Supporting Information

12-LOX catalyzes the oxidation of 2-arachidonoyl-lysolipids in platelets generating eicosanoid-lysolipids that are attenuated by iPLA$_2\gamma$ knockout

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Running Title: *Eicosanoid-lysophospholipids are attenuated by iPLA$_2\gamma$ knockout*

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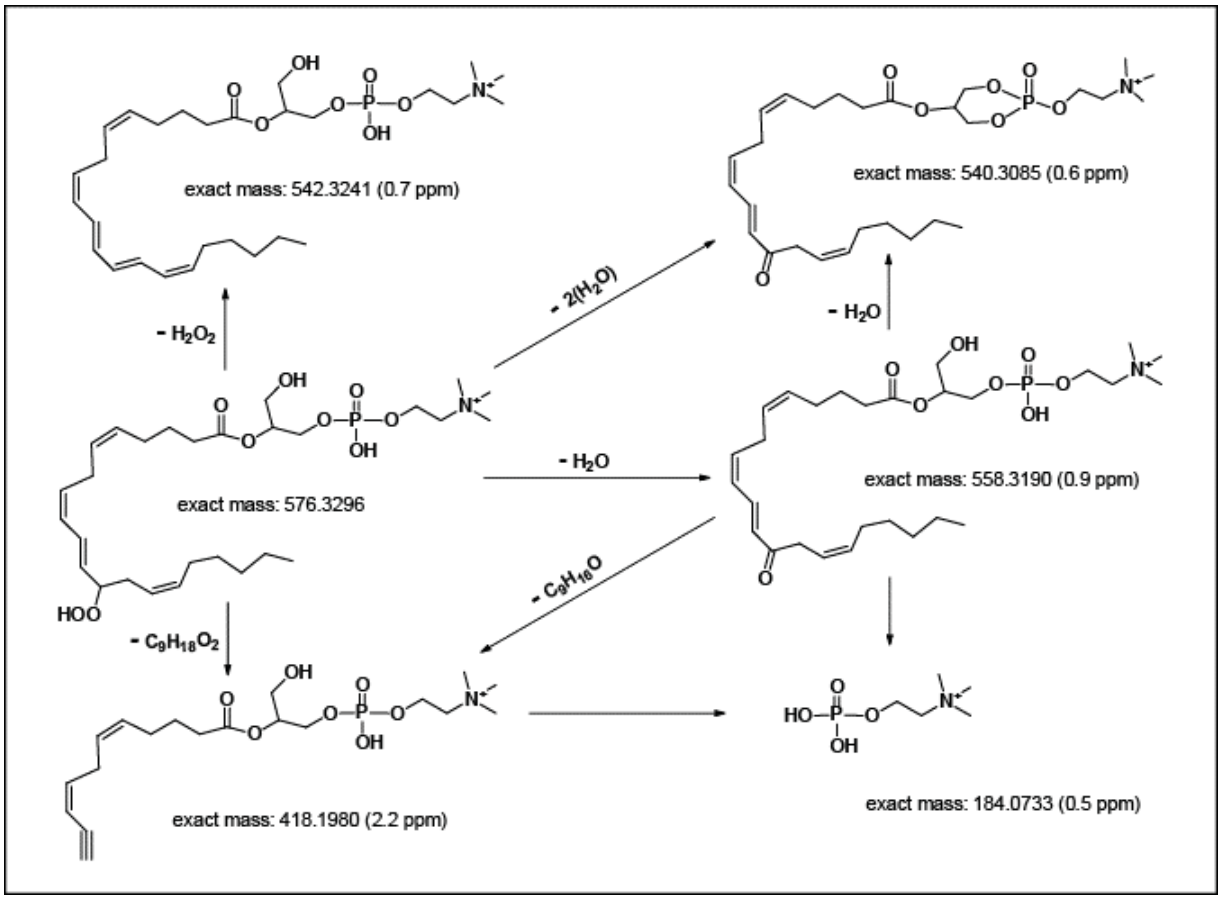
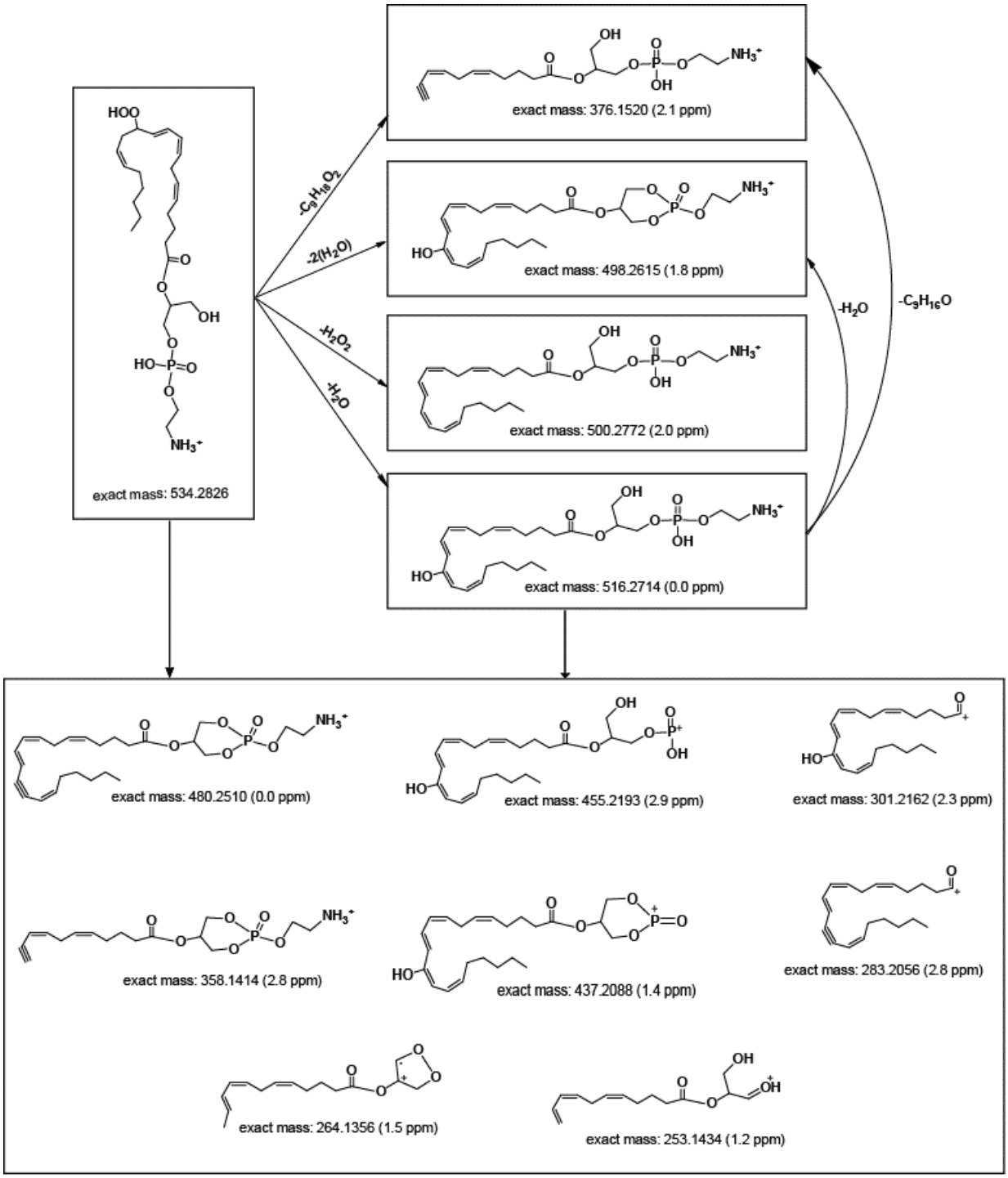
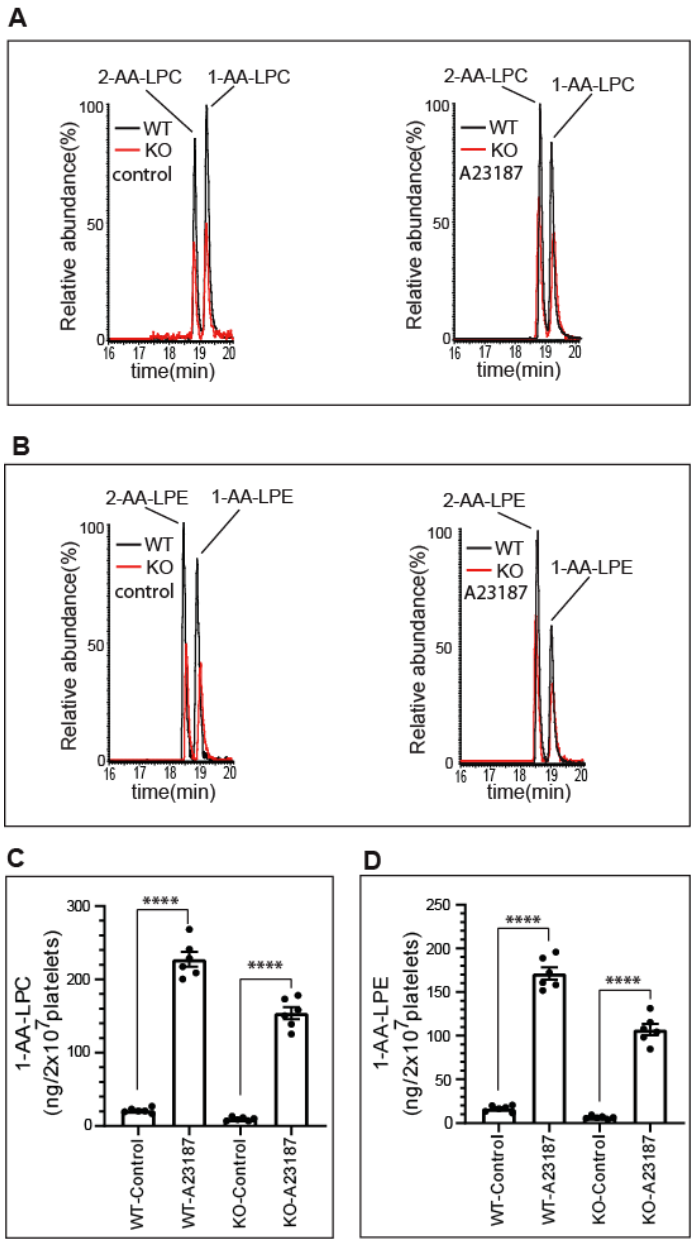


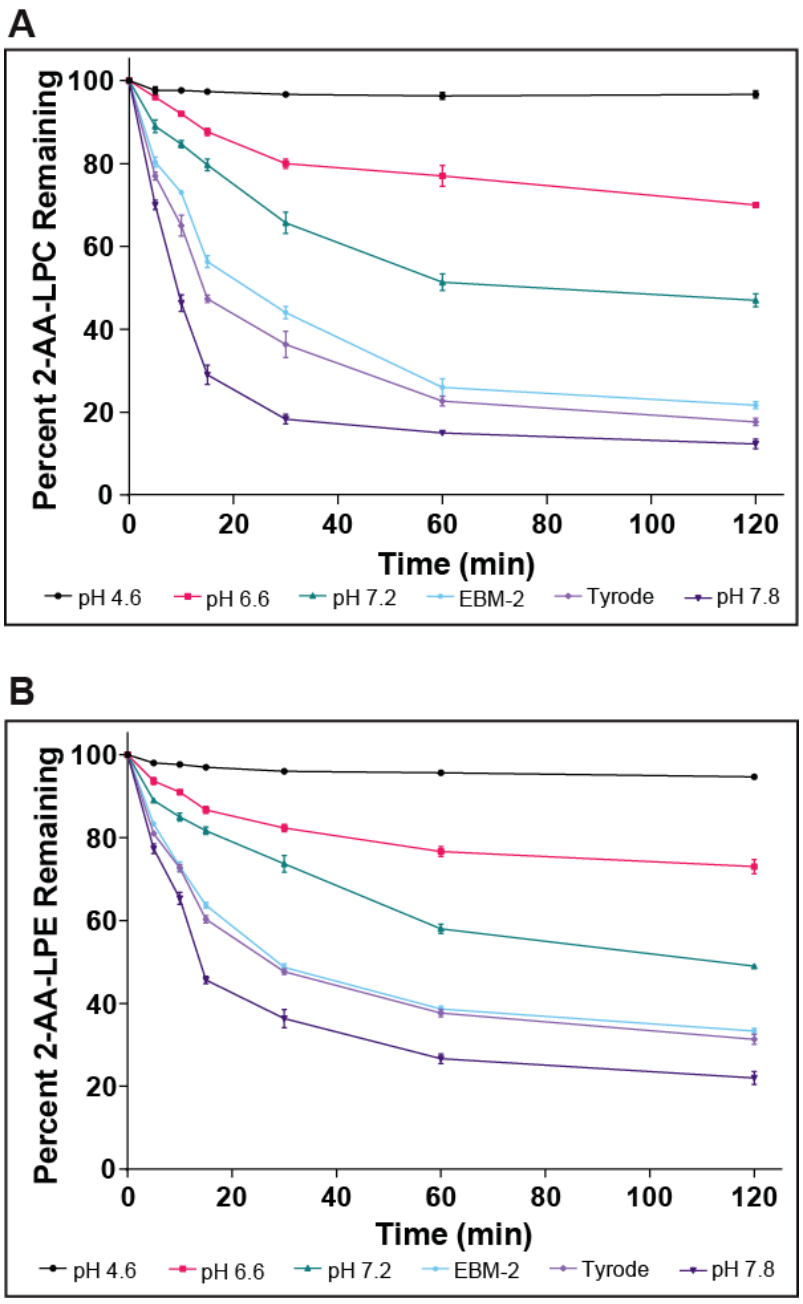
Supplementary Fig. 1. Proposed fragmentation pathways upon collision induced dissociation (CID) leading to the observed accurate mass product ions for 12-hydroperoxyl-eicosatetrataenoic-lysophosphatidylcholine (12-HpETE-LPC). The product ion *m/z* 418.1980 is derived from the unique fragmentation occurring at the labile hydroperoxide bond. Exact masses are calculated theoretical masses; numbers in parentheses indicate the measured mass error in parts per million (ppm) relative to the calculated theoretical mass.



Supplementary Fig. 2. Proposed fragmentation pathways upon collision induced dissociation (CID) leading to the observed accurate mass product ions for 12-hydroperoxyl-eicosatetrataenoic-lysophosphatidylethanolamine (12-HpETE-LPE). The product ion *m/z* 376.1520 is derived from the unique fragmentation occurring at the labile hydroperoxide bond. Exact masses are calculated theoretical masses; numbers in parentheses indicate the measured mass error in parts per million (ppm) relative to the calculated theoretical mass.



Supplementary Fig. 3. Production of 1-AA-LPC and 1-AA-LPE in murine platelets from wild-type (WT) and iPLA$_2\gamma$ knockout (KO) mice stimulated with calcium ionophore A23187. Washed platelets were incubated in the presence or absence of calcium ionophore A23187 at 37°C for 15 min followed by extraction of lipids using a modified Bligh and Dyer procedure and analysis by HPLC-MS/MS. **A**, Representative of LC/MS traces for 1-AA-LPC in the absence (**Control**) or presence (**A23187**) of calcium ionophore A23187 in platelets from WT or KO mice; **B**, Representative of LC/MS traces for 1-AA-LPE in the absence (**Control**) or presence (**A23187**) of calcium ionophore A23187 in platelets from WT or KO mice; **C**, content of 1-AA-LPC in the absence (**Control**) or presence (**A23187**) of calcium ionophore A23187 in platelets from WT or KO mice; **D**, content of 1-AA-LPE in the absence (**Control**) or presence (**A23187**) of calcium ionophore A23187 in platelets from WT or KO mice. Data are expressed as mean values ± SEM of six independent replicates. Statistical analyses were performed using an unpaired two-tailed Student *t* test. Statistically significant *p* values are indicated above the bracketed data sets (**** $p<0.0001$).



Supplementary Fig. 4. pH dependent kinetics of *sn*-2 to *sn*-1 acyl migration for 2-AA-LPC and 2-AA-LPE to 1-AA-LPC and 1-AA-LPE (respectively). For each reaction, 2-AA-LPC or 2-AA-LPE was added to 1.0 ml of the selected medium or buffer (EBM-2 culture medium, Tyrode's buffer, 0.05 M Citrate-sodium citrate buffer (pH 4.6 and pH 6.6) or 0.05 M Tris-HCl buffer (pH7.2 and pH7.8) to give a final concentration of 10 μM, and the resulting mixture was incubated at 37°C for the indicated times. The quantities of 1- or 2-AA-lysophospholipids were then determined for the indicated time points by LC-MS. Plots of remaining 2-AA-LPC (**A**) or 2-AA-LPE (**B**) are shown as a percent of total 1- and 2-AA-LPC or 1- and 2-AA-LPE versus time, respectively. Each curve represents the mean ± SEM from three replicate experiments.

Supplementary Table 1. Measured apparent K_m and V_{max} values for 12-HpETE-lysophospholipid products formed upon oxidation of 2-AA-LPC and 2-AA-LPE with human platelet-type 12-LOX (h12LOX) or mouse platelet-type 12-LOX (m12-LOX). Data are expressed as mean values ± SEM of three replicates.

Enzyme	Substrate	Product	K_m (μM)	V_{max} (nmol $s^{-1}mg^{-1}$)
m12-LOX	2-AA-LPC	12-HpETE-LPC	14 (±4)	48 (±6)
m12-LOX	2-AA-LPE	12-HpETE-LPE	13 (±2)	33 (±5)
h12-LOX	2-AA-LPC	12-HpETE-LPC	12 (± 3)	50 (±8)
h12-LOX	2-AA-LPE	12-HpETE-LPE	9 (±1)	27 (±6)