE	1.4	0.4	5	1.9	0.3	8
12-HETE	0.05	0.01	5	0.02	0.004	8
15-HETE	0.1	0.03	5	0.13	0.02	8
19-HETE	0.9	0.3	5	1	0.3	8
20-HETE	1.1	0.2	3	0.8	0.1	4
9-HODE	1	0.2	7	1.1	0.1	8
13-HODE	0.14	0.03	3	0.09	0.02	4
19,20-EpDPE	1.9	0.3	7	2.6	0.6	8

	Control			EpoxI		
Oxylipin	[pg/ml]	s.e.m	n	[pg/ml]	s.e.m	n
6-keto PGF_{1a}	283	81	7	404	61	8
TXB_2	113	30	5	60	12	8
PGF_{2a}	123	21	7	103	10	8
PGD_2	71	33	7	59	11	8
PGE_2	109	33	7	109	17	8
5-HETE	45	6	5	26	4	8
8-HETE	42	12	5	46	12	8
11-HETE	114	33	5	155	24	8
12-HETE	1187	237	5	475	95	8
15-HETE	78	23	5	101	16	8
19-HETE	36	12	5	40	12	8
20-HETE	23	4	3	17	2	4
9-HODE	18943	3789	7	20837	1894	8
13-HODE	24659	5284	3	15852	3523	4
19,20-EpDPE	142	22	7	195	45	8

Supplemental Methods: Mass spec analysis

Eicosanoid metabolites were extracted from exudates by solid-phase extraction and quantified by HPLC-MS/MS. Briefly, 1 ml exudates was spiked with internal standard (3 ng PGE_2-d_4 , 10,11-DiHN, and 10(11)-EpHep (Cayman)) acidified with 100µl of 1% acetic acid in 50% methanol and applied over HyperSep Retain SPE columns (Thermo Scientific, Bellefonte, PA) that had been prewashed with ethyl acetate, 100% methanol, and 0.1% acetic acid in 5% methanol. Columns were washed twice with 0.1% acetic acid in 5% methanol, dried under low pressure vacuum, and eluted with 0.5 ml of methanol and 1 ml of ethyl acetate into glass tubes containing 2 µl 30% glycerol. Samples were dried under gentle nitrogen flow and reconstituted in 50 µL of 30% ethanol. Triplicate 10 µl injections were performed. Online LC of extracted samples was performed with an Agilent 1200 series capillary HPLC (Agilent Technologies, Santa Clara, CA). Separations were achieved using a Halo C18 column (2.7 µm, 100 × 2.1 mm; MAC-MOD Analytical, Chadds Ford, PA), which was held at 50°C and a flow rate of 400 µl/min. Mobile phase A was 0.1% acetic acid in 85:15 water:acetonitrile. Mobile phase B was 0.1% acetic acid in acetonitrile. Gradient elution was used and the mobile phase was varied as follows: 20% B at 0 min, ramp from 0 to 5 min to 40% B, ramp from 5 to 7 min to 55% B, ramp from 7 to 13 min to 64% B. From 13 to 19 min the column was flushed with 100% B at a flow rate of 550 µ l/min before being returned to starting conditions and equilibrated for 6 min. Electrospray ionization MS/MS was used for detection. Analyses were performed on an MDS Sciex API 3000 equipped with a TurboIonSpray source (Applied Biosystems, Foster City, CA). Desolvation gas was heated to 425°C at a flow rate of 6 l/ min. All analytes were monitored simultaneously in a scheduled multiple reaction monitoring experiment as negative ions at parent ion-product ion mass/charge ratio transitions as listed below. Peak area and analyte concentrations were performed on Analyst 1.5.1 Software (Applied Biosystems, Foster City, CA) based on analyte peak area:internal standard peak area ratios of samples compared to a standard curve for each analyte. Peaks were scored blind to determine those that were present or absente that were not. Parental EETs, THOMEs, PGB_2, and 17,18-EPETE were all below detection).

The m/z transitions for each analyte were as follows:

Analytes	**m/z**
6ketoPGF1a	369.1 > 163.1
TXB2	369.1 > 168.7
PGF2a	353.1 > 309.1
PGE2	351 > 270.9
PGD2	351 > 270.91
19,20-DiHDPA	361.2 > 273.2
17,18-DHET	335.2 > 247.2
12,13-DHOME	313.3 > 128.8
9,10-DHOME	313.3 > 170.5
14,15-DHET	337.3 > 206.8
11,12-DHET	337.3 > 166.9
8,9-DHET	337.3 > 126.7
5,6-DHET	337.3 > 144.4
13-HODE	295 > 195.1
9-HODE	295 > 170.7
20-HETE	319.1 > 289
19-HETE	319.1 > 230.6
15-HETE	319.1 > 218.7
12-HETE	319.1 > 178.6
11-HETE	319.1 > 166.8
8-HETE	319.1 > 154.5
5-HETE	319.1 > 114.6
19(20)-EpDPE	343.2 > 281.1
17(18)-EpETE	317.1 > 258.7
12(13)-EpOME	295 > 194.8
9(10)-EpOME	295 > 170.9
14(15)-EET	319.1 > 218.9
11(12)-EET	319.1 > 207.5
8(9)-EET	319.1 > 150.9
5(6)-EET	319.1 > 191
Internal Standards	
d4-PGE2	355 > 275
10,11-DiHN	329.2 > 310.9
10,11-EpHep	283.3 > 264.9

Supplemental methods: RT-PCR

Amplification was performed in a Techgene Techne TC-312 thermal cycler programmed for an initial denaturation of 5min at 94°C, followed by 25-35 cycles of 1min at 94°C, 1min at 57-60°C and 2min at 72°C, and a final extension of 7min at 72°C. 12µl of the PCR reaction was subjected to electrophoresis on 1.5% agarose gel and the products visualized by ethidium bromide staining. The gel image was captured with a Gel doc 1000 BIORAD digital camera.

PCR primers used:

Ly6C #1: TGCCACTGTGCCTGCAACCT
Ly6C #2: TGATTGGCACACCAGCAGGGC

ccr2 #1: GCTTGATGAAGGGGCCACCACAC
ccr2 #2: ACACAGCCACCACCCAAGTGAC

cx3cR1 #1: TGCTCACGGCGGCATGTCTG
cX3cr1 #2: TGTGACCCGAGACAGCGGGA

ccl2 #1: TGCTGTTCACAGTTGCCGGCT
ccl2 #2: GGGGTCAGCACAGACCTCTCTCTT

cyP2j5 #1: GGCCATGGCGTCTGCCCTTT
CYP2J5 #2: TGGCTTCCACCAGGTGATGGGT

CYP2j6 #1: CCACCGCATCTGTGCTGTCCC
CYP2J6 #2:
TGAAAAGGCATGCCACTAAGCTCAT

CYP2j9 #1:
ACTACAGGCAGTGTCCTTGGGGAA
CYP2J9 #2: TCGGAGAAGAGTGACTGGGCGA

CYP2J13 #1:
AAGTGCTCAAGCGCTCAAGTGCC
CYP2J13 #2:
CGTTCCCCAAAGGTGATGGAGCA

CYP2C29 #1:
ACTGTGTCAGTAAGGAGGTTCGCT
CYP2C29 #2: GTGGGGTCACAGGGTGAGCC

CYP2C38 #1: GGACAGGAGCCGCATGCCCTAT
CYP2C38 #2: GCTGGTAATGGGGTGGCAGAGTGA

CYP2C39 #1: GGCCAGTGATGCCTGATCACAAT
CYP2C39 #2: AGTGGGCCGTGAGCCCAAAT

cyp2c44 #1: CCCGCCAACCCTCGGGATTAC
CYP2C44 #2: GGCTCCACCAGAGCCTTTAGGG

CYP2C50 #1: GCTGCGTGATAGCAAAGAGTTCCC
CYP2C50 #2: ATGTCCCAGCTGGAGCCAGG

CYP2C54 #1: GAGCACCCCCTGGTTGCAGG
CYP2C54 #2: TGGAAAGCAGTGGAGCCAGGC

CYP2C55 #1: AAGCTGCCATGGATCCAGTCCTG
CYP2C55 #2: GAAGCGCCGAAGCTCCTTCCAT

CYP2A1 #1: GGGCTTGCCCTTCATTGGGCA
CYP2A1 #2: CCTGGCTGACTGGTTCGAAGTGG

CYP2U1 #1: CAGCTTCGACGAGGACTACC
CYP2U1 #2: CAGCTGCTCTCCCATACACA

cyp2s1 #1: GCACTGAGGTCTTCCCTCTG
CYP2S1 #2: TGCCTTGATACGTGGTGTGT

b-Actin #1: GTGGGGCGCCCCAGGCACCA
b-Actin #2:CTCCTTAATGTCACGCACGATTT