Supporting Information

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SI Materials and Methods

Antibodies, Cytokines, Growth Factors, Inhibitors, and Reagents. Rabbit polyclonal Akt, ERK1/2, IκBα, phospho-Ser 473 Akt, phospho-Tyr 1068 EGFR, phospho-Tyr-416 Src and Src antibodies and horseradish peroxidase or FITC-conjugated anti-rabbit and Cy3-conjugated anti-mouse secondary antibodies were from Cell Signaling Technology. Rabbit polyclonal active (phospho-) ERK1/2 antibody was from Promega. Mouse monoclonal active caspase-3 and E-cadherin antibodies were from BD Biosciences. Rabbit polyclonal EGFR and phospho-Tyr-1248 ErbB2 antibodies were from Upstate USA . Rabbit polyclonal Fyn and Yes and antibodies were from Santa Cruz Biotechnology. Murine TNF, IL-1α, and IFN-γ were from Pepro Tech. Murine EGF was a gift from Stanley Cohen (Vanderbilt University). AG1478, TAPI-1, CGP, PP1, and PP2 were from Calbiochem. Insulin, transferrin, and selenous acid were from BD Biosciences.

ApopTag *in situ* apoptosis detection kit (TUNEL) and ApopTag *in situ* oligo ligation kit were from Intergen Company. DC protein assay was from Bio-Rad Laboratories. Mouse EGFR and mouse ErbB2 SMARTpool siRNA were from Dharmacon. pcDNA3.1/Zeo vector and Lipofectamine 2000 were from Invitrogen. Protease and phosphatase 1 and 2 inhibitor cocktails were from Sigma. Sulforhodamine multicaspase activity kit was from Biomol International. VectashieldMounting Medium and Antigen Unmasking Solution were from Vector Laboratories.

Cell Line Preparation. The young adult mouse colon (YAMC) cell line is a conditionally immortalized murine colon epithelial cell line isolated from the H-2K^{b}–tsA58 mouse expressing a heat-labile simian virus 40 large T antigen with an IFN-γ-inducible promoter (Immortomouse, Charles River Laboratories).

To prepare the mouse colon epithelial (MCE) cell line with EGF receptor (EGFR) knockout (EGFR$^{-/-}$MCE), EGFR$^{-/-}$ mice were mated with the Immortomouse. Mice homozygous for deletion of the *EGFR* gene and carrying the heat-labile *SV40* gene were killed, and colon epithelium was prepared to derive the EGFR$^{-/-}$MCE cell line, as we have reported previously (42).

Cell Culture. YAMC and EGFR$^{-/-}$MCE cells were maintained in RPMI 1640 media supplemented with 5% FBS (vol/vol), 5 units/ml murine IFN-γ, 100 units/ml penicillin and streptomycin, 5 μg/ml insulin, 5 μg/ml transferrin, and 5 ng/ml selenous acid on collagen-coated plates at 33°C (permissive condition) with 5% CO_2. Before all experiments, cells were serum-starved for 16–18 h in RPMI medium 1640 containing 0.5% FBS (vol/vol) and 100 U/ml penicillin and streptomycin (no IFN-γ) at 37°C (nonpermissive conditions).

Cells of the human colonic epithelial carcinoma cell line HT29 were grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS (vol/vol) and 100 U/ml penicillin and streptomycin at 37°C. HT29 cells were serum starved (0.5%) for 16–18 h before experiments.

Preparation of Cell Lysates. After treatment, cell monolayers were rinsed twice with cold PBS and then were scraped into cell lysis buffer [50 mM Tris·HCl (pH 7.4), 120 mM NaCl, 1% Nonidet P-40) with protease and phosphatase 1 and 2 inhibitor cocktails. The scraped suspensions were centrifuged (14,000 × *g* for 10 min) at 4°C, and the protein concentration was determined with the DC Protein Assay. Equal amounts of cellular lysate proteins were mixed with Laemmli sample buffer and were separated by SDS-polyacrylamide gel electrophoresis for Western blot analysis.

Immunohistochemistry. Antigen retrieval of formalin-fixed sections was performed using the Antigen Unmasking Solution (Vector Labs) by boiling for 15 min. Sections were blocked using 10% goat serum and stained with anti-active caspase 3 or were double-stained with anti-EGFR-Tyr-1068 and anti-E-cadherin antibodies at 4°C overnight. Then, FITC-labeled anti-rabbit and/or Cy3-labeled anti-mouse antibodies were incubated with sections at room temperature for 1 h. Sections were mounted using Vectashield Mounting Medium containing DAPI and were observed by fluorescence microscopy. FITC and DAPI images were taken from the same field.

Apoptosis Assays. Apoptosis was detected in colon sections by ApopTag *In Situ* Oligo Ligation Kit staining using T4 DNA ligase following manufacturer's guidelines (5) and was observed by differential interference contrast microscopy with relative apoptosis determined by counting cells in at least 100 colonic glands.

Apoptosis in cell lines was detected by three methods. ApopTag *in situ* apoptosis detection kits (TUNEL) were used, and DAPI staining was performed as described previously (5). Caspase activity in living cells was detected using the sulforhodamine multicaspase activity kit, according to manufacturer's guidelines. The slides were observed by fluorescence microscopy, and apoptosis was determined by counting at least 500 cells.

Fig. S1. Src, but not MMP, inhibitors block TNF transactivation of EGFR and ErbB2 in intestinal epithelial cells. YAMC cells were treated with TNF (100 ng/ml) for 2 h or with EGF (10 ng/ml) for 5 min in the presence or absence of 1-h pretreatment with Src inhibitors, 0.2 μM CGP, 1 μM PP1, or 1 μM PP2 (*A*) or with 10 nM MMP inhibitor TAPI-1 (*B*) . Inhibitors were maintained during the entire course of TNF treatment. Cellular lysates were prepared to detect EGFR and ErbB2 phosphorylation as described in Fig. 1.

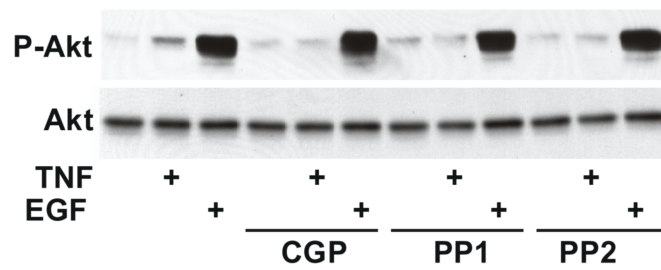

Fig. S2. Src is required for Akt activation in intestinal epithelial cells treated with TNF. YAMC cells were treated with TNF (100 ng/ml) for 2 h or with EGF (10 ng/ml) for 5 min in the presence or absence of 1-h pretreatment with the Src inhibitors 0.2 μM CGP, 1 μM PP1), or 1 μM PP2. Akt activation was detected as described in Fig. 7.

Fig. S3. TNF differentially activates Akt in EGFR$^{-/-}$MCE and KSR$^{-/-}$MCE cells. Cells were treated with TNF (100 ng/ml) for the indicated times or with EGF (10 ng/ml) for 5 min. Akt and EGFR activation were detected as described in Figs. 1 and 7, respectively. IκBα, KSR, EGFR, and Akt expression levels were detected using anti-IκBα, anti-KSR, anti-EGFR, and anti-Akt antibodies, respectively.