SUPPORTING INFORMATION

# Phospholipid ozonation products activate the 5-lipoxygenase pathway in macrophages

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**Figure S1**: Dose response of the release of AA upon the addition of 16:0/9al-PC (closed squares) and POPC (closed triangles) to resident peritoneal macrophages for 1h at 37°C. After incubation AA was analyzed by LC-MS/MS and quantified using standard isotope dilution. Results shown are averages ± SEM (n=3) from three independent experiments. * $p< 0.05$; ** $p<0.01$; *** $p<0.0001$.

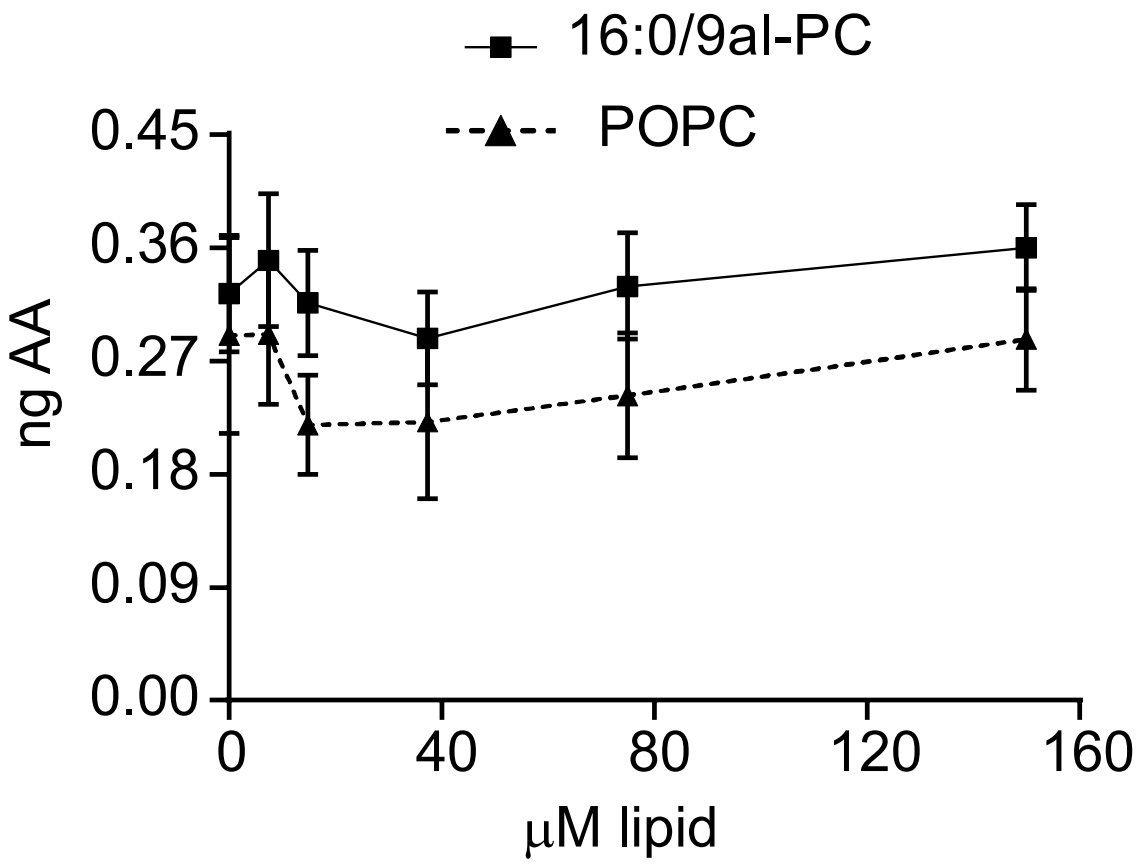

**Figure S2**: Negative ion mass spectrum of the products of POPG exposed to 3 ppm ozone for 3 minutes. The $[M-H]^-$ of POPG at *m/z* 747.6 disappeared after this treatment and 3 major peaks were observed as products. These products were characterized by collisionally activating each ozonization product observed and compared to fragment ions found in literature. The ozonization product at *m/z* 637.5 was determined to be 16:0/9al-PG and the product at *m/z* 669.5 was the methanol hemiacetal of 16:0/9al-PG. The ion present at *m/z* 653.5 is 16:0/9COOH-PG.

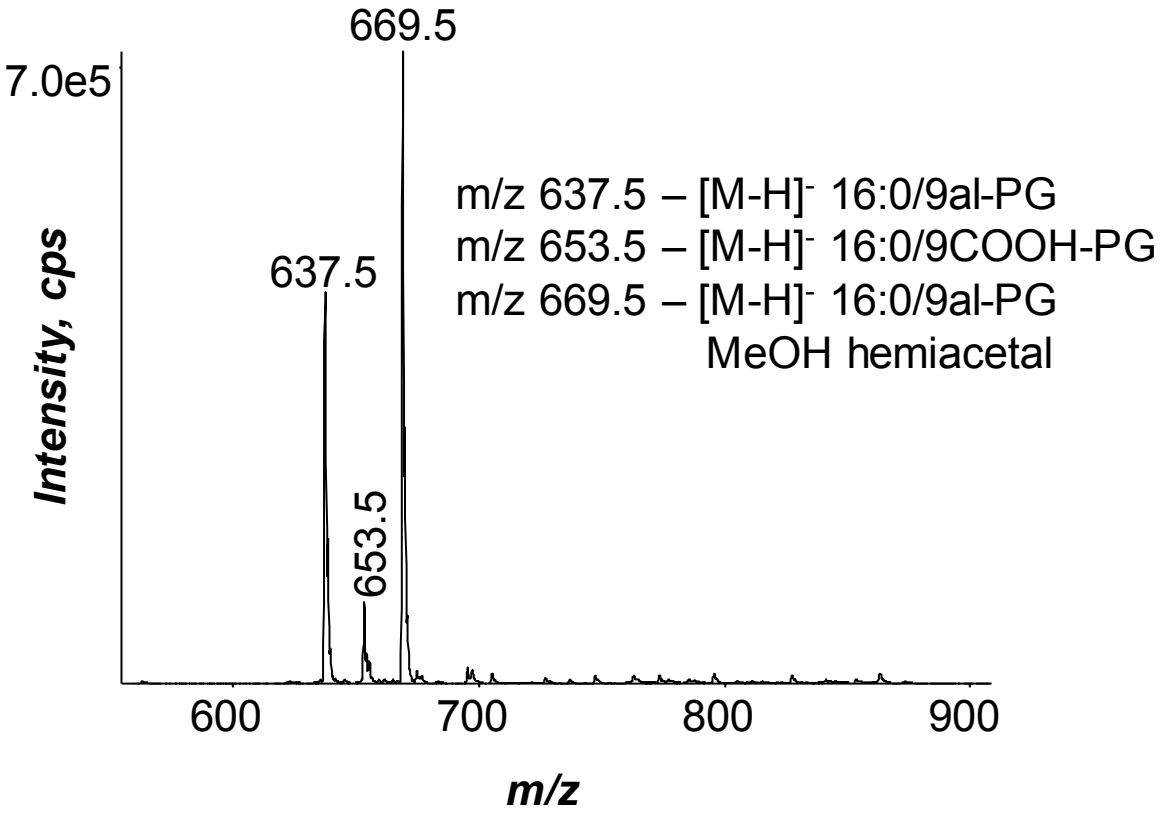

**Figure S3**: Dose response of the release of AA upon the addition of 16:0/9al-PC (closed squares) and POPC (closed triangles) for 3 min followed by the addition of zymosan (25 particles per cell) to resident peritoneal macrophages for 1h at 37°C. After incubation, the AA was analyzed by LC-MS/MS and quantified using standard isotope dilution. Results shown are averages ± SEM (n=3) from three independent experiments. * p< 0.05; ** p<0.01; *** p<0.0001.

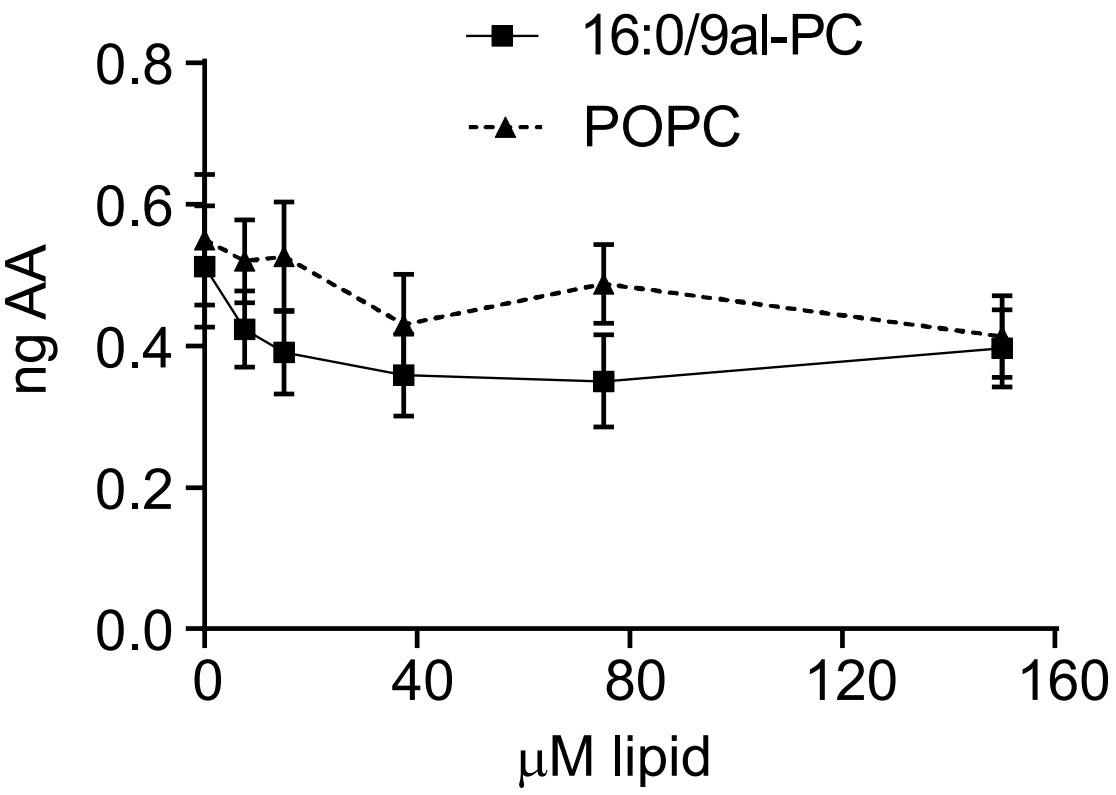

**Figure S4**: Time course of the production of A) 5-LO products ($LTC_4$, 6-*trans*-$LTB_4$, $LTB_4$, and 5-HETE) and B) COX products ($PGE_2$ and $TXB_2$) with the addition of 16:0/9al-PC (37.5 μM) to resident peritoneal macrophages either before, after, or at the same time as zymosan (25 particles per cell) for 1h at 37°C. After incubation, the eicosanoids ($LTC_4$, 6-*trans*-$LTB_4$, $LTB_4$, 5-HETE, $PGE_2$, and $TXB_2$) were analyzed by LC-MS/MS and quantified using standard isotope dilution. Results shown are averages ± SEM (n=3) from three independent experiments. * $p< 0.05$; ** $p<0.01$; *** $p<0.0001$.

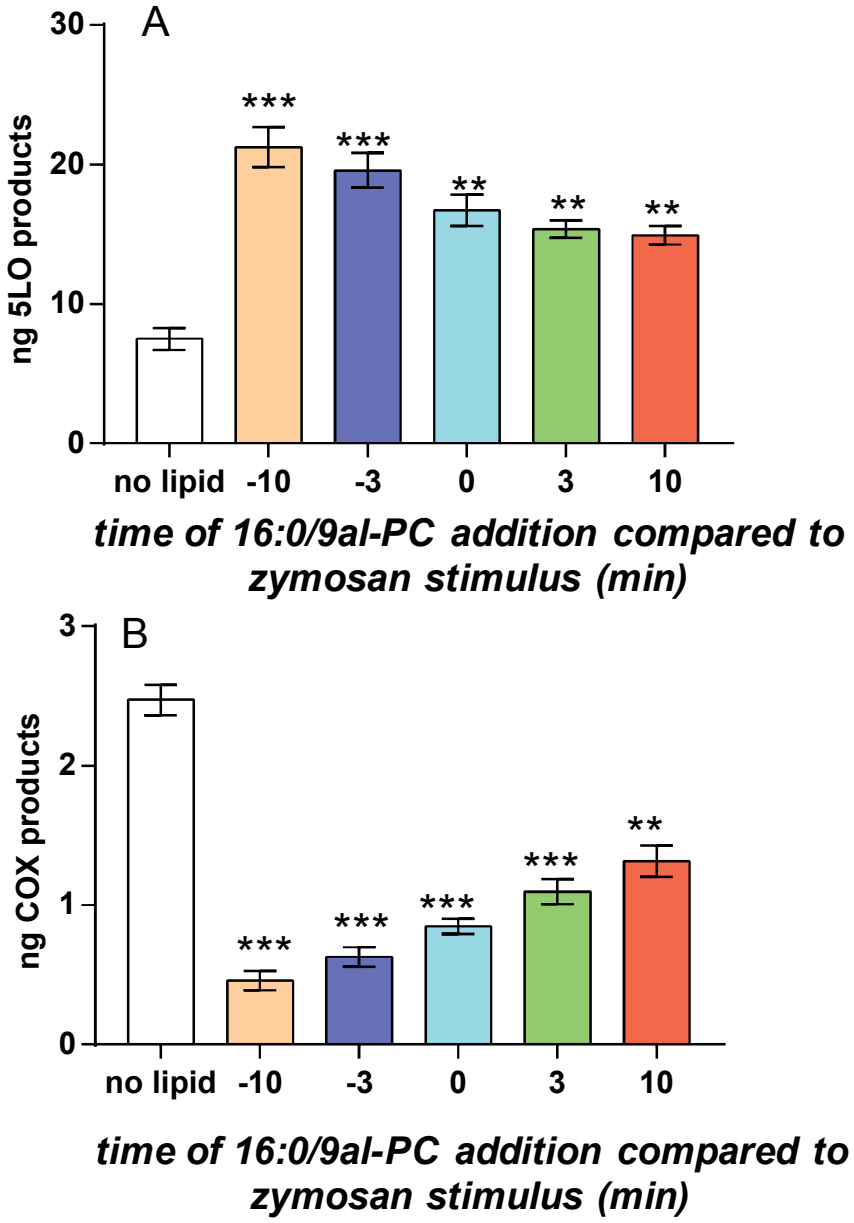

**Figure S5**: The effect of 16:0/9al-PC on intracellular calcium levels was examined in cells isolated from the peritoneal cavity loaded with indo-1 AM. Stimulation with PAF (100 nM) induced transient calcium flux, but no calcium flux was initiated by 16:0/9al-PC (7.5-75 µM). The arrow indicates when stimulus was added. The calcium flux was determined by monitoring the change in indo2/indo1 fluorescence. This response is representative of three experiments.

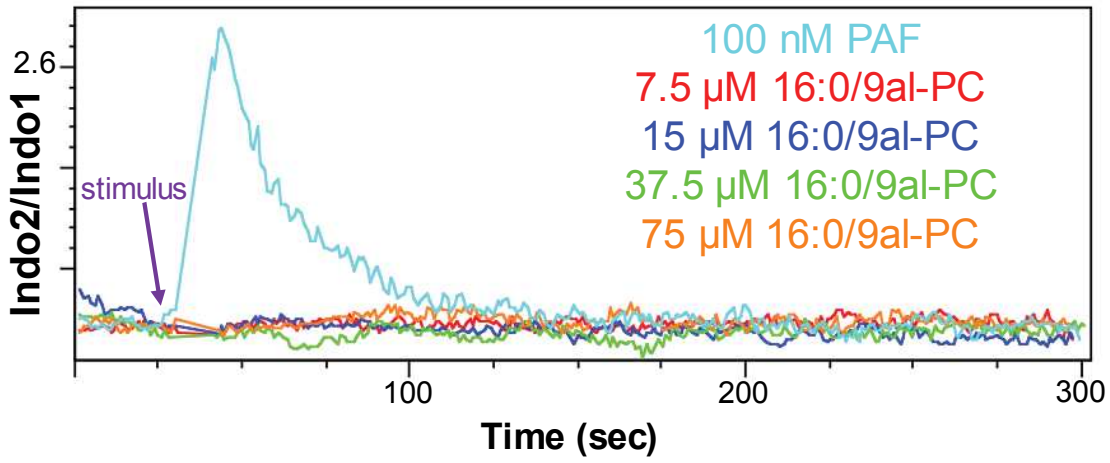

**Figure S6**: Production of 5-LO products ($LTC_4$, 6-*trans*-$LTB_4$, $LTB_4$, and 5-HETE) in resident peritoneal macrophages after a 30 min incubation at 37°C with zymosan (25 particles per cell), 16:0/9al-PC (37.5 μM), or 16:0/9al-PC and zymosan (25 particles per cell). After incubation, the eicosanoids ($LTC_4$, 6-*trans*-$LTB_4$, $LTB_4$, and 5-HETE) were analyzed by LC-MS/MS and quantified using standard isotope dilution. Results shown are averages ± SEM (n=3) from three independent experiments. * $p< 0.05$; ** $p<0.01$; *** $p<0.0001$.