

XPC restrains cancer stemness by modulating STAT1-SOX2 signaling in non-small cell lung cancer

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Abstract

Cancer stem cells (CSCs) drive tumor initiation, therapeutic resistance, and disease relapse, yet the molecular mechanisms sustaining CSC maintenance remain incompletely defined. Xeroderma pigmentosum complementation group C (XPC) is a core DNA damage recognition factor in nucleotide excision repair, and its loss is linked to cancer predisposition. Beyond its canonical role in genome maintenance, XPC has emerged as a transcriptional co-regulator capable of modulating gene expression. Here, we identify XPC as a critical suppressor of CSC phenotypes in non-small cell lung cancer (NSCLC). Genetic depletion of XPC enhances CSC self-renewal and tumor-initiating capacity, whereas inducible XPC expression restricts the CSC population. Transcriptomic and functional analyses reveal that XPC negatively regulates the stemness factor SOX2. Mechanistically, XPC selectively suppresses STAT1 activation by inhibiting phosphorylation at Tyr701, physically associates with STAT1 in the nucleus, and limits STAT1 recruitment to the SOX2 promoter. Loss of XPC results in elevated STAT1 signaling, increased SOX2 transcription, and CSC expansion. Notably, STAT1 signaling is highly activated in CSC-enriched populations and is required for CSC self-renewal. Collectively, these findings uncover a DNA repair-independent function of XPC mediated through a previously unrecognized XPC-STAT1-SOX2 axis, redefining XPC as a regulator of tumor cell plasticity and highlighting STAT1 signaling as a potential therapeutic vulnerability in XPC-deficient tumors.

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 80–85% of all lung cancer cases¹. Despite advances in targeted therapies and immunotherapy, long-term survival remains poor, largely due to high rates of tumor recurrence, metastasis, and therapeutic resistance. These clinical challenges underscore an urgent need to define the cellular and molecular mechanisms that sustain tumor persistence following treatment.

Mounting evidence supports a central role for cancer stem cells (CSCs) in driving tumor initiation, relapse, and metastatic dissemination^{2–4}. CSCs possess enhanced self-renewal capacity, resistance to conventional therapies, and the ability to regenerate heterogeneous tumor populations^{5,6}. In lung cancer, CSC-enriched populations have been linked to poor prognosis and treatment failure⁷; however, the molecular pathways that govern CSC maintenance and expansion remain incompletely understood. Elucidating these mechanisms is critical for the development of durable therapeutic strategies that effectively eliminate CSCs and prevent tumor relapses.

Xeroderma pigmentosum complementation group C (XPC) is a key DNA damage recognition factor in the nucleotide excision repair (NER) pathway, essential for maintaining genomic integrity⁸. Germline loss of XPC causes extreme UV sensitivity and cancer predisposition, and XPC deficiency has traditionally been viewed as a driver of tumorigenesis through impaired DNA repair^{9–11}. Notably, allelic loss of XPC is common in human lung cancers, as revealed by patient genomic analyses¹¹. Moreover,

immunohistochemistry studies demonstrate that many lung cancer patients exhibit low or absent XPC expression¹² (<https://www.proteinatlas.org/ENSG00000154767-XPC/cancer/lung+cancer#IHC>), highlighting the high prevalence of XPC downregulation in clinical disease. Importantly, reduced XPC expression is associated with adverse clinical outcomes: in a cohort of 126 NSCLC patients, XPC-low tumors correlated with shorter median survival¹³, and analysis of 1,432 lung cancer cases using kmplot.com revealed that high XPC expression is a significant predictor of improved overall survival¹². Together, these observations support a tumor-suppressive role for XPC in NSCLC and underscore its potential clinical relevance.

Beyond its canonical role in DNA repair, emerging evidence suggests that XPC also functions as a transcriptional co-regulator by directly binding to promoter regions to either enhance or repress target gene transcription^{14–17}. Notably, XPC has been identified as a core component of the stem cell coactivator complex, which is selectively required for NANOG transcription and the maintenance of self-renewal in mouse embryonic stem cells (ESCs) and the human embryonal carcinoma cell line NTERA-2¹⁶. These findings raise the intriguing possibility that XPC may influence stemness-related transcriptional programs in cancer. However, whether and how XPC regulates CSC maintenance in NSCLC remains unknown.

In this study, we investigate the role of XPC in controlling CSC phenotypes in NSCLC. We provide evidence that XPC functions as a suppressor of CSC maintenance and tumor-initiating capacity, and we define a previously unrecognized mechanism by which XPC links transcriptional regulation to stemness signaling. Our findings reveal a novel role for XPC in modulating CSC biology and suggest that loss of XPC contributes to NSCLC relapses through mechanisms extending beyond defective DNA repair.

Results

XPC restricts the CSC subpopulation in NSCLC cells

Given the established tumor-suppressive role of XPC in NSCLC and its reported function in regulating stemness-related transcription in ESCs, we hypothesized that XPC may influence CSC phenotypes in a context-dependent manner. To test this, we examined XPC expression in CSC-enriched populations of NSCLC cells. NSCLC cell lines were cultured in serum-free 3D Tumor Sphere Medium in suspension for at least 12 days, while bulk cells were maintained under standard adherent conditions. Notably, XPC expression was consistently reduced in spheroid cultures compared with adherent cells across all three lines (**Fig. S1**), suggesting that XPC may play an opposing role in regulating stemness in CSCs relative to embryonic stem cells.

To further investigate the role of XPC in regulating cancer stemness, we generated stable XPC knockdown (KD) A549 and H1299 cell lines using two independent XPC shRNAs (Fig. 1A, E). XPC depletion markedly increased CSC properties, evidenced by enhanced sphere formation and higher tumor-initiating cell frequency (TICf) in limiting dilution xenografts (Fig. 1B-D, F-H). Conversely, we

established a Tet-On inducible XPC expression system in H1299 cells (Fig. 1I). Doxycycline (Dox)-induced XPC overexpression significantly reduced sphere formation and TICf in H1299-pTRE3G-XPC cells, with no effect on sphere formation in parental H1299 cells (Fig. 1J-L). Because sphere formation and limiting dilution xenotransplantation assays are well-established functional measures of CSC abundance^{18,19}, these results demonstrate that XPC suppresses stemness and restricts the CSC population in NSCLC cells.

XPC suppresses SOX2 expression in NSCLC cells

To identify stemness-associated genes regulated by XPC, we performed RNA-seq analysis in A549 cells transfected with pooled XPC siRNA or control siRNA. Heatmap visualization revealed clear segregation between siXPC- and siControl-transfected cells, indicating that XPC modulates specific transcriptional programs (**Fig. S2A**). Specifically, XPC KD resulted in a limited yet reproducible set of differentially expressed genes, with 113 genes upregulated and 131 downregulated. Notably, among core stemness transcription factors (OCT4, SOX2, KLF4, c-MYC, and NANOG)^{20,21}, SOX2 was the only gene significantly upregulated following XPC depletion (Fig. 2A). This was validated at the mRNA level by qRT-PCR in A549 and H441 cells (Fig. 2B, C) and at the protein level by immunoblotting in multiple NSCLC cell lines using independent XPC siRNAs or shRNAs (Fig. 2D; **Fig. S3A, B**). Conversely, ectopic expression of XPC in H441 and H1299 cells markedly suppressed SOX2 (Fig. 2E), with similar inverse regulation confirmed in HCC827 cells (**Fig. S3C, D**). Consistent with these findings, SOX2 promoter reporter assays showed increased activity upon XPC knockdown and decreased activity upon XPC overexpression (Fig. 2F; **Fig. S3E, F**). Analysis of TCGA lung cancer datasets further revealed a significant inverse correlation between XPC and SOX2 mRNA in patient tumors (Fig. 2H). Collectively, these results demonstrate that XPC negatively regulates SOX2 and restricts CSC phenotypes, at least in part, through repression of SOX2 expression, a master regulator of stemness.

XPC inversely regulates STAT1 phosphorylation in NSCLC cells

To identify signaling pathways downstream of XPC, we performed KEGG-based Gene Set Enrichment Analysis (GSEA) on RNA-seq data from A549 cells transfected with XPC or control siRNA. XPC depletion altered multiple pathways, including positive enrichment of “Spliceosome”, negative enrichment of “Nicotine addiction”, “Ribosome”, “Chemical carcinogenesis-reactive oxygen species”, “Neuroactive ligand-receptor interaction”, and “JAK-STAT signaling pathway” (**Fig. S2B**). Among the altered pathways, we focused on JAK-STAT signaling, due to its established role in stem cell and CSC regulation^{22,23}. Unexpectedly, GSEA indicated negative enrichment of JAK-STAT signaling in XPC-depleted cells (**Fig. S2C**), despite XPC downregulation being associated with CSC expansion. Given that KEGG JAK-STAT gene sets encompass signaling mediated by seven distinct STAT family members²⁴, suppression of specific STAT-dependent transcriptional programs, particularly STAT3-driven signaling, may account for the observed negative enrichment and mask transcriptional programs mediated by other STAT proteins.

Consistent with this interpretation, Hallmark gene set analysis revealed negative enrichment of “IL6-JAK-STAT3 signaling” in XPC-depleted cells (**Fig. S2D**).

To pinpoint which STAT proteins are regulated by XPC, we manipulated XPC levels and analyzed IFN β -induced STAT phosphorylation. XPC KD in A549 cells enhanced, whereas XPC overexpression in H1299 cells suppressed, IFN β -induced phosphorylation of STAT1 at Tyr701 without affecting STAT1 Ser727, STAT2 Tyr690, or STAT3 Tyr705 (Fig. 3A, B). We next examined the effect of XPC on STAT1 phosphorylation at Tyr701 in the absence of IFN β stimulation. Basal STAT1 Tyr701 phosphorylation was also elevated in unstimulated XPC-depleted A549 and H441 cells (**Fig. S4A, B**). Together, these results indicate that XPC selectively suppresses STAT1 activation by inhibiting phosphorylation at Tyr701, without affecting STAT2 or STAT3 activation.

XPC interacts with STAT1

To elucidate how XPC regulates CSC phenotypes and STAT1 signaling, we sought to identify XPC-interacting proteins using quantitative proteomics combined with immuno-affinity purification. H1299 cells were transfected with FLAG-XPC or empty vector (EV), and immunoprecipitates were analyzed by mass spectrometry (Fig. 3C). Among proteins enriched in FLAG-XPC samples, we detected known XPC interactors Centrin 2 and RAD23B^{25,26}, validating the approach (Fig. 3D). Notably, we found STAT1 is among these XPC-interacting proteins (Fig. 3D). Reciprocal co-immunoprecipitation of nuclear lysates confirmed this interaction: FLAG-XPC co-purified with endogenous STAT1 in H1299 cells, and endogenous XPC co-purified with STAT1 in A549 cells (Fig. 3E, F). Taken together, these data indicate that XPC interacts with STAT1 in the nucleus, suggesting that XPC may inhibit STAT1 phosphorylation through protein interaction.

STAT1 signaling is highly activated in CSC-enriched populations and is required for CSC self-renewal

JAK/STAT signaling has been implicated in stem cell and CSC maintenance^{23,27–30}. While STAT3 is well-studied, the role of STAT1 remains unclear. To assess STAT1 activation in CSCs, we enriched CSCs using spheroid culture as described above. Immunoblotting revealed increased STAT1 phosphorylation in spheroid cells compared with bulk cells (Fig. 4A), accompanied by elevated expression of STAT1 target genes ISG54, ISG56, IFNA1, and IFNB (Fig. 4B), indicating enhanced STAT1 signaling in CSCs.

To determine whether STAT signaling functionally contributes to CSC maintenance, we inhibited JAK-STAT signaling with the JAK1/2 inhibitor ruxolitinib in A549 and H1299 cells. Treatment significantly reduced sphere formation, demonstrating impaired self-renewal (Fig. 4C, D). These results indicate that STAT1 signaling is highly activated in CSCs and is functionally required to sustain their self-renewal.

STAT1 directly mediates XPC-dependent regulation of SOX2 expression

Activated STAT1 has been associated with increased SOX2 expression in breast cancer cells²⁹ but a causal relationship remains unclear. To investigate the regulatory role of STAT1 in SOX2 expression in NSCLC cells, we modulated JAK-STAT signaling and found that IFN β -mediated activation increased SOX2 expression, whereas JAK1/2 inhibition with ruxolitinib reduced SOX2 levels (Fig. 5A, B). To further determine whether SOX2 is specifically regulated by STAT1, we generated stable STAT1-KD A549 and H441 cell lines using distinct STAT1-targeting shRNAs. All STAT1-KD lines showed a marked decrease in SOX2 expression (Fig. 5C), indicating that STAT1 is an upstream regulator of SOX2 in NSCLC cells.

Given that XPC knockdown elevates SOX2, we tested whether this depends on JAK-STAT signaling. In A549 cells with stable XPC KD, ruxolitinib treatment blocked SOX2 upregulation (Fig. 5D). Similarly, XPC depletion in STAT1-deficient A549 and H441 cells failed to induce SOX2 expression (Fig. 5E). Together, these results demonstrate that XPC suppresses SOX2 expression through inhibition of STAT1 activation.

XPC limits STAT1 binding to the SOX2 promoter

Because pSTAT1 functions as a transcription factor and SOX2 is a STAT1 target, we investigated whether STAT1 directly binds the SOX2 promoter. STAT1 DNA-binding motifs from JASPAR were scanned across ± 1.5 kb of the SOX2 transcription start site, revealing several putative binding sites (Fig. 6A, B). To further validate direct STAT1 binding, we re-analyzed published STAT1 ChIP-seq data from H358 NSCLC cells and revealed clear STAT1 occupancy at the SOX2 promoter³¹. STAT1 binding was observed at four distinct regions: approximately 1 kb and 600 bp upstream of the transcription start site (TSS), 200 bp downstream of the TSS, and directly at the TSS, indicating multiple potential regulatory sites for STAT1-mediated transcriptional control of SOX2 and suggesting a direct regulatory relationship (Fig. 6C).

To experimentally validate STAT1 binding to the SOX2 promoter, FLAG-STAT1 or empty vector was transiently expressed in A549 cells, and ChIP-PCR was performed using primers spanning the putative binding sites. FLAG-STAT1 was enriched at P1, P2, and P4, but not P3, confirming specific binding to the SOX2 promoter (Fig. 6D, E).

We next examined whether XPC regulates STAT1 occupancy at the SOX2 promoter. ChIP assays in XPC-KD A549 cells showed markedly increased STAT1 binding at P1 and P4 compared with controls (Fig. 6F). These results demonstrate that STAT1 directly binds the SOX2 promoter and that XPC suppresses SOX2 expression, at least in part, by limiting STAT1 recruitment.

Discussion

In this study, we identify XPC as a novel suppressor of CSC phenotypes in NSCLC. XPC limits CSC self-renewal and tumor-initiating capacity, at least in part by repressing the stemness regulator SOX2. Mechanistically, XPC interacts with STAT1 in the nucleus, inhibits its phosphorylation at Tyr701, and restricts STAT1 binding at the SOX2 promoter, revealing a previously unrecognized DNA repair-independent role of XPC in transcriptional regulation of stemness. Together, these findings define an XPC-STAT1-SOX2 regulatory axis, highlighting a novel mechanism by which a canonical DNA repair protein modulates CSC phenotypes.

Although XPC was initially recognized for its role in NER⁸, emerging evidence demonstrates multiple non-canonical functions, including enhancing DNA damage-induced apoptosis¹⁷, stabilizing p53³², and maintaining ESC pluripotency¹⁶. Beyond DNA repair, XPC can act as a transcriptional co-regulator by directly binding to promoter regions to either activate or repress target gene transcription^{14–17}. Notably, in ESCs, XPC primarily acts as a transcriptional activator, sustaining expression of pluripotency genes such as NANOG¹⁶. In contrast, in NSCLC, XPC acts as a context-dependent transcriptional repressor, limiting CSC self-renewal through nuclear interaction with STAT1, inhibition of STAT1 phosphorylation, and restriction of STAT1 recruitment to the SOX2 promoter. This context-specific divergence likely reflects differences in cofactor availability, chromatin landscape, and STAT family balance, highlighting XPC's versatility in transcriptional regulation.

Tumor cells reside in the tumor microenvironment (TME), which consists of a diverse group of cells, including cancer cells, immune cells, fibroblasts, etc. Various cytokines secreted by these cells, e.g., interferons (IFNs) and interleukins (ILs), can regulate cancer cell plasticity and stemness by activating the JAK-STAT pathway, which has been associated with tumor growth and progression, as well as the maintenance of CSC subpopulations [Reviewed in^{33,34}]. Among the STAT family members, STAT1 and STAT3 play particularly important but distinct roles in tumorigenesis³⁵. STAT3 is well-established as a driver of CSC programs, promoting self-renewal and stemness-associated transcriptional networks³⁶, whereas the role of STAT1 is more context-dependent³⁷, often mediating IFN responses that can either support or restrain stemness depending on cellular context.

Our findings reveal that XPC interacts with STAT1 and inversely regulates STAT1 phosphorylation in NSCLC cells, identifying a novel mechanism by which XPC suppresses CSC phenotypes. Interestingly, the negative regulation of pSTAT1 by XPC appears to occur primarily in the nucleus, consistent with the predominantly nuclear localization of XPC³⁸. Because STAT1 phosphorylation is typically initiated in the cytoplasm following cytokine stimulation, it is unlikely that XPC directly inhibits phosphorylation at the receptor or JAK level. Instead, XPC may interact with nuclear pSTAT1 and promote its dephosphorylation, thereby limiting STAT1-dependent transcription of stemness genes such as SOX2. This mechanism highlights a potential crosstalk between XPC and STAT family signaling in controlling CSC maintenance and warrants further investigation.

SOX2 is a master regulator of stemness, essential for self-renewal in embryonic and cancer stem cells^{39,40}. Aberrant SOX2 expression is also a defining feature of CSCs in multiple tumor types, where it promotes tumor initiation, self-renewal, and resistance to apoptosis⁴¹. In NSCLC, elevated SOX2 expression in stem-like cells enhances self-renewal and expansion of CSC populations, consistent with its role as a driver of stemness and tumor aggressiveness⁴². Prior studies have implicated pathways such as Hedgehog⁴³, Notch⁴⁴, and STAT3⁴³ in promoting SOX2 transcription in various cancers⁴⁵. These pathways can either directly activate the SOX2 promoter or cooperate with epigenetic regulators to maintain an open chromatin state at SOX2 regulatory regions. Activated STAT1 has also been associated with increased SOX2 expression in breast cancer cells²⁹. However, a causal relationship has not been established. Our study provides direct evidence that STAT1 mediates SOX2 expression in NSCLC CSCs. We demonstrate that STAT1 binds to the SOX2 promoter and promotes its transcription, highlighting a novel mechanism by which STAT1 contributes to CSC maintenance. This finding is particularly intriguing given the context-dependent roles of STAT1 in cancer, where it has been reported to function as either a tumor suppressor or a tumor promoter depending on cellular context, signaling inputs, and tumor type^{37,46}. Our findings place STAT1 in a pro-stemness role in NSCLC by directly coupling cytokine-responsive signaling to transcriptional activation of a core stemness regulator.

Importantly, we identify XPC as a negative regulator of SOX2 expression through modulation of STAT1 activity. XPC interacts with STAT1 in the nucleus, inhibits STAT1 phosphorylation, and restricts STAT1 occupancy at the SOX2 promoter. As a result, XPC suppresses transcription of SOX2, limits CSC self-renewal, and reduces tumor-initiating capacity. These findings reveal a previously unrecognized DNA repair-independent function of XPC in controlling stemness through transcriptional regulation.

Collectively, our findings define a novel XPC-STAT1-SOX2 regulatory axis that integrates cytokine-responsive signaling with transcriptional control to govern CSC phenotypes in NSCLC. In CSC-enriched populations, STAT1 is hyperactivated and directly binds the SOX2 promoter, driving SOX2 transcription to sustain stemness and self-renewal. XPC functions as a critical negative regulator by physically interacting with STAT1 in the nucleus, selectively suppressing STAT1 phosphorylation at Tyr701, and limiting STAT1 recruitment to the SOX2 promoter (Fig. 7A). Loss of XPC removes this inhibitory constraint, resulting in enhanced STAT1 activity, elevated SOX2 expression, and expansion of the CSC compartment (Fig. 7B). Through this mechanism, XPC acts as a molecular brake on CSC maintenance, uncoupling STAT1 signaling from SOX2-driven stemness programs.

This study establishes a previously unrecognized, DNA repair-independent role for XPC in the transcriptional regulation of CSC phenotypes. By linking a canonical DNA repair factor to STAT1-dependent control of a core stemness gene, our work provides new mechanistic insight into CSC regulation and tumor plasticity. Clinically, CSCs drive tumor progression, metastasis, and therapeutic resistance, and approximately half of NSCLC patients exhibit low XPC expression. These data suggest that XPC may serve as a biomarker for CSC abundance and tumor aggressiveness, and that therapeutic

strategies targeting the XPC-STAT1-SOX2 axis could benefit a substantial patient population, either alone or in combination with existing treatments.

Materials and Methods

Cell culture and spheroid culture

A panel of NSCLC cell lines, including A549, H1299, H441, H460, H1650 and HCC827 were obtained from ATCC (Manassas, VA, USA), authenticated by short tandem repeat profiling and routinely tested negative for mycoplasma contamination. Cells were cultured in RPMI1640 supplemented with 10% FBS at 37°C in humidified atmosphere of 5% CO₂ in air. For spheroid culture, single-cell suspensions were cultured in serum-free 3D Tumor Sphere Medium XF (PromoCell, Heidelberg, Germany) in ultra-low attachment dishes (Corning, NY, USA) for at least 12 days.

shRNA, siRNA, plasmid, transfection, and lentivirus packaging

shRNA and siRNAs targeting human XPC, including shXPC-1, shXPC-2, XPC siRNA SMARTpool (siXPC-p), siXPC-1 and siXPC-2, and a non-targeting scrambled siRNA were described previously¹². STAT1 shRNAs were purchased from MilliporeSigma (shSTAT1-1: TRCN0000004264; shSTAT1-2: TRCN0000004267; shSTAT1-3: TRCN0000004268. St. Louis, MO, USA). pRc-CMV-STAT1-FLAG was obtained from Addgene (plasmid # 8691, Watertown, MA).

To generate the p3XFLAG-CMV-14-XPC expression construct, full-length human XPC cDNA was amplified by PCR from the pXPC3 plasmid (provided by Dr. R. Legerski, MD Anderson Cancer Center) and subcloned into the p3XFLAG-CMV-14 vector (MilliporeSigma) using standard molecular cloning procedures. For construction of the Tet On-inducible pLVX-TRE3G-XPC plasmid, XPC cDNA was subcloned from pXPC3 into the pLVX-TRE3G vector (Takara Bio, Mountain View, CA, USA). All constructs were verified by restriction enzyme digestion and Sanger sequencing.

siRNAs and plasmid constructs were transfected into cells using Lipofectamine 2000 (Fisher Scientific) according to the manufacturer's instructions. Lentiviral shRNA plasmids were packaged into lentivirus and used to infect target cells. Briefly, Lenti-X 293T cells (Takara Bio) were co-transfected with shRNA lentiviral plasmids together with the packaging plasmids psPAX2 and pMD2.G (Addgene) using Lipofectamine 2000. Culture medium was replaced 6–8 h after transfection. Viral supernatants were collected at 48 and 72 h post-transfection, cleared by centrifugation, and filtered through a 0.45-µm filter. Target cells were infected with lentiviral supernatant in the presence of polybrene (8 µg/mL).

Establishment of stable transfection cell lines

To establish a cell line stably expressing Tet On-inducible XPC, H1299 cells were first transduced with the pLVX-Tet3G vector (Takara Bio), selected with G418 at 500 µg/ml. The G418 resistant cells were

transduced with pLVX-TRE3G-XPC followed by selection with 1 µg/ml puromycin. The successfully stable transfection was confirmed using immunoblotting after cultured in the presence of Doxycycline. Stable knockdown cell lines were generated using the lentiviral shRNA constructs described above. Target cells were infected with lentiviral particles in the presence of polybrene, and stably transduced cells were selected with puromycin.

SOX2-Luciferase reporter assay

A549 and H1299 cells were co-transfected with 100 ng pRL-TK Renilla luciferase control (Promega, Madison, WI, USA) and 500 ng pGL3-SOX2 reporter plasmid (Addgene). Meanwhile, A549 cells were transfected with siCtrl or siXPC (100 nM), whereas H1299 cells were transfected with empty vector or p3XFLAG-CMV-14-XPC plasmid. 48h after transfection, luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Luminescence was recorded on a GloMax Discover Microplate Reader (Promega), and firefly luciferase activity was normalized to Renilla luciferase activity.

Sphere formation assay

The sphere-forming capacity of NSCLC cells was evaluated using a semi-solid TumorSphere formation assay as previously described⁴⁷. Briefly, A total of 1,000 cells was mixed with semisolid media (MethoCult H4100; STEMCELL Technologies Inc., Vancouver, BC, Canada) containing serum-free 3D Tumor Sphere Medium XF and plated in 6-well Ultra-Low Attachment plates (Corning). After 12 days, tumorspheres exhibiting a symmetric morphology and containing more than 50 cells were counted. In parallel, 100 cells per well were seeded into standard 6-well plates and cultured in RPMI 1640 supplemented with 10% FBS to determine colony formation efficiency. Sphere formation rates were quantified and normalized to the corresponding colony formation rates to account for differences in cell viability and plating efficiency.

In vivo limiting dilution assay (LDA)

Tumor-initiating cell frequency (TICf) was determined using an in vivo LDA. Cells in logarithmic growth phase were harvested, counted, and resuspended in a 1:1 mixture of sterile phosphate-buffered saline (PBS) and growth factor-reduced Matrigel (Corning). Decreasing numbers of cells (1×10^6 , 1×10^5 , or 1×10^4) were injected subcutaneously into nude mice (The Jackson Laboratory, Bar Harbor, ME, USA) in a total volume of 100 µL per injection. Mice were monitored regularly for tumor formation, and tumor incidence was recorded over a 2-week period. TICf was calculated using Extreme Limiting Dilution Analysis (ELDA) software based on the proportion of mice that developed tumors at each cell dose. All experiments were performed in accordance with the protocol approved by the Ohio State University, which stipulates a maximal allowable tumor diameter of 1.5 cm. The maximal tumor size was not exceeded during the study.

RNA sequencing (RNA-seq) and analysis

A549 cells were transiently transfected with pooled XPC siRNA or control siRNA. After 48 h, total RNA was extracted using the Total RNA Purification Kit (Norgen Biotek Corporation, Thorold, ON, Canada) according to the manufacturer's instructions. mRNA libraries were prepared using the KAPA mRNA HyperPrep Kit (Roche Sequencing Solutions, Wilmington, MA, USA) for paired-end sequencing. Libraries were sequenced on an Illumina NovaSeq 6000 at the Center for Medical Genomics, Indiana University (Indianapolis, IN, USA). The pre-processing of raw reads was done with FastQC v0.11.8, and the reads containing adapters, ploy-N, or any bases with Phred quality scores lower than 20 were removed with TrimGalore v0.6.10 to generate clean reads. The clean reads were aligned to the human reference genome (hg38) using STAR v2.7.10a and gene quantified with featureCounts. Differential expression analysis was conducted with DESeq2. Gene Set Enrichment Analysis (GSEA) was performed using the clusterProfiler package v3.18.0. RNA-seq data generated in this study have been deposited in the NCBI BioProject database under accession number PRJNA1405773.

Publicly available database analyses

Lung cancer transcriptomic datasets were obtained from The Cancer Genome Atlas (TCGA). Correlation between XPC and SOX2 mRNA expression levels in TCGA lung cancer samples was analyzed and visualized using Xenabrowser (<https://xenabrowser.net>). In addition, publicly available STAT1 ChIP-seq datasets were retrieved from the Cistrome Data Browser and analyzed to evaluate STAT1 binding at the SOX2 gene promoter.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cells using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Complementary DNA (cDNA) was generated from 1.5 µg of total RNA using a reverse transcription kit (Thermo Fisher Scientific) in a 20 µL reaction volume. The resulting cDNA was diluted to a final volume of 60 µL with DEPC-treated water, and 2.5 µL of diluted cDNA was used for quantitative PCR amplification with Fast SYBR Green PCR Master Mix (Thermo Fisher Scientific) on a QuantStudio 3 Real-Time PCR system (Thermo Fisher Scientific). Gene expression levels were normalized to 18S rRNA, and primer sequences are provided in Supplementary **Table S1**.

Western blot analysis

Whole-cell lysates were prepared by SDS lysis buffer, and immunoblot analyses were performed following previously established protocols⁴⁸. The primary antibodies used in this study were listed in **Table S2**. The original western blots were included in Supplementary Materials.

Immunoprecipitation-Mass Spectrometry (IP-MS)

H1299 cells were transiently transfected with p3XFLAG-CMV-14-XPC constructs or empty vector. 48 h after transfection, cells were harvested and lysed by sonication in lysis buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM KCl, 420 mM NaCl, 2 mM EDTA, 1% Triton X-100, 2.5 mM β-glycerophosphate, 1 mM Na₃VO₄, 1 mM DTT, 1 mM PMSF, and a complete protease inhibitor cocktail. Lysates were cleared by centrifugation, and protein concentrations were determined using a BCA assay. For immunoprecipitation,

equal amounts of total protein (1 mg) were incubated with anti-FLAG M2 magnetic beads (MilliporeSigma) for 1 h at room temperature with gentle rotation to capture FLAG-tagged protein complexes. Beads were then washed extensively with Tris-buffered saline (TBS). Following the final wash, bound proteins were subjected to on-bead digestion with sequencing-grade trypsin. Peptide samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an LTQ Orbitrap mass spectrometer in Center for Proteomics and Bioinformatics, Case Western Reserve University. Raw MS data were processed using standard proteomics workflows and searched against the human protein database using the Sequest search engine, with trypsin specified as the protease and up to two missed cleavages permitted. Protein quantification was performed by spectral counting. Only proteins identified with high confidence ($\geq 99\%$) and supported by at least two peptides with $\geq 95\%$ confidence were reported. Proteins identified in FLAG immunoprecipitates from FLAG-XPC transfected cells were compared with those identified in empty vector transfected cells to define specific XPC-interacting proteins. The MS proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD073425.

Co-immunoprecipitation (co-IP)

H1299 cells were transiently transfected with p3XFLAG-CMV-14-XPC constructs or empty vector control for 48 h. These cells, along with A549 cells, were harvested and the nuclei were isolated using Nuclei Isolation Kit (NUC101, MilliporeSigma). Nuclei were lysed in lysis buffer as described in the IP-MS study. For IP of FLAG-tagged XPC from H1299 cells, equal amounts of total protein (1 mg) from FLAG-XPC or empty vector transfected H1299 cells were incubated with anti-FLAG M2 magnetic beads for 1 h at room temperature with gentle rotation. For IP of endogenous STAT1 from A549 cells, equal amounts of total protein (1 mg) were incubated with 5 μ g of either rabbit anti-STAT1 antibody or normal rabbit IgG (Cell Signaling Technology, Danvers, MA, USA), together with Protein A magnetic beads (Cell Signaling Technology) overnight at 4°C. Following IP, beads were washed, resuspended in 2× Laemmli sample buffer, boiled for 10 min, and subjected to immunoblotting with anti-XPC and anti-STAT1 antibodies (Cell Signaling Technology).

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was conducted as previously described⁴⁹. Briefly, H1299 cells transiently transfected with pRc-CMV-STAT1-FLAG or empty vector, and A549 cells stably expressing XPC or control shRNA, were fixed with 1% formaldehyde for 10 min at room temperature. Crosslinking was quenched by adding 120 mM glycine for 5 min. Cells were washed with PBS, harvested using a cell scraper, and pelleted by centrifugation at 2,500 rpm for 10 min at 4°C. ChIP was conducted using the ChIP-IT Express Enzymatic Kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's instructions.

For H1299 cells, anti-FLAG M2 magnetic beads were used to immunoprecipitate FLAG-tagged STAT1-bound DNA. For A549 cells, endogenous STAT1-bound DNA was immunoprecipitated using rabbit anti-STAT1 antibody or normal rabbit IgG (Cell Signaling Technology) as a negative control. Immunoprecipitated DNA was purified using phenol/chloroform extraction and analyzed by quantitative

PCR with primers targeting the SOX2 promoter (Supplementary Table S1). Relative enrichment of FLAG-tagged STAT1 at the SOX2 promoter was calculated as fold enrichment over empty vector-transfected cells. For endogenous STAT1, enrichment at the SOX2 promoter was determined by comparing shXPC-expressing cells to shCtrl cells.

Statistical analysis

Data are presented as means $\pm$ standard deviation (SD). Two-sample t-tests were used for comparisons between two groups, and one-way ANOVA was applied for comparisons among multiple groups. TICf was analyzed using a generalized linear model⁵⁰. A two-sided P value < 0.05 was considered statistically significant.

Declarations

Ethics declarations

The authors declare no competing interests.

Availability of Data and Materials

RNA-Sequencing files were deposited in the NCBI BioProject database under accession number PRJNA1405773. The Mass Spectrometry proteomics data were deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD073425.

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References

1. Bray, F. *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 74, 229–263 (2024). <https://doi.org/10.3322/caac.21834>
2. Dean, M., Fojo, T. & Bates, S. Tumour stem cells and drug resistance. *Nat. Rev. Cancer* 5, 275–284 (2005). <https://doi.org/nrc1590> [pii];10.1038/nrc1590 [doi]
3. Nguyen, L. V., Vanner, R., Dirks, P. & Eaves, C. J. Cancer stem cells: an evolving concept. *Nat. Rev. Cancer* 12, 133–143 (2012). <https://doi.org/nrc3184> [pii];10.1038/nrc3184 [doi]
4. Eramo, A. *et al.* Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death. Differ* 15, 504–514 (2008). <https://doi.org/4402283> [pii];10.1038/sj.cdd.4402283 [doi]
5. Chu, X. *et al.* Cancer stem cells: advances in knowledge and implications for cancer therapy. *Signal Transduct Target Ther* 9, 170 (2024). <https://doi.org/10.1038/s41392-024-01851-y>
6. Lee, H. *et al.* Cancer stem cells: landscape, challenges and emerging therapeutic innovations. *Signal Transduct Target Ther* 10, 248 (2025). <https://doi.org/10.1038/s41392-025-02360-2>
7. Lathia, J., Liu, H. & Matei, D. The Clinical Impact of Cancer Stem Cells. *Oncologist* 25, 123–131 (2020). <https://doi.org/10.1634/theoncologist.2019-0517>
8. Legerski, R. & Peterson, C. Expression cloning of a human DNA repair gene involved in xeroderma pigmentosum group C. *Nature* 359, 70–73 (1992).
9. Cleaver, J. E., Thompson, L. H., Richardson, A. S. & States, J. C. A summary of mutations in the UV-sensitive disorders: xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. *Hum Mutat* 14, 9–22 (1999). [https://doi.org/10.1002/\(SICI\)1098-1004\(1999\)14:1%3C9::AID-HUMU2%3E3.0.CO;2-6](https://doi.org/10.1002/(SICI)1098-1004(1999)14:1%3C9::AID-HUMU2%3E3.0.CO;2-6)
10. Friedberg, E. C. *et al.* Defective nucleotide excision repair in xpc mutant mice and its association with cancer predisposition. *Mutat Res* 459, 99–108 (2000). [https://doi.org/10.1016/s0921-8777\(99\)00068-3](https://doi.org/10.1016/s0921-8777(99)00068-3)
11. Hollander, M. C. *et al.* Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc Natl Acad Sci U S A* 102, 13200–13205 (2005). <https://doi.org/10.1073/pnas.0503133102>

12. Cui, T. *et al.* XPC inhibits NSCLC cell proliferation and migration by enhancing E-Cadherin expression. *Oncotarget* 6, 10060–10072 (2015). <https://doi.org/3542> [pii]
13. Wu, Y. H. *et al.* Reduced XPC messenger RNA level may predict a poor outcome of patients with nonsmall cell lung cancer. *Cancer* 110, 215–223 (2007). <https://doi.org/10.1002/cncr.22743> [doi]
14. Le, M. N. *et al.* NER factors are recruited to active promoters and facilitate chromatin modification for transcription in the absence of exogenous genotoxic attack. *Mol. Cell* 38, 54–66 (2010). [https://doi.org/S1097-2765\(10\)00211-X](https://doi.org/S1097-2765(10)00211-X) [pii];10.1016/j.molcel.2010.03.004 [doi]
15. Frechet, M. *et al.* Overexpression of matrix metalloproteinase 1 in dermal fibroblasts from DNA repair-deficient/cancer-prone xeroderma pigmentosum group C patients. *Oncogene* 27, 5223–5232 (2008). <https://doi.org/10.1038/onc.2008.153>
16. Fong, Y. W. *et al.* A DNA repair complex functions as an Oct4/Sox2 coactivator in embryonic stem cells. *Cell* 147, 120–131 (2011). [https://doi.org/S0092-8674\(11\)01008-7](https://doi.org/S0092-8674(11)01008-7) [pii];10.1016/j.cell.2011.08.038 [doi]
17. Wang, Q. E., Han, C., Zhang, B., Sabapathy, K. & Wani, A. A. Nucleotide excision repair factor XPC enhances DNA damage-induced apoptosis by downregulating the antiapoptotic short isoform of caspase-2. *Cancer Res* 72, 666–675 (2012). <https://doi.org/0008-5472.CAN-11-2774> [pii];10.1158/0008-5472.CAN-11-2774 [doi]
18. Clarke, M. F. *et al.* Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res* 66, 9339–9344 (2006). <https://doi.org/0008-5472.CAN-06-3126> [pii];10.1158/0008-5472.CAN-06-3126 [doi]
19. Stewart, J. M. *et al.* Phenotypic heterogeneity and instability of human ovarian tumor-initiating cells. *Proc. Natl. Acad. Sci. U. S. A* 108, 6468–6473 (2011). <https://doi.org/1005529108> [pii];10.1073/pnas.1005529108 [doi]
20. Boyer, L. A. *et al.* Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 122, 947–956 (2005). <https://doi.org/10.1016/j.cell.2005.08.020>
21. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676 (2006). <https://doi.org/10.1016/j.cell.2006.07.024>
22. Herrera, S. C. & Bach, E. A. JAK/STAT signaling in stem cells and regeneration: from Drosophila to vertebrates. *Development* 146 (2019). <https://doi.org/10.1242/dev.167643>
23. Dolatabadi, S. *et al.* JAK-STAT signalling controls cancer stem cell properties including chemotherapy resistance in myxoid liposarcoma. *Int J Cancer* 145, 435–449 (2019). <https://doi.org/10.1002/ijc.32123>
24. Hu, X., Li, J., Fu, M., Zhao, X. & Wang, W. The JAK/STAT signaling pathway: from bench to clinic. *Signal Transduct Target Ther* 6, 402 (2021). <https://doi.org/10.1038/s41392-021-00791-1>
25. Masutani, C. *et al.* Purification and cloning of a nucleotide excision repair complex involving the xeroderma pigmentosum group C protein and a human homologue of yeast RAD23. *EMBO J* 13, 1831–1843 (1994).

26. Araki, M. *et al.* Centrosome protein centrin 2/caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J Biol Chem* 276, 18665–18672 (2001). <https://doi.org/10.1074/jbc.M100855200>
27. Kroon, P. *et al.* JAK-STAT blockade inhibits tumor initiation and clonogenic recovery of prostate cancer stem-like cells. *Cancer Res* 73, 5288–5298 (2013). <https://doi.org/10.1158/0008-5472.CAN-13-0874>
28. Park, S. Y. *et al.* The JAK2/STAT3/CCND2 Axis promotes colorectal Cancer stem cell persistence and radioresistance. *J Exp Clin Cancer Res* 38, 399 (2019). <https://doi.org/10.1186/s13046-019-1405-7>
29. Qadir, A. S. *et al.* CD95/Fas Increases Stemness in Cancer Cells by Inducing a STAT1-Dependent Type I Interferon Response. *Cell Rep* 18, 2373–2386 (2017). <https://doi.org/10.1016/j.celrep.2017.02.037>
30. Doherty, M. R. *et al.* Interferon-beta represses cancer stem cell properties in triple-negative breast cancer. *Proc Natl Acad Sci U S A* 114, 13792–13797 (2017). <https://doi.org/10.1073/pnas.1713728114>
31. Fan, Y. *et al.* FXR1 regulates transcription and is required for growth of human cancer cells with TP53/FXR2 homozygous deletion. *Elife* 6 (2017). <https://doi.org/10.7554/eLife.26129>
32. Wu, Y. H. *et al.* p53 dysfunction by xeroderma pigmentosum group C defects enhance lung adenocarcinoma metastasis via increased MMP1 expression. *Cancer Res* 70, 10422–10432 (2010). <https://doi.org/0008-5472.CAN-10-2615> [pii];[10.1158/0008-5472.CAN-10-2615](https://doi.org/10.1158/0008-5472.CAN-10-2615) [doi]
33. Martin-Hijano, L. & Sainz, B., Jr. The Interactions Between Cancer Stem Cells and the Innate Interferon Signaling Pathway. *Front Immunol* 11, 526 (2020). <https://doi.org/10.3389/fimmu.2020.00526>
34. Dorritie, K. A., Redner, R. L. & Johnson, D. E. STAT transcription factors in normal and cancer stem cells. *Adv Biol Regul* 56, 30–44 (2014). <https://doi.org/10.1016/j.jbior.2014.05.004>
35. Avale, L., Pensa, S., Regis, G., Novelli, F. & Poli, V. STAT1 and STAT3 in tumorigenesis: A matter of balance. *JAKSTAT* 1, 65–72 (2012). <https://doi.org/10.4161/jkst.20045>
36. Godugu, D., Chilamakuri, R. & Agarwal, S. STAT3 axis in cancer and cancer stem cells: From oncogenesis to targeted therapies. *Biochim Biophys Acta Rev Cancer* 1880, 189461 (2025). <https://doi.org/10.1016/j.bbcan.2025.189461>
37. Meissl, K., Macho-Maschler, S., Muller, M. & Strobl, B. The good and the bad faces of STAT1 in solid tumours. *Cytokine* 89, 12–20 (2017). <https://doi.org/10.1016/j.cyto.2015.11.011>
38. Van der Spek, P. J. *et al.* XPC and human homologs of RAD23: Intracellular localization and relationship to other nucleotide excision repair complexes. *Nucleic Acids Res* 24, 2551–2559 (1996).
39. Fong, H., Hohenstein, K. A. & Donovan, P. J. Regulation of self-renewal and pluripotency by Sox2 in human embryonic stem cells. *Stem Cells* 26, 1931–1938 (2008). <https://doi.org/10.1634/stemcells.2007-1002>

40. Boumahdi, S. *et al.* SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. *Nature* 511, 246–250 (2014). <https://doi.org:10.1038/nature13305>
41. Mamun, M. A., Mannoor, K., Cao, J., Qadri, F. & Song, X. SOX2 in cancer stemness: tumor malignancy and therapeutic potentials. *J Mol Cell Biol* 12, 85–98 (2020). <https://doi.org:10.1093/jmcb/mjy080>
42. Singh, S. *et al.* EGFR/Src/Akt signaling modulates Sox2 expression and self-renewal of stem-like side-population cells in non-small cell lung cancer. *Mol Cancer* 11, 73 (2012). <https://doi.org:10.1186/1476-4598-11-73>
43. Li, J. *et al.* Super-Enhancer Driven LIF/LIFR-STAT3-SOX2 Regulatory Feedback Loop Promotes Cancer Stemness in Head and Neck Squamous Cell Carcinoma. *Adv Sci (Weinh)* 11, e2404476 (2024). <https://doi.org:10.1002/advs.202404476>
44. Faltings, L. J. *et al.* SOX2 commands LIM homeobox transcription factors in choroid plexus development and tumorigenesis. *Neuro Oncol* 27, 2006–2022 (2025). <https://doi.org:10.1093/neuonc/noaf085>
45. Zhang, S., Xiong, X. & Sun, Y. Functional characterization of SOX2 as an anticancer target. *Signal Transduct Target Ther* 5, 135 (2020). <https://doi.org:10.1038/s41392-020-00242-3>
46. Wang, S. *et al.* A feedforward loop between STAT1 and YAP1 stimulates lipid biosynthesis, accelerates tumor growth, and promotes chemotherapy resistance in mutant KRAS colorectal cancer. *Commun Biol* 8, 1278 (2025). <https://doi.org:10.1038/s42003-025-08740-2>
47. Han, C. *et al.* DDB2 Suppresses Tumorigenicity by Limiting the Cancer Stem Cell Population in Ovarian Cancer. *Mol. Cancer Res* 12, 784–794 (2014). <https://doi.org:1541-7786.MCR-13-0638> [pii];10.1158/1541-7786.MCR-13-0638 [doi]
48. Cai, S. *et al.* ERK inactivation enhances stemness of NSCLC cells via promoting Slug-mediated epithelial-to-mesenchymal transition. *Theranostics* 12, 7051–7066 (2022). <https://doi.org:10.7150/thno.73099>
49. Lavudi, K. *et al.* ALDH1A1 promotes PARP inhibitor resistance by enhancing retinoic acid receptor-mediated DNA polymerase theta expression. *NPJ Precis Oncol* 7, 66 (2023). <https://doi.org:10.1038/s41698-023-00411-x>
50. Hu, Y. & Smyth, G. K. ELDA: extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. *J. Immunol. Methods* 347, 70–78 (2009). [https://doi.org:S0022-1759\(09\)00195-1](https://doi.org:S0022-1759(09)00195-1) [pii];10.1016/j.jim.2009.06.008 [doi]

Figures

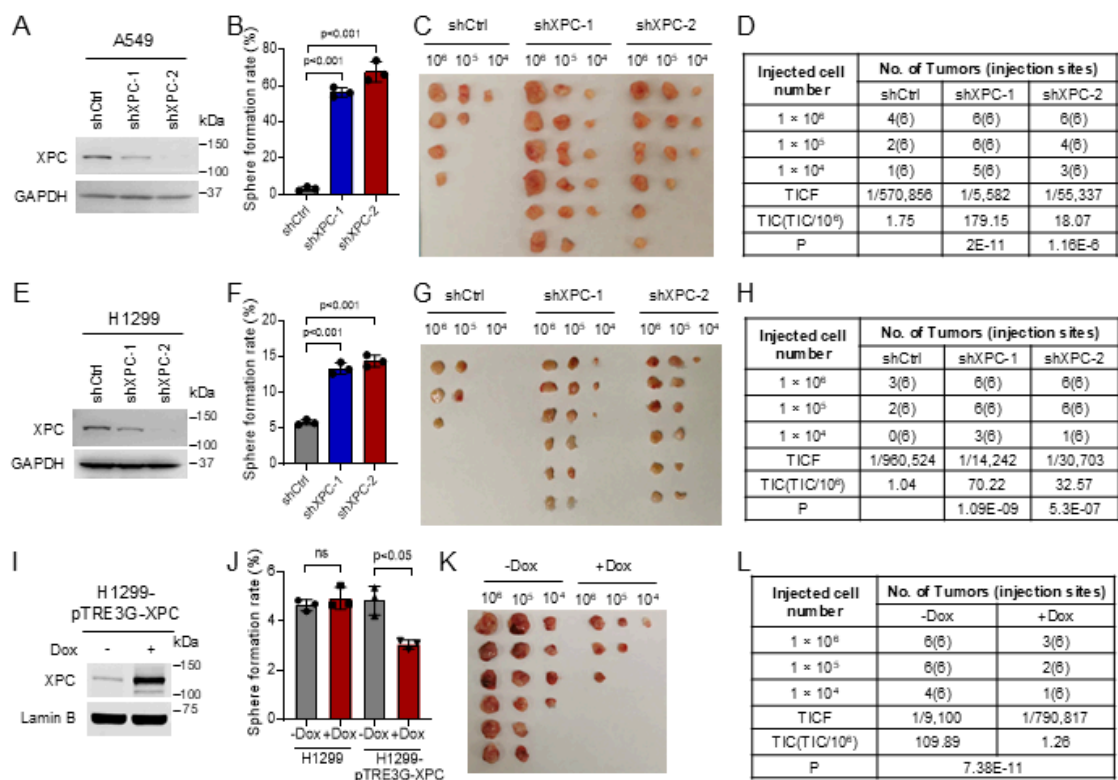


Figure 1

XPC suppresses the CSC population in NSCLC cells. A-H.Downregulation of XPC enhances sphere formation capacity and tumorigenicity in NSCLC cells. A549 (A-D) and H1299 (E-H) cells were stably transfected with distinct XPC shRNA or control shRNA (A, E), sphere forming ability was assessed using the semisolid sphere formation assay (B, F); the frequency of tumor initiating cells (TICf) was estimated by xenotransplantation limiting dilution assays (C, D, G, H). **I-L.** Overexpression of XPC reduces sphere

formation and tumorigenic potential in H1299 cells. H1299 cells harboring a Tet-inducible XPC expression vector (H1299-pTRE3G-XPC) were treated with or without Doxycycline (Dox) for 12 days to induce XPC expression (I). Sphere forming ability (J) and TICf (K, L) were analyzed as described above. N = 3, bar: SD, **: P < 0.01.

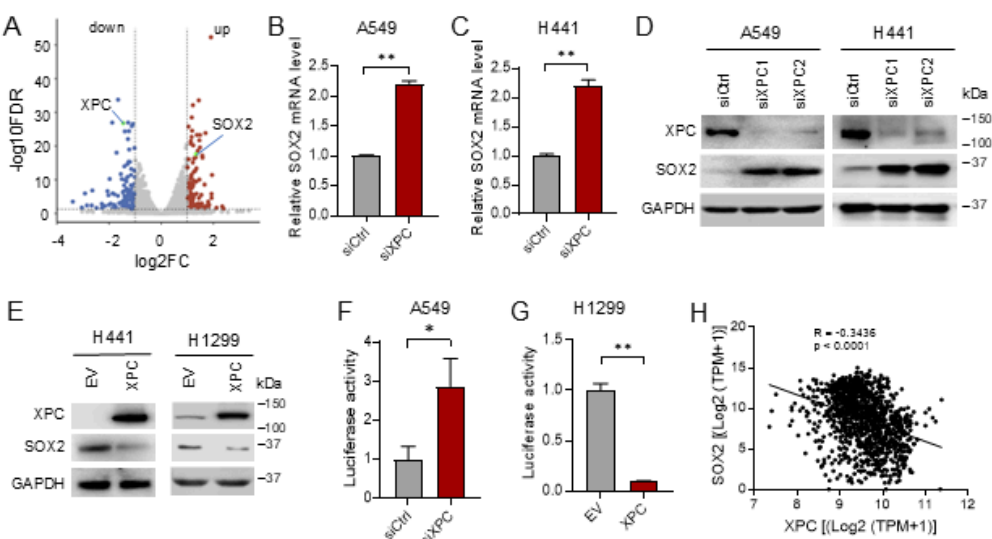


Figure 2

XPC negatively regulates SOX2 expression. **A.** RNA-seq analysis reveals increased SOX2 expression in A549 cells following XPC knockdown (KD). A549 cells were transfected with pooled XPC siRNA or control siRNA for 48 h, total RNA was isolated and subjected to RNA-Seq analysis. A volcano plot illustrates differentially expressed genes (Cutoff: $|\text{Log}_2\text{FC}| \geq 1$, $\text{FDR} \leq 0.05$). **B, C.** qRT-PCR validation of SOX2 upregulation upon XPC KD in A549 (B) and H441 (C) cells. **D.** Immunoblot analysis confirms increased SOX2 protein levels following XPC KD in A549 and H441 cells. **E.** XPC overexpression in H441 and H1299 cells reduces SOX2 protein expression. **F, G.** XPC KD enhances, whereas XPC overexpression suppresses, SOX2 promoter activity. A549 cells transfected with siXPC or control siRNA, and H1299 cells transfected with an XPC expression vector or empty vector, were co-transfected with the pGL3-SOX2 luciferase reporter and the pRL-TK Renilla control plasmid. Luciferase activity was measured 48 h after transfection. **H.** TCGA analysis demonstrates an inverse correlation between XPC and SOX2 mRNA expression in lung cancer patients ($n = 1,325$). $N = 3$, bar: SD, **: $P < 0.01$.

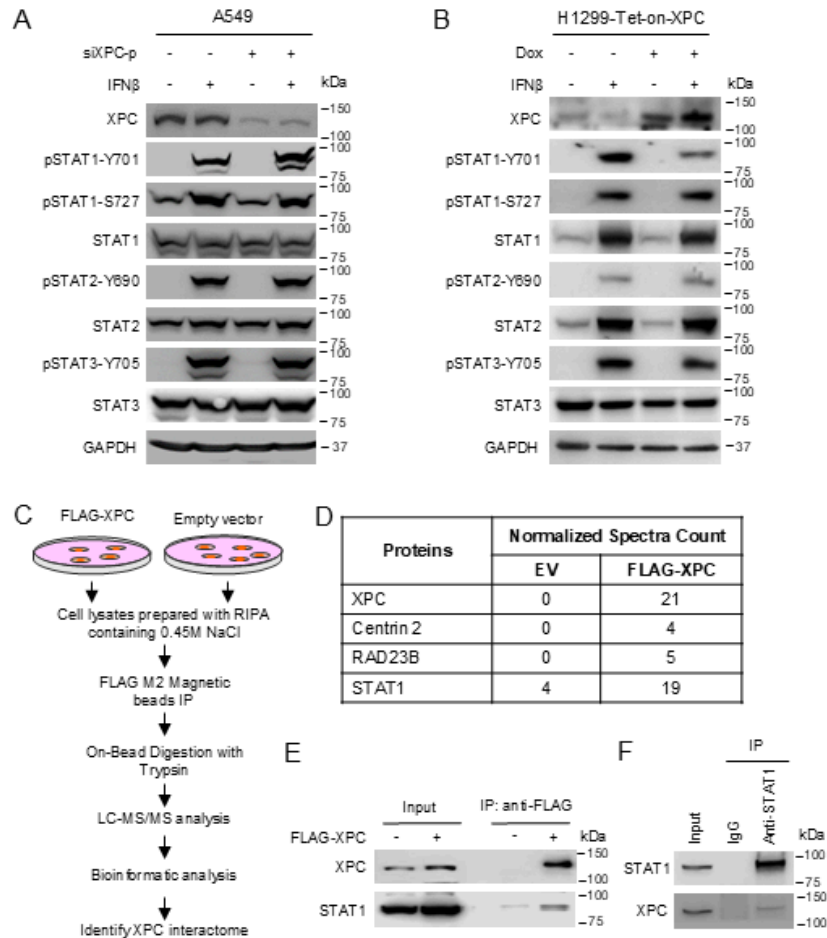


Figure 3

XPC suppresses STAT1 phosphorylation and interacts with STAT1 in NSCLC cells. **A, B.** XPC inhibits IFN β -induced STAT1 phosphorylation at Tyr 701. A549 cells were transfected with pooled siXPC for 48 h (B), and H1299-pTRE3G-XPC cells were treated with Dox (0.2 μ g/ml) for 48 h to induce XPC expression (B). Cells were then treated with IFN β (1,000 U/ml) for an additional 24 h. Phosphorylated and total STAT1, STAT2, and STAT3 levels were determined by immunoblotting. **C.** Workflow for XPC

immunoprecipitation (IP) proteomics. **D.** IP-mass spectrometry identifies STAT1 among XPC-interacting proteins in H1299 expressing FLAG-tagged XPC. **E.** Co-IP of FLAG-XPC confirms interaction with STAT1 in nuclear lysates of H1299 expressing FLAG-tagged XPC. **F.** Reciprocal co-IP with an anti-STAT1 antibody validates endogenous XPC-STAT1 interaction in A549 cells.

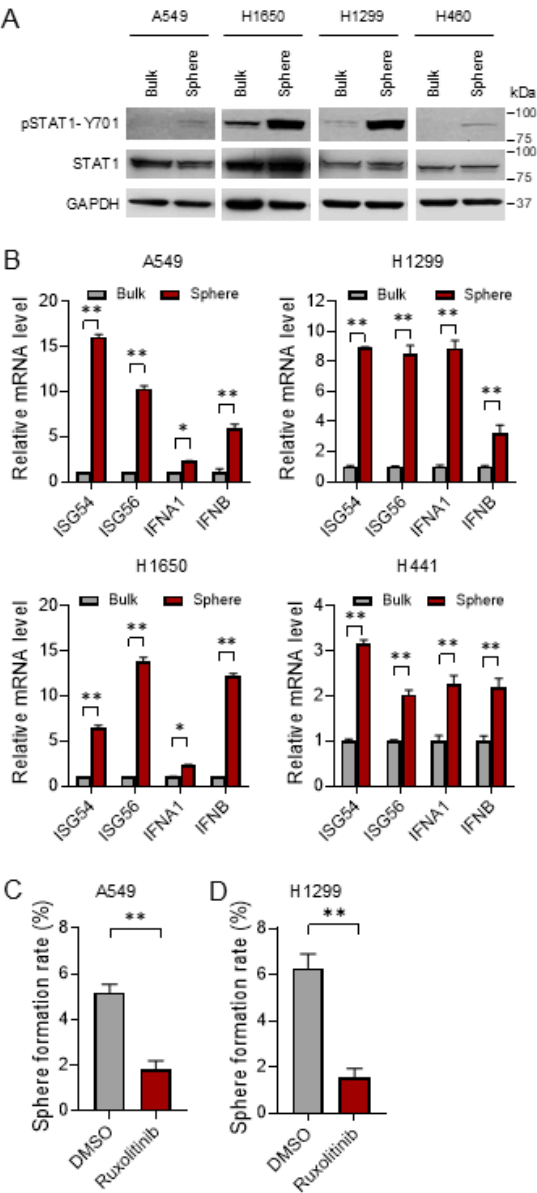


Figure 4

NSCLC CSCs possess enhanced STAT signaling. A. A panel of NSCLC cell lines were cultured either under standard adherent conditions with complete medium (Bulk) or under spheroid culture conditions in serum-free medium to enrich for stem-like cancer cells (Sphere). Phosphorylated STAT1 at Tyr 701 (pSTAT1-Y701) and total STAT1 expression were evaluated by immunoblotting. **B.** The expression of a panel of STAT1 target genes was analyzed by qRT-PCR (B). **C, D.** A549 (C) and H1299 (D) cells were treated with Ruxolitinib (5 μ M) for 24 h, 1,000 cells were seeded and sphere forming ability was assessed using the semisolid sphere formation assay. n = 3, bar: SD, **: P < 0.01.

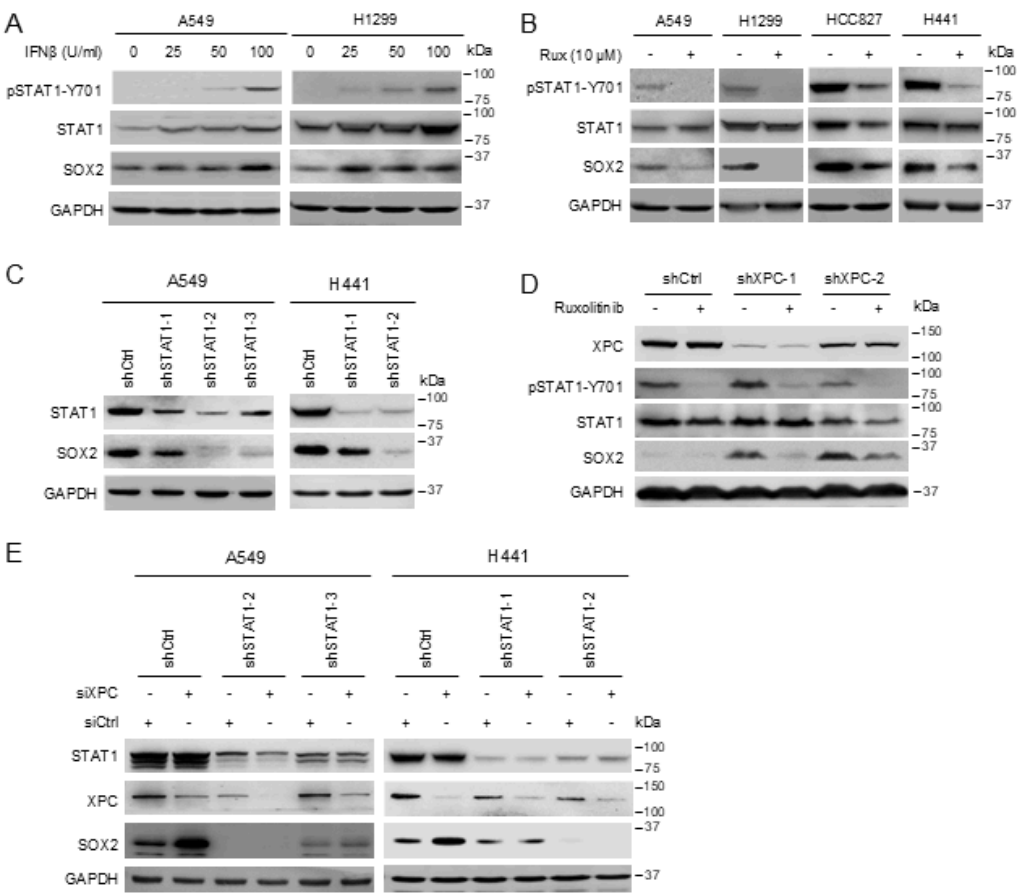


Figure 5

XPC downregulates SOX2 through inhibition of STAT1 signaling. **A.** IFN β -induced JAK–STAT activation increases SOX2 expression. A549 and H1299 cells were treated with increasing doses of IFN β for 48 h. Whole-cell lysates were prepared and analyzed by immunoblotting to assess SOX2 expression. **B.** Pharmacological inhibition of JAK–STAT signaling reduces SOX2 expression. A panel of NSCLC cell lines was treated with ruxolitinib (10 μ M) for 48 h, followed by immunoblotting analysis of SOX2 expression. **C.** STAT1 knockdown decreases SOX2 expression. A549 and H441 cells stably expressing distinct STAT1 shRNAs were analyzed by immunoblotting to determine SOX2 protein levels. **D.** Inhibition of JAK–STAT signaling attenuates XPC knockdown–induced SOX2 upregulation. A549 cells stably expressing XPC shRNA or control shRNA were treated with ruxolitinib (10 μ M) for 48 h, and SOX2 expression was examined by immunoblotting. **E.** STAT1 knockdown blocks XPC knockdown–induced SOX2 upregulation. A549 and H441 cells stably expressing STAT1 shRNA or control shRNA were transiently transfected with pooled XPC siRNA or control siRNA for 48 h, followed by immunoblotting analysis of SOX2 expression.

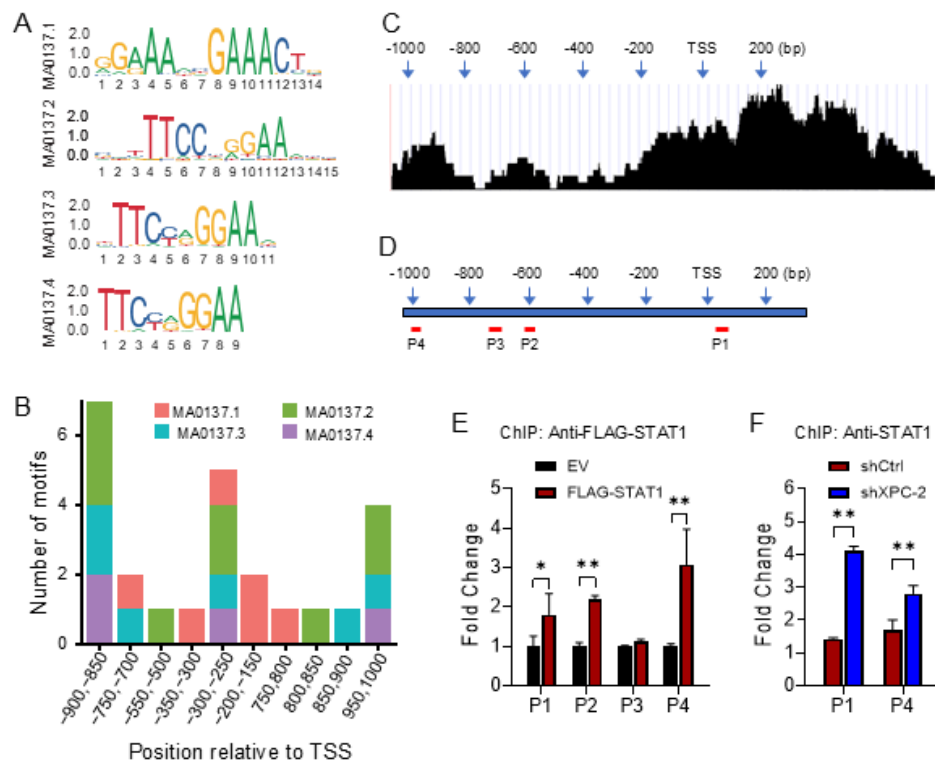


Figure 6

XPC negatively regulates STAT1 recruitment to the SOX2 promoter. **A.** STAT1 DNA-binding motifs identified from the JASPAR database. **B.** Predicted STAT1 binding sites within the SOX2 promoter region. **C.** Analysis of publicly available ChIP-seq data from the Cistrome database indicates STAT1 binding at the SOX2 promoter. The STAT1 ChIP-seq dataset was derived from H358 lung cancer cells. **D.** Schematic representation of the human SOX2 promoter showing the regions amplified by ChIP-qPCR primer sets. **E.**

STAT1 is enriched at the SOX2 promoter. H1299 cells were transfected with empty vector or FLAG-tagged STAT1 for 48 h, followed by ChIP using anti-FLAG M2 Magnetic beads. **F.** XPC downregulation enhances STAT1 occupancy at the SOX2 promoter. ChIP assays were performed in A549 cells stably expressing XPC shRNA or corresponding control cells using the anti-STAT1 antibody or normal IgG to assess STAT1 binding to the SOX2 promoter region. N = 3, bar: SD, **: P < 0.01.

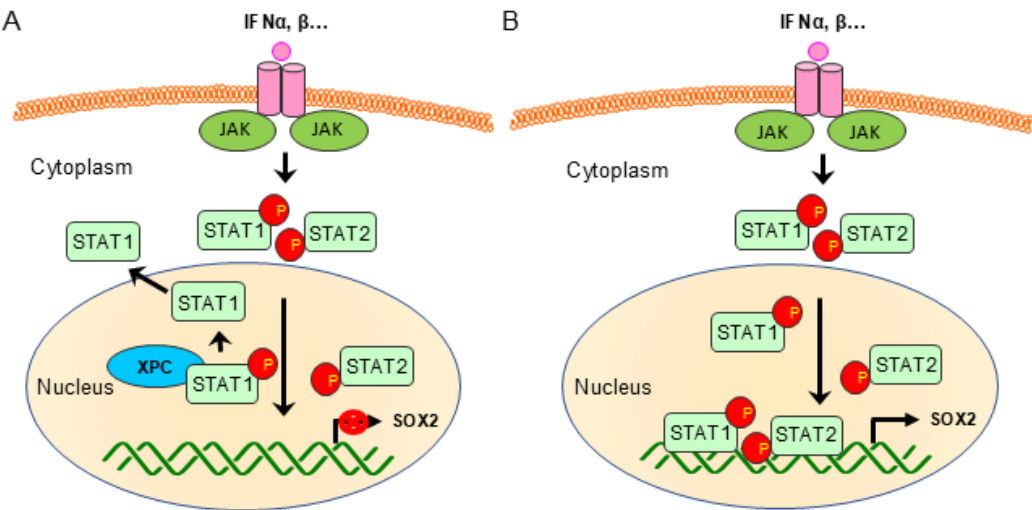


Figure 7

Model of XPC-STAT1-SOX2 signaling in cancer stemness regulation. A. In XPC-proficient cells, XPC interacts with nuclear pSTAT1, promoting STAT1 dephosphorylation and thereby limiting its chromatin binding. As a result, STAT1 recruitment to the SOX2 promoter is reduced, leading to suppression of SOX2 transcription and stemness-associated gene expression. **B.** In XPC-deficient cells, the absence of XPC prevents pSTAT1 dephosphorylation, resulting in sustained nuclear pSTAT1 accumulation. Active pSTAT1 binds to the SOX2 promoter, driving SOX2 transcription and promoting stemness-related programs.

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