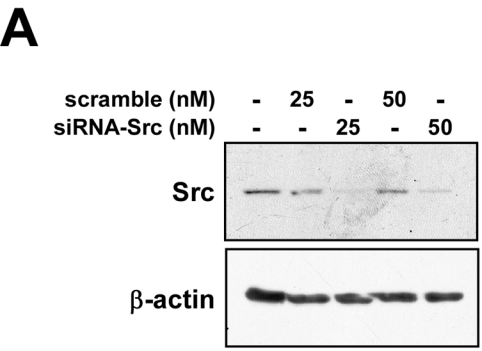
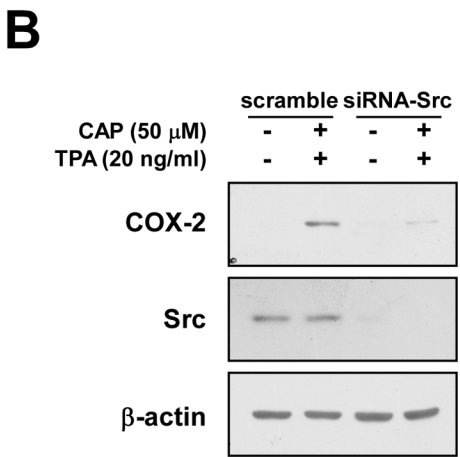
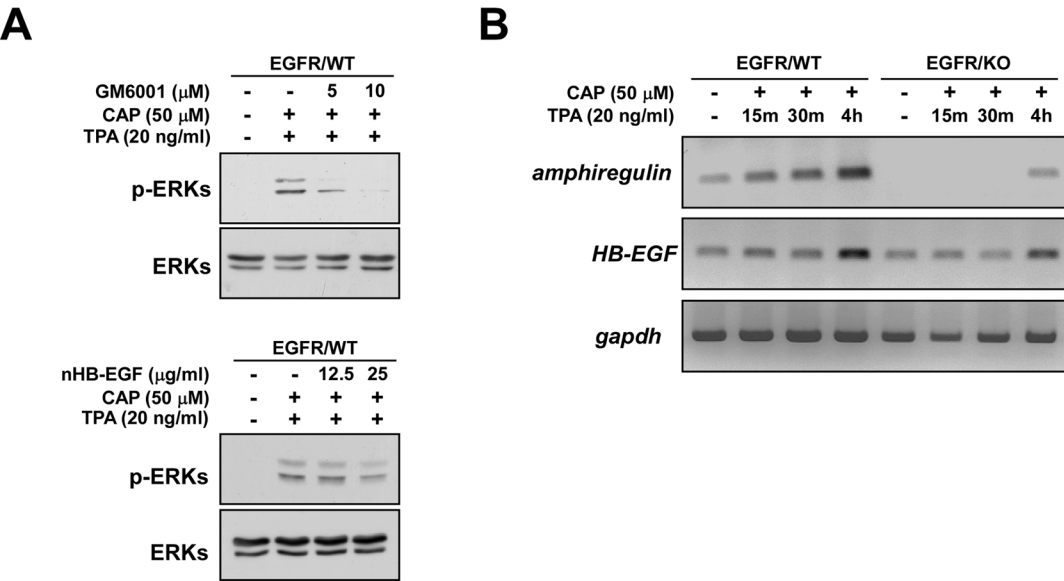
Supplemental Figure 1

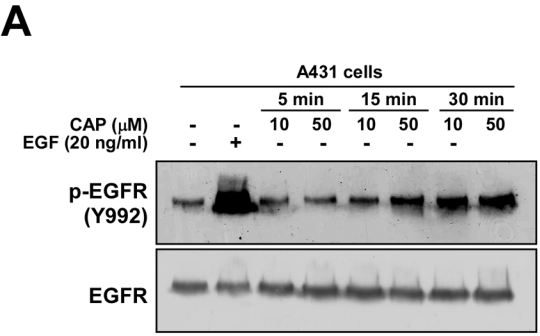


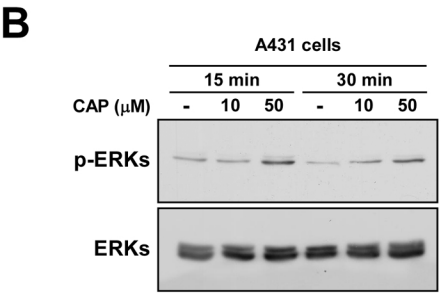


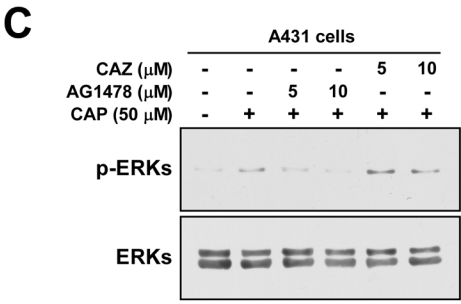
Supplemental Figure 2

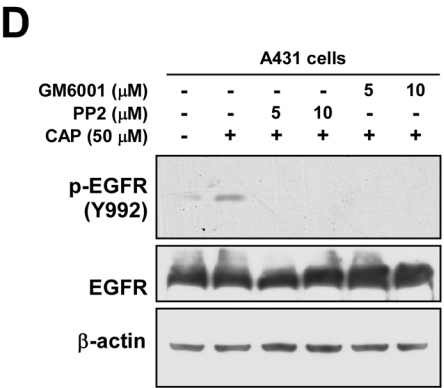


Supplemental Figure 3









Supplemental Figure 1. Effects of siRNA-Src on COX-2 expression in EGFR/WT cells. *A,* EGFR/WT cells were grown to about 50% confluence, and transfected with siRNA-Src or scramble (negative control) using Lipofectamine 2000. The final concentrations of siRNA transfected were 25 or 50 nM. Cells were then grown for 72 h in complete medium, and disrupted to determine the level of knockdown of Src by Western blot. *B,* Transfection with siRNA-Src has an inhibitory effect on TPA/capsaicin-induced COX-2 expression and Src phosphorylation. When cells reached 70% confluence, 50 nM siRNA-Src or scramble was transfected using Lipofectamine 2000. After transfection, cells were grown for 48 h in complete medium and starved for 24 h. After starvation, cells were treated with 50 μM capsaicin (CAP) for 30 min and then treated with 20 ng/ml TPA for 4 h. Cells were disrupted and the abundance of each protein was assessed by Western blot.

Supplemental Figure 2. Involvement of MMP-mediated EGFR transactivation signaling in TPA/capsaicin-induced EGFR downstream activation. *A,* Cells were treated with GM6001 (an MMP inhibitor) or a neutralizing antibody against HB-EGF at the indicated concentration for 30 min before being treated with 50 μM capsaicin. At 30 min after capsaicin treatment, cells were treated with 20 ng/ml TPA for 15 min. Cells were then disrupted and the phosphorylation of ERKs was determined by Western blot as described in Materials and Methods. *B,* TPA/capsaicin induces EGF-like ligand production more strongly in EGFR/WT cells compared to EGFR/KO cells. EGFR/WT and KO MEFs were treated with 50 μM capsaicin for 30 min, followed by 20 ng/ml of TPA at the indicated concentrations. mRNA levels of amphiregulin and HB-EGF, which are major EGF-like ligands produced by MMP activation, were measured using RT-PCR as described in Materials and Methods.

Supplemental Figure 3. Effect of capsaicin on the activation of EGFR and its downstream activity in A431 human skin adenocarcinoma cells. *A* and *B,* capsaicin induces phosphorylation of EGFR and downstream ERKs in a time-dependent manner. Cells were treated with capsaicin (0, 10, or 50 μM) for the indicated time and lysed to measure phosphorylation and total abundance of EGFR and ERKs. Treatment with EGF for 5 min served as a positive control in *A*. *C,* capsaicin-induced ERKs phosphorylation is dependent on EGFR activation, but independent of TRPV1 activation. Cells were treated with AG1478 (an EGFR inhibitor) or capsazepine (a TRPV1 antagonist) at the indicated concentrations for 1 h before being treated with 50 μM capsaicin. After being treated with capsaicin for 30 min, cells were lysed and the expression of each protein was analyzed by Western blot. *D,* The capsaicin-induced EGFR activation is dependent on activation of Src and MMP. Cells were treated with PP2 (a Src inhibitor) or GM6001 (an MMP inhibitor) at the indicated concentrations for 1 h before being treated with 50 μM capsaicin. After being treated with capsaicin for 30 min, cells were lysed and the expression of each protein was analyzed by Western blot.