SUPPLEMENT

EOSINOPHIL CYSTEINYL LEUKOTRIENE SYNTHESIS MEDIATED BY EXOGENOUS SECRETED PHOSPHOLIPASE A_2 GROUP X

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SUPPLEMENTAL RESULTS

Supplemental Table 1: Transfected HEK293T cell-based LPS assay.

Sample	Polymixin*	IL-8 pg/ml (SEM)
Untreated	N	105.6 (6.6)
LPS	N	>3937.5
$sPLA_2$-X (100 nM)	N	122.6 (19.8)
Untreated	Y	107.2 (9.5)
LPS	Y	105.5 (13.9)
$sPLA_2$-X (100 nM)	Y	102.2 (15.6)

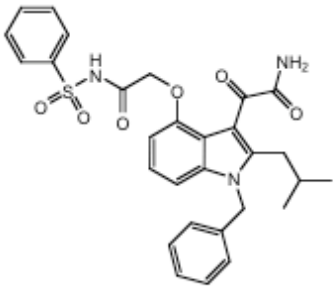
* Polymixin B binds to LPS and eliminates IL-8 expression.

Supplemental Table 2: Selectivity of $sPLA_2$-X inhibitor (ROC-0929) and structurally similar control inhibitor (ROC-0428) for the 9 mammalian sPLA2s [IC50 (nM)*].

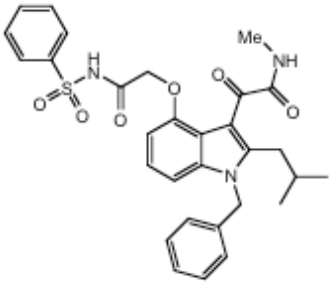
	Inhibitor	
$sPLA_2$	**ROC-0929**	**ROC-0428**
hGIB	>1600	nd
hGIIA	>1600	nd
hGIID**	700±200	nd
hGIIE	>1600	nd
hGIIF	>1600	nd
hGIII	>1600	nd
hGV	>1600	nd
hGX	20±10	6600±900
hGXIIA	>1600	nd

*IC50 values obtained using fluorometric assay with pyrene-labeled phosphatidylglycerol as substrate.
**IC50 value obtained using radiolabeled *E. coli* membrane assay.

ROC-0929



ROC-0428



Supplemental Table 3: Selectivity of inhibitors for recombinant human sPLA$_2$-X (9.7 - 22.8 nM)

Inhibitor	ROC-0929		ROC-0428	
Concentration	667 nM	33 nM	1667 nM	167 nM
% Inh (SD)	85.9 (8.3)	43.0 (7.4)	20.7 (3.0)	4.3 (9.3)

SUPPLEMENTAL FIGURE LEGENDS:

Supplemental Figure 1. Effect of cPLA$_2\alpha$ inhibitors on sPLA$_2$-V-mediated eosinophil CysLT synthesis. *A*, Treatment of eosinophils with 100 nM exogenous sPLA$_2$-V for 20 min (black bar) increased CysLT synthesis over buffer control (Control, $^{*}P=0.06$); in agreement with prior evidence, the cPLA$_2\alpha$ inhibitors Pyr-2 and Wyeth-2 failed to significantly decrease sPLA$_2$-V-mediated CysLT synthesis by eosinophils. *B*, Because of the broad range of basal and stimulated CysLT production from the 3 eosinophil donors used for these studies, plots of the individual data are shown to confirm that the same result of an increase in CysLT synthesis that was not suppressed by cPLA$_2\alpha$ inhibitors occurred in all the subjects.

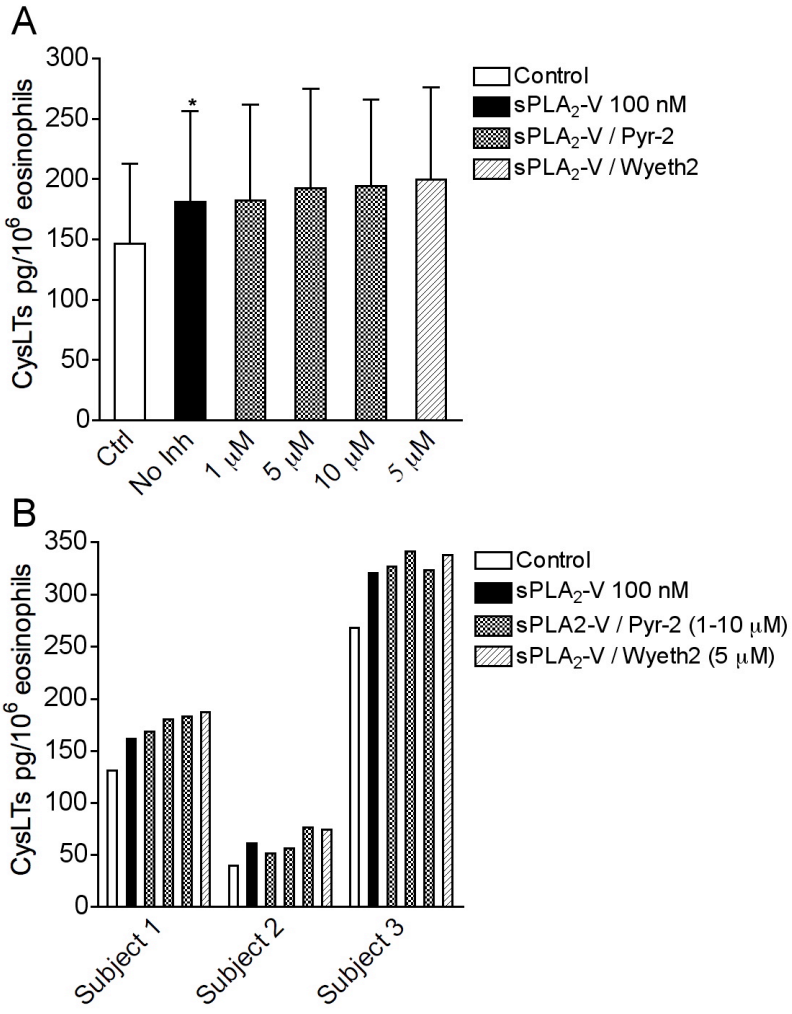
Supplemental Figure 2. Additional details of the activation of CysLT synthesis in fMLP stimulated eosinophils by sPLA$_2$-X. *A,* Individual eosinophil donor results for the CysLT production in eosinophils mediated by fMLP (10 nM) alone, or with the addition of sPLA$_2$-X. Exogenous sPLA$_2$-X at concentrations of 10 nM and 100 nM increased CysLT synthesis over fMLP alone in all subjects. *B*, Similarly, eosinophils from each donor treated with a higher concentration of fMLP (100 nM) made significant quantities of CysLTs that was further increased in all subjects by the addition of sPLA$_2$-X at concentrations of 10 nM and 100 nM.

Supplemental Figure 3. Full analysis of the exogenous sPLA$_2$-X-mediated generation of lysophospholipid species by eosinophils. *A-E*, Treatment of eosinophils with sPLA$_2$-X (100 nM) initiated the generation lysophosphatidylcholine (LysoPC) species (A), lysophosphatidylethanolamine (LysoPE) species (B), lysophosphatidylserine (lysoPS) species (C), lysophosphatidylinositol (LysoPI) species (D), and lysophosphatidylglycerol (LysoPG) species (E) relative to control conditions (white bar) and relative to eosinophils treated with fMLP (100 nM). *F*, Neither sPLA$_2$-X or fMLP caused significant release of lysophosphatidic acid (LysoPA) species by eosinophils. $^{*}P\leq0.01$ and $^{\dagger}P\leq0.05$ overall. $^{\ddagger}P\leq0.01$ and $^{\S}P\leq0.05$ versus fMLP.

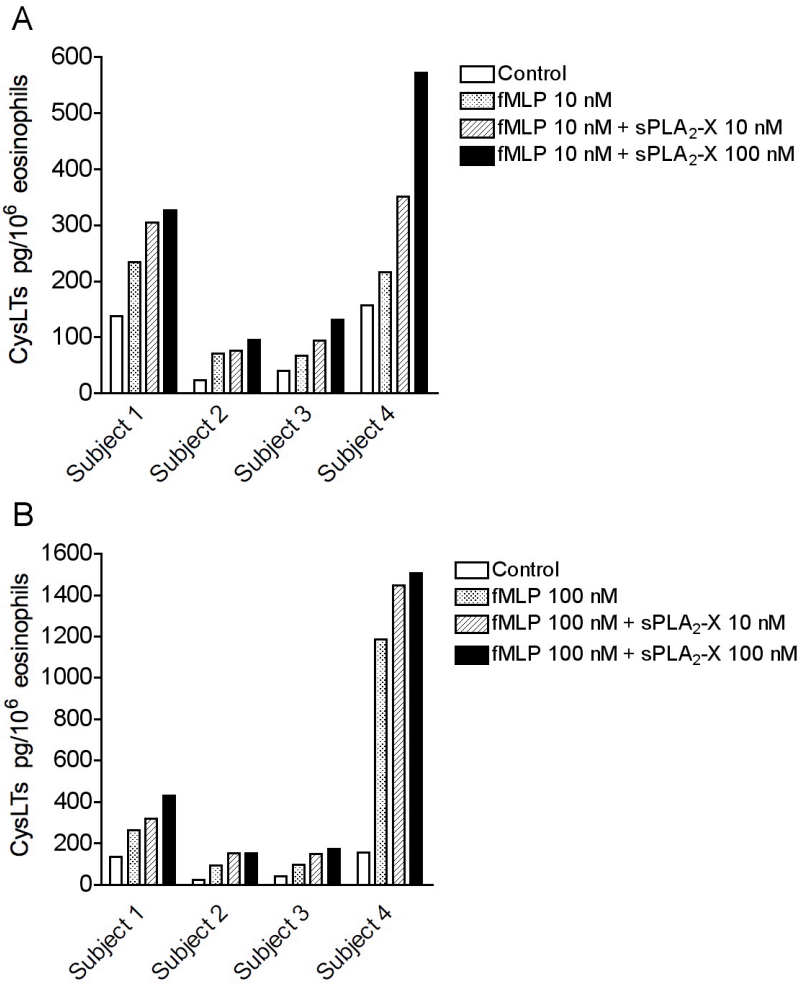
Supplemental Figure 4. Exogenous sPLA$_2$-X-mediated generation of plasmenyl lysophospholipids and lyso-platelet activating factor by eosinophils. *A-C*, Treatment of eosinophils with sPLA$_2$-X (100 nM) initiated the generation of plasmenyl LysoPC species (A), plasmenyl LysoPE species (B), and lyso-platelet activating factor (LysoPAF) species (C) relative to control conditions (white bar) and relative to eosinophils treated with fMLP (100 nM). $^{*}P\leq0.01$ and $^{\dagger}P\leq0.05$ overall. $^{\ddagger}P\leq0.01$ and $^{\S}P\leq0.05$ versus fMLP.

Supplemental Figure 5. Control images for confocal microscopy. The first column of images (A) shows eosinophils that have been stimulated with fMLP, but have not been labeled with either a primary or secondary antibody. The second column (B) shows eosinophils that have been labeled with a Cy3-anti-rabbit antibody, but have not been treated with a primary antibody. The third column (C) shows eosinophils that have been labeled with rabbit IgG at the same concentration as the antibodies used to detect cPLA$_2$ and 5-LO, and labeled with a Cy3-anti-rabbit antibody.

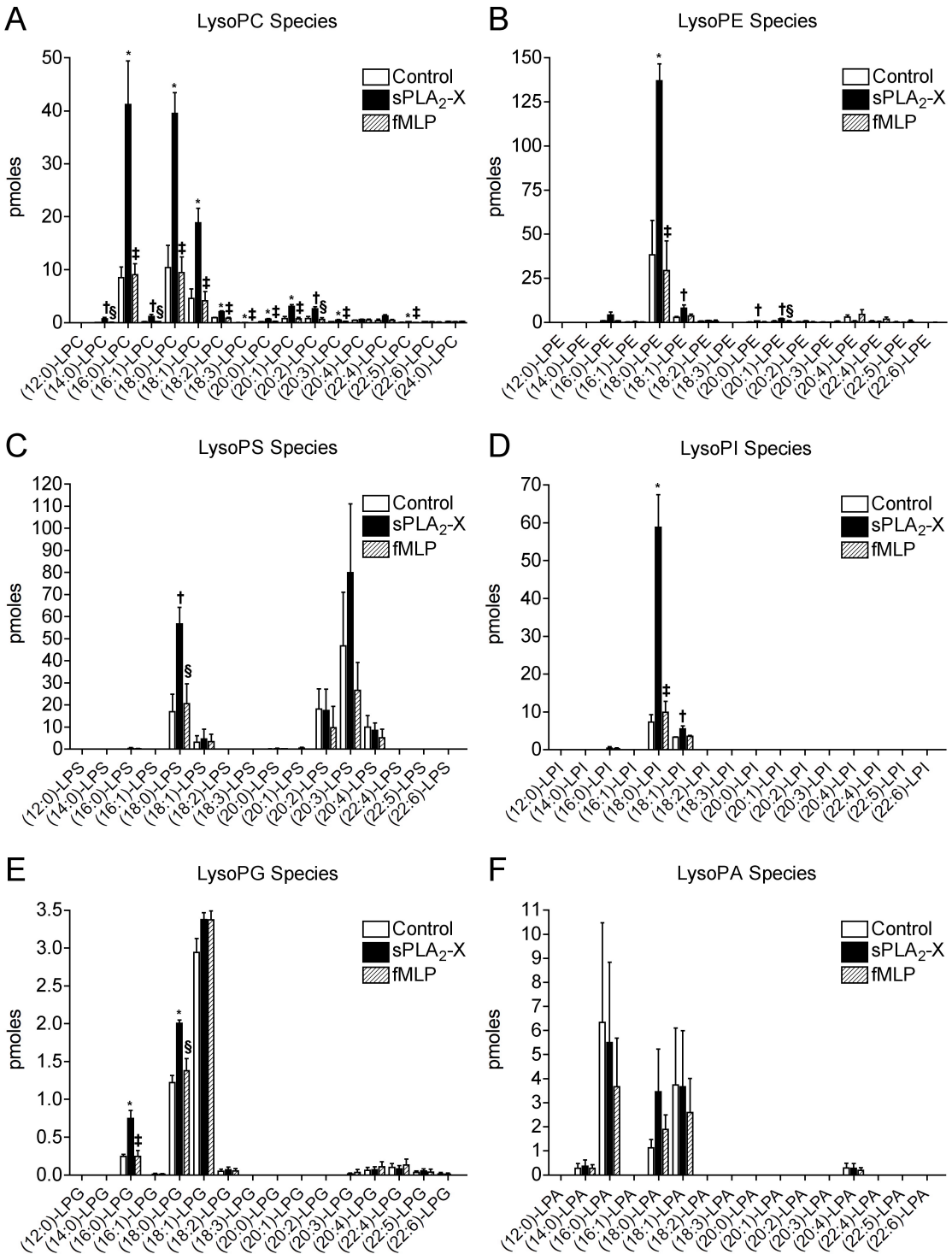
Supplemental Figure 1



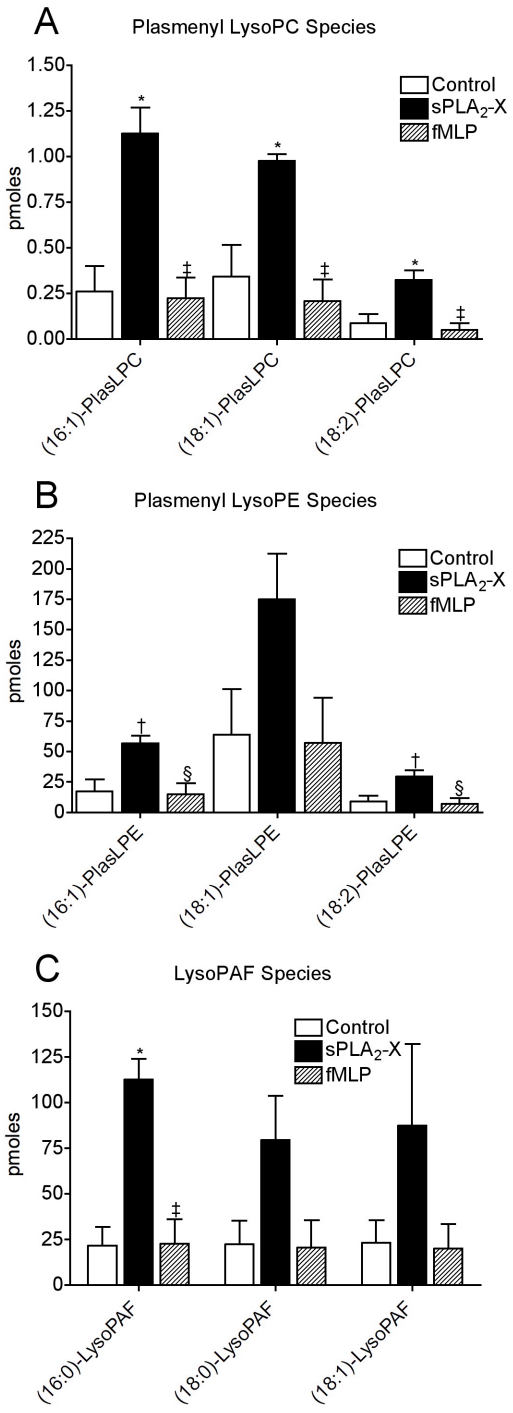
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5