## Cell lines

The TE, HCE cell lines and FLO-1 cells were cultured in DMEM (Corning) supplemented with 10% FBS (Sigma) and 1x penicillin/streptomycin (Invitrogen) in 5% CO2 as described previously. OE19, OE33, EsoAd1 and SKGT4 were cultured in RPMI (Gibco) supplemented with 10% FBS (Sigma) and 1x antibiotic-antimycotic (Gibco). NCI-N87 cells were cultured in DMEM/F12 1/1 (Gibco) supplemented with 10% FBS (Sigma) and 1x antibiotic-antimycotic (Gibco). FEF3 and FEF3303 cell lines were cultured in DMEM supplemented with 15% FBS (HyClone) and 1x penicillin/streptomycin in 5% CO2. The ESCC, EAC, gastric cancer and FEF cell lines were described previously (1–4)

The ESCC-Fb-1 and ESCC-CAF-J1 cell lines were generated from esophageal biopsies. The tissues were incubated in of dispase/collagenase/Y27632 solution for 45min at 37oC (vortexed every 10 minutes and mechanically disrupted with a P1000 pipette tip). Preparations were next incubated for 10 minutes at 37oC in 0.25% trypsin/EDTA. Soybean trypsin inhibitor (Sigma) was used to inactivate trypsin. The resulting cell suspension and tissue fragments were forced through a 70 μm strainer, centrifuged, resuspended in fibroblast media supplemented with fungizone (Gibco) and gentamicin (Gibco) and seeded into one well of a 6-well plate. The culture media (DMEM supplemented with 15% FBS (HyClone) and 1x penicillin/streptomycin) was replaced every 48 hours or as needed.

## 2D co-culture of esophageal cancer cells and fibroblasts

For co-culture experiments, the same culture medium was used for all cell lines (epithelial and fibroblast): DMEM (Corning) supplemented with 10% FBS (HyClone) and 1x penicillin/streptomycin (Invitrogen). The tumor cells and fibroblasts were seeded a 1:3 ratio and maintained at 37oC 5% CO2 for 48 or 72 hours. No transwell inserts or other barriers were used to separate the cells, except for the experiment depicted in Fig. S2A. Conditioned media were purified from debris and floating cells by centrifugation (800g – 10’) and stored frozen at -80oC.

## 3D tumoroid culture

The cells were trypsinized, mixed with matrigel (BD) at a 1:5 volume ratio and seeded at 3000 cells/well. After matrices solidified, growth media were added (CnT Prime 3D Barrier Media for Epidermal Models, ZenBio) and replaced every 48-72 hours. For immunohistochemistry (IHC), tumoroids were recovered on day 12 from matrigel with dispase digestion, fixed overnight in 4% paraformaldehyde and embedded in 2% Bacto-Agar:2.5% gelatin before dehydration and embedding in paraffin. Tumoroid size was quantified by taking brightfield images and measuring the cross-section area using ImageJ (5).

## 3D organotypic culture

TE11 cells were grown in organotypic culture as described previously (6), with the following modifications. Transwell inserts (30mm, PET, 0.4μm pore; Millipore) were used to support the 3D collagen/matrigel matrices, containing 27% Nutragen bovine collagen solution type I (Advanced Biomatricx), 18% matrigel (BD), 1× minimal essential medium with Earle’s salts (BioWhittaker), 1.68 mM L-glutamine (Cell- gro), 10% fetal bovine serum (Hyclone), 0.15% sodium bicarbonate (BioWhittaker), and 2×104 FEF3303 fibroblasts. On day 7 of culture, 2.5x105 epithelial cells were seeded on top of the matrices. On day 15, the cultures were fixed in Zn-buffered formalin (Thermo) for 2 hours at 4oC before dehydration and embedding in paraffin.

## Xenograft tumors

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania and the Wistar Institute. A total of 6x106 cells (all epithelial, or 5x106 epithelial cells with 106 fibroblasts), resuspended in 50 μl Matrigel, was injected subcutaneously into rear flanks of athymic nude mice (6-10 weeks old, Taconic or Charles River). Tumors were measured twice a week after becoming palpable. For therapeutic studies, tocilizumab or human IgG control were delivered intraperitoneally at 10mg/kg three times a week.

For therapeutic studies using patient-derived xenograft (PDX) tumor models (7, 8), non-obese diabetic/severe combined immunodeficient/interleukin-2 receptor γ-chain-deficient (NSG) mice were used. Surgical specimens were partitioned for histology, cryopreservation, and implanted within 1 hour of resection. Mice were anesthetized using isoflurane, and a 5 mm incision was made in the flank. Tumor tissue was placed in the subcutaneous pocket created, which was closed by suturing.  Tumors were passaged using the same procedure when they reached ~1cm3.

## Generation of IL-6 knockout cell lines

The IL-6 CRISPR/Cas9 KO (h) kit (contains a pool of three gRNA sequences: TCTCATTCTGCGCAGCTTTA, GTACCTCATTGAATCCAGAT, CACTACTCTCAAATCTGTTC) was purchased from Santa-Cruz biotechnology and used according to the manufacturer’s instructions. The resulting single cell-derived clones were expanded, and the levels of IL-6 in cell culture conditioned media were measured by ELISA and compared to wild-type TE11 conditioned medium, as well as to DMEM (**Fig. S4**). The stability of knockout was confirmed by ELISA for up to passage 12 post-transfection.

## Real-time qPCR

RNA was isolated using the RNAqueous-4PCR kit (Ambion). cDNA was generated using oligo-dT primers and Superscript II Reverse Transcriptase (Life Technologies). Real-time PCR was performed using validated SYBR Green primers and ABI7000 and ABI StepOne instruments (Applied Biosystems). The primer sequences can be found in **Table S5**.

For qPCR analysis of STAT3- and ERK1/2-target genes, resected tumors were placed in RNAlater solution (ThermoFisher, Waltham, MA) and stored at -20**o**C, after which RNA was harvested using RNeasy (Qiagen).  cDNA was generated with TaqMan Reverse Transcription Reagents (ThermoFisher), and quantitated on a QuantStudio 6 Real-Time PCR System (ThermoFisher). The STAT3 target gene panel was selected based on the results of STAT3 ChIP-Seq (David Frank lab, unpublished data, GEO submission pending). The ERK1/2 target genes were selected based on published research (9).

## Flow cytometry (FACS)

Cells were labeled with CFDA-SE (Invitrogen; 1 or 0.1 μM for epithelial cells or fibroblasts, respectively) for 30 minutes at 37oC, quenched for 30 minutes at 37oC, trypsinized and seeded into co- or mono-cultures. After 72 hours, the cells were trypsinized, resuspended in PBS with 1% BSA and 0.01% sodium azide. Samples were analyzed on a FACScalibur (BD) or Accuri (BD). Data were analyzed using FlowJo (Treestar).

## Histology

Clinical materials were obtained from informed-consent patients according to the Institutional Review Board standards and guidelines. ESCC tissue samples were obtained as surgical biopsies from Kagoshima University Hospital, as described previously (10). EAC and gastric cancer tissue samples were obtained as surgical biopsies from Dr. Kenneth K. Wang, M.D. at the Mayo Clinic (Rochester, MN; IRB protocol 15-009292) and Dr. Matthew D. Stachler M.D., Ph.D. at the Brigham and Women’s Hospital (Boston, MA; IRB protocol 2012P002411). HNSCC tissue samples were obtained as biopsies from Dr. Devraj Basu, M.D., Ph.D. at the University of Pennsylvania (IRB protocol 417200).

The list of antibodies and specific details can be found in **Table S6**. For IL-6 IHC, the antibody from Abcam was used in all experiments; the antibody from R&D was used to confirm staining specificity. For IHC staining, the slides with paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated, followed by heat-mediated antigen retrieval at the appropriate pH (for pH6.0: 10mM Sodium Citrate; for pH9.0: 10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20). Endogenous peroxidases were blocked with 3% H2O2 solution, followed by sequential incubation at room temperature with avidin, biotin and protein blocking solutions (Thermo Scientific Starting Block T20 Blocking Buffer). Incubation with primary antibodies was carried out at 4oC overnight, followed by 30 minute incubations at 37oC with a biotinylated secondary antibody (Vector labs, 1:200 dilution) and the ABC reagent (Vector labs). DAB reagent (Vector labs) was used to develop the reaction, followed by a counter-stain with Gills #2 hematoxylin, dehydration and mounting. All histological samples were evaluated and scored by a pathologist (AKS) in a blinded fashion.

*LADL Fluorescent In Situ Hybridization*

Stellaris RNA FISH probes for human IL-6 ( BioSearch Technologies cat. no: VSMF-20534-5) were used to perform RNA FISH on deparaffinized FFPE biopsied tissue sections as previously described (11). For each field of view, 30 z-section images spaced at 0.33 um were acquired on a Nikon Ti-E widefield microscope using a 100x 1.4 NA objective and a Pixis cooled CCD camera (Princeton Instruments).

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