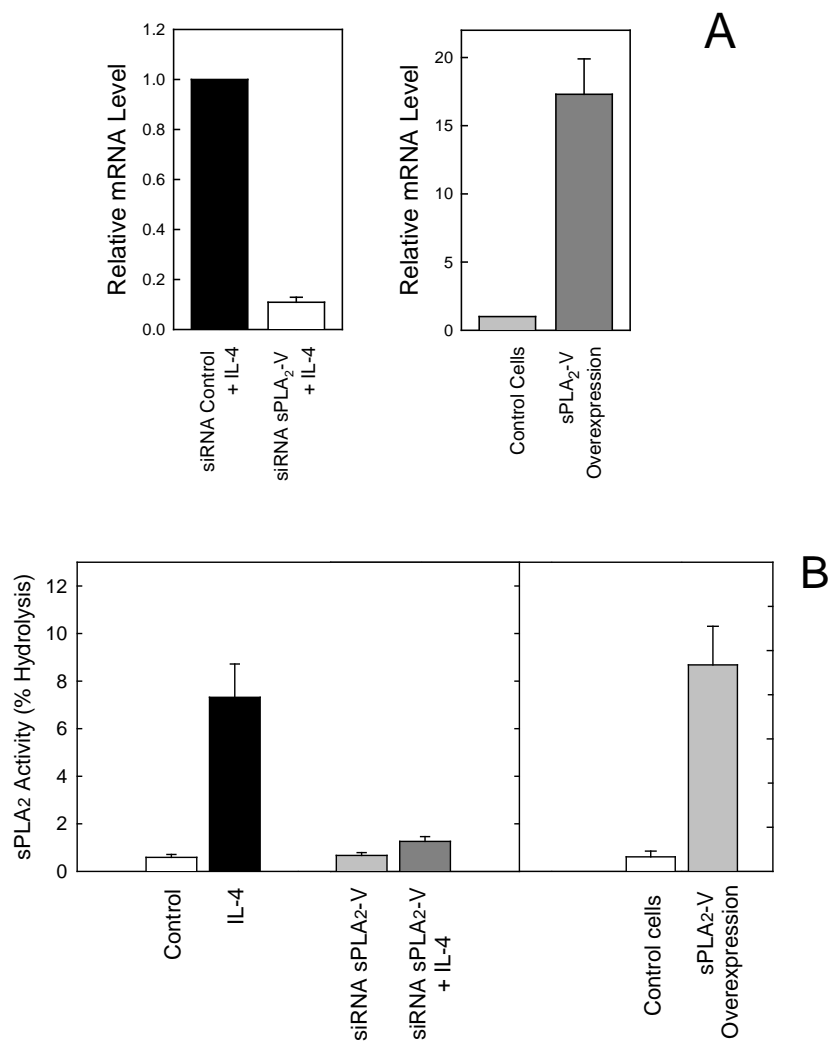
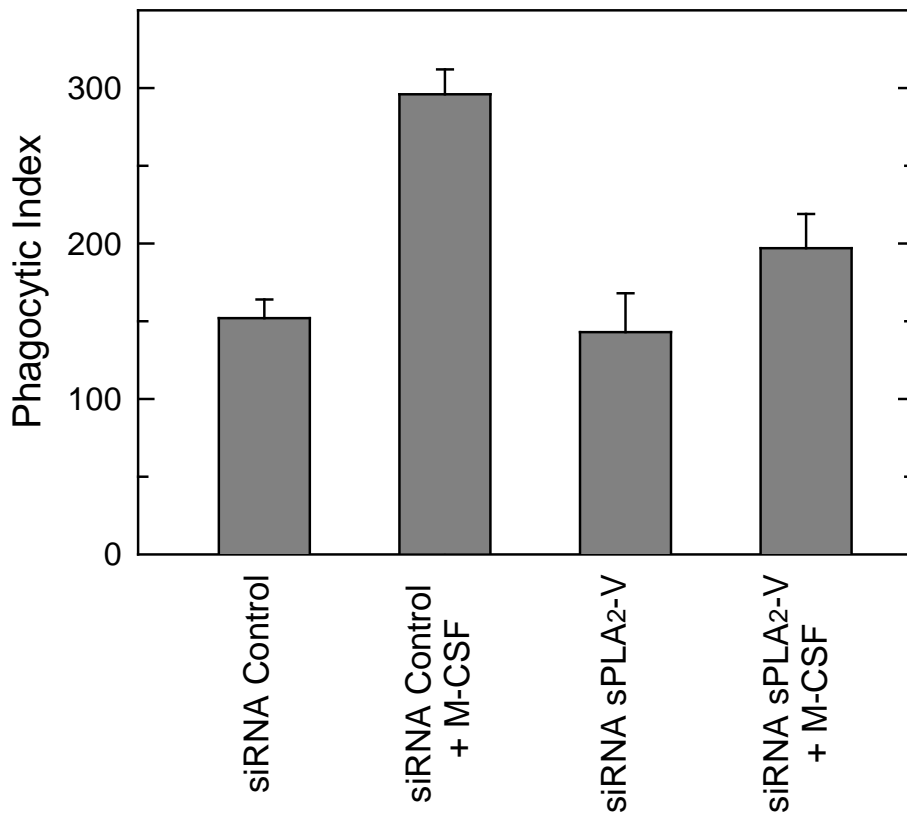


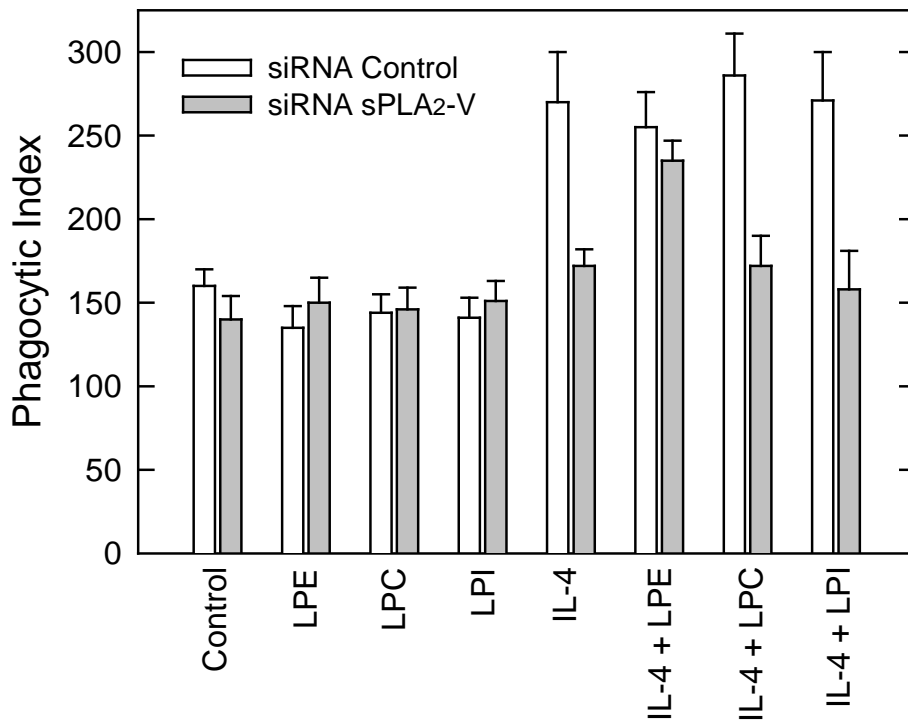
Supplemental Figure 1 – A) Expression of PLA₂ forms in human macrophages, as determined by qPCR. B) Effect of a 24-h treatment with 500 U/ml IFN γ plus 10 ng/ml LPS (green bars) or 1,000 U/ml IL-4 (orange bars) on the expression level of PLA₂ forms, as determined by qPCR. Data are average of three independent experiments with triplicate determinations (mean \pm S.E.M.).



Supplemental Figure 2 – A) qPCR analysis of sPLA₂-V levels. Left panel: cells were transfected with control siRNA (closed bar) or siRNA against sPLA₂-V (open bar), and after a 24-h treatment with 1,000 U/ml IL-4, the levels of mRNA expression for sPLA₂-V were analyzed by qPCR. Right panel: mRNA expression levels for sPLA₂-V were analyzed in macrophages overexpressing an empty plasmid (light gray bar) or a plasmid containing human sPLA₂-V (dark gray bar). Data are representative of at least three independent experiments. Error bars represent \pm SEM. (n=3). B) sPLA₂ activity of human macrophage homogenates. Left panel: homogenates were prepared from untreated cells without (open bar) or with (gray bar) siRNA to sPLA₂-V, or cells treated with 1,000 U/ml IL-4 without (black bar) or with (dark gray bar) siRNA to sPLA₂-V, as indicated. Right panel: homogenates were prepared from cells overexpressing a plasmid containing sPLA₂-V (gray bar) or an empty vector (open bar). PLA₂ activity in the homogenates was assayed using a [³H]AA-labeled natural membrane substrate, as detailed in Materials and Methods. Data are shown as means \pm SEM of three independent determinations from different homogenate samples.



Supplemental Figure 3 – sPLA₂-V is involved in the phagocytosis of zymosan by M-CSF-treated macrophages. Human macrophages, either untreated (control) or treated with 50 ng/ml M-CSF for 24 h, and treated with siRNA control or siRNA for sPLA₂-V, as indicated, were analyzed for phagocytosis of fluorescent zymosan particles by confocal microscopy, and a phagocytic index was calculated by dividing the number of phagosomes by the total number of cells in a field, which was multiplied by the percentage of phagocytosing cells. Data are shown as means ± SEM of three independent experiments.



Supplemental Figure 4 – Effect of various lysophospholipids on the phagocytosis of zymosan by IL-4-treated macrophages. Human macrophages, either untreated or treated with 1,000 U/ml IL-4 for 24 h, as indicated, were analyzed for phagocytosis of zymosan particles. The cells were treated with siRNA control or siRNA for sPLA₂-V as indicated. Where indicated, LPE, LPC or LPI (5 μM) were added. A phagocytic index was calculated by dividing the number of phagosomes by the total number of cells in a field, which was multiplied by the percentage of phagocytosing cells. The average of three independent experiments with triplicate determinations is shown (mean ± S.E.M.).