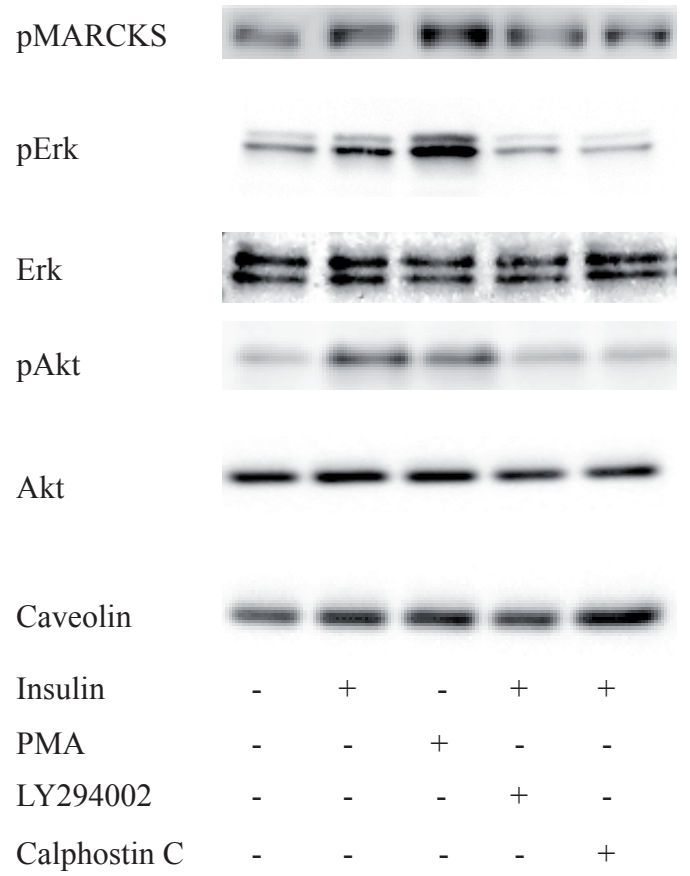
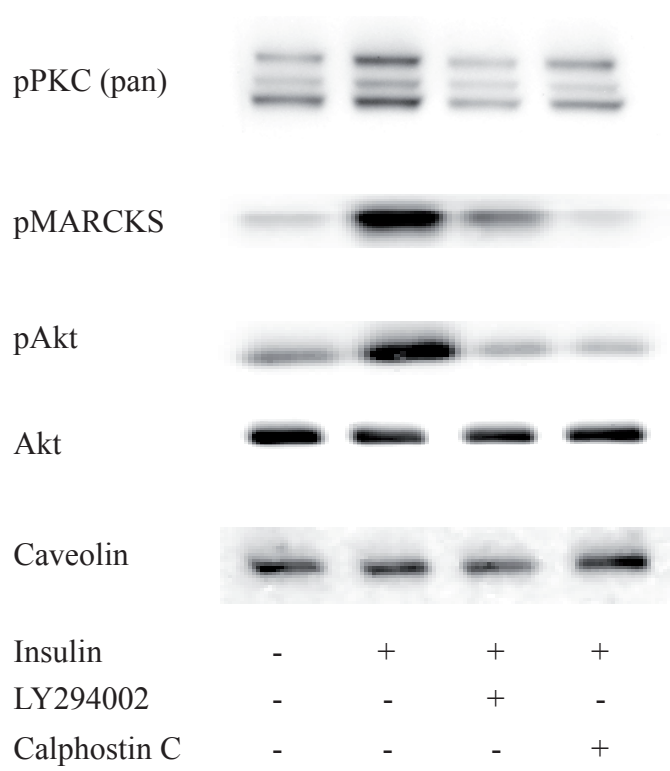


Supplemental Figure 2



Supplemental Figure 1: Validation of MARCKS siRNA targeting construct. Panel A shows the results of immunoblots prepared from endothelial cells transfected with control or MARCKS siRNA targeting constructs and probed with antibodies as shown. The MARCKS siRNA construct used throughout these studies (described in Experimental Procedures) is compared in this figure both with control siRNA as well as a second independent MARCKS targeting construct (labeled MARCKS#2). The MARCKS#2 duplex siRNA has the sequence 5'-AGCGAATGGGCAGGAAAAT-dtdt-3', corresponding to nucleotides 90-108 of the bovine MARCKS mRNA. The antibodies shown here were all obtained from Cell Signaling, with the exception of the MARCKS antibody, which is from CellTec (Vancouver, BC); the caveolin-1 antibody is from BD Transduction Labs. Panel B shows the results of wound healing assays analyzed in endothelial cells transfected with control or MARCKS, or MARCKS#2 siRNA duplex siRNAs, showing that two independent MARCKS targeting constructs each attenuate directed endothelial cell migration. Additional details of the protocols used are presented in the Experimental Procedures section of the text.

Supplemental Figure 2: Effects of kinase inhibitors on phosphorylation of MARCKS, PKC, ERK1/2, and kinase Akt. This figure shows immunoblots analyzed in endothelial cells incubated with kinase inhibitors and then treated for 15 min with insulin or phorbol ester (PMA), and then probed with antibodies as shown. The protein kinase C (PKC) inhibitor calphostin C (100 nM) or the PI3K inhibitor LY294002 (10 μ M) were added one hour prior to the addition of insulin (100 nM), PMA (10 nM), or vehicle. Following cell treatments, cell lysates were resolved by SDS-PAGE and analyzed in immunoblots probed with antibodies directed against phospho-PKC (pPKC), phospho-MARCKS (pMARCKS), phospho-Akt (pAKT), Akt, phospho-ERK1/2, total ERK1/2, or caveolin, as shown. These data are representative blots of 3 similar experiments that yielded equivalent results.