SUPPLEMENTARY MATERIALS AND METHODS

siRNA transfection

SUM149 cells were transfected with Lipofectamine 2000 (Invitrogen) using control, non-targeting siRNA (Ambion) and gene-specific silencer select siRNAs (Ambion) as specified in Supplementary Table 5 according to manufacture instructions. The sense and anti-sense strands of the siRNAs are indicated.

Immunoblotting

Cells for immunoblotting were lysed in RIPA buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 1mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with 25 mM β-glycerophosphate, 2 mM sodium vanadate and complete protease inhibitor cocktail (Roche). Protein concentrations were determined by BCA Protein Assay Kit (ThermoFisher Scientific) and equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (Immobilon-P, Millipore). Membranes were blocked for 1 h at room temperature (RT) with 3% BSA in TBST then incubated overnight with antibodies as indicated and exposed to secondary HRP-coupled anti-mouse or anti-rabbit antibodies (GE Healthcare Life Sciences) at 1:10,000 for 1 h at RT. Antibodies against: E-cadherin, Snail, Slug, pSTAT3 (Tyr705), STAT3, GAPDH (all from Cell Signaling), Fibronectin (Sigma), Vimentin (Calbiochem), N-cadherin (BD Biosciences) were used.

Immunohistochemistry

All kits and developing substrates were obtained from Vector Laboratories. Paraffin-embedded tumor sections (4 μm) were de-paraffinized in xylene and ethanol, rehydrated and subjected to antigen retrieval by microwaving for 30 min in antigen unmasking solution. 5% H_2O_2 was used to block endogenous peroxidase activity for 30 min at RT. Protein blocking of non-specific epitopes was carried out using 1.5% normal horse serum. Slides were incubated with primary antibodies for CD68 (Dako), CD163 (Leica Biosystems), IL-8 (BD Biosciences), pJAK2 (Abcam), pSTAT3 (Cell Signaling) overnight at 4°C (Table S6). After washing with PBS-T, slides were incubated with secondary antibody for 2 h at RT and DAB staining was carried out according to manufacturer instructions (Vectastain ABC kit, Vector Laboratories). Slides were counterstained with hematoxylin.

Quantitative RT-PCR

Total RNA was extracted from cells with Trizol reagent (Ambion), according to manufacturer instructions. Equal amounts of total RNA from each sample (1 μg) were reversed-transcribed with the Goscript Reverse Transcription kit (Promega) in accordance with manufacturer instructions. Quantitative PCR was performed using SYBR Green PCR Master Mix (Bio-rad) with the 7500 Fast Real-time PCR thermocycler (Applied Biosystems). All reactions were carried out in a 20 μl reaction

volume in triplicate. Gene expression was normalized to GAPDH. qPCR primers used are indicated (Supplementary Table 6).

ELISA and cytokine arrays

The Human Cytokine Antibody Array V kit (Raybiotech) was used to detect cytokines, chemokines and growth factors in cell-free culture supernatants according to manufacturer instructions. Briefly, the arrays were blocked, incubated with 1 mL of CM overnight, followed by incubation with biotin-conjugated detection antibodies for 2 h and HRP-linked secondary antibodies for 2 h. Membranes were then incubated with chemiluminescent substrate and exposed to x-ray film. Quantitative array analysis was performed using the densitometry software, Image J (NIH). For ELISA, cytokines and chemokines were detected in cell-free culture supernatants using commercial ELISA kits from Biolegend (IL-8), Raybiotech (GRO-α) and Assay Biotech (GRO-β).

Cell Invasion Assay

Invasion assays were performed using 24-well Biocoat Matrigel invasion chambers (BD Biosciences) with 8 μm pore size filters. $5x10^4$ SUM149 cells were placed into the upper chamber in 100 μl RPMI serum-free medium. RPMI medium supplemented with 10% FBS (600 μl) was placed in the lower chamber as a chemoattractant. After incubation for 24 h, the invaded cells that crossed the insert were fixed with methanol, stained with crystal violet, visualized and counted with Image J.