Supporting Information

Inhibition of TNF-α-Induced Inflammation by Andrographolide via Down-Regulation of the PI3K/Akt Signaling Pathway

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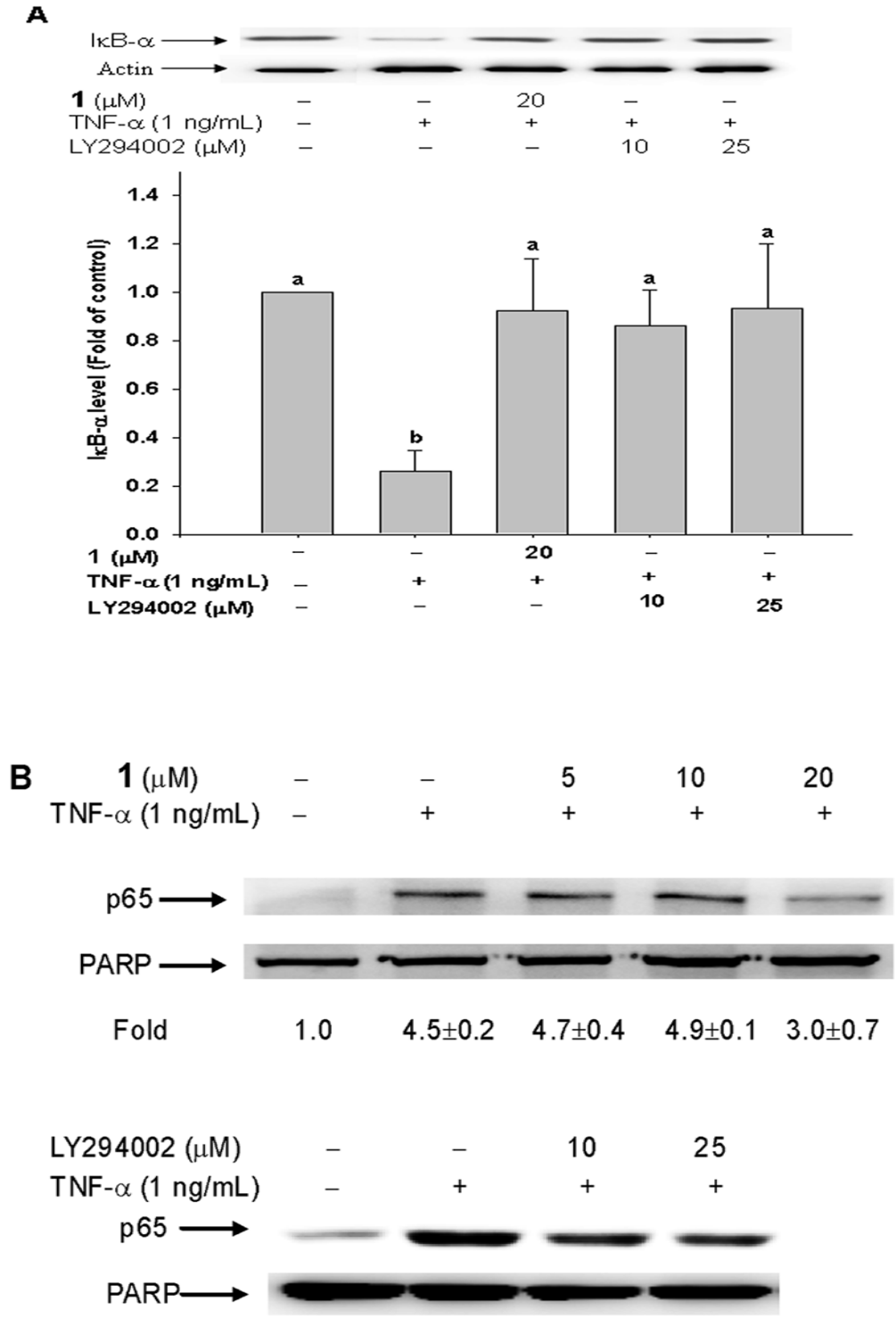
inflammatory conditions.

Figure S1



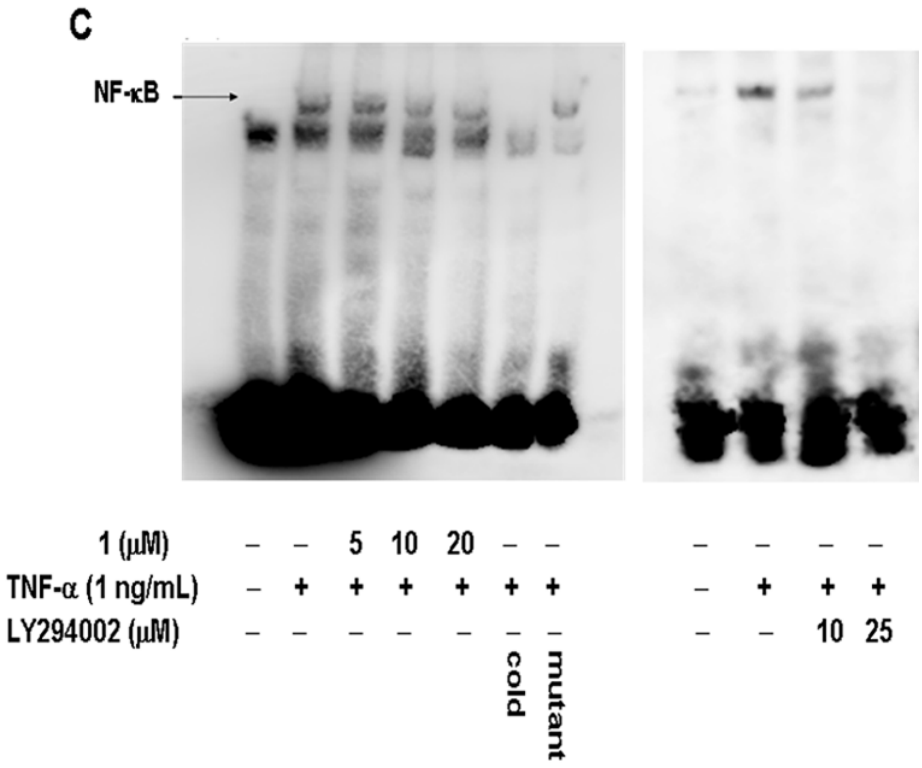


Figure S1. Effects of andrographolide (**1**) and LY294002 on NF-κB activation in TNF-α-stimulated HUVEC cells. Cells were pretreated with indicated concentrations of andrographolide (**1**) and LY294002 for 16 h before being challenged with 1 ng/mL TNF-α for an additional 15 min (for IκB-α) and 30 min (for p65 nuclear protein). Western blot analysis was used to measure the protein expression (A and B for IκB-α and p65 nuclear protein, respectively) and EMSA was used to measure NF-κB nuclear protein DNA-binding activity (C). The unlabeled double-stranded oligonucleotides of NF-κB and mutant NF-κB were added for the competition assay and specificity assay, respectively. Bands were detected by using streptavidin-horseradish peroxidase and

were developed using a SuperSignal West Pico kit (Pierce Chemical Co). Values are means ± SD of three independent experiments, and values not designated by the same letter are significantly different ($p < 0.05$).

Figure S2

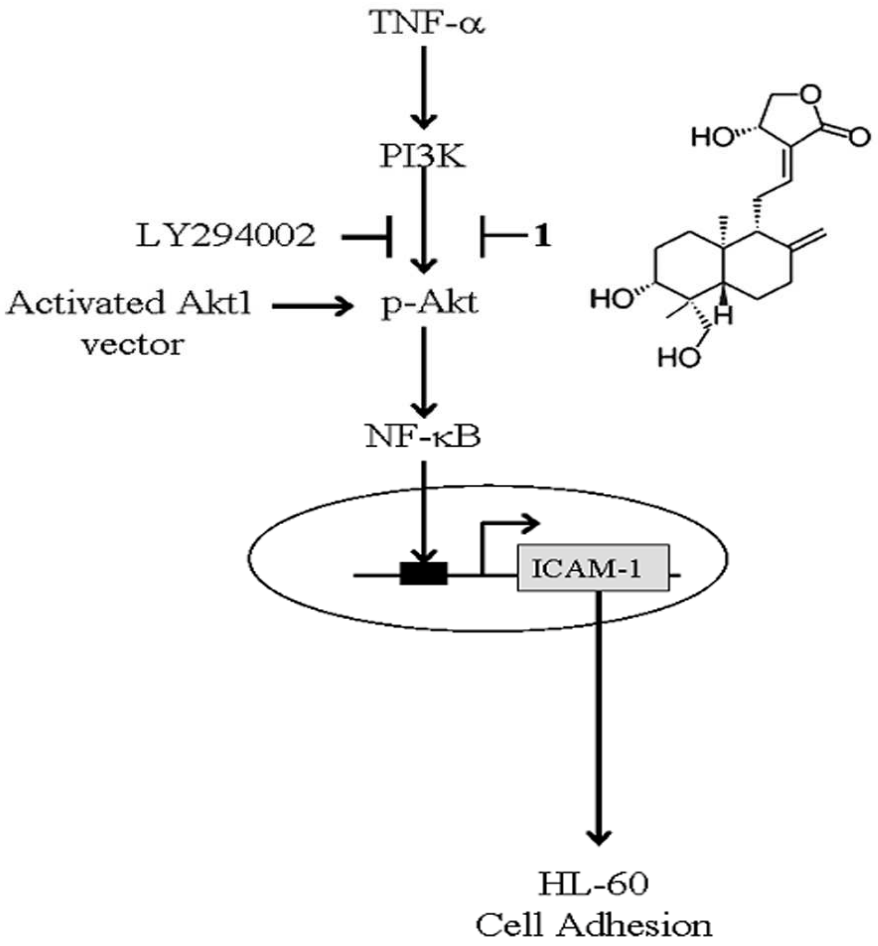


Figure S2. Diagram showing pathways that mediate the inhibition of expression of ICAM-1 and adhesion of HL-60 cells to HUVEC cells by andrographolide (**1**) under

inflammatory conditions. Compound **1** inhibits TNF-α-induced PI3K/Akt pathway and downstream target NF-κB activation which lead to reduced ICAM-1 expression and subsequent HL-60 cell adhesion.