## Supplemental Experimental Procedure

*Animal Experiments*

Mice on a 129X1/SvJ x C57BL/6 background with targeted disruption in the *Ephx2* gene were obtained from Chris Sinal, Dalhousie University, Halifax, NS, Canada under a National Cancer Institute Material Transfer Agreement 1-16268-04 (1). These mice backcrossed onto a C57BL6 genetic background five generations prior to use in this study, kept and bred in house (2). All procedures were conducted in accordance with approved IACUC protocols. *EPHX2*- null or wild type mice (25-30 g) were treated with sEH inhibitors, 1-(1-methyl-sulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea, TUPS (3). TUPS was given via drinking water (10 mg/L in 1% PEG) for seven days. Water consumption was daily monitored and the concentration of TUPS was adjusted to water consumption to achieve similar plasma drug levels. A second set of animals was treated with 1% PEG alone (vehicle). Blood was collected early in the morning from lethal injection of sodium pentobarbital (100 mg/kg BW ip) to mutant mice and their wild-type controls by cardiac puncture in EDTA-rinsed syringes. Blood samples were collected from the right ventricle of each animal, and plasma was separated (10 min, 400 x *g*) and stored at –80°C until analysis. Liver samples were collected, frozen immediately and were kept at -80ºC. TUPS was extracted from blood samples as described previously and the plasma concentration was analyzed by liquid chromatography coupled mass spectroscopy LC-MS/MS (4).

*Oxylipin extraction and analysis*

Oxylipins were isolated by solid phase extraction on 60 mg Waters Oasis-HLB cartridges (Milford, MA), as described earlier (2). Analytes in a 20 µl extract aliquot were separated by HPLC and residues were detected by negative mode electrospray ionization (2, 5). Multiple reaction monitoring was performed on a Quattro Ultima tandem quadrpole mass spectrometer (Micromass, Manchester, UK). Analytes were quantified using internal standard method, as previously described (5). Reagent blanks showed background levels below detection limit, and analytical replicates showed precision to be within 15% for greater than 80% of the analytes present at levels greater than ten times the method detection limits.

## Supplemental Results

*Plasma oxylipins*

Plasma CYP- and sEH- dependent oxylipin profile from both female and male of *Ephx2*-null and wild type mice is shown in Figure S1 and S2, respectively. Compared with their wild type counterparts, both female and male mutated mice showed elevated ratio levels of plasma linoleate and arachidonate epoxides to their vicinal diols (Fig. S1.C and S2.C). Inhibition of sEH in wild types animals results in decrease of both arachidonate and linoleate diols and increase their precursor epoxides (Fig. S1 A). Whereas, no effect was observed in null-sEH animals in the presence of sEH inhibitor, TUPS (Fig. S1 B). In females wild types mice, inhibition of sEH has a higher effect on epoxides and diols of linoleic acid than arachidonic acid (Fig. S2 A).

## References


1. Sinal, C. J., Miyata, M., Tohkin, M., Nagata, K., Bend, J. R., & Gonzalez, F. J. (2000) *J Biol Chem* **275,** 40504-40510.
2. Luria, A., Weldon, S. M., Kabcenell, A. K., Ingraham, R. H., Matera, D., Jiang, H., Gill, R., Morisseau, C., Newman, J. W., & Hammock, B. D. (2007) *J Biol Chem* **282,** 2891-2898.
3. Jones, P. D., Tsai, H. J., Do, Z. N., Morisseau, C., & Hammock, B. D. (2006) *Bioorg Med Chem Lett* **16,** 5212-5216.
4. Watanabe, T., Schulz, D., Morisseau, C., & Hammock, B. D. (2006) *Anal Chim Acta* **559,** 37-44
5. Newman JW, Watanabe T, Hammock BD. *J Lipid Res.* 2002;43:1563–1578.

## FIGURE LEGENDS

**Figure S1.** Arachidonic acid (AA) and linoleic acid (LA) metabolites in plasma samples from wild type **(A)** and sEH-null **(B)** male mice in the absence and presence of sEH inhibitor, TUPS. Plasma sample from each genotype was extracted and subjected to LC/MSMS analysis as described in Supplemental Experimental Procedure. Data are presented as AVG±SEM of linoleate and arachidonate P450 metabolites. EpOME, epoxyoctadecenoic acid; DHOME, dihydroxyoctadecenoic acid; EET, epoxyeicosatrienoic acid; DHET, dihydroxyeicosatrienoic acid.

**Figure S2.** Arachidonic acid (AA) and linoleic acid (LA) metabolites in plasma samples from wild type **(A)** and sEH-null **(B)** female mice in the absence and presence of sEH inhibitor, TUPS. Plasma sample from each genotype was extracted and subjected to LC/MSMS analysis as described in Supplemental Experimental Procedure. Data are presented as AVG±SEM of linoleate and arachidonate P450 metabolites. EpOME, epoxyoctadecenoic acid; DHOME, dihydroxyoctadecenoic acid; EET, epoxyeicosatrienoic acid; DHET, dihydroxyeicosatrienoic acid.

**Figure S1.**

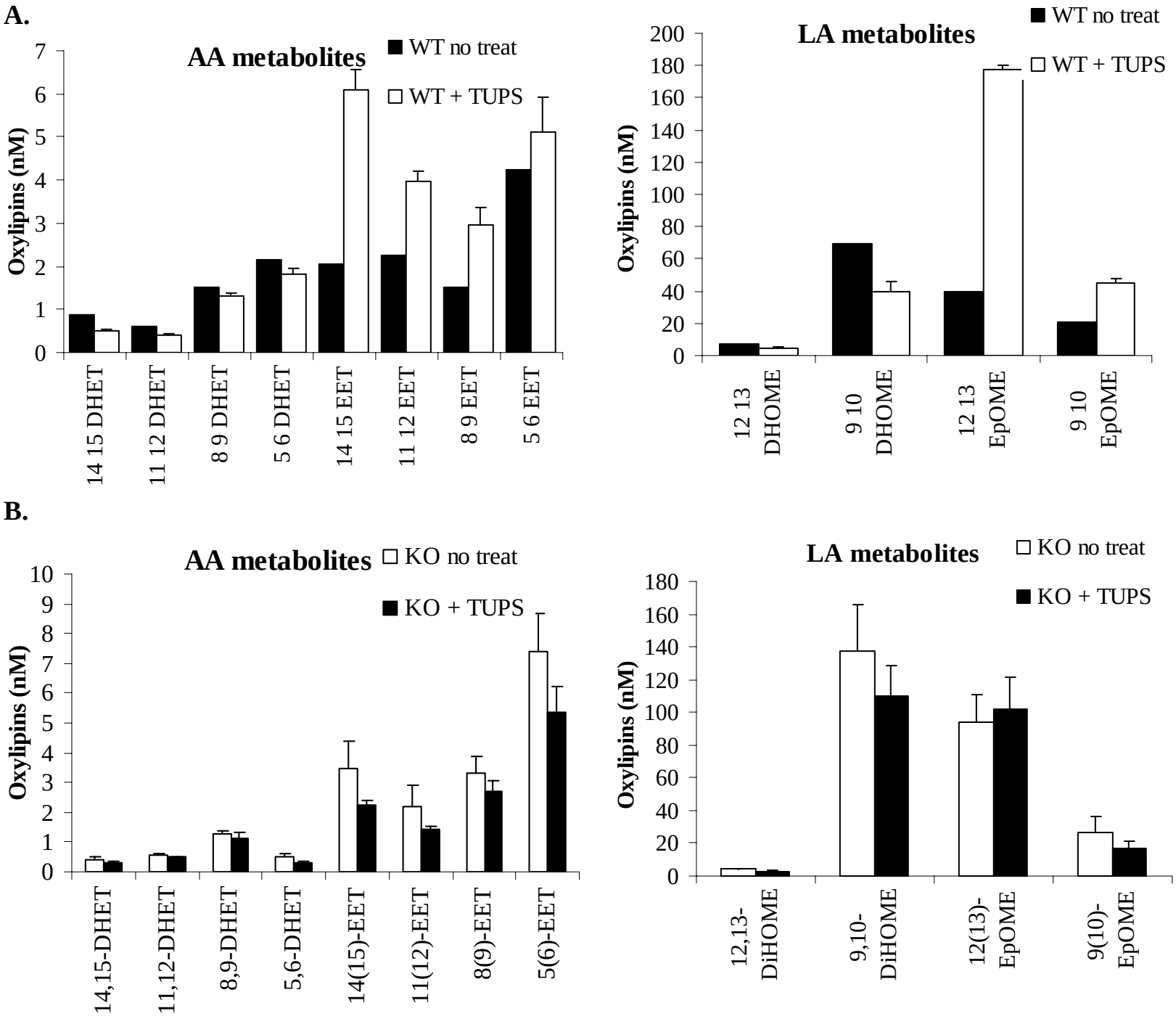
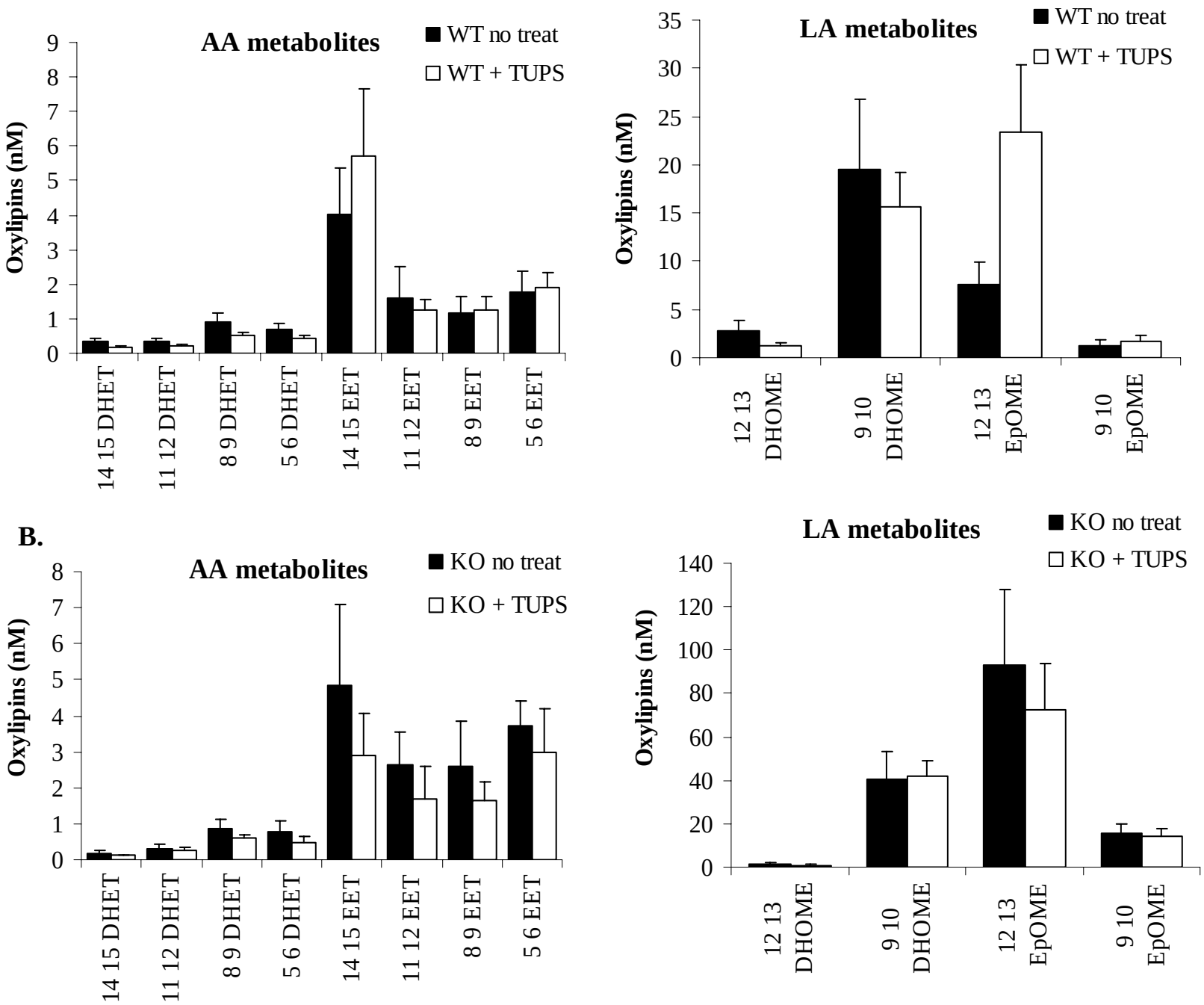

A.
AA metabolites
WT no treat
WT + TUPS
Oxylipins (nM)
0
1
2
3
4
5
6
7
14 15 DHET
11 12 DHET
8 9 DHET
5 6 DHET
14 15 EET
11 12 EET
8 9 EET
5 6 EET
LA metabolites
WT no treat
WT + TUPS
Oxylipins (nM)
0
20
40
60
80
100
120
140
160
180
200
12 13 DHOME
9 10 DHOME
12 13 EpOME
9 10 EpOME
B.
AA metabolites
KO no treat
KO + TUPS
Oxylipins (nM)
0
1
2
3
4
5
6
7
8
9
10
14,15-DHET
11,12-DHET
8,9-DHET
5,6-DHET
14(15)-EET
11(12)-EET
8(9)-EET
5(6)-EET
LA metabolites
KO no treat
KO + TUPS
Oxylipins (nM)
0
20
40
60
80
100
120
140
160
180
12,13-DiHOME
9,10-DiHOME
12(13)-EpOME
9(10)-EpOME

**Figure S2.**


A.
AA metabolites
WT no treat
WT + TUPS
Oxylipins (nM)
0
1
2
3
4
5
6
7
8
9
14 15 DHET
11 12 DHET
8 9 DHET
5 6 DHET
14 15 EET
11 12 EET
8 9 EET
5 6 EET
LA metabolites
WT no treat
WT + TUPS
Oxylipins (nM)
0
5
10
15
20
25
30
35
12 13 DHOME
9 10 DHOME
12 13 EpOME
9 10 EpOME
B.
AA metabolites
KO no treat
KO + TUPS
Oxylipins (nM)
0
1
2
3
4
5
6
7
8
14 15 DHET
11 12 DHET
8 9 DHET
5 6 DHET
14 15 EET
11 12 EET
8 9 EET
5 6 EET
LA metabolites
KO no treat
KO + TUPS
Oxylipins (nM)
0
20
40
60
80
100
120
140
12 13 DHOME
9 10 DHOME
12 13 EpOME
9 10 EpOME