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**Shayaq Ul Abeer Rasool, Arshad A. Pandith, Farooq Ahmad Ganie, Qurat ul Aein, Usma Manzoor, Dil Afroze, Aadil Manzoor Baba, Zubaida Rasool, Nazir Ahmad Dar & Showkeen Muzamil**

# IL-11–IL6ST–STAT3 signaling defines a convergent inflammatory axis in esophageal cancer subtypes

Shayaq Ul Abeer Rasool[1], Arshad A. Pandith[*1], Farooq A. Ganie[2], Quratul Aein[1], Usma Manzoor[1], Dil Afroze[1], Adil M. Baba[4], Zubaida Rasool[3], Nazir A. Dar[4], Showkeen Muzammil[5]

[1] Advanced Center for Human Genetics, Sher I Kashmir Institute of Medical Sciences (SKIMS), Srinagar-190011, J&K, India
[2] Department of Cardiovascular and Thoracic Surgery, Sher-I-Kashmir Institute of Medical Sciences(SKIMS), Srinagar-190011, J&K, India
[3] Department of Pathology, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Srinagar-190011, J&K, India
[4]Department of Biochemistry, University of Kashmir, Srinagar-190011, J&K, India
[5] Department of Biochemistry, Sher-e-Kashmir University of Agricultural Sciences and
Technology, Shuhama, J&K, India

[*]**Corresponding author**

Arshad A. Pandith, Ph.D.

Associate Professor

Advanced Center for Human Genetics,
Sher I Kashmir Institute of Medical Sciences (SKIMS), Srinagar-190011, J&K, India
E-mail address: arshaajiz@gmail.com

arshad.pandith@skims.ac.in

Tel: 0194-2401014 (Ext: 2414)

**Abstract**

**Introduction:** Esophageal cancer (EC) is an aggressive malignancy characterized by poor survival outcomes and strong links to chronic inflammatory signaling. Interleukin-11 (IL-11) has emerged as a cytokine of interest in tumorigenesis and therapy resistance. This study investigated the expression, prognostic implications and functional relevance of IL-11 signaling in EC.

**Methodology:** IL-11 pathway components were analyzed in 50 surgically resected EC and adjacent normal tissues using RT-qPCR and immunohistochemistry (IHC). Histological subtype-stratified analyses were performed for esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Functional assays in KYSE-410 cells evaluated the effect of IL-11 neutralization on viability, migration, and pathway activation. RNA-sequencing, reverse-phase protein array (RPPA), clinical, and survival data from the TCGA-ESCA cohort were analyzed to validate pathway activation and prognostic associations. Additionally, murine RNA-sequencing datasets following anti-IL-11 or anti-IL-11 receptor treatment were examined to assess downstream transcriptional effects

**Results:** IL-11, COX-2, and STAT3 mRNA levels were significantly upregulated in tumors compared with matched normal epithelium ($p < 0.05$) accompanied by increased nuclear phosphorylation of STAT3 (Tyr705). TCGA analyses confirmed elevated expression of IL-11 pathway components in EC. Elevated IL11 expression showed a trend toward reduced overall survival and was significantly associated with poorer disease-free survival. While IL11, STAT3, and PTGS2 expression did not differ significantly between EAC and ESCC, receptor-level signaling components IL6ST (gp130) and JAK1 were significantly higher in EAC,

with RPPA data demonstrating increased pSTAT3 (Tyr705) and e-cadherin levels in this subtype. Downstream transcriptional analyses revealed subtype-specific STAT3 programs, with proliferation and invasion genes enriched in ESCC and survival-associated mediators elevated in EAC. Neutralization of IL11 significantly reduced cell viability ($IC_{50}$ = 1 µg/mL), impaired migration, and suppressed pSTAT3 activation.

**Conclusion:** These findings identify the IL-11/IL6ST/JAK1/STAT3/COX-2 axis as a central inflammatory signaling pathway in esophageal cancer. Although ligand expression is comparable across subtypes, receptor-level signaling in EAC suggests pathway sensitization rather than ligand abundance as a key determinant of signaling intensity. Functional inhibition of IL-11 attenuates tumor-promoting phenotypes, supporting IL-11 signaling as a biologically relevant and potentially pan-histologic therapeutic target in esophageal cancer.

**Keywords:** Esophageal Cancer, Interleukin-11, STAT3, COX-2, Inflammation, Targeted Therapy, Cytokine Signaling

## Introduction

Esophageal cancer (EC) is a highly aggressive malignancy and a leading cause of cancer-related mortality worldwide, with an estimated 604,000 new cases and 544,000 deaths annually[1, 2]. The disease exhibits significant geographical and demographic disparities, with higher incidence rates in Eastern Asia, parts of Africa, and the Middle East, often referred to as the esophageal cancer belt [3, 4] The two predominant histological subtypes; esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), differ in epidemiology, risk factors, and molecular pathogenesis[5]. While ESCC is more prevalent in developing regions and associated with tobacco use, alcohol consumption, and dietary carcinogens, EAC has seen a sharp rise in Western populations, largely attributed to obesity, gastro-esophageal reflux disease (GERD), and Barrett’s esophagus[6, 7]. Despite advancements in multimodal treatment strategies, including surgery,

chemotherapy, and immunotherapy, the overall 5-year survival rate remains dismal, below 20% in most cases due to late-stage diagnosis and therapeutic resistance [8, 9].

At the molecular level, chronic inflammation plays a critical role in EC pathogenesis, driving tumor initiation and progression through cytokine-mediated signaling pathways[10, 11]. Among these, the interleukin-11 (IL-11)/STAT3 signaling axis has emerged as a key regulator of tumorigenesis in various cancers, including gastric and colorectal malignancies. IL-11 binding activates the gp130/JAK axis, leading to phosphorylation of STAT3 at Tyr705, which transcriptionally induces COX-2 and other inflammation-linked genes. The pathway is implicated in promoting cell proliferation, survival, angiogenesis, and immune evasion[12, 13]. Elevated IL-11 expression has been linked to chemoresistance, epithelial-to-mesenchymal transition (EMT), and tumor progression[14-16]; however, its role in esophageal cancer remains poorly understood.

Despite growing evidence of IL-11's oncogenic potential in gastrointestinal tumorigenesis [17], there is a lack of comprehensive studies evaluating its functional role and therapeutic relevance in EC. Current immunotherapeutic approaches, including immune checkpoint inhibitors, have shown promise but are often limited by resistance mechanisms and variable patient response rates. Targeting the IL-11/STAT3 pathway offers a novel therapeutic avenue to suppress tumor growth, enhance treatment efficacy, and overcome resistance in EC[13].

In this study, we investigated the expression and functional significance of IL-11 signaling in esophageal cancer. We analyzed the expression of IL-11 pathway components in tumor and adjacent normal tissues from patients with esophageal cancer. Because esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) differ markedly in etiology, molecular architecture, and inflammatory signaling, we further performed histology-specific analyses within our cohort and in

TCGA-ESCA datasets to determine whether activation of the IL-11/COX-2/STAT3 signaling axis differed between subtypes. We also examined the functional consequences of IL-11 neutralization using anti-IL-11 antibodies in esophageal cancer cells to evaluate its effects on cellular proliferation, migration, and downstream signaling pathways. To further characterize pathway activity, we analyzed the expression of canonical STAT3 downstream transcriptional targets and performed correlation analyses between IL-11 signaling components, STAT3 activation markers, and downstream effector genes. Finally, we assessed the association between IL11 expression and clinical outcomes using TCGA survival datasets. Collectively, this study aims to provide mechanistic and preclinical insights into the role of IL-11 signaling in esophageal cancer and to explore its potential as a therapeutic target.

## Materials and Methods

### Patient Recruitment and Sample Collection

The present study was conducted in Advanced Centre for Human Genetics, Sher-i-Kashmir Institute of Medical Sciences, (SKIMS), Srinagar (J&K, India). Fifty patients diagnosed with esophageal carcinoma were recruited from the CVTS and Surgical Oncology Departments at SKIMS. Among the recruited cohort, 23 patients were diagnosed with ESCC and 27 with EAC. Ethical approval was obtained, and informed consent was obtained from all participants. Histologically confirmed EC (ages 38–70) with no prior treatment were included in the study. Patients with prior malignancies, immunosuppressive therapy, autoimmune diseases, or active infections were excluded.

Tumor and adjacent normal tissues collected via surgical resection or biopsy were snap-frozen in liquid nitrogen, or stored in RNAlater (ThermoFisher Scientific USA) for RNA and protein extraction or fixed in 10% formalin for histology.

### Histopathological and Immunohistochemical (IHC) Analysis

Formalin-fixed paraffin-embedded (FFPE) tumor blocks were analyzed by IHC using 4 µm sections for immunohistochemical evaluation. Antigen was retrieved in citrate buffer (pH 6.0) for 15 minutes in EZ Retriever system (BioCare Medical, Walnut Creek, CA). Deparaffinized slides (sections) were subjected to endogenous peroxidase blocked by 3% $H_2O_2$ in methanol for 10 minutes. Slides were incubated with respective antibodies against human anti-pSTAT3 (1:100, CST, USA), anti-IL-11 (1:50, R&D Systems, UK #AF218), and anti-COX-2 (1:100, CST, USA) followed by appropriate HRP-conjugated secondary antibodies. Detection was achieved using DAB (3,3'-diaminobenzidine) substrate and counterstained with hematoxylin. Staining intensity and percentage of positively stained tumor cells were scored semi-quantitatively by an independent pathologist blinded to clinical data. The scoring method based on percent of cell nuclei that were positive: 0 (no staining), 1 (10–25%), 2 (26–50%), or 3 (> 50%).

**RNA Extraction, cDNA Synthesis, and qPCR**

Total RNA was isolated from 50 tumor and adjacent normal tissues using TRIzol (QIAGEN, Germany), followed by chloroform phase separation, isopropanol precipitation, ethanol washing, and resuspension in RNase-free water. RNA integrity was verified by 1% agarose gel electrophoresis and Nanodrop spectrophotometry (OD260/OD280 ratio).

DNase treatment was performed with DNase I (ThermoFisher, USA) to remove genomic DNA contamination. cDNA synthesis was conducted using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit, and primer specificity was confirmed via NCBI BLAST and qPCR amplification of GAPDH. For qRT-PCR of COX-2, STAT3 and IL-11 mRNA expression, the PCR amplification was performed on Rotor-Gene Q accessories (Qiagen, Germany) using Maxima SyBR Green /ROX qPCR Master Mix (2x) with an internal control GAPDH as per the protocol. The sequence of primers used is given in the supplementary Table 1. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Expression data (RT-qPCR and IHC scores) were additionally analyzed after

stratifying samples into ESCC (n = 23) and EAC (n = 27) to examine subtype-specific trends. All assays were performed in triplicates, and melt-curve analysis confirmed primer specificity.

**Cell Culture and Functional Assays**

Cell Culture: KYSE-410 esophageal squamous carcinoma cells were maintained in DMEM + 10% FBS + 1% penicillin-streptomycin at 37°C, 5% $CO_2$. Cells were treated with anti-IL-11 antibodies (1–5 µg/mL) for 24–72 hours. Anti-IL-11 neutralizing antibodies were supplied in aqueous buffer and did not require DMSO. Therefore, untreated negative controls and doxorubicin, 0.1 µg/mL as positive control were used in all antibody-based assays

Cell Viability Assay: KYSE-410 cells were seeded in 96-well plates (5,000 cells/well) and treated with anti-IL-11 neutralizing antibodies (1–5 µg/mL) for 24-48 hours. Cells were fixed with methanol, stained with 0.5% crystal violet for 30 minutes, and washed. Dye was solubilized in 33% acetic acid and absorbance was measured at 570 nm using a microplate reader. $IC_{50}$ values were calculated using GraphPad Prism.

Wound Healing Assay: Cell migration was assessed by creating a scratch in a confluent monolayer of KYSE-410 cells in 6-well plates. Following serum starvation (6 h), a sterile pipette tip was used to create the scratch. Cells were then treated with anti-IL-11 antibodies and monitored over 24–48 hours. Images were captured at 0, 24, and 48 hours; the relative wound closure was quantified using ImageJ software.

**Western Blot Analysis**

Cells were lysed in RIPA buffer (ThermoFisher Scientific, India) with protease and phosphatase inhibitors. Protein concentration was determined using the Bradford assay. Equal amounts of protein (30 µg) were resolved on SDS-PAGE and transferred onto PVDF membrane (Millipore). Membranes were blocked with 3% BSA and incubated overnight at 4°C with anti-pSTAT3 (1:1000, CST, USA), anti-IL-11 (1:500,

R&D Systems, USA), and anti-GAPDH (1:2000, CST, USA #2118) primary antibodies. This was followed by HRP-conjugated secondary antibody incubation. Bands were visualized using ECL detection (Bio-Rad, USA) and quantified via ImageJ software.

**In Silico Stratified Expression, Clinical, and Survival Analyses Using TCGA-ESCA**

To comprehensively evaluate IL-11 signaling across esophageal cancer subtypes and validate experimental findings, transcriptomic, proteomic, clinical, and survival data were analyzed from The Cancer Genome Atlas Esophageal Carcinoma (TCGA-ESCA) cohort. RNA-sequencing expression data (HTSeq-FPKM normalized counts) and corresponding clinical annotations were obtained through cBioPortal for Cancer Genomics and the Genomic Data Commons (GDC) data portal. Patients were stratified based on histological subtype into esophageal adenocarcinoma (EAC; n = 87) and esophageal squamous cell carcinoma (ESCC; n = 94), according to TCGA pathological annotations.

Gene expression analyses included IL11, IL6ST (gp130), JAK1, STAT3, and PTGS2 (COX-2). Prior to intergroup comparisons, data distribution was assessed using the Anderson–Darling normality test. To examine transcriptional consequences of STAT3 activation, RNA-sequencing expression data of canonical STAT3 downstream targets (*CCND1, BCL2L1, MMP2, MMP9, PIM1, BIRC5, SOCS3, VEGFA*) were extracted and compared between EAC and ESCC cohorts. Expression values were obtained as log2-transformed RSEM normalized counts unless otherwise stated. Normally distributed variables were analyzed using independent Student's *t*-tests, while non-normally distributed data were compared using the Mann–Whitney U test.

To assess pathway activation at the protein level, Reverse Phase Protein Array (RPPA) data for phosphorylated STAT3 (Tyr705) and CDH1 were retrieved from the TCGA-ESCA RPPA dataset via cBioPortal. Expression levels were compared between EAC, n = 48; ESCC, n = 78 using the

Mann–Whitney U test following confirmation of non-normal distribution. To provide independent in vivo validation of IL-11 pathway effects, publicly available RNA-sequencing datasets (NCBI Gene Expression Omnibus GSE128940) from murine models treated with anti-IL-11 or anti-IL-11 receptor antibodies were analyzed. Differentially expressed genes associated with inflammatory signaling, extracellular matrix remodeling, EMT, and proliferation were extracted and visualized as heatmaps.

Clinicodemographic variables, including age at diagnosis, body weight, race, smoking status, alcohol consumption, lymph node involvement, and family history of esophageal cancer, were compared between EAC and ESCC using appropriate parametric or non-parametric statistical tests depending on data distribution.

Overall survival (OS) and disease-free survival (DFS) were evaluated using Kaplan–Meier survival analysis. Patients were stratified by histological subtype, and survival differences were assessed using the log-rank test. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated using Cox proportional hazards models, with EAC designated as the reference group. GEPIA2 was used for exploratory visualization, whereas statistical comparisons were performed on TCGA data obtained via cBioPortal and GDC.



**Statistical Analysis:**

All statistical analyses were performed using GraphPad Prism v10.0 and the VassarStats computational platform. Data are presented as mean ± standard deviation (SD) or median with interquartile range, as appropriate. Normality of continuous variables was assessed using the Anderson–Darling test. Parametric comparisons were performed using paired or unpaired Student’s *t*-tests, while non-parametric data were analyzed using Mann–Whitney U or Kruskal–Wallis tests. One-way ANOVA followed by Tukey’s post hoc test was used for multi-group comparisons in functional assays. Associations between molecular

markers were evaluated using Spearman's rank correlation. Survival analyses were conducted using Kaplan–Meier curves with log-rank tests and hazard ratios (HRs) derived from Cox proportional hazards models. A two-tailed *p*-value < 0.05 was considered statistically significant.

## Results

### 4.1 Patient Demographics and Clinical Features

Among 50 esophageal cancer (EC) patients included in the study, 72% were male and 28% female, with a mean age of 56.3 ± 8.7 years. Histologically, 27 patients (54%) had esophageal adenocarcinoma (EAC) and 23 patients (46%) presented with esophageal squamous cell carcinoma (ESCC). A majority was chronic smokers (64%), and resided in rural regions (86%). Dietary evaluation revealed that 58% regularly consumed red chili wur (a known irritant), while 50% had high intake of Noon Chai (salted tea). Only 2% reported a family history of EC. Tumor staging based on AJCC criteria indicated that 62% were diagnosed at Stage III or higher. A detailed distribution of clinicodemographic characteristics are provided in Supplementary Table 2.

### 4.2 IL-11, STAT3, and COX-2 are overexpressed in esophageal cancers

RT-qPCR analysis demonstrated overexpression of IL-11, PTGS-2 and STAT3 in 72%, 76% and 86% of tumors respectively. We found significant upregulation of IL-11 (2.9 ±1.47; p<0.001), COX-2 (2.8 ±1.29, *p*< 0.001), and STAT3 (1.72 ±0.48, *p*< 0.01) transcripts in tumor tissues compared to matched adjacent normal tissues (Figure 1A). These findings indicate consistent activation of the IL-11/STAT3/COX-2 inflammatory axis in esophageal cancer.

### 4.3 Subtype-stratified expression analysis in the local cohort

To address the biological heterogeneity of EC subtypes, IL-11, COX-2, and STAT3 mRNA expression levels were analyzed separately for ESCC

(n = 23) and EAC (n = 27). Both histological subtypes demonstrated higher expression of all three genes in tumor tissue relative to matched adjacent normal tissues. The subtype fold change comparisons of IL-11(2.91±1.4 vs 2.95±1.58; p=0.93), COX-2 (2.9±1.27 vs 2.76±1.34; p=0.71) and STAT3 (1.77±0.39 vs 1.66±0.58; p=0.43) in EAC vs ESCC were statistically insignificant. (Figure 1B-D) These findings suggest that IL-11 pathway activation is a shared feature of esophageal cancer rather than subtype-restricted phenomenon.

### 4.4 In Silico Validation of IL-11/STAT3/COX-2 Axis Using TCGA-ESCA

To validate the experimental findings and examine clinical relevance, we analyzed transcriptomic data from TCGA-ESCA cohort. IL-11 was significantly upregulated in EC tissues compared to normal esophageal tissue ($p< 0.001$), alongside increased expression of COX-2 (*PTGS2*, $p< 0.01$) and STAT3 ($p<0.05$). (Figure 2A-C) The expression of IL6ST were comparable between (logFC >0.05) in ESCA compared to paired normal tissue (Figure 2D). To further delineate signaling events downstream of IL6ST, expression of Janus kinase 1 (JAK1), the principal kinase mediating gp130-dependent STAT3 phosphorylation, was evaluated. JAK1 expression was significantly higher in esophageal cancer compared to normal tissue (median log2(TPM+1): 5.82 vs 5.20; p = 0.00517; Figure 3E). Although the IL11 remained elevated in all pathological states of ESCA; highest expression was observed in Stage II tumors (median log2(TPM+1): 1.53) but the differences across stages was not significant (Stage I–IV, p = 0.196). This indicates early and sustained activation of IL11 during EC progression (Figure 2F). Correlation analysis showed a positive association between IL-11 and COX-2 (r = 0.45, p < 0.001), STAT3 (r = 0.162, p 0.023), IL6ST (r = 0.16, p 0.025) and JAK 1 (r = 0.196, p = 0.007) expression (Supplementary Figures 1 a-d). Kaplan-Meier survival analysis demonstrated that patients with high IL-11 expression had poor overall survival (OS logrank p=0.24 HR =1.3) and disease-free survival (DFS logrank p=0.049, HR= 1.6, p(HR)=0.052)

compared to those with lower IL-11 expression (Figure 3A-B). The hazards ratio indicated a 30-60% increased risk of mortality in IL-11 high expressers, supported by negative impact in pancancer survival analysis suggest its potential as a prognostic biomarker (Figure 3E). The analysis of combined signature of all five tested genes (IL11, IL6ST, JAK1, STAT3, PTGS2) also impacted OS (HR 1.4 ; logrankP=0.18) and DFS negatively (HR 0.78 ; logrank P=0.3 ). (Figures 3 C and D)

## 4.5 Immunohistochemical validation of IL-11 pathway activation

IHC analysis of FFPE tumor sections revealed strong cytoplasmic and membranous staining for IL-11, and predominant nuclear staining of phosphorylated STAT3 (Tyr705) in cancer cells (Figure 4A–D). Semi quantitative scoring indicated IL-11 positivity in 82% of tumors (score ≥2), COX-2 positivity in 74%, pSTAT3 positivity in 70%. Staining intensity correlated moderately with mRNA levels, and high IL-11 IHC scores were associated with higher histological grade and advanced tumor stage ($p < 0.05$), supporting its role in disease progression. Adjacent normal tissues showed weak to moderate physiological staining, while negative controls showed no DAB signal consistent with baseline cytokine receptor signaling and confirming staining specificity.



## 4.6 Anti-IL-11 neutralization reduces esophageal cancer cell viability and migration

Treatment of KYSE-410 ESCC cells with anti-IL-11 neutralizing antibodies (1–5 µg/mL) resulted in a dose-dependent decrease in cell viability. At 1 µg/mL, cell survival was reduced by 42% at 48 hours ($IC_{50}$ = 1 µg/mL), and at 5 µg/mL, viability dropped by more than 70% compared with untreated controls ($p < 0.01$) (Figure 5A-B). Doxorubicin (0.1 µg/mL) served as a positive control and demonstrated expected cytotoxicity.

In wound healing assays, IL-11 inhibition significantly impaired cellular migration. Quantification revealed a significant reduction in wound closure at 24 and 48 hours post-anti-IL-11 treatment compared with controls (Figure 5F-G), highlighting a role of IL-11 signaling in promoting tumor invasive phenotypes.

### 4.7 IL-11 blockade affects STAT3 activation

Western blotting of KYSE-410 cells treated with anti-IL-11 antibodies showed a dose-dependent reduction in pSTAT3 protein levels. At 1 µg/ml concentration, a slight decrease in p-STAT3 levels compared to the untreated control. At 4 µg/ml**,** anti-IL-11 treatment led to a more substantial decrease in both markers, compared to untreated controls. (Figure 5C-E). Doxorubicin served as a positive control. These findings indicate that IL-11 drives the activation of key inflammatory and proliferative mediators in EC, and its inhibition may reverse oncogenic signaling.

### 4.8 TCGA-Based Comparison of IL-11 Pathway Components between EAC and ESCC

To determine whether IL-11 pathway activation differs between esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC), TCGA-ESCA log transformed RNA seq V2 RSEM data were stratified by histological subtype (EAC, n = 87; ESCC, n = 94). Comparative analysis revealed no statistically significant differences in IL11 (8.68±1.73 vs. 8.52±1.94; p = 0.466), COX-2/PTGS2 (8.68±1.73 vs. 8.52±1.94; p = 0.569), or STAT3 (12.6±0.47 vs. 12.69±0.52; p = 0.711) transcript levels between EAC and ESCC (Figure 6A–C), indicating comparable ligand and effector gene expression across subtypes.

In contrast, IL6ST (gp130), the shared signal-transducing receptor for IL-6 family cytokines, showed a modest but statistically significant increase in EAC compared to ESCC (median expression 9.31 vs 9.20; $p < 0.036$) (Figure 6D). Also JAK1 showed significantly elevated expression in EAC

compared with ESCC (mean log2 RSEM: 12.28±0.37 vs 11.80±0.52; $p < 0.0001$). These findings suggest that while IL-11 ligand and downstream effector transcripts are similarly expressed, receptor-level differences may contribute to differential pathway activation between EC subtypes. (Figure 6E). The coordinated upregulation of IL6ST and JAK1 in EAC, provides a mechanistic basis for enhanced STAT3 phosphorylation observed in this subtype.

### 4.9 Differential STAT3 Activation and Clinical Characteristics in EAC and ESCC

To assess whether receptor-level differences translate into altered downstream signaling, RPPA-based analysis of phosphorylated STAT3 (Tyr705) was performed using TCGA-ESCA data (EAC, n = 48; ESCC, n = 78). pSTAT3 Tyr705 levels were significantly higher in EAC compared to ESCC (median RPPA score 0.168 vs −0.1348; $p < 0.0007$) (Figure 6F), indicating enhanced STAT3 activation in EAC despite similar STAT3 transcript levels.

Clinicodemographic comparison revealed that EAC patients were significantly older at diagnosis (66.64 ± 11.92 vs 58.26 ± 10.18 years; $p < 0.001$) and had higher body weight (86.27 ± 19.78 vs 64.46; $p < 0.001$) compared to ESCC patients (Supplementary Figures 2A–B). Primary lymph node involvement was more frequent in EAC (94.59%) than ESCC (68.48%; $p < 0.001$). Alcohol consumption and smoking ($p<0.01$) were significantly associated with ESCC, whereas family history of esophageal cancer did not differ between subtypes (Supplementary Figures 3A-D). The risk factors were distinct for EAC vs ESCC. The prevalence of barett’s esophagus, reflux history, tumour involvement site, columnar mucosa dysplasia and metaplasia were significantly different between the two histological subtypes (Supplementary Figures 4A-F).

Survival analysis demonstrated no significant differences in overall survival (OS) or disease-free survival (DFS) between EAC and ESCC (OS log-rank $p = 0.58$; DFS log-rank $p = 0.69$), suggesting that despite

distinct clinico-demographic profiles and differential STAT3 activation, clinical outcomes remain broadly comparable (Supplementary Figure 5A-B).

### 4.10 Differential expression and correlation analysis of STAT3 downstream transcriptional targets in esophageal cancer

To further investigate the functional transcriptional consequences of IL-11–STAT3 pathway activation, expression of canonical STAT3 downstream target genes was examined in the TCGA-ESCA RNA-sequencing dataset stratified by histological subtype (EAC, n = 87; ESCC, n = 94). Comparative analysis revealed distinct subtype-specific transcriptional programs despite similar expression of upstream IL-11 signaling components.

ESCC tumors demonstrated relatively higher expression of proliferation- and invasion-associated genes including CCND1 (13.03 ± 1.51 vs. 12.18 ± 1.01), MMP9 (8.87 ± 1.97 vs. 7.93 ± 1.86), MMP2 (12.10 ± 1.46 vs. 11.43 ± 1.41), BIRC5 (9.09 ± 0.79 vs. 8.40 ± 0.84), SOCS3 (11.19 ± 1.05 vs. 10.62 ± 1.03), and PIM1 (10.79 ± 0.94 vs. 9.86 ± 1.04) compared with EAC tumors. These genes are associated with cell-cycle progression, extracellular matrix remodeling, and tumor invasiveness, indicating stronger proliferative and matrix-remodeling programs in ESCC.

In contrast, EAC tumors showed relatively higher expression of survival- and angiogenesis-associated mediators including BCL2L1 (11.68 ± 0.63 vs. 10.61 ± 0.58) and VEGFA (11.76 ± 0.94 vs. 11.20 ± 0.96) compared with ESCC tumors, suggesting enhanced anti-apoptotic signaling and angiogenic activity in this subtype (Figure 7).

To determine whether these transcriptional differences were reflected at the protein level, reverse-phase protein array (RPPA) data from the TCGA-ESCA cohort were examined. Analysis of the epithelial marker CDH1 (E-cadherin) revealed significantly higher protein expression in EAC compared with ESCC (0.73 ± 0.82 vs. 1.23 ± 1.12, $p < 0.01$),

indicating subtype-specific differences in epithelial differentiation and supporting the transcriptional divergence observed between EAC and ESCC.

To further evaluate pathway connectivity, correlation analyses were performed between IL11 signaling components and STAT3-regulated transcriptional targets using TCGA-ESCA data. STAT3 expression showed significant positive correlations with multiple canonical downstream targets including CCND1 ($\rho = 0.335$, $p = 1.63\times10^{-6}$), BCL2 ($\rho = 0.365$, $p = 1.49\times10^{-7}$), VEGFA ($\rho = 0.30$, $p = 2.1\times10^{-5}$), SOCS3 ($\rho = 0.312$, $p = 9.07\times10^{-6}$), and MMP2 ($\rho = 0.258$, $p = 2.69\times10^{-4}$), supporting active STAT3-driven transcriptional programs in esophageal cancer. Notably, IL11 expression demonstrated a strong positive correlation with the combined STAT3 target gene signature ($\rho = 0.477$, $p = 1.7\times10^{-12}$), indicating that tumors with elevated IL11 expression exhibit enhanced activation of downstream oncogenic pathways. Furthermore, IL11 expression correlated with IL6ST ($\rho = 0.182$, $p = 0.0109$) and JAK1 ($\rho = 0.183$, $p = 0.0105$), while both IL6ST ($\rho = 0.225$, $p = 0.012$) and JAK1 ($\rho = 0.35$, $p = 6.85\times10^{-5}$) correlated with phosphorylated STAT3 (Tyr705), supporting a receptor-level mechanism of STAT3 activation in esophageal cancer (Supplementary Table S3).

Collectively, these findings suggest that although upstream IL-11 pathway components are comparably expressed across esophageal cancer subtypes, the downstream transcriptional outputs of STAT3 activation diverge between EAC and ESCC, reflecting distinct biological programs related to proliferation, invasion, and survival.

### 4.11 Independent murine RNA-seq datasets demonstrate reversal of inflammatory and EMT programs following IL-11 inhibition

To examine whether IL-11 signaling regulates inflammatory and mesenchymal transcriptional programs in vivo, we analyzed publicly available RNA-sequencing data from murine models treated with

neutralizing anti-IL-11 or anti-IL-11 receptor antibodies [18]. Differential expression analysis demonstrated down regulation of canonical inflammatory mediators (Ptgs2, Il6, Ccl2, Tnfa), EMT-associated genes (Snai1, Zeb1, Vim) and extracellular matrix remodeling enzymes (Mmp2, Mmp9) following IL-11 pathway inhibition (Figure 8A). Genes associated with proliferative signaling, including Ccnd1, were also suppressed following IL-11 pathway inhibition. Gene set enrichment analyses demonstrated global reversal of inflammatory, fibrotic, proliferative and mesenchymal transcriptional signatures, consistent with suppression of IL-11–dependent signaling programs (Figure 8 B). The inflammatory response was most drastically affected by both treatments, with anti-IL11 showing a 41% stronger inhibitory effect in this category compared to Anti-IL11RA (Figure 8 C). These findings provide independent transcriptomic evidence that IL-11 signaling contributes to inflammatory and pro-tumorigenic transcriptional programs in vivo.

**Discussion:**

Our study highlights the significant role of IL-11 in esophageal cancer by demonstrating its overexpression in tumor samples compared to adjacent normal tissues. This finding aligns with a growing body of literature indicating that IL-11 is often implicated in promoting inflammation and tumor progression across various cancer types. The IL-11 receptor complex, composed of IL-11Rα and the shared gp130 subunit, initiates downstream activation of JAK kinases upon ligand binding, which in turn phosphorylates STAT3 at Tyr705[15, 19]. This leads to STAT3 dimerization, nuclear translocation, and activation of genes implicated in oncogenesis, proliferation-associated targets including BCL2L1 (Bcl-XL), CCND1 (cyclin D1), migration and EMT-related targets include MMPs, and EMT transcription factors such as SNAIL and TWIST and pro-angiogenic factors like VEGFA[20]. Our data show that IL-11 also upregulates COX-2, enhancing prostaglandin mediated inflammation and angiogenesis which is critical for EC cell survival and metastasis. Thus,

the dual phenotypic effects observed in KYSE-410 cells are consistent with the pleiotropic nature of STAT3 signaling

Importantly, our subtype-stratified analyses indicate that activation of the IL-11 signaling axis is not restricted to a specific histological subtype of esophageal cancer. Both EAC and ESCC demonstrated comparable overexpression of IL11, STAT3, and COX-2 at the transcript level in both the local cohort and TCGA-ESCA dataset. These findings suggest that IL-11–driven inflammatory signaling represents a shared molecular feature of esophageal carcinogenesis, despite the distinct etiologic origins of EAC and ESCC. This convergence on a common cytokine-driven pathway may partially explain why these subtypes exhibit overlapping clinical outcomes despite divergent risk-factor profiles.

Interleukin-11 expression correlates with poor prognosis and promotes tumor progression in renal cell carcinoma [21]. In colorectal cancer, IL-11-driven STAT3 activation was shown to be essential for tumor regeneration after chemotherapy, emphasizing its role in therapeutic resistance [22]. Similarly studies have demonstrated that IL-11 levels are predictive of poor response to immune checkpoint blockade in gastrointestinal tumors, suggesting that IL-11 may contribute to an immunosuppressive tumor microenvironment [23, 24]. Similarly, in pancreatic cancer, IL-11 was shown to facilitate fibrosis, tumor growth and metastasis, underscoring its potential as a therapeutic target [25].

Our RT-qPCR results demonstrated significant overexpression of IL-11, COX-2, and p-STAT3 in esophageal cancer tissues. The correlation of IL-11 with COX-2, a key enzyme involved in prostaglandin synthesis and inflammation, further reinforces the notion that IL-11 contributes to a pro-inflammatory microenvironment conducive to tumor progression. Previous studies have established that COX-2 is frequently overexpressed in various cancers and is associated with enhanced angiogenesis, tumor growth, and metastasis [26, 27]. The concordance between our gene expression analyses and protein expression results further solidifies the

role of IL-11 in esophageal cancer. Our findings indicate that IL-11 and its associated signaling pathways may play a pivotal role in sustaining tumor cell viability and promoting cancer cell migration, aligning with studies suggesting that IL-11 enhances epithelial-to-mesenchymal transition (EMT) processes in cancer cells [28].

Our correlation analysis between IL-11 and STAT3, as well as COX-2, elucidates potential mechanistic links in esophageal cancer progression. The significant correlation of IL-11 with COX-2 ($R = 0.44$, $p < 0.001$) indicates that IL-11 may promote an inflammatory milieu that supports tumor growth and metastasis. This is consistent with findings by Wu et al. who reported that IL-11 induces COX-2 expression through the STAT3 pathway in breast cancer[29]. Moreover, the correlation of IL-11 with p-STAT3 further emphasizes the potential for IL-11 to activate oncogenic signaling pathways that facilitate cancer progression. The proposed mechanistic model illustrating IL-11 signaling through IL11RA/IL6ST, activation of JAK1, phosphorylation of STAT3, and induction of COX-2, leading to enhanced protumorigenic processes as summarized in Figure 9.

Consistent with this interpretation, RPPA-based proteomic analysis demonstrated significantly higher phosphorylation of STAT3 at Tyr705 in EAC compared to ESCC, despite similar total STAT3 mRNA expression across subtypes. Because STAT3 phosphorylation represents the functionally active state of the pathway, these findings indicate that EAC tumors exhibit enhanced IL-11/IL6ST–dependent STAT3 activation rather than differential STAT3 expression per se. Similar discrepancies between total STAT3 expression and pSTAT3 levels have been reported in other inflammation-driven malignancies and highlight the necessity of integrating proteomic data to accurately assess pathway activity.

Notably, while IL11 ligand and downstream effector transcripts did not differ significantly between EAC and ESCC, our TCGA-based analyses revealed a modest but significant upregulation of IL6ST (gp130) in EAC.

IL6ST serves as the shared signal-transducing receptor subunit for IL-6 family cytokines and is essential for downstream JAK–STAT3 activation. Elevated IL6ST expression in EAC suggests enhanced receptor availability and signaling sensitivity rather than increased ligand abundance as a determinant of pathway activation. This receptor-level regulation provides a mechanistic explanation for subtype-specific differences in downstream signaling intensity and underscores the importance of evaluating pathway activation beyond transcript-level analyses alone. Our observation of increased JAK1 expression in esophageal cancer, with significantly higher levels in EAC compared to ESCC, further strengthens this receptor-level sensitization model. JAK1 is the primary kinase responsible for transmitting IL6ST-dependent signals to STAT3. Its upregulation provides a mechanistic explanation for the enhanced pSTAT3 activation observed in EAC despite similar STAT3 transcript levels. Similar amplification of receptor–kinase modules driving STAT3 activation has been reported in other inflammation-driven malignancies, emphasizing the importance of evaluating pathway components beyond ligand abundance. Differential expression patterns of STAT3 target genes revealed that ESCC tumors were enriched for proliferation- and invasion-associated programs, whereas EAC tumors showed relatively higher expression of survival- and angiogenesis-related mediators. These findings suggest that although upstream IL-11 signaling components are shared across EC subtypes, the biological outputs of STAT3 activation may diverge between EAC and ESCC.

In our in vitro experiments using the ESCC KYSE-410 cell line, the administration of anti-IL-11 neutralizing antibodies demonstrated a dose-dependent decrease in cell viability, corroborating earlier findings that targeting IL-11 signaling can inhibit cancer cell proliferation[30]. The reduction in cell migration, observed in the wound healing assay, mirrors results from other studies where IL-11 was implicated in enhancing migratory capabilities in cancer cells. Our results also align with preclinical studies where IL-11 neutralizing antibodies reduced tumor

burden in murine models of gastric and pancreatic cancer[31]. The studies have indicated IL 11 inhibition extends lifespan which can be in part because of decrease in cancer development [32]. This supports the feasibility of targeting IL-11 therapeutically. Importantly, in a phase I clinical trial of the STAT3 inhibitor TTI-101 (NCT03195699), favorable pharmacokinetics and tolerability were reported, and early antitumor activity was observed, particularly in head and neck and gastrointestinal cancers[33].The observed correlation between IL-11 and COX-2 expression is particularly relevant because COX-2 inhibitors, such as celecoxib, have demonstrated additive or synergistic effects when combined with chemotherapeutics in colorectal and gastric cancer clinical trials[34]. However some studies show addition of celecoxib to standard chemotherapy did not improve OS or PFS rates but can increase hematologic toxicity of patients with cancer[35]While COX-2 inhibition alone has yielded mixed outcomes due to cardiovascular side effects, targeting upstream IL-11 signaling may provide a more tumor-specific anti-inflammatory strategy with broader therapeutic impact. Furthermore, IL-11's role in promoting epithelial-to-mesenchymal transition through STAT3 activation of SNAI1, ZEB1, and TWIST1 has been reported in hepatocellular and gastric cancers [36] In our in vitro assays, the inhibition of cell migration and viability following IL-11 blockade reinforces the idea that IL-11 actively drives EMT and metastatic potential in EC. Given IL-11’s established role in fibroblast activation and stromal remodeling[37], it is plausible that IL-11 signaling contributes not only to epithelial tumor cell plasticity but also to the creation of a permissive tumor microenvironment that supports invasion, immune evasion, and therapeutic resistance in esophageal cancer. The identification of JAK1 upregulation also expands the translational relevance of our findings, as JAK1 represents a clinically actionable node within the IL-11 signaling cascade. Selective JAK1 inhibitors upadacitinib, filgotinib, and abrocitinib are already approved for inflammatory and hematologic conditions [38-41], and their potential repositioning in esophageal cancer, either alone or in combination with

IL-11 blockade, warrants investigation, particularly in EAC where IL6ST–JAK1–STAT3 signaling appears most pronounced.

The differential efficacy and toxicity profiles observed with COX-2 inhibitors highlight the limitations of targeting downstream inflammatory mediators in isolation[42]. Our findings suggest that upstream blockade of IL-11 signaling may offer a more selective and comprehensive strategy by simultaneously suppressing STAT3 activation, COX-2 induction, EMT, and migratory phenotypes. Furthermore, IL-11 pathway inhibition via blocking IL11/IL11RA demonstrated suppression of inflammatory, fibrotic, proliferative and mesenchymal transcriptional programs, supporting the functional relevance of IL-11 signaling in regulating tumor-promoting pathways. Therefore, targeting the initiating cytokine–receptor interaction, IL-11 inhibition has the potential to attenuate multiple oncogenic outputs while minimizing off-target effects associated with broader anti-inflammatory therapies.

Our TCGA-based survival analyses indicate that elevated IL-11 expression is associated with poorer overall and disease-free survival in esophageal cancer overall, supporting its role as a marker of aggressive inflammatory signaling in various malignancies [43, 44]. The use of median and quartile cutoffs for stratifying patients based on IL-11 expression provides a framework for personalized treatment strategies, enabling clinicians to identify high-risk patients who may benefit from targeted therapies. These insights warrant urgent evaluation of IL-11 inhibitors in patient-derived xenograft (PDX) and syngeneic murine models of EC to validate in vivo efficacy and optimize dosage strategies.

While our findings are compelling, certain limitations must be acknowledged. The reliance on a single cell line for in vitro studies may not capture the heterogeneity present in esophageal cancer. Future studies incorporating multiple cell lines and patient-derived organoids representing diverse genetic backgrounds are essential to validate the generalizability of our findings. We were unable to assess the effects of

anti-IL-11 treatment on tumor growth and metastasis in murine models and validate our findings in vivo which is critical to establish pharmacokinetics, treatment response dynamics, and potential off-target effects. We show the associations that are correlative but the precise mechanism is not explored. The circulating IL-11 levels in plasma or assess its potential as a minimally invasive biomarker has not been evaluated in our study. Correlating serum IL-11 expression with clinical parameters could offer significant translational value.

**Conclusion:** Our study establishes IL-11 as a pro-tumorigenic cytokine in esophageal cancer, demonstrating significant overexpression in patient tumor samples and association with adverse survival outcomes. Through integrated transcriptomic, proteomic and functional analyses, we delineate the role of IL11-IL6ST–JAK1-STAT3 as a key inflammatory pathway contributing to tumor cell viability and invasiveness in EC. Subtype-stratified analyses further reveal distinct transcriptional programs downstream of STAT3 activation, with ESCC enriched for proliferation and invasion-associated genes and EAC exhibiting relatively higher expression of survival and angiogenesis mediators. Functional neutralization of IL-11 significantly reduced cancer cell proliferation and migration while suppressing downstream oncogenic signaling, supporting the biological relevance of this pathway. Collectively, these findings highlight IL-11 signaling as a promising therapeutic target in esophageal cancer. Future studies should prioritize in vivo validation and combinatorial regimens to translate IL-11-targeted approaches into clinical application.

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**Conflict of interest:** All the authors declare no conflict of interest.

**Ethical approval:** All procedures done involving human participants were done in compliance with the ethical standards of the institutional and/ or national research committee and with the 1964 Helsinki

declaration and its later amendments or comparable ethical standards. The ethical sanction was attained from Institutional Ethical Committee. The study protocol was approved by the Ethics Committee of SK Institute of Medical Sciences (IEC/SKIMS protocol # RP 16/2020)

**Consent to participate:** Approvals of the patients who participated in this study were obtained through a native written information consent form.

**Data availability:** The datasets generated and/or analysed during the current study are available in the Zenodo repository: https://doi.org/10.5281/zenodo.19217128

TCGA-ESCA RNA-sequencing and RPPA datasets analysed in this study are publicly available from the Genomic Data Commons (GDC) data portal (https://portal.gdc.cancer.gov/) and cBioPortal for Cancer Genomics (https://www.cbioportal.org/). Murine RNA-sequencing datasets were obtained from the Gene Expression Omnibus (GEO) under accession number GSE128940, from which processed data were derived. All processed datasets used in the study are publicly available in the Zenodo repository.



## Author contributions

S.A.R: Data interpretation, Experimentation, Drafting the manuscript, Statistical analysis, Result interpretation for revision, A.A.P,: Design of the study, conceived the work and drafting the manuscript, performed the experiments, supervision, acquisition of funds and evaluation of results F.A.G: Provided samples. QA: Experimentation, U.M., Experimentation, D.A: Logistic support, supervision and reviewed the manuscript Z.R.: Histopathology N.A.D., A.M.B., Cell line experimentation S.M., Assisted to conduct experiments

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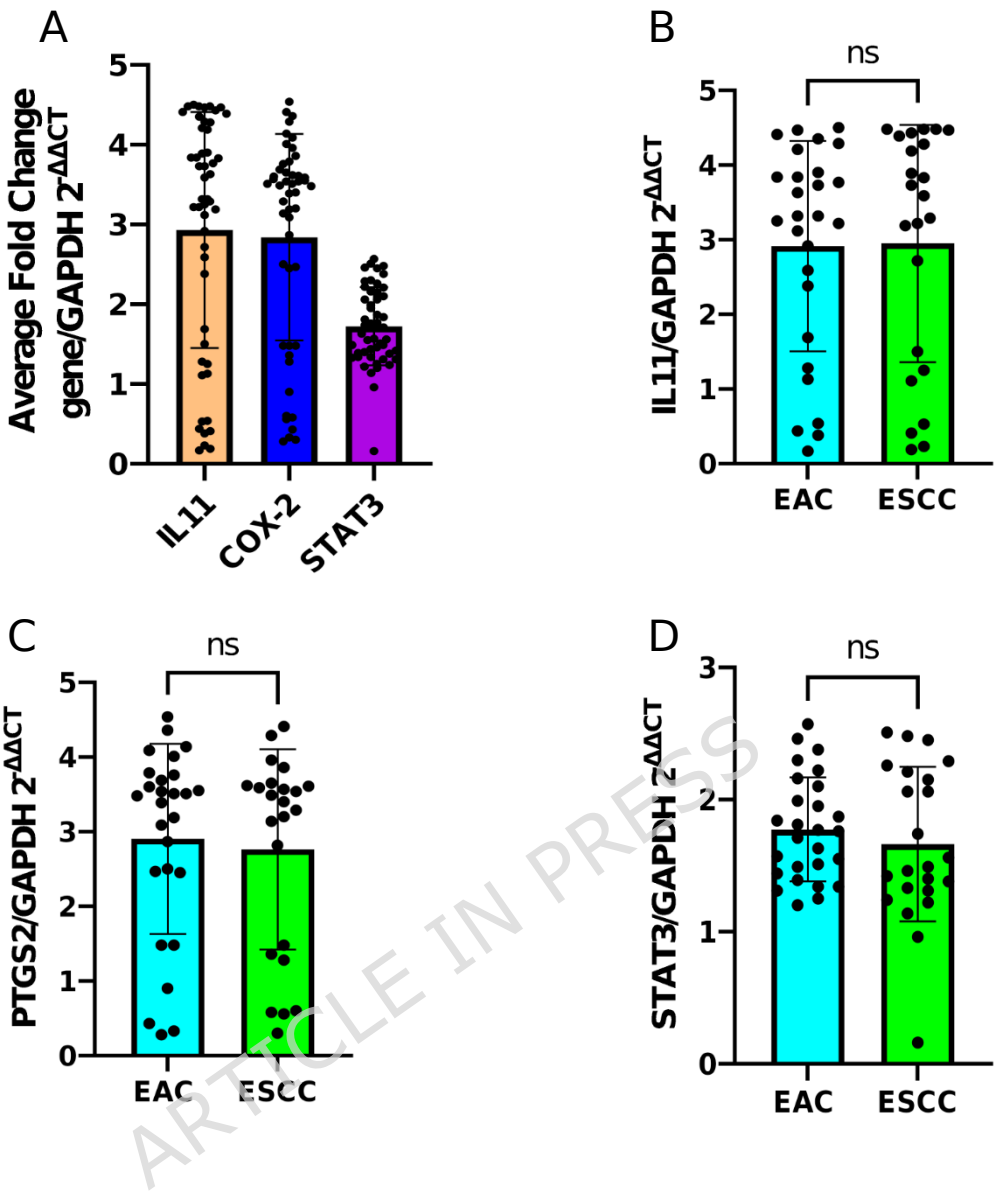





**Figure 1. Differential expression of IL-11 pathway components in esophageal cancer and histological subtypes**

(A) RT-qPCR analysis of IL11, PTGS2 (COX-2), and STAT3 mRNA expression in paired esophageal cancer (EC) tumor tissues and adjacent normal tissues (n = 50). Gene expression was normalized to GAPDH and calculated using the $2^{-}\Delta\Delta Ct$ method. Tumor tissues showed significant upregulation of IL11 (~2.9-fold), PTGS2 (~2.8-fold), and STAT3 (~1.7-fold) compared with matched normal tissues.

(B–D) Subtype-stratified comparison of IL11 (B), PTGS2 (C), and STAT3 (D) expression between esophageal adenocarcinoma (EAC; n = 27) and esophageal squamous cell carcinoma (ESCC; n = 23) samples. No statistically significant differences were observed between subtypes. Data are presented as mean ± SD. P values were calculated using independent Student's *t*-test. $p < 0.05$ was considered statistically significant.

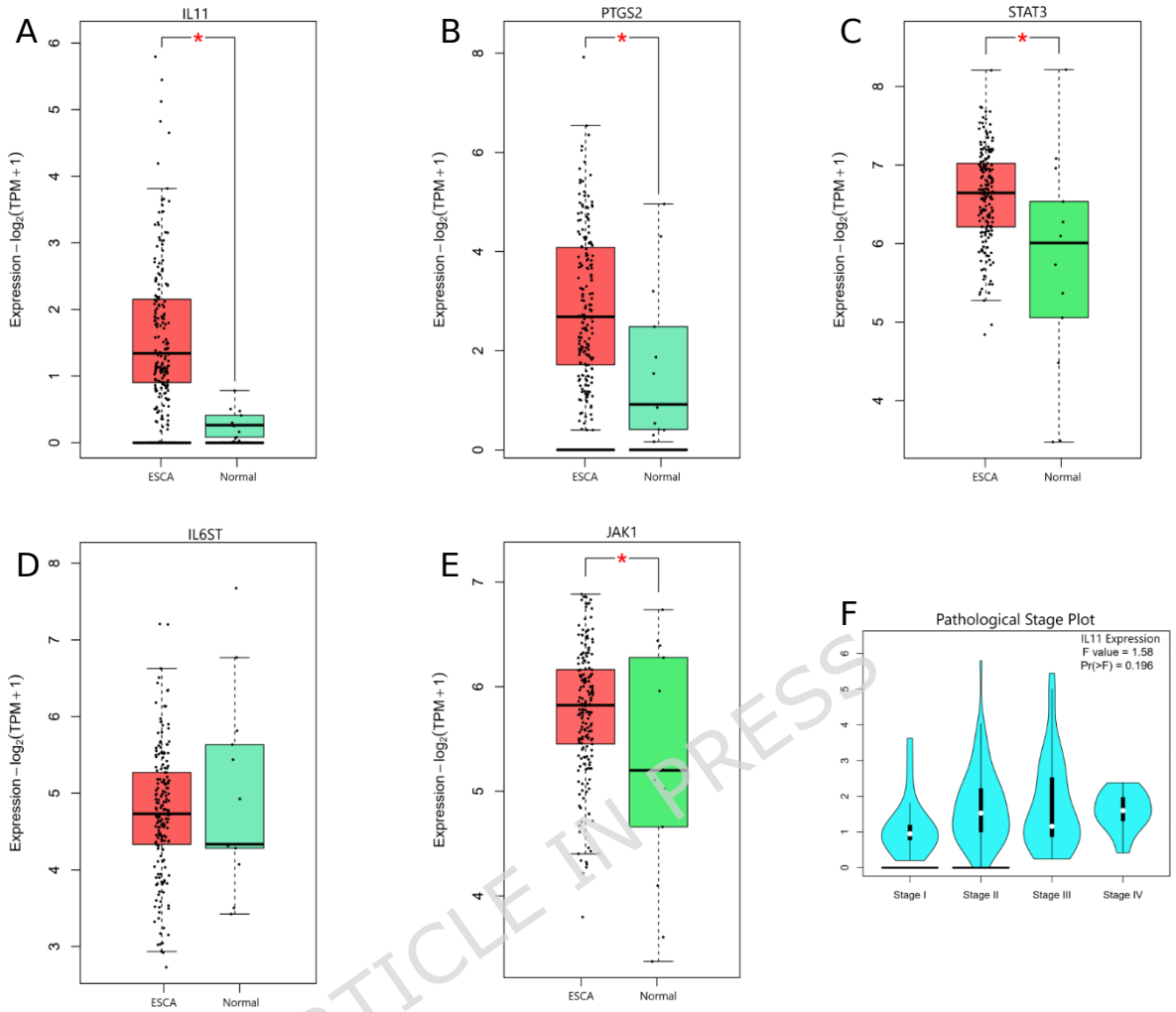



**Figure 2. In silico expression profiling of IL-11 signaling components in esophageal carcinoma versus normal esophageal tissues using TCGA-ESCA data**

(A) IL-11 ($p < 0.001$) (B) PTGS2 expression ($p < 0.05$) (C) STAT3 expression ($p < 0.05$) (D) IL6ST expression($p > 0.05$) (E) JAK1 expression ($p < 0.05$) (F) IL11 expression stratified by pathological tumor stage (Stage I–IV). IL11 expression was elevated across all stages, with highest levels observed in Stage II tumors; however, differences were not statistically significant. Expression values are shown as $\log_2$-transformed normalized counts. ESCA n=182, matched normal epithelium n=13 . Differential expression analysis was performed using TCGA thresholds ($\log_2$ fold change ≥ 0.5; $*p < 0.05$).

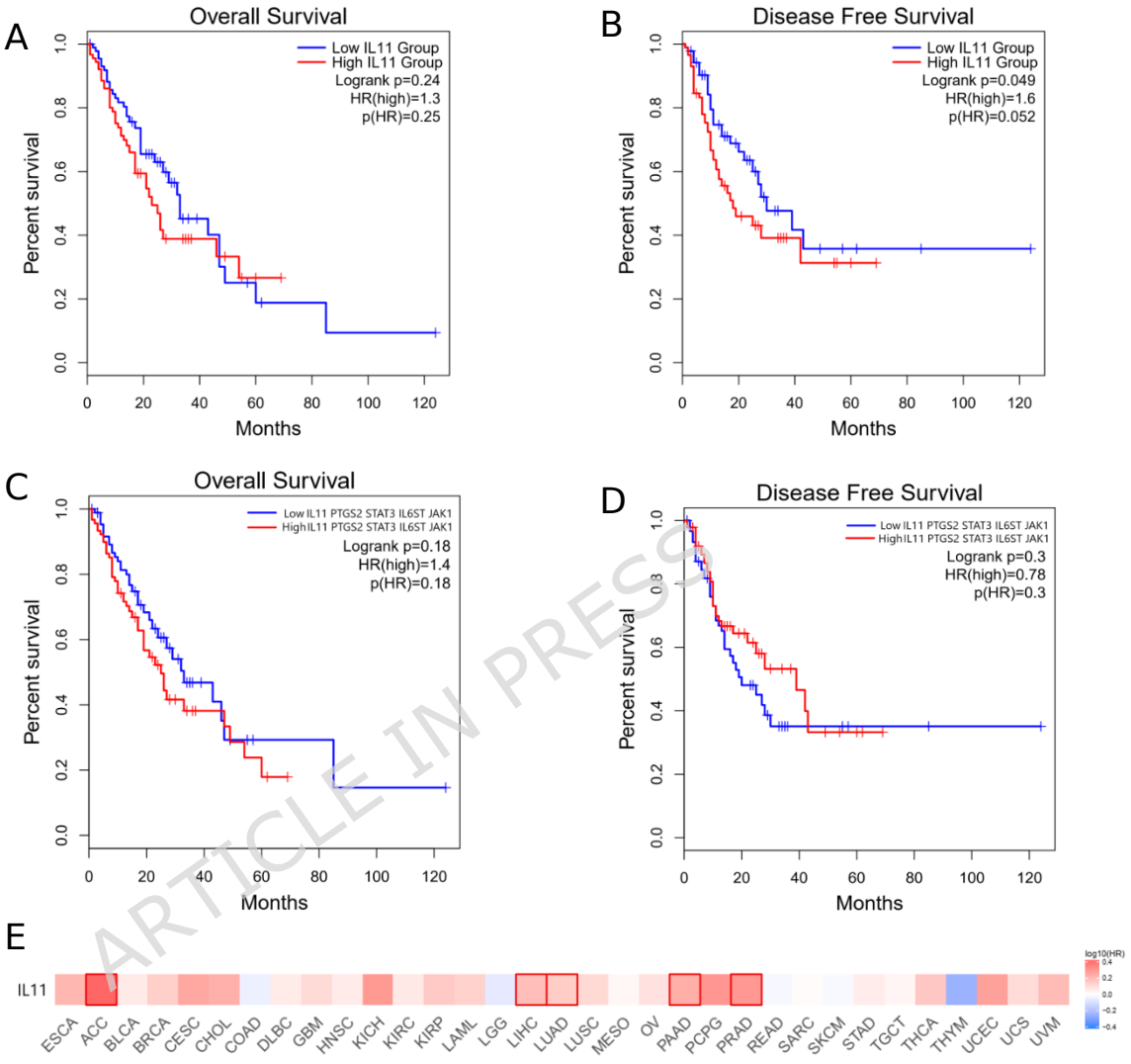



**Figure 3. Association of IL-11 expression with survival outcomes in esophageal cancer**

(A–B) Kaplan–Meier survival analyses demonstrating that patients with high IL11 expression (above median) exhibit significantly reduced overall survival (OS) (A) and disease-free survival (DFS) (B) compared with low-expression groups.
(C–D) Kaplan–Meier analysis of OS (C) and DFS (D) based on combined expression of IL11, STAT3, PTGS2 (COX-2), IL6ST, and JAK1 gene signature in TCGA-ESCA cohort.
(E) Hazard ratio heatmap depicting DFS-associated risk across cancer types, highlighting IL-11 as a poor prognostic indicator in esophageal cancer. Survival differences were assessed using log-rank tests.

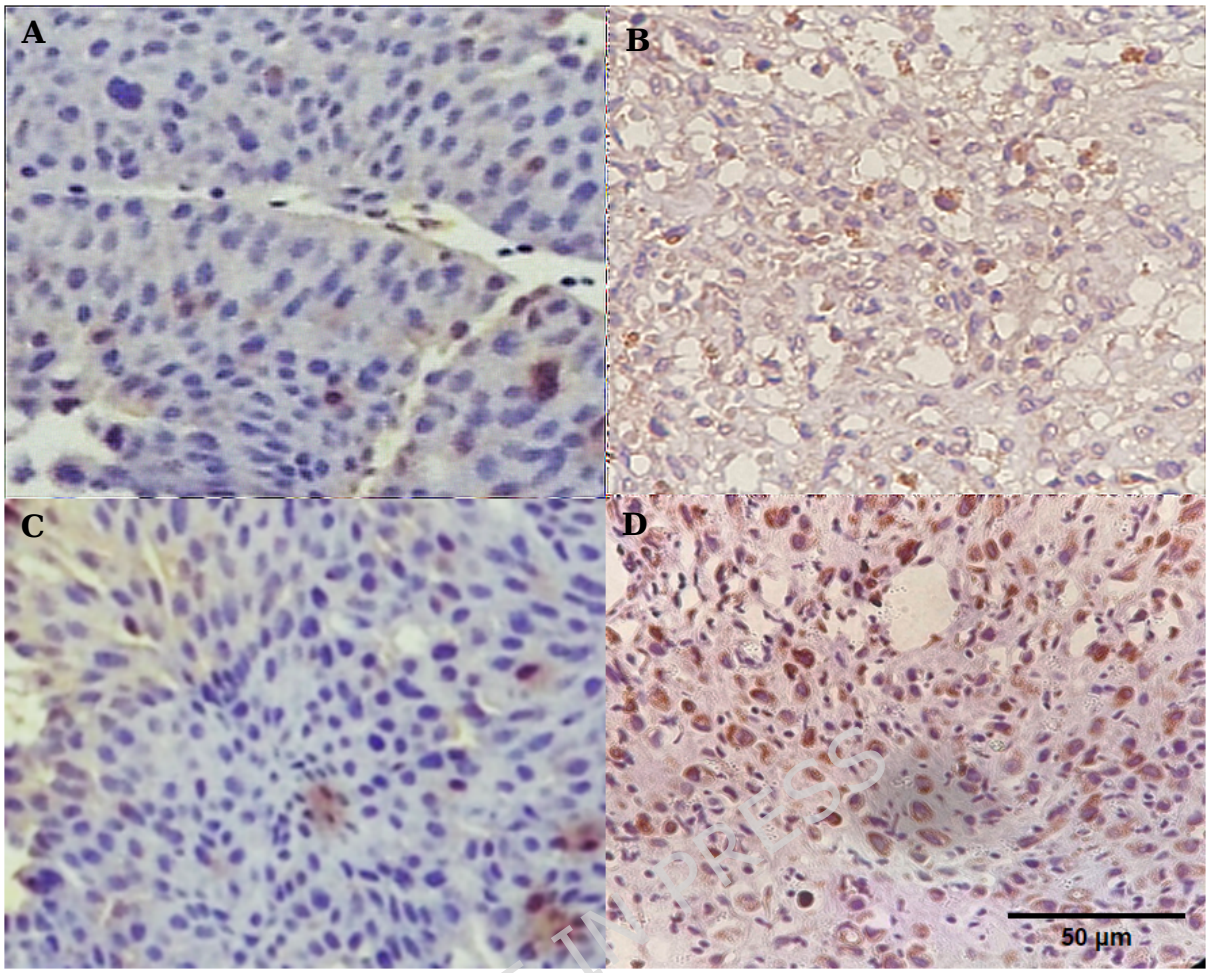



**Figure 4. Immunohistochemical validation of IL-11 pathway activation in esophageal cancer tissues**

Representative immunohistochemical staining of IL-11 signaling components in adjacent normal and tumor tissues. (A) Weak COX-2 expression in adjacent normal epithelium.(B) Strong cytoplasmic COX-2 expression in tumor tissue. (C) Low STAT3 expression in adjacent normal tissue. (D) Prominent nuclear STAT3 staining in tumor cells, indicating active STAT3 signaling. All images were captured at 40× magnification. Brown staining represents positive immunoreactivity (DAB), and nuclei are counterstained with hematoxylin.

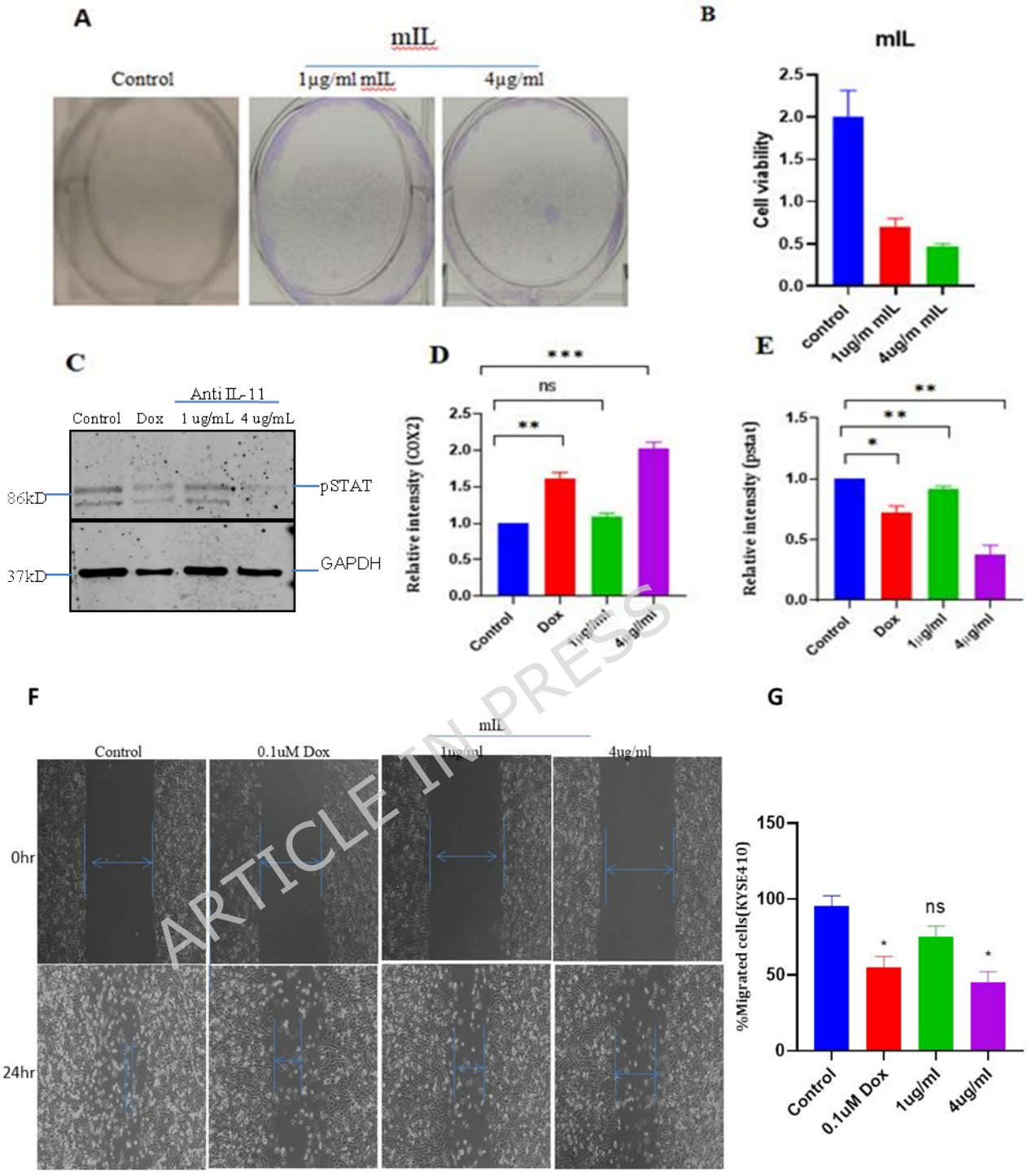



**Figure 5: Effect of anti-IL-11 neutralizing antibody on ESCC cells.**

(A) Crystal violet assay showing dose-dependent reduction in in KYSE-410 cell viability after 24–48 h of anti-IL-11 treatment. (B) Quantification of cell viability following anti-IL-11 treatment. (C–E) Western blot analysis and corresponding densitometric quantification demonstrating decreased expression of phosphorylated STAT3 (Tyr705) following anti-IL-11 treatment. (F-G) Wound-healing assay showing impaired cell migration at 24 hours after IL-11 neutralization. Data are presented as

mean ± SD. Statistical significance was determined using one-way ANOVA. *$p < 0.05$, **$\mathbf{p} < 0.01$, ***$\boldsymbol{p} < 0.001$.

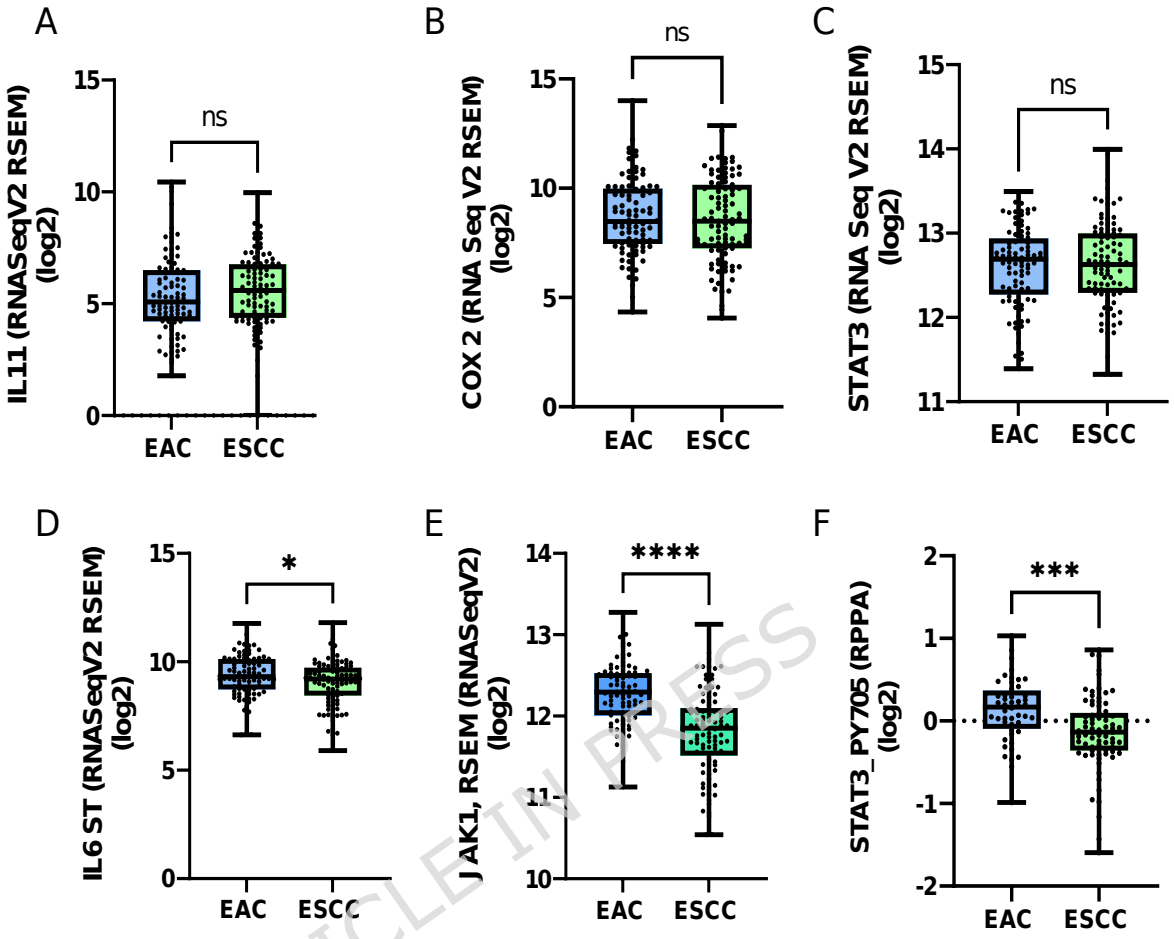



**Figure 6. Histological subtype-specific expression of IL-11 pathway components in TCGA-ESCA**

(A-F) Comparison of IL11, PTGS2 (COX-2), STAT3, IL6ST (gp130), and JAK1 expression between esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC) samples from TCGA-ESCA dataset. While IL11, PTGS2, and STAT3 expression levels were comparable between subtypes, IL6ST and JAK1 expression were significantly higher in EAC, indicating enhanced receptor-level signaling capacity. Statistical comparisons were performed using independent Student's *t*-test or Mann–Whitney U test, as appropriate. ns not significant; *P<0.05; **P<0.01; ***P<0.001

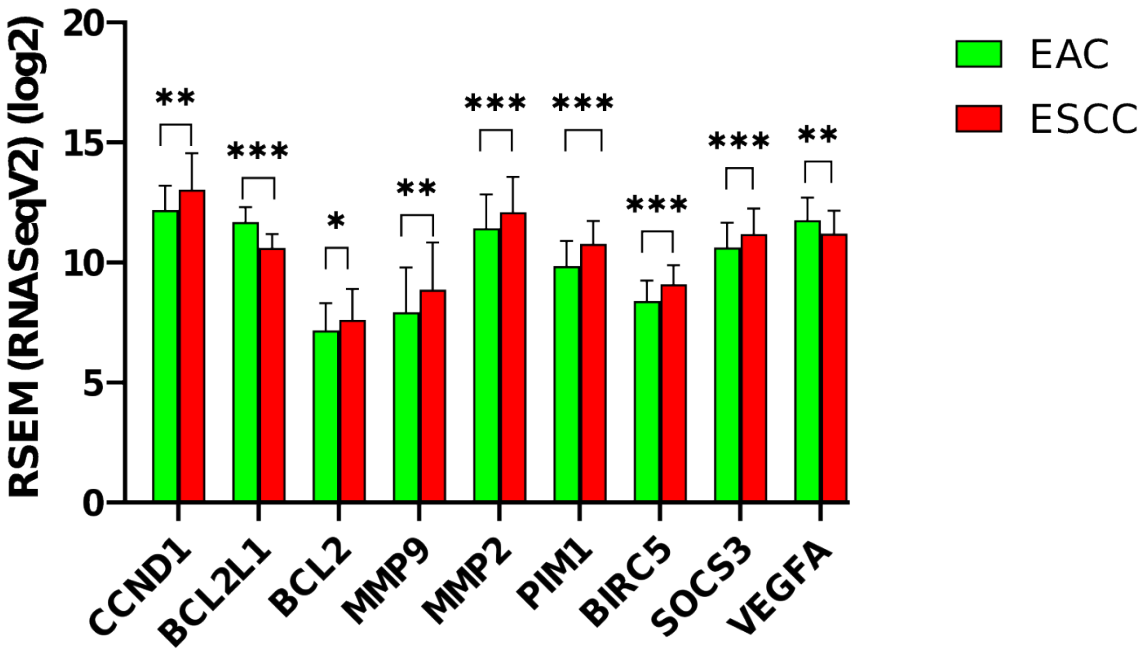
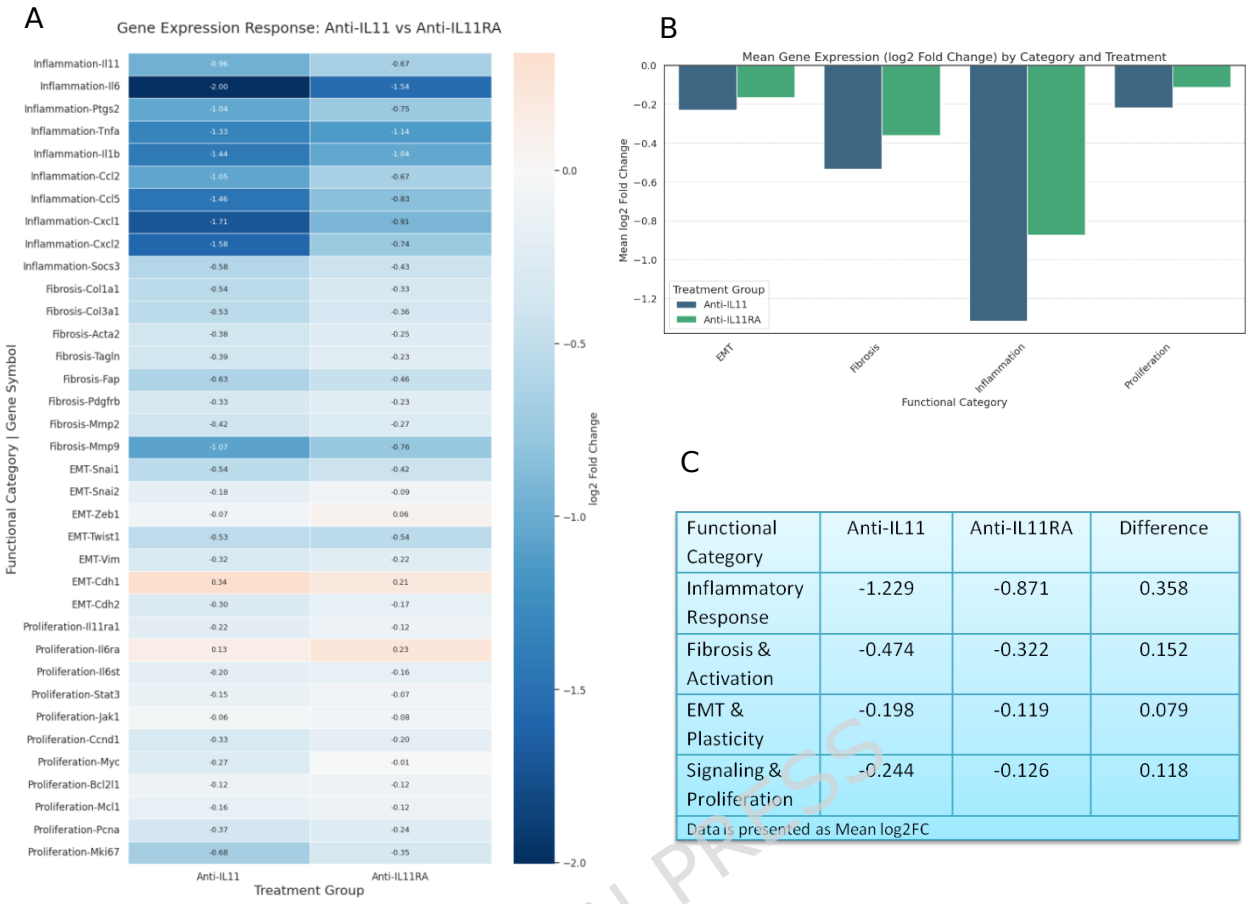



**Figure 7. Differential expression of STAT3 downstream transcriptional targets in TCGA-ESCA tumors.**

Bar chart showing RNA-seq expression of canonical STAT3-regulated genes in esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). Genes associated with proliferation and matrix remodeling (CCND1, MMP2, MMP9, BIRC5, PIM1) are enriched in ESCC, whereas survival- and angiogenesis-associated genes (BCL2L1 and VEGFA) show higher expression in EAC. Expression values represent log2-transformed normalized RNA-seq counts from TCGA-ESCA. P-values calculated by student independent T test. *P<0.05; **P<0.01; ***P<0.001




C

| Functional Category | Anti-IL11 | Anti-IL11RA | Difference |
|---|---|---|---|
| Inflammatory Response | -1.229 | -0.871 | 0.358 |
| Fibrosis & Activation | -0.474 | -0.322 | 0.152 |
| EMT & Plasticity | -0.198 | -0.119 | 0.079 |
| Signaling & Proliferation | -0.244 | -0.126 | 0.118 |

Data is presented as Mean log2FC

**Figure 8. Transcriptomic reversal of inflammatory, proliferative, and mesenchymal programs following IL-11 pathway inhibition in murine models.**

(A) Heatmap showing differential expression of representative genes associated with inflammatory signaling (*Ptgs2, Il6, Ccl2, Tnfa*), epithelial–mesenchymal transition (*Snai1, Zeb1, Vim*), extracellular matrix remodeling (*Mmp2, Mmp9*), and proliferative pathways (*Ccnd1*) in murine RNA-sequencing datasets following treatment with neutralizing anti-IL-11 or anti-IL-11 receptor (IL11RA) antibodies. Colors represent relative changes in gene expression compared with control conditions.
(B) Quantitative comparison illustrating the overall decrease in expression of genes belonging to the indicated functional pathways after inhibition of IL-11 signalling.
(C) Mean percentage reduction in pathway activity calculated from aggregated gene expression changes within each functional category, demonstrating broad suppression of inflammatory, fibrotic, proliferative, and mesenchymal transcriptional signatures following IL-11 pathway blockade.

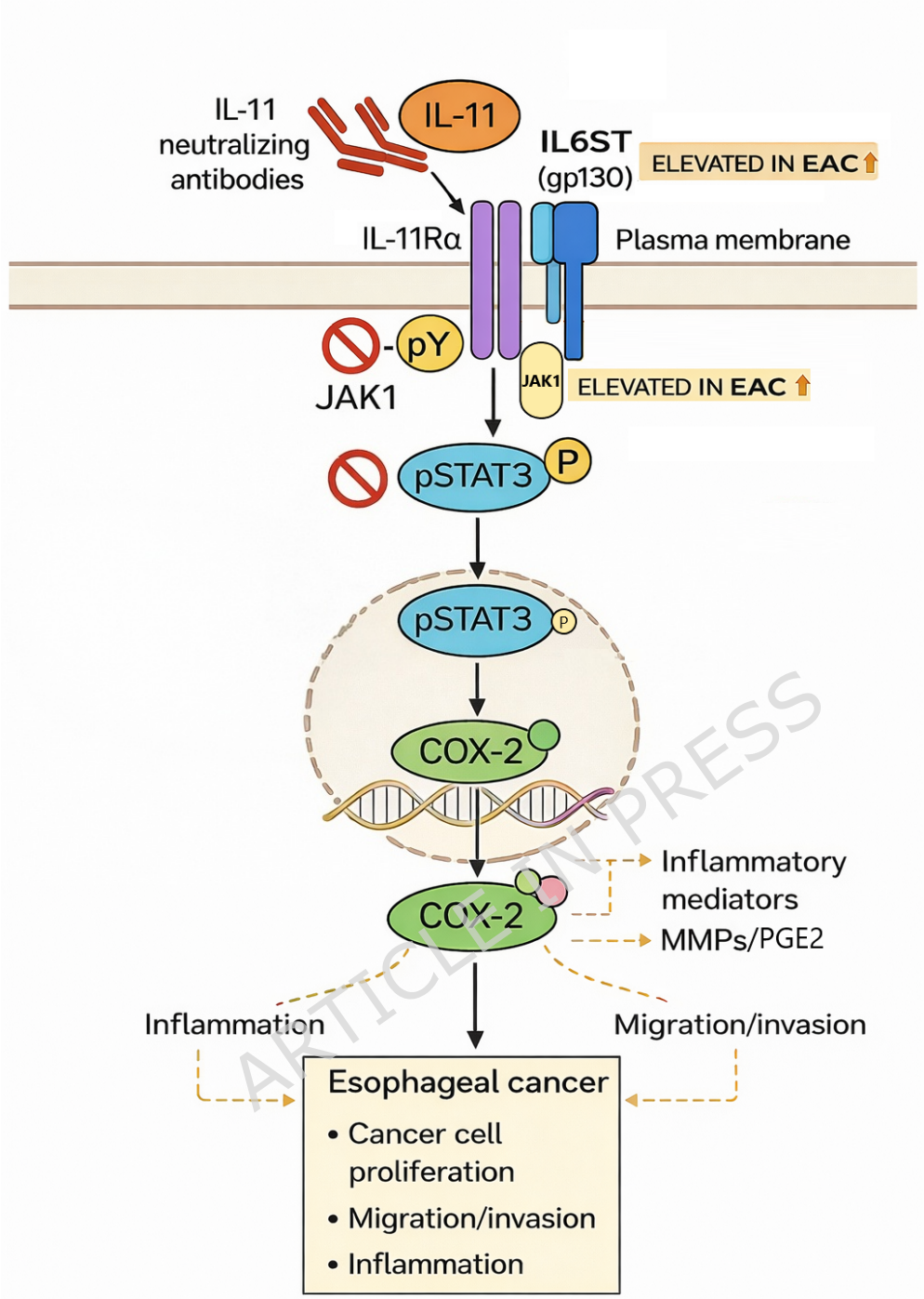



**Figure 9. Schematic representation of IL-11–mediated signaling in esophageal cancer**

Proposed mechanistic model illustrating IL-11 signaling through IL11RA/IL6ST, activation of JAK1, phosphorylation of STAT3, and induction of COX-2, leading to enhanced tumor cell survival, proliferation, migration, and inflammation. Elevated IL6ST and pSTAT3 signaling in EAC relative to ESCC is highlighted. Therapeutic inhibition of IL-11 signaling disrupts this oncogenic axis and may attenuate tumor initiation and progression.