



IGSF9 promotes immunotherapy resistance in colon cancer by orchestrating an immunosuppressive tumor microenvironment and enables combinatorial targeting strategies

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ABSTRACT

Colon cancer (CC), a significant global health burden with high incidence and mortality, is often accompanied by an immunosuppressive tumor microenvironment (TME) that limits the efficacy of immunotherapy. Immunoglobulin superfamily member 9 (IGSF9), a cell surface protein involved in cell adhesion and signaling, has been shown to promote tumor progression and regulate TME in other cancers, but its role in CC remains poorly understood. This study integrated multi-omics analyses, single-cell sequencing, and preclinical models to explore IGSF9's function in CC and its correlation with immunotherapy resistance. The results showed that IGSF9 was significantly upregulated in CC tumors, positively correlated with advanced tumor stages and poor prognosis such as reduced overall survival in colon adenocarcinoma (COAD). High IGSF9 expression was correlated with an immunosuppressive TME characterized by increased infiltration of regulatory T cells (Tregs), cancer-associated fibroblasts (CAFs), and reduced immune cell infiltration; it was also linked to lower tumor mutational burden (TMB) and microsatellite instability (MSI), predicting poor response to anti-PD-1 immunotherapy in clinical datasets. Single-cell and spatial transcriptomics revealed that IGSF9 was predominantly expressed in malignant epithelial cells, correlated with epithelial-mesenchymal transition (EMT) pathways. Drug sensitivity analysis identified Doramapimod, a MAPK inhibitor, which combined with anti-PD-1 therapy significantly enhanced tumor regression in mouse models by reducing Treg infiltration. In conclusion, this study establishes IGSF9 as a prognostic and predictive biomarker for immunotherapy resistance in CC and suggests that targeting IGSF9-associated KRAS/MAPK pathways with Doramapimod may offer a novel combination strategy to overcome TME-mediated resistance, warranting further clinical investigation for personalized CC treatment.

1. Introduction

Colon cancer (CC), the third most common malignancy worldwide, poses a significant global health burden, with an estimated 2.2 million new cases and 1.1 million deaths annually [1,2]. Despite advancements in treatment, patients with advanced or metastatic CC often face poor prognosis [3], particularly those with immunosuppressive tumor microenvironments (TME) that limit the efficacy of immunotherapy [4,5].

Nonetheless, the efficacy of immunotherapy exhibits marked heterogeneity across cancer types [6]. Despite substantial breakthroughs in the field, a considerable subset of patients remains unresponsive to

immunotherapeutic interventions [7,8]. This disparity underscores the critical need to dissect the biological determinants of treatment response [9]. TME has emerged as a central regulator of immunotherapy outcomes, with its complex architecture dictating the balance between anti-tumor immunity and immune evasion [10]. Dynamic interactions among tumor cells, immune cell subsets (e.g., T cells, macrophages), and stromal elements (e.g., fibroblasts, endothelial cells) within the TME orchestrate either permissive or suppressive microenvironments for immune effector function [11,12].

Immunoglobulin superfamily member 9 (IGSF9) [13], a cell surface protein involved in cell adhesion and signaling, has emerged as a

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potential regulator of tumor biology. Previous studies have demonstrated that IGSF9 promotes lung cancer invasion and metastasis through GSK-3 β [14]. Additionally, a research team validated in mice that IGSF9 blocks the efficacy of immunotherapy by inhibiting T-cell proliferation [15]. These findings collectively indicate that IGSF9 exhibits tumor-promoting functions and influences treatment efficacy by regulating the TME.

TME in CC is highly heterogeneous, with immunosuppressive features such as reduced immune cell infiltration and elevated levels of regulatory T cells (Tregs) and cancer-associated fibroblasts (CAFs) contributing to treatment resistance [16,17]. IGSF9 has been shown to modulate TME components, including CD8⁺ T cells proliferation which are linked to diminished anti-tumor immune responses [15]. However, in CC, which is primarily characterized by immunosuppressive TME, how IGSF9 orchestrates TME reprogramming remains elucidated.

This study aims to characterize the role of IGSF9 in the immunosuppressive TME of CC, investigate its correlation with immunotherapy resistance, and explore potential therapeutic interventions to overcome this resistance. Our analytical approach begins with an initial pan-cancer discovery phase to establish the broader context of IGSF9 dysregulation, followed by colon cancer-focused validation, which includes single-cell transcriptomics, spatial profiling, and preclinical therapeutic experiments. By integrating multi-omics analyses, single-cell sequencing, and preclinical models, we seek to establish IGSF9 as a prognostic and predictive biomarker and identify novel combination therapies for CC patients with high IGSF9 expression.

2. Methods

2.1. Data sources

Expression profiles and clinical datasets for TCGA, GTEx, and CCLE were obtained from the UCSC XENA platform (<https://xenabrowser.net/datapages/>). Tumor tissue data were sourced from TCGA, whereas normal tissue annotations were supplemented by GTEx. This integrated approach effectively addressed the limitation of insufficient or missing normal tissue samples in select TCGA cohorts. For tumor cell line expression analysis, data were retrieved from the CCLE repository.

2.2. Data preprocessing and normalization

For transcriptomic data obtained from TCGA, we utilized the log2 (TPM+1) normalized expression values provided by the UCSC XENA platform, which had been uniformly processed using the TOIL pipeline to ensure cross-sample comparability. For GTEx normal tissue data, the same TOIL-processed log2(TPM+1) values were used to enable direct comparison with TCGA tumor samples. When integrating TCGA and GTEx data for tumor-normal comparisons, we note that batch effects may exist despite uniform processing; therefore, we applied ComBat (from the R package, version 3.46.0) batch correction when performing differential expression analyses across datasets to minimize technical variation while preserving biological signals.

For single-cell RNA sequencing analyses, we utilized pre-processed and annotated datasets from the IMMUCan and TISCH databases, which apply standard quality control filtering (removing cells with <200 genes, >20 % mitochondrial content), SCTransform normalization, and Harmony integration for batch correction across samples.

For spatial transcriptomics data from the CROST database, spot-level expression matrices were normalized using SCTransform and spatially-aware deconvolution algorithms as implemented in the original database processing pipelines.

For the immunotherapy cohorts (GSE61676 and GSE135222), we used the processed expression matrices provided by the Gene Expression Omnibus (GEO), which were normalized using the robust multi-array average (RMA) method for microarray data (GSE61676) and DESeq2-normalized counts for RNA-seq data (GSE135222), respectively.

Drug sensitivity data from GDSC2 were analyzed using pre-computed IC50 values and cell line expression data (log2(TPM+1)) provided by the database, ensuring consistency in cross-study comparisons.

2.3. Enrichment analysis

Pearson correlation analysis was applied to explore the relationship between the target gene and all other genes across 33 different cancer types. After obtaining the correlation data, the 50 genes with the strongest positive and negative correlations were selected to create heatmaps illustrating these associations. To further characterize the biological significance, functional enrichment analyses based on Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were performed using the *clusterProfiler* package (version 4.14.4). In this analysis, gene sets were assembled from the top 300 genes showing the highest positive correlations (including the target gene) and used for pathway enrichment, with results displayed as bubble plots and bar graphs.

For gene set-level evaluation, Gene Set Enrichment Analysis (GSEA) was performed across all 33 cancer types using *clusterProfiler*, integrating annotations from GO, KEGG, and Reactome databases. In parallel, Gene Set Variation Analysis (GSVA, version 2.0.5) [18] was conducted with the updated HALLMARK gene sets (50 canonical pathways) from the Molecular Signatures Database (MsigDB) to assess pathway activity patterns. GSVA scores were generated for each tumor type, and Pearson correlation coefficients between the target gene's expression and pathway scores were calculated. These correlations were visualized in an integrated heatmap to reveal key functional relationships.

2.4. Mutation, copy number, and methylation analysis

The cBioPortal platform (<http://www.cBioPortal.org>) was employed to systematically explore the mutation and amplification landscapes of the target gene across various cancer types. Structural variant data were examined to uncover characteristic patterns of genetic alterations. For each cancer type, Pearson correlation analysis was conducted to evaluate the relationship between gene expression levels (log2-transformed TPM values) and copy number variation (CNV) z-scores. In parallel, promoter methylation (derived from HM450 array data available in cBioPortal) was analyzed for its correlation with gene expression using Pearson's method, given that promoter hypermethylation is typically associated with reduced transcriptional activity. The findings were presented through graphical plots, emphasizing statistically significant correlations ($p < 0.05$) while excluding tumor types without relevant CNV or methylation data from the analyses.

2.5. ESTIMATE analysis

The ESTIMATE package in R was applied to compute stromal scores, immune scores, overall ESTIMATE scores (calculated as the combined stromal and immune scores), and tumor purity levels within cancer tissues [19]. The associations were illustrated using various visual formats, including correlation line graphs, radar charts, lollipop plots, and heatmaps summarizing pairwise correlations, with detailed statistics provided in supplementary files for further examination.

2.6. TIMER2.0 analysis

To characterize immune cell infiltration, the Tumor Immune Estimation Resource 2.0 (TIMER2.0, <http://timer.cistrome.org/>) was utilized. This web-based platform integrates several immune deconvolution methods [20]. Using the "Immune Association" module, the gene symbol (IGSF9) was entered, and correlations with specified immune cell populations were visualized through the "Gene" function. A

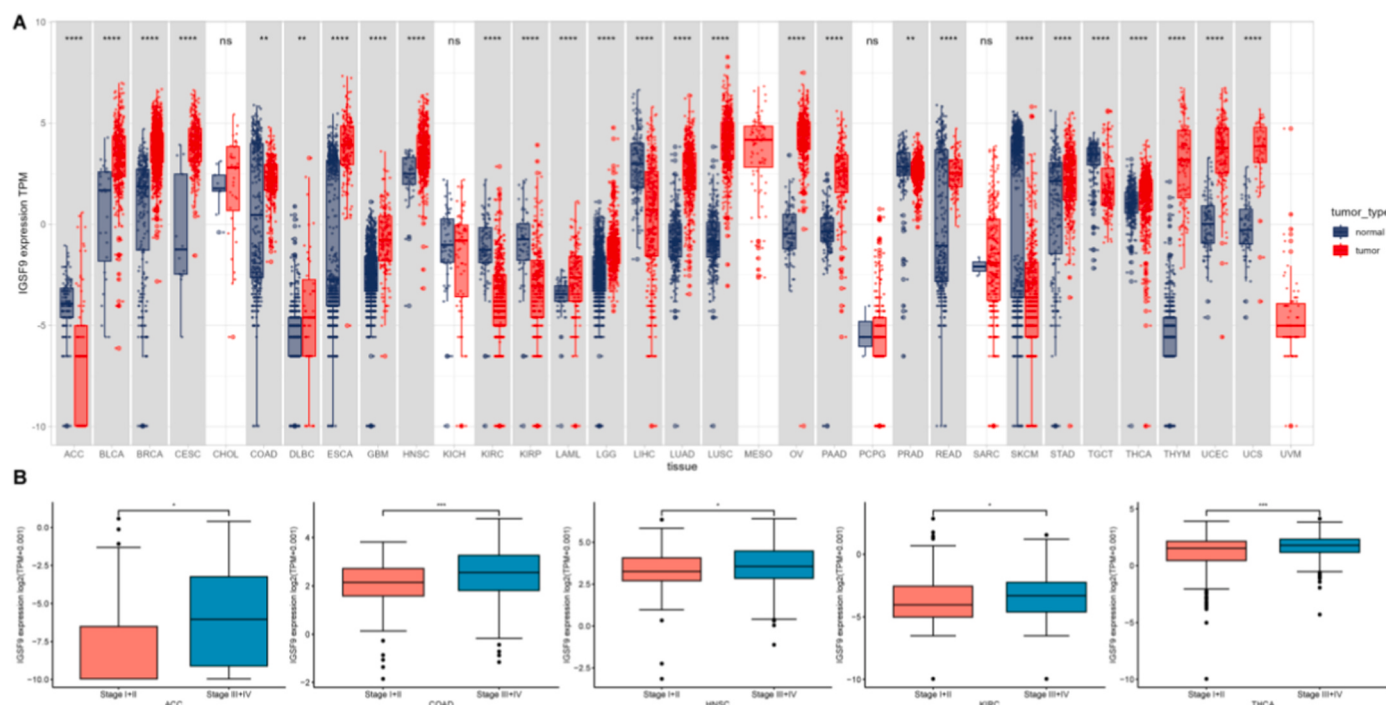


Fig. 1. Differential Expression of IGSF9 in Normal and Tumor Tissues and Across Tumor Stages. (A) mRNA expression of IGSF9 in normal tissues (GTEx) and tumor tissues (TCGA) across 33 cancer types. (B) IGSF9 expression in stage-specific tumor tissues (stages I-II vs. III-IV) for ACC, COAD, HNSC, KIRC, and THCA.

total of 21 immune cell subsets—including various T cell types, macrophages, and dendritic cells—were systematically evaluated for their infiltration levels and correlation with IGSF9 expression.

2.7. CIBERSORT and ImmuCellAI

CIBERSORT [21] and ImmuCellAI (<http://bioinfo.life.hust.edu.cn/ImmuCellAI/#/>) algorithms were applied to estimate the abundance of 26 immune cell populations based on TCGA transcriptomic data. Relationships between cell infiltration levels and gene expression were displayed through heatmaps and circular correlation plots.

2.8. Gene mutation analysis

Somatic mutation characteristics between tumors with high versus low gene expression levels were analyzed using the *maftools* R package (version 2.18.0). Differentially mutated genes (DMGs) were identified via Wilcoxon rank-sum tests, and statistically significant genes (adjusted $p < 0.05$) were illustrated using forest plots. These plots detailed the mutation types (e.g., missense, nonsense) and effect sizes, supplemented with bar charts that summarized mutation burden (mutations per megabase) for each group.

2.9. Drug resistance analysis

Data from the Genomics of Drug Sensitivity in Cancer (GDSC2, <https://www.cancerrxgene.org/>) database were leveraged to explore links between gene expression and sensitivity (IC50 values) to 198 anticancer agents.

2.10. Cell culture and animal experiments

Animal experiments were approved by the Animal Care and Ethics Committee of Jinhua Central Hospital. Female Balb/c mice (4–6 weeks old) were obtained from Changzhou Cavens Model Animal Co., Ltd., and acclimated for a week. CT26 cells were cultured to 70–80 % confluence overnight in serum-supplemented medium. Cells were collected by

centrifugation at 1150 rpm for 5 min, rinsed twice in cold PBS, counted, and resuspended at $\sim 3 \times 10^6$ cells/mL. Mice were prepared and injected subcutaneously with 1×10^6 CT26 cells in 200 μ L PBS. When tumors reached volumes of 150–250 mm³ (average ~ 200 mm³) on day 12 post-inoculation, mice were randomly allocated into four groups: (1) saline control, (2) Doramapimod (10 mg/kg, $n = 4$), (3) anti-PD-1 (30 mg/kg, $n = 4$), and (4) combination therapy ($n = 4$). Treatments were given intraperitoneally every 3 days, for a total of five doses.

2.11. Multiplex immunofluorescence staining

Multiplex staining was conducted with validated antibodies against murine CD8A (rabbit monoclonal, Abcam, ab237723) and FOXP3 (rabbit polyclonal, Abcam, ab20034). The Opal™ 7-color Automated IHC Kit (PerkinElmer) was used following the manufacturer's instructions. Slides were scanned on the Vectra® 3 imaging platform, and marker expression was quantified using inForm® software (version 2.4).

2.12. Statistical analysis

All analyses were performed in R (version 4.2.3). Categorical variables were analyzed by Pearson's chi-squared or Fisher's exact tests (where applicable), while continuous variables were compared using Student's *t*-test or Wilcoxon rank-sum test depending on normality (assessed via Shapiro-Wilk test). Kaplan-Meier survival curves with log-rank tests were used to assess overall survival (OS), progression-free interval (PFI), disease-free interval (DFI), and disease-specific survival (DSS), using the *survival* and *survminer* R packages. Univariate Cox regression identified variables associated with survival ($p < 0.05$), which were then entered into multivariate Cox models. Forest plots summarized hazard ratios (HR) and 95 % confidence intervals (CI) from univariate Cox analyses of the target gene across OS, DSS, DFI, and PFI. All *p*-values were two-sided. For multiple comparison corrections, we employed the following approaches: (1) In differential expression analyses across multiple cancer types, the Benjamini-Hochberg method was used to control the false discovery rate (FDR), with adjusted $p < 0.05$ considered statistically significant; (2) In pathway enrichment analyses

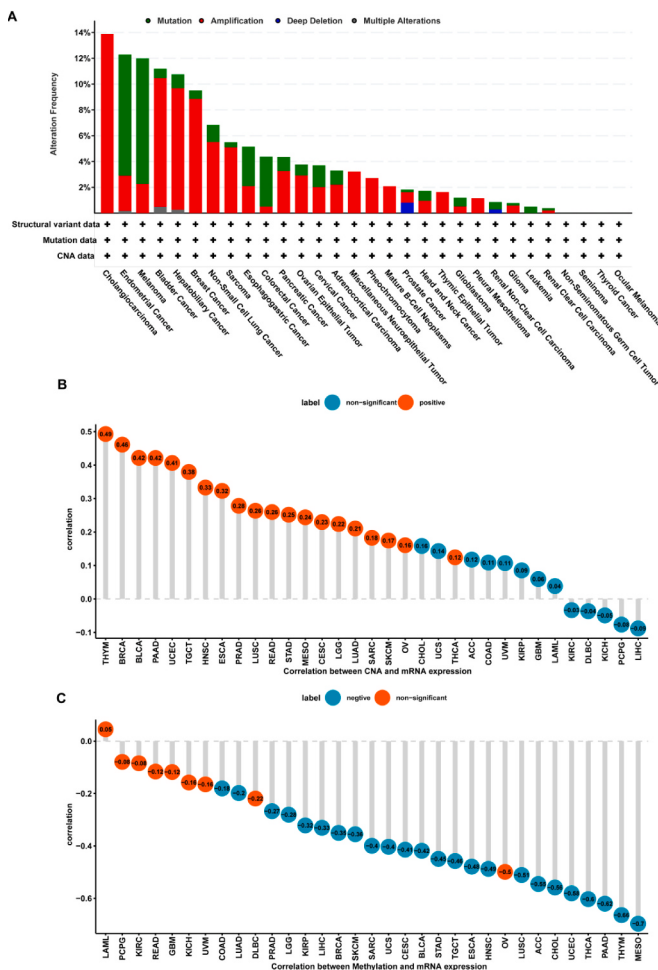


Fig. 2. Pan-Cancer Genetic Alterations of IGSF9. (A) Genetic alteration profiles of IGSF9 (mutations, amplifications, deletions) in TCGA pan-cancer datasets, visualized via cBioPortal. (B) Correlation between IGSF9 mRNA expression and copy number variation (CNV) in pan-cancer. (C) Correlation between IGSF9 mRNA expression and promoter methylation levels in pan-cancer.

(GO, KEGG, GSEA), adjusted p-values corrected by the Benjamini-Hochberg method were reported, with a significance threshold set at adjusted $p < 0.05$; (3) For gene mutation analysis using maftools, adjusted p-values (FDR-corrected) were used as stated in the original text; (4) For correlation analyses (Pearson and Spearman), survival analyses (Kaplan-Meier and Cox regression), and immune infiltration correlations, nominal p-values < 0.05 were considered statistically significant, as these analyses were typically performed within specific cancer types rather than across multiple comparisons.

3. Results

3.1. Differential expression of IGSF9 in tumor versus normal tissue samples

By integrating RNA expression data from TCGA and GTEx databases, we analyzed IGSF9 expression across 33 tumor types. IGSF9 exhibited significant differential expressions in 27 cancers. Elevated IGSF9 expression was observed in tumor tissues compared to normal counterparts in cancers such as bladder urothelial carcinoma (BLCA), breast cancer (BRCA), cervical squamous cell carcinoma (CESC), colon adenocarcinoma (COAD), diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma (GBM), head and neck

squamous cell carcinoma (HNSC), acute myeloid leukemia (LAML), lower grade glioma (LGG), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic cancer (PAAD), rectal cancer (READ), gastric cancer (STAD), thyroid cancer (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS). Conversely, higher IGSF9 levels were seen in normal tissues relative to tumors in adrenocortical carcinoma (ACC), clear cell renal carcinoma (KIRC), papillary renal carcinoma (KIRP), hepatocellular carcinoma (LIHC), prostate cancer (PRAD), skin melanoma (SKCM), and testicular germ cell tumors (TGCT). For cancers such as cholangiocarcinoma (CHOL), kidney chromophobe (KICH), mesothelioma (MESO), pheochromocytoma/paraganglioma (PCPG), sarcoma (SARC), and uveal melanoma (UVM), expression differences were not statistically significant, likely due to limited normal controls (Fig. 1A).

Stage-specific analysis (Fig. 1B) showed that IGSF9 expression was significantly higher in advanced (stage III/IV) than early-stage (I/II) tumors in ACC, COAD, HNSC, KIRC, and THCA, suggesting its potential role as a diagnostic and staging biomarker.

3.2. IGSF9 alterations and genomic associations in pan-cancer

Data from cBioPortal revealed that IGSF9 alterations—including mutations, amplifications, and deletions—were widespread across cancers. Amplifications were most frequent in cholangiocarcinoma (CHOL), while deep deletions occurred mainly in prostate cancer (PRAD) and non-clear cell renal carcinomas. Certain tumors, such as thyroid cancer, ocular melanoma, seminomas, and non-seminomatous germ cell tumors, showed no detectable alterations (Fig. 2A).

Analysis of copy number variation (CNV; Fig. 2B) and promoter methylation (Fig. 2C) demonstrated significant CNV-expression correlations in ~66 % of tumor types, including THYM, BRCA, and BLCA. Promoter methylation levels were inversely associated with IGSF9 expression in about the most tumor types, except LAML, PCPG, KIRC, READ, GBM, KICH, UVM, DLBC, and OV.

3.3. IGSF9 as a prognostic indicator in multiple cancers

Through univariate Cox proportional hazards modeling, we assessed the prognostic value of *IGSF9* expression in relation to OS, DSS, PFI, and DFI. Elevated *IGSF9* levels were consistently linked to poorer OS in LGG, ACC, PAAD, COAD, THYM, SKCM, and MESO (Fig. 3A). Similarly, high *IGSF9* expressions were a risk factor for DSS in cancers including LGG, ACC, PAAD, BLCA, COAD, and HNSC. For DFI, elevated *IGSF9* expression predicted adverse outcomes in PAAD and ACC (Fig. 3C), while in PFI analyses, *IGSF9* overexpression was associated with unfavorable prognosis in LGG, PAAD, ACC, KIRC, PRAD, GBM, and COAD (Fig. 3D). Collectively, these results underscore the significant impact of *IGSF9* on survival outcomes across multiple cancer types, highlighting its potential as a robust prognostic biomarker.

3.4. Pathway and functional associations of IGSF9

We conducted correlation analyses between *IGSF9* expression and the transcriptome across tumors, identifying both positively and negatively co-expressed gene sets. Enrichment of these gene sets through HALLMARK analyses (Fig. S1) revealed significant correlations between *IGSF9* expression and key oncogenic pathways, particularly the KRAS and Notch signaling cascades, suggesting potential associations with cell cycle dynamics, DNA replication fidelity, and apoptosis. It should be noted that these correlational findings do not establish direct mechanistic involvement of *IGSF9* in these pathways. In COAD specifically (Fig. 4A), strong correlations were found between *IGSF9* expression and KRAS pathway activity, suggesting a potential association that may warrant further mechanistic investigation to inform treatment strategies for KRAS-mutant CC.

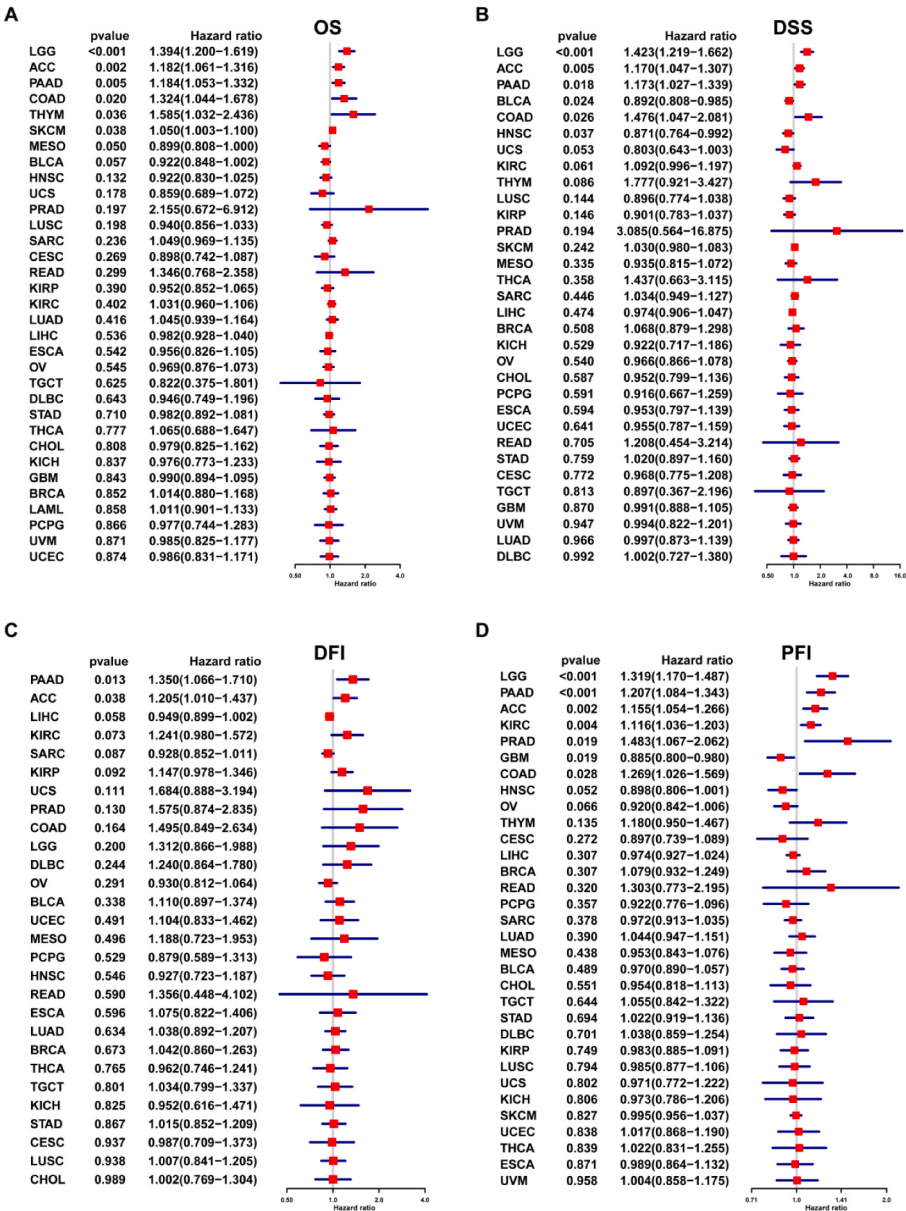


Fig. 3. Prognostic Value of IGSF9 in Pan-Cancer. (A–D) Univariate Cox regression analysis of IGSF9 expression for overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) in pan-cancer.

In addition, we showed 50 genes with a positive correlation to *IGSF9* expression (Fig. 4B) and genes with a negative correlation to *IGSF9* expression (Fig. 4C). GO and KEGG pathway analyses revealed that *IGSF9* was associated with biological processes such as selective autophagy and ERBB signaling (Fig. 4D), as well as pathways like VEGF-mediated angiogenesis (Fig. 4E), reinforcing its role in tumor progression and microenvironment modulation.

3.5. Immune microenvironment and *IGSF9* expression

To comprehensively understand the regulatory function of *IGSF9* within the TME across various cancer types, we initiated our analysis by leveraging an immunotherapy - related gene signature (Fig. S2A). The findings revealed that EMT1 is linked to nearly all cancer types and is up-regulated in most tumors. In COAD, we observed that EMT1 and EMT3 are both up-regulated in patients with high *IGSF9* expression, suggesting a potential association between *IGSF9* and tumor metastasis in COAD [22]. Additionally, the expression of *IGSF9* is significantly

correlated with mismatch repair [23] and antigen processing machinery [24], indicating that *IGSF9* could potentially serve as a biomarker for immunotherapy in COAD patients.

ESTIMATE analyses (Fig. S2B) indicated that higher *IGSF9* expression correlated with lower immune and stromal scores in most tumor types, while showing a positive association in THCA. Focusing on COAD (Fig. 5), the expected negative correlation between tumor purity and immune/stromal content was disrupted in high-*IGSF9* tumors, highlighting a complex relationship between *IGSF9* expression and immune cell exclusion within the tumor microenvironment.

3.6. The relationship between *IGSF9* within pan-cancer immune cell infiltration

To gain deeper insight into the specific TME components associated with *IGSF9* expression, we systematically examined immune cell infiltration patterns using both ImmuCellAI and TIMER2 platforms. Our analyses revealed that *IGSF9* expression was markedly correlated with

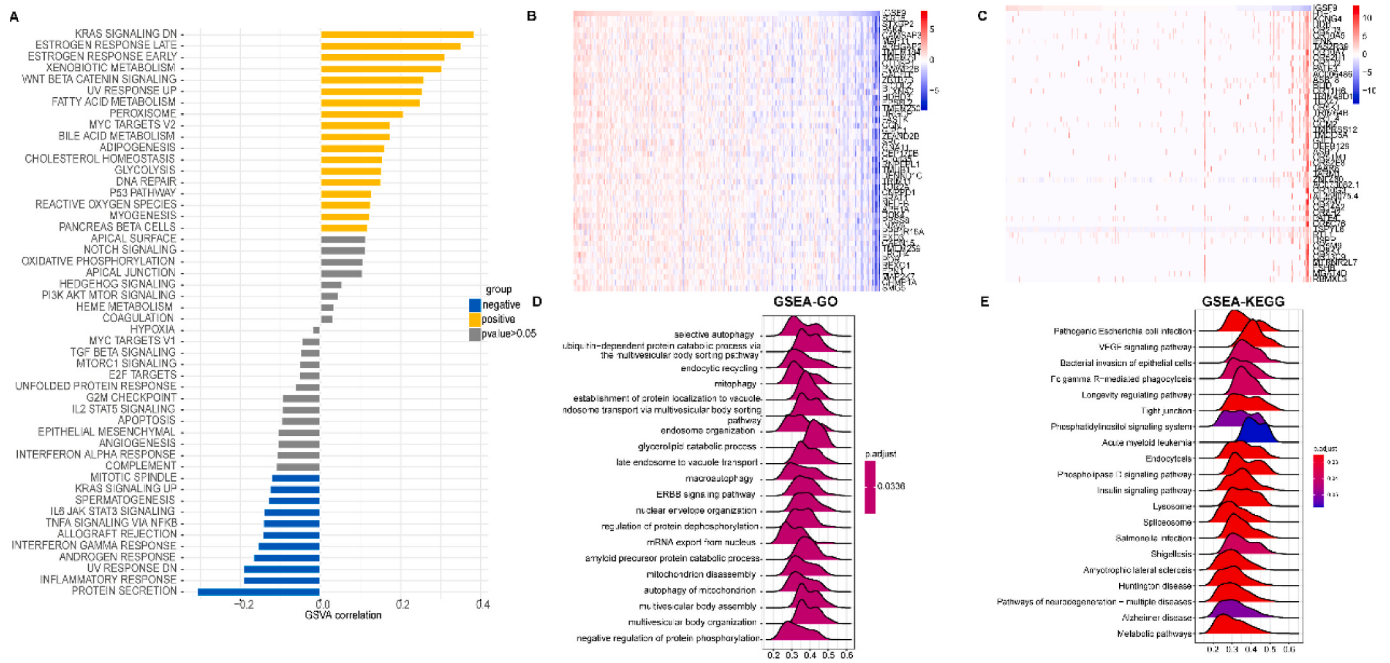


Fig. 4. Pathway Enrichment Analysis of IGSF9 in Pan-Cancer and COAD. (A) Pathway enrichment specific to COAD, highlighting KRAS signaling and EMT-associated pathways. (B–C) Top 50 positively and negatively correlated genes with IGSF9 in pan-cancer. (D–E) GO and KEGG pathway enrichment via GSEA for IGSF9-associated genes, identifying links to autophagy, ERBB signaling, and VEGF pathways.

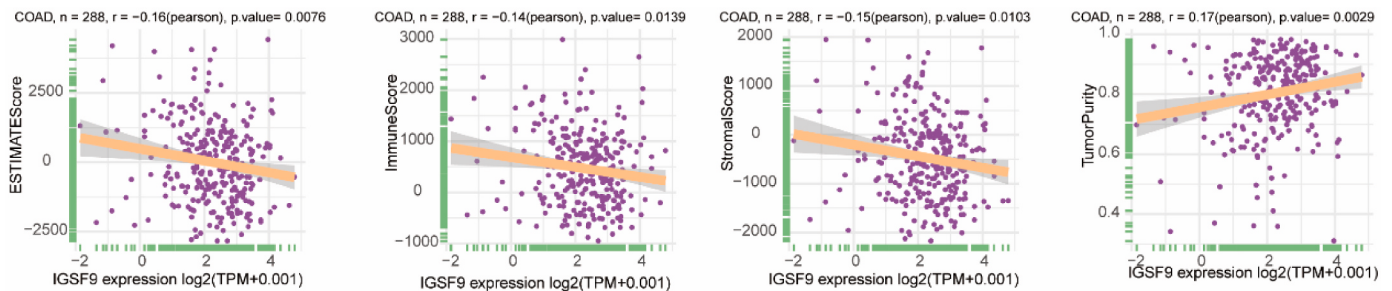


Fig. 5. IGSF9 Correlates with Immunosuppressive Tumor Microenvironment (TME) in COAD. Correlations between IGSF9 expression and ESTIMATE-derived stromal score, immune score, and tumor purity in COAD.

infiltration levels of various immune cell subsets across multiple cancer types, including CD8⁺ naive T cells, Th17 cells, neutrophils, monocytes, as well as certain CD4⁺ naive T cell populations (Fig. S3A). To further validate and refine these associations, TIMER2 analysis was performed, which consistently demonstrated a robust positive correlation between IGSF9 expression and neutrophil infiltration across nearly all tumor types, corroborating the ImmuCellAI findings. In the context of COAD, IGSF9 expression showed significant positive associations not only with neutrophil infiltration but also with elevated levels of regulatory T cells (Tregs) and cancer-associated fibroblasts (CAFs) (Fig. S3B). These results suggest that IGSF9 expression is associated with features of an immunosuppressive TME and epithelial-mesenchymal transition (EMT)-related signatures in COAD. However, these correlational findings do not establish that IGSF9 directly regulates these immune cell populations or EMT processes. The observed associations may reflect common upstream regulators or parallel biological programs rather than direct causal relationships. It is important to note, however, that due to the inherent heterogeneity of immune landscapes across different malignancies, the strength and nature of these correlations varied to some extent among tumor types.

3.7. High expression of IGSF9 is associated with poor response to immunotherapy

Given the close correlation between IGSF9 and the heterogeneity of the immune microenvironment, we investigated its relationship with immunotherapy efficacy. Tumor mutational burden (TMB) [25] and microsatellite instability (MSI) status [26] are two clinical indicators closely linked to immunotherapy response. Analysis of two independent datasets revealed that COAD patients with high IGSF9 expression had lower TMB levels (Fig. 6A), and MSI scores (Fig. 6B) were near the lowest across all tumors analyzed. Spearman correlation analyses further showed negative correlations between IGSF9 and both TMB and MSI (Fig. 6C and D), suggesting that CC patients with high IGSF9 expression are likely non-responsive to immunotherapy.

We performed exploratory correlation analyses using two publicly available immunotherapy datasets to assess whether IGSF9 expression patterns observed in colon cancer may have broader implications for immunotherapy response across cancer types. It is important to note the characteristics and limitations of these cohorts: GSE61676 comprises 64 patients with advanced NSCLC treated with bevacizumab plus erlotinib combination therapy, representing an anti-angiogenic and EGFR-targeted regimen rather than immune checkpoint blockade.

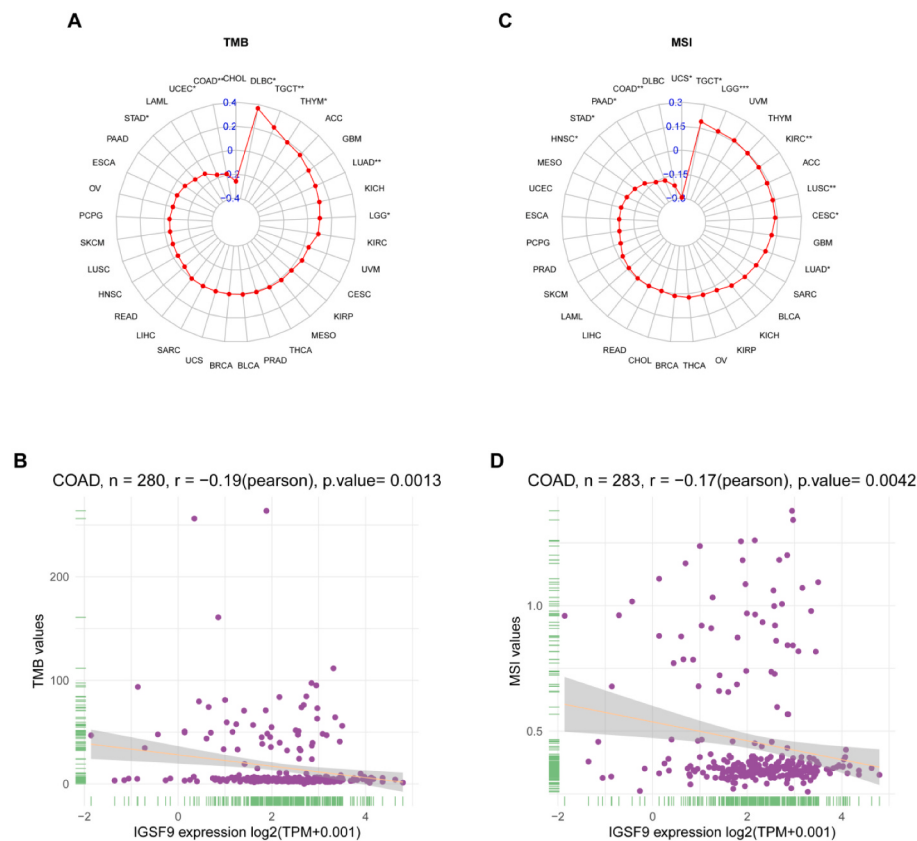


Fig. 6. IGSF9 Expression and Immunotherapy Predictive Biomarkers. (A–B) Correlations between IGSF9 and tumor mutational burden (TMB) in pan-cancer and COAD. (C–D) Correlations between IGSF9 and microsatellite instability (MSI) in pan-cancer and COAD.

GSE135222 includes 121 melanoma patients treated with anti-PD-1/PD-L1 monotherapy or combination immunotherapy, with heterogeneous treatment protocols. In the GSE61676 dataset [27] (Fig. 7A), patients with high IGSF9 expression showed significantly more non-responders to immunotherapy compared to low IGSF9 expressers. Survival analysis also indicated shorter survival duration after immunotherapy in high IGSF9 patients. Similarly, in the GSE135222 dataset [28] (Fig. 7B), all high IGSF9 expression patients experienced relapses after immunotherapy and had significantly shorter survival times. These cross-cancer exploratory analyses provide preliminary evidence suggesting that IGSF9 may serve as a biomarker associated with treatment resistance; however, the lack of colon cancer-specific immunotherapy cohorts substantially limits the direct clinical applicability of these findings to colon cancer patients. Prospective validation in dedicated colon cancer immunotherapy trials is essential before any clinical implementation.

3.8. Single-cell and spatial transcriptomics reveal IGSF9 is predominantly expressed in colorectal cancer epithelium

To localize IGSF9 expression, we analyzed its distribution across single-cell datasets of multiple CRC samples using the IMMCan database [29] (Fig. 8A). Boxplot analysis showed IGSF9 was primarily expressed in endothelial and epithelial cells (Fig. 8B). We then selected the GSE146771 dataset with high IGSF9 expression for UMAP dimensionality reduction and cell type annotation (Fig. 8C), mapping IGSF9 expression onto the UMAP plot. Results demonstrated that IGSF9 was predominantly expressed in malignant tumor epithelium.

Subsequent validation using spatial transcriptomics data from GSE146771 further supported these findings. Following spatial transcriptomics annotation (Fig. 9A), colocalization of “Malignant” cell clusters with IGSF9 expression was observed, reconfirming the single-cell data (Fig. 9B). To quantify cellular expression patterns, Spearman

correlation analysis was performed. Consistent with spatial localization, IGSF9 expression positively correlated with malignant tumor epithelial cells (Fig. 9C) but negatively correlated with endothelial cells (Fig. 9D), fibroblasts (Fig. 9E), and proliferative T cells (Tprolif, Fig. 9F). Data from the ProteinAtlas database (Fig. S4) also confirmed high IGSF9 expression in malignant epithelium of CC.

3.9. Doramapimod sensitizes immunotherapy responses in IGSF9-high CC patients

In an effort to explore potential precision therapeutic strategies for CC patients exhibiting high IGSF9 expression, we conducted a comprehensive drug sensitivity screening based on data from the GDSC2 database (Fig. S5). This analysis highlighted Doramapimod—a potent MAPK pathway inhibitor—as the most promising agent for IGSF9-high CC, consistent with our earlier observations linking IGSF9 dysregulation to aberrant activation of the KRAS signaling axis. To experimentally validate these *in silico* findings and assess the potential synergy between Doramapimod and immunotherapy, we established a subcutaneous xenograft model using CT26 tumor-bearing Balb/c mice (Fig. 10A). Treatment with Doramapimod alone significantly impeded tumor growth, while the combination of Doramapimod and anti-PD-1 therapy yielded even greater tumor regression compared to either monotherapy (Fig. 10B and C).

Further examination of the tumor immune microenvironment through histological analysis (Fig. 10D) revealed that combined treatment substantially reduced the infiltration of FOXP3⁺ Tregs relative to either single-agent group (Fig. 10E). Concurrently, this regimen significantly enhanced the infiltration of cytotoxic CD8⁺ T cells (Fig. 10F). Comparative analyses across the different treatment arms underscored Doramapimod's capacity to diminish immunosuppressive Treg populations, thereby amplifying the efficacy of anti-PD-1 therapy through

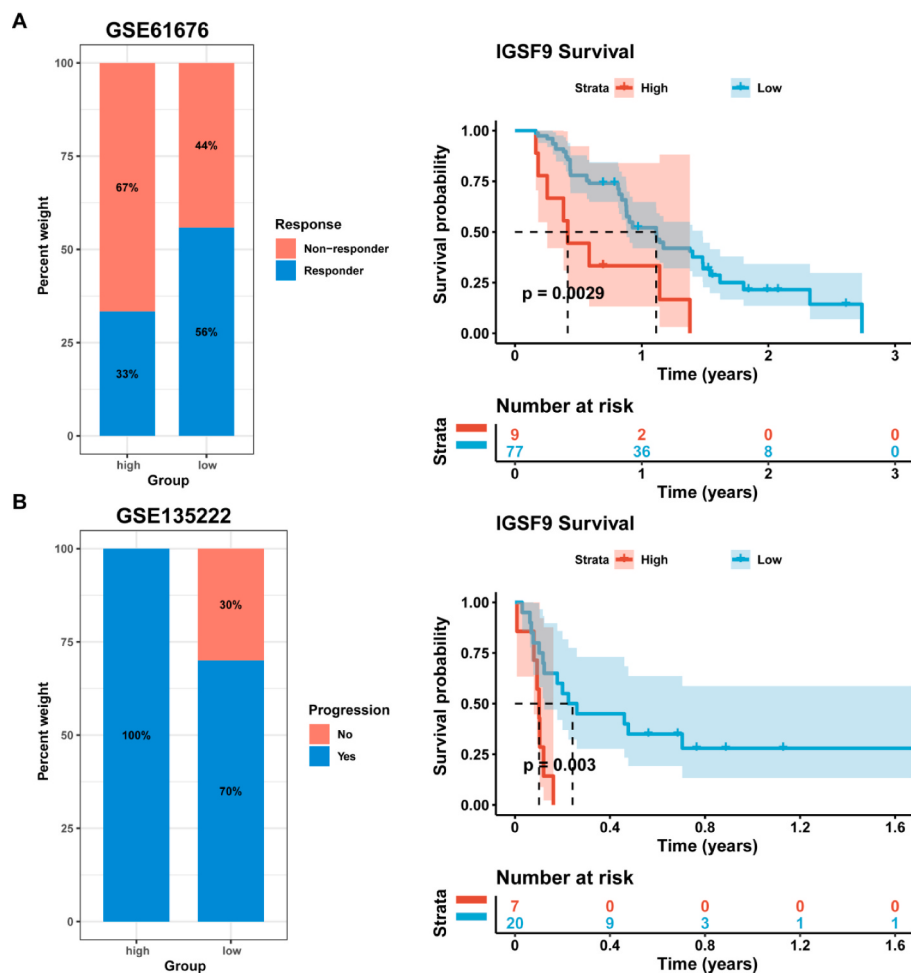


Fig. 7. IGSF9 Expression and Immunotherapy Efficacy in Clinical Datasets. (A) Response to combination therapy (bevacizumab + erlotinib) in GSE61676 linked to IGSF9 expression. (B) Survival outcomes with anti-PD-1/PD-L1 therapy in GSE135222.

increased CD8⁺ T cell recruitment and activation. Collectively, these findings support Doramapimod as a promising adjunctive agent capable of sensitizing *IGSF9*-high CC tumors to immune checkpoint blockade, likely through modulation of the tumor's immunosuppressive microenvironment.

4. Discussion

Our study, through an integrated pan-cancer discovery and CC-focused validation approach, demonstrates that IGSF9 is a promising biomarker particularly relevant to CC biology. While our initial pan-cancer analyses revealed IGSF9 dysregulation across multiple malignancies, we specifically prioritized colon cancer for in-depth characterization based on: (1) the significant upregulation and prognostic value of IGSF9 in COAD, (2) the strong correlation with immunosuppressive TME features, and (3) the clinical need for novel biomarkers in colon cancer immunotherapy. The subsequent single-cell, spatial transcriptomics, and preclinical therapeutic experiments were therefore focused exclusively on CC to provide mechanistic and translational insights specific to this malignancy. These findings align with previous reports showing IGSF9 promotes tumor progression by enhancing EMT and suppressing T-cell proliferation in lung cancer [14]. In COAD, high IGSF9 expression is associated with reduced immune cell infiltration and an immunosuppressive TME, characterized by increased Tregs and CAFs. These results extend prior observations in mouse models where IGSF9 blocked immunotherapy efficacy by inhibiting T-cell function, highlighting its conserved role in creating the immune desert-like

microenvironment across species.

Notably, our single-cell and spatial transcriptomics analyses reveal IGSF9 is predominantly expressed in malignant epithelial cells of CC, rather than immune or stromal cells. This epithelial-specific expression pattern contrasts with some immune checkpoint molecules [30] (e.g., PD-L1, which is often expressed on both tumor and immune cells), suggesting IGSF9 may exert its immunosuppressive effects indirectly, such as secreting factors that recruit immunosuppressive myeloid cells or modulating the epithelial-stromal crosstalk.

Functional enrichment analyses reveal correlations between IGSF9 expression and KRAS and Notch signaling pathways, which are known to be critical for cell cycle regulation and epithelial-mesenchymal transition (EMT) [31]. While these associations are intriguing, we acknowledge that enrichment-based analyses cannot establish direct mechanistic involvement. The correlation between IGSF9 and KRAS pathway activity may reflect co-regulation by shared upstream factors, parallel activation in aggressive tumor phenotypes, or indirect relationships through intermediate molecular mediators. Direct experimental validation—such as IGSF9 knockdown/overexpression studies with assessment of KRAS/ERK phosphorylation, rescue experiments, and co-culture systems—would be required to establish mechanistic causality. This finding is consistent with a recent study showing IGSF9 activates GSK-3 β / β -catenin signaling to promote lung cancer metastasis. Interestingly, Hui et al. found that IFN- γ is a key cytokine that induces IGSF9 expression, which may further explain why IGSF9 is upregulated in the tumor microenvironment [32]. In COAD, the correlation between IGSF9 and KRAS pathways may explain treatment resistance in

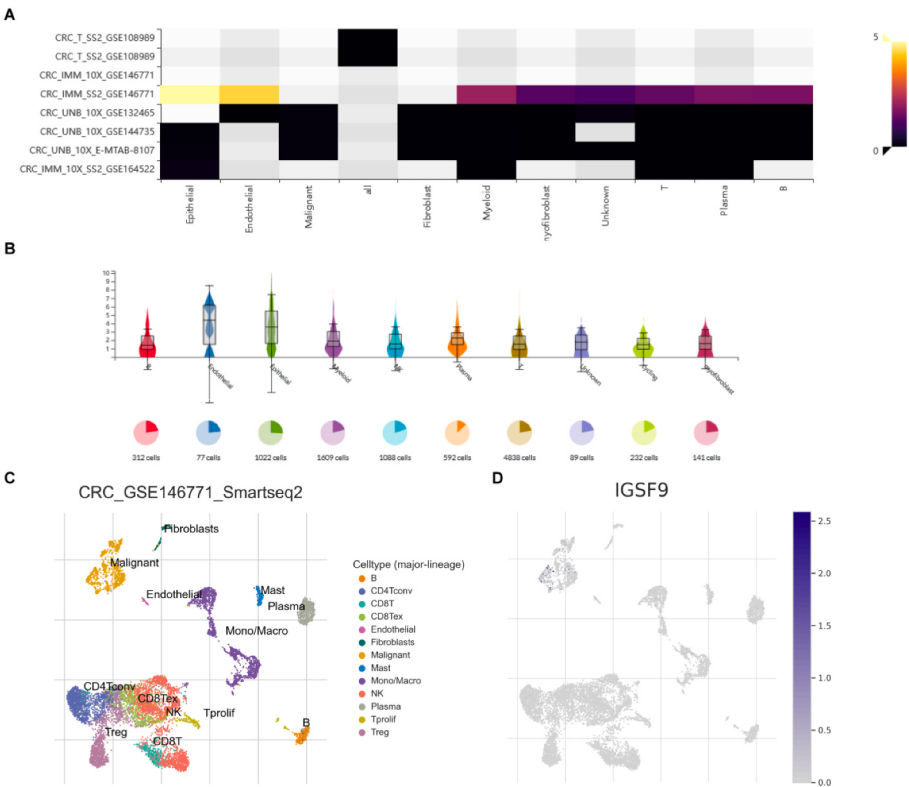


Fig. 8. Single-Cell RNA Sequencing Analysis of IGSF9 in COAD. (A) IGSF9 expression across single-cell datasets of colorectal cancer (CRC) via IMMUCan database. (B–C) Expression in GSE146771 dataset, showing enrichment in malignant epithelial cells using TISCH database.

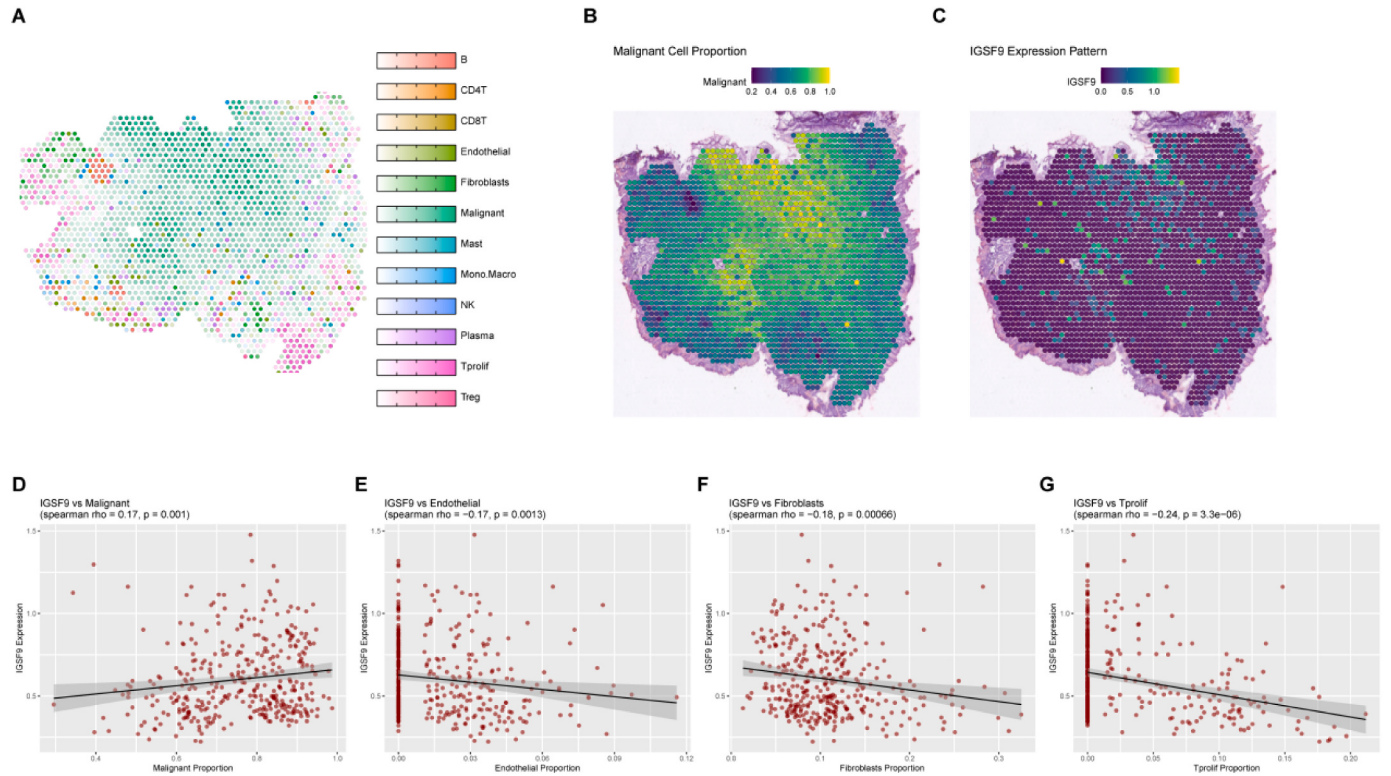


Fig. 9. Spatial Transcriptomics of IGSF9 in COAD. (A) Cellular annotation of COAD tissue via spatial transcriptomics (GSE146771 from CROST database). (B–C) Localization of malignant tumor cells and IGSF9 expression. (D–G) Correlations between IGSF9 and cell types (malignant epithelium, endothelial cells, fibroblasts, proliferative T cells).

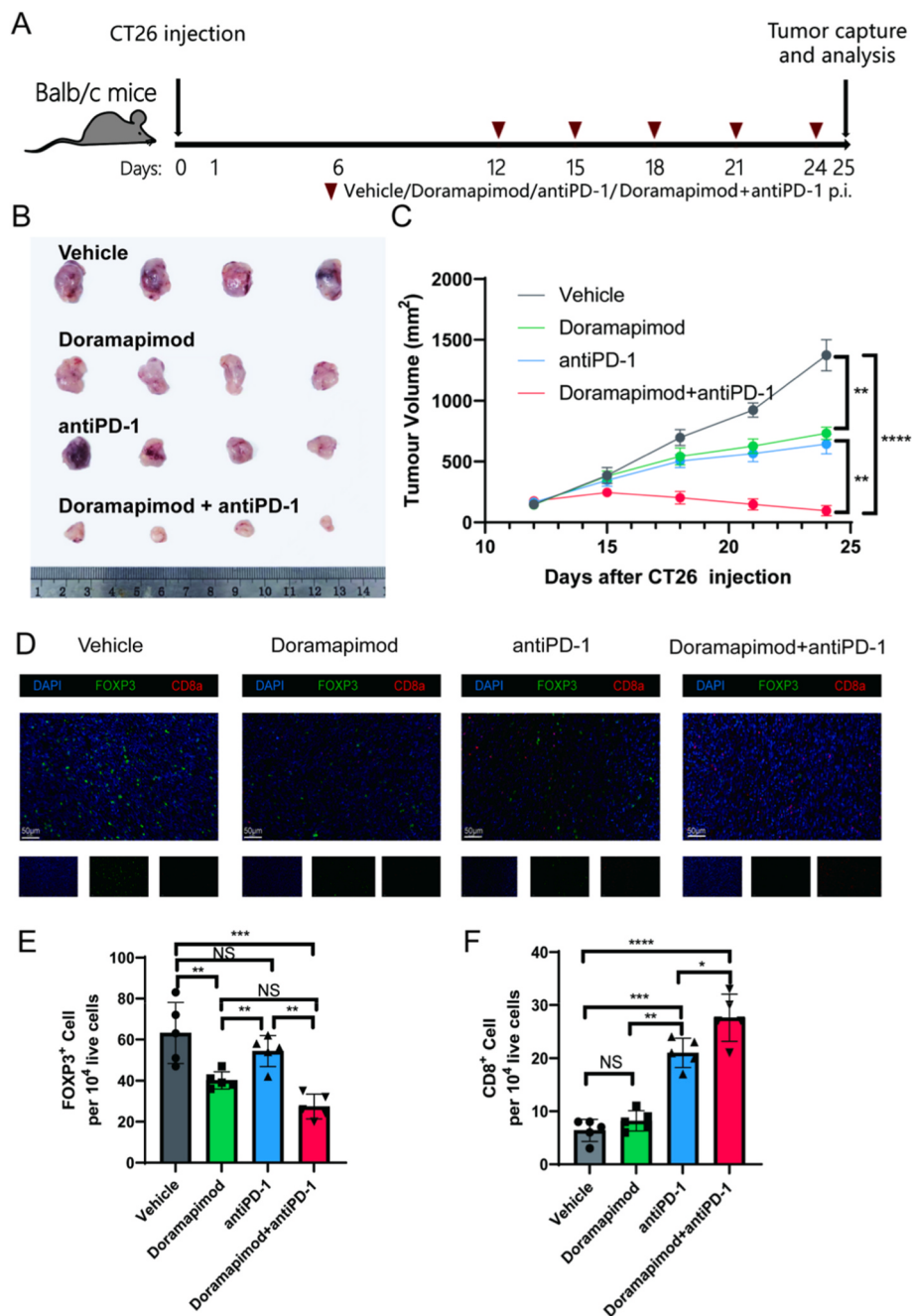


Fig. 10. In vivo experiments. (A) Schematic flowchart depicting the establishment of tumor-bearing mice and treatment protocols. (B, C) Representative physical images and quantitative analysis of tumor size dynamics, with $n = 4$ mice per group. (D) Multiplex immunofluorescence staining images of tumor sections post-treatment, with scale bars indicating 50 μm . (E, F) Statistical quantification of FOXP3⁺ Tregs (E) and CD8⁺ T (F) cell infiltration levels, analyzed using the Wilcoxon signed-rank test for statistical significance.

KRAS-mutated tumors, as KRAS mutations are known to drive immunosuppressive TME remodeling [33]. Our drug sensitivity analysis further identifies Doramapimod [34], a MAPK inhibitor targeting KRAS downstream pathways, as a potential therapeutic agent for IGSF9-high CC. Combined with anti-PD-1 therapy, Doramapimod reduces Treg infiltration and enhances tumor regression in mouse models, providing preclinical validation for targeting IGSF9-associated pathways to overcome immunotherapy resistance.

A key finding of this study is the strong association between high IGSF9 expression and poor response to immunotherapy in CC. Patients with high IGSF9 exhibit lower TMB and MSI scores, which are established predictors of immunotherapy efficacy [35,36]. This is further

validated in two independent immunotherapy datasets, where high IGSF9 expression correlates with increased non-responders and shorter survival after treatment. These results are consistent with mechanistic studies showing IGSF9 suppresses T-cell infiltration and promotes immune evasion, highlighting its role as a predictive biomarker for immunotherapy resistance. Notably, while previous studies have focused on immune checkpoint molecules (e.g., PD-1/PD-L1) in TME [37,38], our work identifies IGSF9 as a novel regulator of immunosuppression of TME that acts through distinct mechanisms. For example, unlike PD-L1, which directly interacts with T-cell surface receptors, IGSF9 may indirectly inhibit immunity by recruiting immunosuppressive cell populations (e.g., neutrophils, Tregs) and activating EMT

programs. This suggests that IGSF9 could serve as a complementary biomarker to PD-L1, particularly in PD-L1-negative tumors where alternative resistance mechanisms are prevalent.

Doramapimod (BIRB 796) is a potent and selective p38 MAPK inhibitor that has been evaluated in multiple clinical trials for inflammatory conditions, including rheumatoid arthritis and Crohn's disease [39, 40]. Pharmacokinetic studies have demonstrated favorable oral bioavailability and a manageable safety profile in humans, with dose-limiting toxicities primarily related to hepatic enzyme elevations at higher doses. However, it is important to note that Doramapimod has not yet been evaluated in oncology clinical trials, and its safety profile in combination with immune checkpoint inhibitors remains to be established. The potential for additive hepatotoxicity when combining Doramapimod with anti-PD-1 agents—which can themselves cause immune-related hepatic adverse events—warrants careful consideration in future clinical trial design. Additionally, the optimal dosing schedule, pharmacokinetic interactions, and long-term safety of this combination require systematic investigation in phase I/II studies before clinical implementation. Our preclinical findings provide a rationale for such clinical development, but we acknowledge that substantial translational work remains necessary.

However, several limitations must be addressed. First, our findings are primarily based on publicly available databases and preclinical models, without validation in prospective clinical trials specific to colon cancer immunotherapy. Notably, the immunotherapy datasets used in this study—GSE61676 and GSE135222—are not colon cancer-specific cohorts. GSE61676 comprises non-small cell lung cancer patients treated with bevacizumab/erlotinib, while GSE135222 includes melanoma patients receiving anti-PD-1/PD-L1 therapy. While these datasets provide preliminary evidence linking IGSF9 expression to immunotherapy outcomes across various cancer types, the absence of large-scale, prospective validation in colon cancer-specific immunotherapy cohorts limits the generalizability of our predictive findings. Future studies using dedicated colon cancer immunotherapy trials with IGSF9 expression profiling are essential to establish its clinical utility as a predictive biomarker. Second, the role of IGSF9 in the TME varies across cancer types, as evidenced by its differential correlation with immune cell infiltration in pan-cancer analyses. Third, the preclinical CT26 murine model used in this study, while providing valuable proof-of-concept evidence, has inherent limitations in replicating the complexity of the human colon cancer microenvironment. Differences in immune system dynamics between mice and humans—including variations in immune cell subsets, checkpoint molecule expression patterns, and cytokine networks—may impact the translatability of our therapeutic findings. The CT26 model represents MSS murine CRC, which may not fully capture the heterogeneity observed in human colon cancer subtypes. Future validation studies utilizing patient-derived xenografts in humanized mouse models or organoid co-culture systems with patient-derived immune cells would provide more clinically relevant evidence to support the therapeutic potential of combining Doramapimod with immune checkpoint inhibitors.

5. Conclusion

In summary, this study establishes IGSF9 as a key regulator of immunosuppressive TME in CC, providing a rationale for its use as a prognostic and predictive biomarker. The combination of MAPK inhibition and immune checkpoint blockade offers a promising strategy to overcome resistance in IGSF9-high tumors, warranting further clinical investigation.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Written informed consent was acquired from every patient, and study was approved by the Clinical Research Ethics Committee of Affiliated Jinhua Hospital, Zhejiang University School of Medicine ((2024) Ethics approval No. (91)).

Declaration of generative AI and AI-assisted technologies in scientific writing

During the preparation of this work, the authors utilized ChatGPT for language editing and polishing. The authors have thoroughly reviewed and edited the output and take full responsibility for the content of this publication.

CRediT authorship contribution statement

Kangfu Dai: Investigation, Supervision, Writing – original draft. **Xingxing Yu:** Project administration, Writing – original draft. **Shicheng Zhou:** Data curation, Formal analysis. **Zhekang Jin:** Methodology, Resources. **Wuzhen Dong:** Project administration, Supervision. **Pengcheng Yu:** Resources. **Chenyang Ge:** Investigation, Supervision. **Zhifeng Zhong:** Methodology, Software. **Jianping Wang:** Resources, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations:

CC - Colon Cancer; TME - Tumor Microenvironment; IGSF9 - Immunoglobulin Superfamily Member 9; TCGA - The Cancer Genome Atlas; GTEx - Genotype-Tissue Expression; CCLE - Cancer Cell Line Encyclopedia; GO - Gene Ontology; KEGG - Kyoto Encyclopedia of Genes and Genomes; GSEA - Gene Set Enrichment Analysis; GSVA - Gene Set Variation Analysis; CNV - Copy Number Variation; OS - Overall Survival; PFI - Progression-Free Interval; DFI - Disease-Free Interval; DSS - Disease-Specific Survival; TMB - Tumor Mutational Burden; MSI - Microsatellite Instability; EMT - Epithelial-Mesenchymal Transition; CAFs - Cancer-Associated Fibroblasts; Tregs - Regulatory T Cells; PD-1 - Programmed Death 1; HNSC - Head and Neck Squamous Cell Carcinoma; LUAD - Lung Adenocarcinoma; LUSC - Lung Squamous Cell Carcinoma; PAAD - Pancreatic Adenocarcinoma; STAD - Stomach Adenocarcinoma; THCA - Thyroid Carcinoma; UCEC - Uterine Corpus Endometrial Carcinoma; ACC - Adrenocortical Carcinoma; KIRC - Kidney Renal Clear Cell Carcinoma; LIHC - Liver Hepatocellular Carcinoma; PRAD - Prostate Adenocarcinoma; SKCM - Skin Cutaneous Melanoma; TGCT - Testicular Germ Cell Tumors; CHOL - Cholangiocarcinoma; KICH - Kidney Chromophobe; MESO - Mesothelioma; PCPG - Pheochromocytoma and Paraganglioma; SARC - Sarcoma; UVM - Uveal Melanoma; DLBC - Diffuse Large B-Cell Lymphoma; LAML - Acute Myeloid Leukemia; LGG - Lower Grade Glioma; GBM - Glioblastoma Multiforme; CESC - Cervical Squamous Cell Carcinoma; OV - Ovarian Serous Cystadenocarcinoma; READ - Rectum Adenocarcinoma; THYM - Thymoma; UCS - Uterine Carcinosarcoma; KRAS - Kirsten Rat Sarcoma Viral Oncogene Homolog; FDX1 - Forkhead Box P3.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrep.2026.102478>.

Data availability

The data that has been used is confidential.

References

- [1] A.B. Benson, et al., NCCN guidelines insights: Colon cancer, version 2.2018, *J. Natl. Compr. Cancer Netw.* 16 (4) (2018) 359–369.
- [2] F. Bray, et al., Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J. Clin.* 74 (3) (2024) 229–263.
- [3] D.I. Tsimigras, et al., Liver metastases, *Nat. Rev. Dis. Primers* 7 (1) (2021) 27.
- [4] D.J. Schaefer, et al., Hemorrhage-activated NRF2 in tumor-associated macrophages drives cancer growth, invasion, and immunotherapy resistance, *J. Clin. Invest.* 134 (3) (2023).
- [5] T. Yue, et al., Hydrogen sulfide creates a favorable immune microenvironment for Colon cancer, *Cancer Res.* 83 (4) (2023) 595–612.
- [6] G.L. Szeto, S.D. Finley, Integrative approaches to cancer immunotherapy, *Trends Cancer* 5 (7) (2019) 400–410.
- [7] A. Kalbasi, A. Ribas, Tumour-intrinsic resistance to immune checkpoint blockade, *Nat. Rev. Immunol.* 20 (1) (2020) 25–39.
- [8] A. Hazini, K. Fisher, L. Seymour, Deregulation of HLA-I in cancer and its central importance for immunotherapy, *J. Immunother. Cancer* 9 (8) (2021).
- [9] J.M. Pitt, et al., Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy, *Ann. Oncol.* 27 (8) (2016) 1482–1492.
- [10] T. Tang, et al., Advantages of targeting the tumor immune microenvironment over blocking immune checkpoint in cancer immunotherapy, *Signal Transduct. Targeted Ther.* 6 (1) (2021) 72.
- [11] O. Elhanani, R. Ben-Uri, L. Keren, Spatial profiling technologies illuminate the tumor microenvironment, *Cancer Cell* 41 (3) (2023) 404–420.
- [12] M.Z. Jin, W.L. Jin, The updated landscape of tumor microenvironment and drug repurposing, *Signal Transduct. Targeted Ther.* 5 (1) (2020) 166.
- [13] C.K. Cheung, et al., The pathogenesis of IgA nephropathy and implications for treatment, *Nat. Rev. Nephrol.* 21 (1) (2025) 9–23.
- [14] H. Luan, et al., IGSF9 promotes tumor invasion and metastasis through GSK-3 β / β -catenin mediated EMT in lung cancer, *Neoplasia* 58 (2024) 101067.
- [15] Y. Liu, et al., Targeting the immunoglobulin IGSF9 enhances antitumor T-cell activity and sensitivity to Anti-PD-1 immunotherapy, *Cancer Res.* 83 (20) (2023) 3385–3399.
- [16] L. Zhang, et al., Single-cell analyses inform mechanisms of myeloid-targeted therapies in Colon cancer, *Cell* 181 (2) (2020) 442–459.e29.
- [17] J. Roelands, et al., An integrated tumor, immune and microbiome atlas of colon cancer, *Nat. Med.* 29 (5) (2023) 1273–1286.
- [18] S. Xu, et al., Using clusterProfiler to characterize multiomics data, *Nat. Protoc.* 19 (11) (2024) 3292–3320.
- [19] K. Yoshihara, et al., Inferring tumour purity and stromal and immune cell admixture from expression data, *Nat. Commun.* 4 (2013) 2612.
- [20] T. Li, et al., TIMER2.0 for analysis of tumor-infiltrating immune cells, *Nucleic Acids Res.* 48 (W1) (2020) W509–w514.
- [21] V. Thorsson, et al., The immune landscape of cancer, *Immunity* 48 (4) (2018) 812–830.e14.
- [22] B. Bakir, et al., EMT, MET, plasticity, and tumor metastasis, *Trends Cell Biol.* 30 (10) (2020) 764–776.
- [23] J. Taieb, et al., Deficient mismatch repair/microsatellite unstable colorectal cancer: diagnosis, prognosis and treatment, *Eur. J. Cancer* 175 (2022) 136–157.
- [24] K. Yang, A. Halima, T.A. Chan, Antigen presentation in cancer - mechanisms and clinical implications for immunotherapy, *Nat. Rev. Clin. Oncol.* 20 (9) (2023) 604–623.
- [25] R.M. Samstein, et al., Tumor mutational load predicts survival after immunotherapy across multiple cancer types, *Nat. Genet.* 51 (2) (2019) 202–206.
- [26] M. Baretti, D.T. Le, DNA mismatch repair in cancer, *Pharmacol. Ther.* 189 (2018) 45–62.
- [27] F. Baty, et al., 24h-gene variation effect of combined bevacizumab/erlotinib in advanced non-squamous non-small cell lung cancer using exon array blood profiling, *J. Transl. Med.* 15 (1) (2017) 66.
- [28] H. Jung, et al., DNA methylation loss promotes immune evasion of tumours with high mutation and copy number load, *Nat. Commun.* 10 (1) (2019) 4278.
- [29] J. Camps, et al., Meta-analysis of human cancer single-cell RNA-seq datasets using the IMMUCAN database, *Cancer Res.* 83 (3) (2023) 363–373.
- [30] H. Jeong, et al., Epithelial-mesenchymal transition induced by tumor cell-intrinsic PD-L1 signaling predicts a poor response to immune checkpoint inhibitors in PD-L1-high lung cancer, *Br. J. Cancer* 131 (1) (2024) 23–36.
- [31] A.P. Deshmukh, et al., Identification of EMT signaling cross-talk and gene regulatory networks by single-cell RNA sequencing, *Proc. Natl. Acad. Sci. U. S. A.* 118 (19) (2021).
- [32] L. Hui, et al., IGSF9-targeted therapy inhibits the progression of acute myeloid leukemia, *Blood Adv* 16 (9) (2025) 4217–4231.
- [33] J. Boumelha, M. Molina-Arcas, J. Downward, Facts and hopes on RAS inhibitors and cancer immunotherapy, *Clin. Cancer Res.* 29 (24) (2023) 5012–5020.
- [34] S. Lin, et al., Single-nucleus RNA sequencing reveals heterogeneous microenvironments and specific drug response between cervical squamous cell carcinoma and adenocarcinoma, *EBioMedicine* 97 (2023) 104846.
- [35] A.M. Leader, et al., Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification, *Cancer Cell* 39 (12) (2021) 1594–1609.e12.
- [36] K. Ganesh, et al., Immunotherapy in colorectal cancer: rationale, challenges and potential, *Nat. Rev. Gastroenterol. Hepatol.* 16 (6) (2019) 361–375.
- [37] M. Wu, et al., Improvement of the anticancer efficacy of PD-1/PD-L1 blockade via combination therapy and PD-L1 regulation, *J. Hematol. Oncol.* 15 (1) (2022) 24.
- [38] H. Zhang, et al., Roles of tumor-associated macrophages in anti-PD-1/PD-L1 immunotherapy for solid cancers, *Mol. Cancer* 22 (1) (2023) 58.
- [39] C. Pargellis, et al., Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site, *Nat. Struct. Biol.* 9 (4) (2002) 268–272.
- [40] S. Schreiber, et al., Oral p38 mitogen-activated protein kinase inhibition with BIRB 796 for active Crohn's disease: a randomized, double-blind, placebo-controlled trial, *Clin. Gastroenterol. Hepatol.* 4 (3) (2006) 325–334.