Tumor-myeloid crosstalk drives therapy resistance in localized bladder cancer

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Abstract

Neoadjuvant cisplatin-based chemotherapy results in pathologic complete response for only a minority of patients with muscle-invasive bladder cancer (MIBC), and mechanisms of resistance and the effects of chemotherapy on the MIBC microenvironment remain incompletely understood. Here, we defined the single-cell and spatial transcriptomes of cancer and immune cells from MIBC patients with extreme responses to cisplatin-based chemotherapy. Tumors with persistent MIBC after chemotherapy harbored cancer cells expressing epithelial-to-mesenchymal programs that were associated with worse overall survival in independent cisplatin-treated bladder cancer cohorts. These cisplatin-resistant tumor cells were infiltrated by macrophages that upregulated tumor permissive programs defined by increased PARP14 expression in spatially-resolved multicellular niches. Macrophage reprogramming through PARP14 inhibition sensitized tumors to cisplatin via downregulation of tumor cell pathways implicated in resistance. Our results demonstrate that cancer cells and macrophages cooperate to promote cisplatin resistance and identify macrophage-directed PARP14 inhibition as a novel therapeutic strategy to sensitize MIBC to cisplatin.

Introduction

Muscle-invasive bladder cancer (MIBC) is a potentially lethal disease state that often involves multimodal treatment with chemotherapy, surgery, and immunotherapy[1,2]. For many years, neoadjuvant cisplatin-based chemotherapy followed by radical cystectomy has been a standard curative-intent treatment for localized MIBC[3-5]. However, only 30% of MIBC patients experience pathologic complete response following neoadjuvant chemotherapy, and patients with residual muscle-invasive tumors at the time of radical cystectomy are more likely to develop metastatic bladder cancer and die from the disease[3]. Recent evidence suggests the combination of cisplatin-based neoadjuvant chemotherapy and immune checkpoint inhibition improves outcomes in MIBC[6], implicating the bladder tumor microenvironment in mechanisms of response and resistance to cisplatin-based treatment. However, chemoimmunotherapy strategies only improve clinical outcomes in a subset of patients with MIBC. Thus, there is an urgent need to dissect the impact of cisplatin on tumor cells and the microenvironment to inform novel therapeutic approaches.

Genomic and transcriptomic characterization of response to neoadjuvant therapies in MIBC has largely focused on bulk-sequenced samples comprised of complex mixtures of tumor and non-tumor cell types[7-11]. Bulk RNA profiling broadly identified several molecular subtypes that reflect tumor heterogeneity, but do not yet guide clinical management[12,13]. Single cell analysis of preclinical bladder cancer models and localized tumors provided insights on the diversity of urothelial and non-urothelial cell types implicated in bladder cancer pathogenesis[14-18]. However, our understanding of how cytotoxic chemotherapy impacts the bladder tumor cells as well as cells in the microenvironment such as macrophages, which are the most abundant type of immune cells and contribute to tumor growth and immune evasion in this disease[19,20], remains limited in MIBC.

We hypothesized that specific interactions between specific urothelial malignant cells and immune cells jointly promote chemotherapy resistance in MIBC. To investigate this hypothesis, we paired complementary single-nucleus RNA sequencing (snRNAseq) and spatial transcriptomic methods in clinically integrated contexts with focused functional

assessments to understand cancer and immune cell multicellular communities implicated in resistance to neoadjuvant cisplatin-based chemotherapy.

Results

Dissecting the cellular landscape of post-cisplatin bladder biopsies.

We performed single-nucleus RNA sequencing (snRNA-seq) of frozen surgical resection specimens from MIBC patients treated with neoadjuvant chemotherapy followed by radical cystectomy (Fig. 1A, Suppl. Table 1). All patients received at least three cycles of cisplatin-based chemotherapy, and no patients had received prior intravesical therapy (e.g. BCG or intravesical chemotherapy), systemic anti-cancer therapy, or radiotherapy. Specimens were defined as “tumor scar” if pathology review indicated pathologic complete response (i.e., ypT0 N0) per established criteria[21], or “persistent tumor” if there was persistent muscle-invasive (≥ypT2) disease and/or clinical progression to metastatic disease (Fig. 1B; Methods).

After quality control, our single-cell atlas included transcriptomes from 59,006 nuclei spanning malignant and non-malignant cell types. To minimize batch effects across patients, tissue collection, and processing, all nuclei were integrated using Harmony[22] and jointly clustered in an unsupervised manner. Clusters were then annotated based on expression of established marker genes[14,23,24](Fig. 1C). We identified 13 cell subsets comprising urothelial cells and cells present in the tumor microenvironment shared among patients, treatment and response groups (Fig. 1D–F, Suppl. Fig. 1A). We confirmed malignant cells in the urothelial compartment of persistent tumors by inferred copy-number alterations (CNAs), which were consistent with genomic profiles from previously described chemotherapy resistant urothelial tumors[11](Suppl. Fig. S1B, S1C). Other identified cell types included non-malignant urothelial cells, immune cells, and diverse stromal cell types (cancer-associated fibroblasts, endothelial cells, pericytes and smooth muscle cells), consistent with the histologic composition of MIBC. snRNA-seq captured expected proportions of epithelial, fibroblast and immune cells across individual tumors and response groups (Fig. 1F). Within the immune cell compartment, we identified lymphoid and myeloid cell fractions in tumor scar and persistent tumors with robust representation for further interrogation.

Tumor-intrinsic programs are implicated in cisplatin resistance and clinical outcome of MIBC

Gene expression signatures derived from bulk RNA profiling of MIBC tumors broadly divide tumors into luminal and basal subtypes, but the implications of these (or other) tumor-intrinsic transcriptional programs in predicting response to neoadjuvant therapies, resistance to cisplatin, and overall survival remain uncertain[13,25-27]. We hypothesized that tumor-intrinsic cellular programs enriched in persistent tumors may identify malignant cell features driving resistance to cisplatin. We first identified 30,413 putative urothelial cells based on cytokeratin (e.g. *KRT5*, *KRT6A*) and *TP63* expression across all profiled samples. We then confirmed the presence of benign urothelial cells in tumor scar samples and urothelial malignant cells in persistent tumors via inferring established CNAs that characterize MIBC[11,12].

We decomposed expression profiles of single nuclei into expression programs present in both the tumor scar of complete responders and in persistent tumors through consensus nonnegative matrix factorization (cNMF[28]) (Fig. 2A, B, Suppl Fig. S2A, Methods). We identified seven programs that reflect cell lineages (Myogenesis, EMT) or cell states (tumor necrosis factor/nuclear factor κB (TNF-NFκB), phosphoinositide 3-kinase (PI3K)/ mammalian target of rapamycin (mTOR), Interferon, drug metabolism, oxidative phosphorylation) (Fig. 2C, Suppl. Table 2). Most programs were detectable in both the tumor scar and persistent tumor cell populations (Fig. S2B). However, the EMT program was significantly enriched in malignant urothelial cells from persistent tumor samples but largely inactive in the tumor scar ($p = 0.039$, two-sided Wilcoxon Rank Sum, Fig. S2C). The cell surface marker *CD44*, previously implicated in bladder tumor invasion and metastasis[29], was the most highly expressed gene in this program. This EMT program was also enriched for genes implicated in cell proliferation and migration (e.g. *VOPP1*)[30,31], as well as genes associated with MIBC basal/squamous differentiation (e.g., *CD44*, *TP63*)[12], a cell phenotype induced by cisplatin in preclinical models of MIBC[32].

Whereas the EMT program was preferentially enriched in the persistent tumor cells, certain transcriptional programs exhibited globally consistent behavior between persistent cells and tumor scar, but harbored distinct biological subbehaviors between groups.

Specifically, the drug metabolism and oxidative phosphorylation (Ox-Phos) programs were expressed in benign urothelial cells in tumor scar as well as in malignant urothelial cells in persistent tumors. The activation of Ox-Phos genes is consistent with cellular activation of stress-related pathways in urothelial cells after exposure to cytotoxic cisplatin-based chemotherapy. However, within the Ox-Phos program, malignant urothelial cells in persistent tumors (Program 5) expressed genes implicated in cell cycle progression and invasion (e.g. *S100A11*)[33], interferon response genes (e.g. *IFITM3*), and HLA class I molecules (e.g. *HLA-A*, *HLA-B*, *B2M*). In contrast, the Ox-Phos program in non-malignant urothelial cells from tumor scar samples (Program 6) was characterized by genes involved in oncogene-induced senescence (*IGFBP7*)[34,35] and cytoskeletal wound healing (e.g. *MYL9*, *TPM2*)[36,37]. Overall, these results suggest cisplatin induces common gene expression programs in both benign and malignant urothelial cells, while also driving distinct tumor-specific programs in non-responders that may reflect interactions with the tumor microenvironment suitable for therapeutic targeting.

To assess the potential predictive and prognostic implications of these malignant-cell-specific programs enriched in persistent tumor compared with tumor scar, we assessed these programs in clinically annotated bulk RNA-seq data from two large, independent clinicogenomic MIBC cohorts: (i) patients with primary locally advanced or metastatic bladder cancer treated with cisplatin-based chemotherapy[38] and (ii) patients with primary MIBC not treated with neoadjuvant therapy from The Cancer Genome Atlas (TCGA)[12]. Bulk tumor samples from the cisplatin treated patients were profiled prior to initiation of systemic chemotherapy. We focused on the three programs (EMT, Ox-Phos usage in persistent tumors, Ox-Phos usage in tumor scar) that were significantly different between the tumor scar and persistent tumors in our discovery cohort ($p < 0.05$, two-sided Wilcoxon Rank Sum, Fig. S2C). We observed an association between pre-treatment EMT score and significantly worse overall survival (OS) in the cisplatin-treated cohort but not in the untreated (TCGA) cohort ($P = 0.004$, log-rank, Fig. 2D). No significant associations were found between Ox-Phos program in persistent tumors or tumor scar and survival in patients in either cohort. We then performed a multivariable Cox regression analysis of OS with age, sex, smoking, tumor stage, and the malignant programs as covariates. The EMT program (HR 2.60; 95% CI 1.39-4.87; $p < 0.01$) remained significantly associated

with shorter OS in the cisplatin treated cohort (Fig. 2E). These results support an association between an EMT gene signature and poor outcomes in MIBC patients treated with cisplatin-based chemotherapy. Altogether, these findings show that tumor-intrinsic urothelial programs from cisplatin-resistant cells exhibit distinct biological states that may have therapeutic implications.

SPP1/PARP14 macrophage polarity defines the microenvironment of cisplatin resistant MIBC

We next examined whether the EMT-enriched persistent tumor cells harbored unique immune cells in the microenvironment. Prior studies have shown that cisplatin is associated with T cell priming, and the combination of cisplatin-based chemotherapy with anti-PD-L1 immune checkpoint blockade has been shown to improve OS in metastatic bladder cancer compared to chemotherapy alone[39]. Thus, we compared the proportions of sub-clustered immune cells in tumor scar and persistent tumor, noting that T cells and tumor-associated macrophages (TAMs) were the immune cell populations most represented in our cohort (Fig. 3A). There was significant increase in memory T cells in the tumor scar compared to persistent tumor ($p = 0.015$, two-sided Wilcoxon Rank Sum), consistent with persistence of anti-tumor immunity following cancer cell death. We also observed a higher proportion of mast cells in tumor scar compared with persistent tumor ($p = 0.033$, two-sided Wilcoxon Rank Sum).

Myeloid cells, specifically TAMs, play an important role in the tumor microenvironment and contribute to treatment resistance in several tumor types[24,40-43]; however, their role in MIBC and cisplatin resistance remains uncertain. TAMs were the most abundant immune cell population across the cohort, though the overall proportion of TAMs in tumor scar compared to persistent tumor was not significantly different (Fig. 3A). Distinct subsets of TAMs are known to exhibit different transcriptional cell states and phenotypes, so we hypothesized that certain TAM subtypes drive selective cisplatin response in MIBC. Five TAM clusters expressed distinct markers implicated in anti-tumor and pro-tumor roles in the microenvironment (Fig. 3B)[44]. The TAM cluster significantly enriched in persistent tumors ($p = 0.022$, two-tailed t-test) exhibited higher expression of the secreted factor *SPP1*, the lipid transport-related gene *APOE,* and *PARP14*, which has

been implicated in macrophage polarization by directing an anti-tumor to pro-tumor phenotype shift [45,46]. The other four macrophage clusters expressed HLA class II genes (e.g. *HLA-DBP1*), pro-inflammatory genes (e.g. *TIMP-3*), and two clusters had classic tumor promoting macrophage polarity markers (e.g. *MRC1* and *PPARG*)[47] (Fig. 3C).

We then examined macrophage transcriptional programs to interrogate their phenotypes in the cisplatin resistance setting. TAMs in persistent tumors demonstrated upregulation of gene sets associated with interferon-γ, which promotes macrophage activation; interferon-α, which has a known role in the regulation of DNA damage response and acquired resistance to cisplatin[48,49]; and an interferon-related DNA damage resistance signature[50] (Fig. 3D). Because SPP1/PARP14-Hi TAMs were the TAM subpopulation most represented in persistent tumors, we hypothesized that this TAM subtype had a phenotype implicated in resistance to cisplatin. Indeed, we found that SPP1/PARP14-Hi TAMs were significantly enriched for interferon-α and DNA damage resistance signature expression compared with other TAM subpopulations (Fig. 3E; Methods). Conversely, the expression of myogenesis and EMT programs enriched in a subset of urothelial malignant cells in persistent tumors were downregulated in SPP1/PARP14-Hi TAMs.

Tissue macrophages adopt distinct phenotypes in response to stimuli that have historically been described as M1 pro-inflammatory and M2 tumor-permissive polarization states[51]. However, there is increasing recognition that macrophages undergo differentiation states along a continuum of phenotypes not fully captured by a strict dichotomization as anti-tumor M1 or pro-tumor M2[52]. HLA-DPB1-Hi macrophages that predominated in the tumor scar strongly expressed genes associated with an anti-tumor function phenotype (*IL1B, HLA-DPB1, CD86*), whereas the SPP1/PARP14-Hi population enriched in persistent tumor expressed a complex mixture of genes representing both pro- and anti-tumor functions, underscoring the phenotypic plasticity of these macrophages in tumors resistant to cisplatin (Fig. 3F). Genes that suggest a tumor promoting phenotype detected in SPP1/PARP14-Hi populations included CTSD, which has been implicated in promoting invasion and metastasis in breast cancer[53].

Overall, our results relate resistance-associated tumor-intrinsic programs, such as EMT, to the surrounding microenvironment characterized by activated SPP1/PARP14-Hi TAMs that together may contribute to cisplatin resistance.

Cancer cell-TAM interactions are spatially organized in MIBC

Given our observation that *CD44* was the most highly expressed gene in a malignant cell program (EMT) associated with worse survival in cisplatin-treated bladder cancer cohorts, as well as our observation of *SPP1* upregulation in TAMs in persistent tumors, we hypothesized that CD44-expressing cancer cells and SPP1-expressing macrophages cooperate to promote cisplatin resistance. We first analyzed our snRNAseq cohort using CellphoneDB[54] to identify candidate cellular crosstalk between urothelial cells and macrophages through known ligand-receptor pairs (Fig. 4A). Non-cytotoxic macrophages (e.g, HLA-DBP1-Hi) expressed *IL-1* and TNF programs that have been implicated in the inflammatory response as well as metalloproteinases that may maintain tissue homeostasis in both the tumor scar and persistent tumors. However, only malignant urothelial cells enriched in the EMT program in persistent tumors were inferred to use *CD44* to engage SPP1 on TAMs, an interaction previously implicated in initiation of bladder cancer metastasis in mouse models[29].

Based on these observations, we hypothesized that urothelial cells and SPP1/PARP14-Hi macrophages define spatially organized multicellular communities in persistent tumors. To further investigate co-localization of macrophages with tumor cells and interrogate cellular crosstalk, we performed *in situ* whole transcriptome sequencing on a subset of persistent tumors that also underwent single cell analysis (PS1, PS3, PS4, PS6; Fig. 4B, Methods). The proportion of urothelial and stromal cells were relatively consistent across patients while immune cell content varied, and the expression of epithelial, stroma and immune cells appropriately mapped the histologic location of these cell types as assessed by genitourinary pathologist review (Fig. 4C, Suppl. Fig. 3A,B and Methods). At the cell subtype level, we found an enrichment of urothelial cells in the epithelial compartment and a predominance of smooth muscle cells in the stromal compartment, consistent with the anatomical location and biology of muscle-invasive bladder cancer (Fig. 4C; Suppl. Fig. S3A; Methods). Moreover, TAMs were the

predominant immune cell population present in the tumor microenvironment (Fig. 4C). Thus, we hypothesized that the location of macrophages is spatially restricted and may occupy a cellular niche with cancer urothelial cells to promote therapy resistance. We therefore applied the snRNA-seq SPP1/PARP14-Hi-specific signatures in spatial gene-expression based clustering to define the location of TAMs in bladder tumors. We confirmed that TAMs were significantly enriched in two spatially defined clusters (IMM_1 and IMM_2, adjusted $p < 0.001$, two-sided Wilcoxon Rank Sum Test with Holm-Bonferroni FDR correction, Fig. 4D). To further examine the spatial co-existence between SPP1/PARP14-Hi TAM and persistent tumor cells, we used SpaGCN[55] to determine the proximity between these two cell types in spatially restricted tumor areas. A spatial domain was composed by the expression of *SPP1* as well as *S100A8*, which drives monocyte differentiation towards pro-tumor TAMs, and *IFI6*, a type-I IFN-stimulated gene expressed in the pro-metastatic tumor microenvironment [48] (Fig. 4B and 4E). We used the same approach to dissect further tumor-TAM crosstalk in macrophage-enriched spatial domains and identified preferential CD44-SPP1 ligand-receptor pairs in those locations (Fig. 4F). These results nominate SPP1/PARP14-Hi TAMs as the most critical immune cell population in cisplatin resistant MIBC. Moreover, they identify multicellular niches of cancer cells and macrophages characterized by the interaction of tumor cells expressing CD44 and TAMs expressing SPP1 in these resistant states.

Macrophage reprogramming through PARP14 inhibition

Macrophages can be therapeutically targeted through: (i) activation blockade, using small molecule inhibitors and antibodies to inhibit their recruitment to the tumor; (ii) depletion of tumor promoting TAMs; (iii) reprogram tumor promoting TAMs to an ant-tumor phenotype[42,44]. Given our finding that SPP1/PARP14-Hi TAMs co-localize with cancer cells in cisplatin-resistant bladder tumors and because PARP14 is implicated in pro-tumor macrophage polarization[45,46], we hypothesized that inhibition of PARP14 could reverse this tumor permissive TAM phenotype and potentiate the anti-tumor effect of cisplatin.

We first tested whether cisplatin exposure impacts PARP14 expression in macrophages. We treated murine RAW264.7 macrophages with increasing doses of

cisplatin and observed a dose-dependent increase in PARP14 expression (Fig. 5A). RBN012759 is a selective inhibitor of PARP14 (PARP14i) that has been shown to reverse pro-tumor macrophage gene expression programs and restore sensitivity to immune checkpoint blockade [46,56]. Treatment of RAW264.7 macrophages with RBN2759 caused a significant increase in PARP14 expression, as has been previously described [46,57], and this effect was further increased by co-treatment with IFNγ, which has also been demonstrated to increase PARP14 expression (Fig. 5B, Suppl Fig. 4A). Together these data demonstrate that cisplatin directly upregulates macrophage PARP14 expression and that PARP14 inhibition with RBN012759 engages PARP14 in macrophages.

We next investigated the effects of cisplatin and PARP14i treatment on tumor growth and microenvironmental changes in a syngeneic immune-competent mouse model of bladder cancer (Fig. 5C). BBN963[58] tumor-bearing mice were randomized to treatment with vehicle, cisplatin, PARP14i, or cisplatin plus PARP14i. Treatments were well tolerated, with no significant changes in mouse body weights across treatment arms (Suppl Fig. 4B, C). Cisplatin monotherapy resulted in a significant decrease in tumor size compared with vehicle control, whereas PARP14i monotherapy did not. Mice treated with cisplatin plus PARP14i demonstrated a significant reduction in tumor volume compared to cisplatin monotherapy (Fig. 5D, E). At the end of the experiment, mice were sacrificed, and bulk RNA sequencing of tumors was performed (Methods). Transcriptional profiling clustered vehicle- and PARP14i-treated together in one group whereas cisplatin and cisplatin plus PARP14i treated tumors clustered together in a separate group (Fig. 5F). We next assessed the expression of genes known to be specifically modulated by RBN012759. We observed a significant increase in known PARP14-regulated genes including antitumor inflammation chemokines and components of innate immunity, such as Cxcl11 and Tlr7, in the PARP14i-treated tumors[46,56] (Fig. 5G, Suppl. Fig. 5). These results revealed that RBN012759 elicited anti-tumor immune gene expression changes consistent with PARP14 inhibition. Cisplatin monotherapy led to increased expression of multiple inflammatory gene sets compared to vehicle (TNFα, type I IFN and IFN-γ) (Fig. 5H), similar to those observed in TAMs and urothelial cancer cells from persistent tumors from MIBC patients (Fig. 2C, 3D-E). The addition of PARP14i to cisplatin resulted in a significant downregulation of EMT gene programs (Fig. 5I) that were increased in the

persistent tumors from MIBC patients (Fig. 2C-E). Moreover, complement and myogenesis pathways were also downregulated in cisplatin plus PARP14i-treated tumors, consistent with suppression of PARP14 leading to anti-tumor effects in both cancer and immune cells[45,46,56] (Suppl Table 3). Collectively these molecular changes suggest that PARP14 inhibition promotes an inflammatory anti-tumor microenvironment that sensitizes bladder tumors to cisplatin.

Discussion

To define cancer and immune cell interactions responsible for cisplatin resistance, we comprehensively analyzed single nuclei and spatial transcriptomes from bladder tumors with and without persistent MIBC after chemotherapy. We uncovered populations of chemotherapy-refractory cancer cells defined by the expression of multiple transcriptional programs. Tumor cell intrinsic EMT gene signatures derived from our single cell dataset were associated with worse overall survival when applied to bulk RNA sequencing data from two independent cohorts of cisplatin treated patients. These results suggest that a transition between epithelial and mesenchymal cell states contributes to therapy resistance and that EMT signatures may identify patients most likely to benefit from cisplatin-based chemotherapy. Within the myeloid cell compartment, a subpopulation of TAMs expressing *SPP1* and *PARP14* was enriched in persistent tumors. These TAMs expressed DNA damage resistance-related genes (ex *CTSL* [59]) as well as type I interferon and cysteine cathepsins that have been previously associated with resistance to cytotoxic chemotherapy [50,60,61]. Furthermore, we observed that SPP1/PARP14-Hi TAMs co-existed in niches with cancer cells expressing EMT programs in persistent tumor samples. Analysis revealed significant cell-cell interactions within multicellular niches, where TAMs express ligands for interferon-γ induced cytokines (IL-18) implicated in macrophage activation as well as chemokines (CXCL16), integrins (ICAM, TIMP2), complement (C1QB) and secreted factors (SPP1) that foster cancer cell survival and resistance to therapy [42]. Macrophages treated with cisplatin exhibited a dose-dependent increase in PARP14 expression, and the addition of PARP14 inhibitor to cisplatin decreased tumor growth and downregulated signaling pathways (e.g. EMT and

complement) in mouse bladder cancer models. Overall, these results support a model of macrophage reprogramming and establish PARP14 as a rationale target to overcome cisplatin resistance in MIBC.

There were several limitations to this study. Bladder cancer is a complex mixture of cancer cells, immune and stroma, and multi-regional sampling would be needed to fully capture tumor heterogeneity. Nonetheless, the cancer-immune cell interactions identified in persistent tumor samples were consistent across patients and anatomic tumor locations. Profiling matched pre- and post-cisplatin treated tumors can also inform the progressive tumor and immune cell phenotypes over cytotoxic chemotherapy. Nevertheless, we validated our findings in pre-treatment samples from bulk-sequencing cohorts, including one longitudinal prospective clinical trial, that confirmed the relevance of single-cell results. Additionally, pure urothelial carcinoma is the most common histologic type of urothelial cancer, so we purposely excluded variant histology tumors given the sparsity of data in cisplatin treated MIBC. Future work could focus on cohorts with variant histology urothelial carcinoma to decipher convergent mechanisms of resistance to therapy. Lastly, our work focused on tumor-immune cell interactions, but the stromal compartment may also play an important role in resistance to therapy in MIBC that should be investigated in future studies.

Our results provide a roadmap for understanding cancer-immune cell interactions and modulation of macrophage phenotypes to sensitize MIBC to cytotoxic chemotherapy. Cisplatin based chemotherapy is a backbone of systemic therapies in MIBC, and the combination of cisplatin and anti-PD-L1 immunotherapy has recently been shown to improve outcomes in both localized MIBC and metastatic urothelial cancer[6]. This work identifies a distinct TAM population that contributes to cisplatin resistance and can be reprogrammed through PARP14 inhibition. This approach could potentially be integrated with anti-PD-1 therapy to further drive anti-tumor responses and improve clinical outcomes.

Legends

Figure 1. Defining the tumor microenvironment of cisplatin-treated MIBC.

(A) Study overview and experimental workflow. Figure created with BioRender.com.

(B) Summary of clinicopathological characteristics, treatment history and molecular single-nucleus and spatial transcriptomic profiling (Visium, 10x Genomics) of tumor samples. Treatment groups: PT, persistent tumor; TS, tumor scar. Treatment history: CG, cisplatin/gemcitabine; ddMVAC, Dose-Dense Methotrexate, Vinblastine, Doxorubicin, and Cisplatin; Nivo, Nivolumab. snRNA-seq, single-nucleus RNA-sequencing.

(C) Heatmap of mean expression selected marker genes (columns) across urothelial and non-urothelial cells (rows) in all samples. NK, natural killer cell; CAF, cancer-associated fibroblast; SMC, smooth muscle cell.

(D) UMAP of epithelial, immune, and stromal cell subpopulations captured across all samples colored by broad cell type. Mono, monocytes; Macro, macrophages; NK, natural killer cell; Plasma, plasma cell; SMC, smooth muscle cell; Mast, mast cell; CAF, cancer-associated fibroblast.

(E) Uniform Manifold Approximation and Projection (UMAP) of malignant and non-malignant cells captured across all samples colored by patient and response to cisplatin.

(F) Bar chart with the proportion of cell types across samples. Cell subsets across persistent tumors and tumor scar either with (left) or without (right) urothelial cells.

Figure 2. Malignant cell programs shared across persistent tumors are prognostic in cisplatin treated bladder cancer.

(A) UMAP of urothelial cells captured across all samples colored by patient and response to cisplatin.

(B) UMAP colored with per-cell relative usage of each of the seven expression programs identified by cNMF in urothelial cells.

(C) Violin plots and heatmaps with distribution of cNFM usage program and expression level of the 15 top program-associated genes in each sample.

(D) Kaplan-Meier analysis of overall survival (OS) in two independent cohorts: cisplatin-treated cohort (left) and TCGA treatment naïve MIBC patients (right). Patients were stratified by high and low EMT score in bulk RNA-seq. $P_{log\text{-}rank}$, log-rank test p value.

(E) Multivariable Cox regression analysis of bulk RNA-seq data from cisplatin cohort incorporating gender, smoking status, age at diagnosis, tumor stage and EMT program expression as covariates. Data is presented as hazard ratio (HR) ± 95% confidence interval (CI).

Figure 3. Tumor-associated macrophages acquire DNA damage resistance phenotypes in cisplatin resistant MIBC.

(A) Boxplots with the proportion of immune cell populations across persistent tumors and tumor scar. Boxplots include dots with proportion of nuclei in individual samples; centerline, median; box limits, upper and lower quartiles; whiskers extending to the interquartile range past upper and lower quartiles. *Two-sided Wilcoxon Rank Sum $p < 0.05$.

(B) UMAP of myeloid cells captured across all samples, labeled and colored by cell type. Bar plots show cell type proportions grouped by response to treatment.

(C) Heatmap of scaled normalized expression for cell type defining genes determined by two-sided Wilcoxon rank-sum test with Bonferroni FDR correction ($q < 0.05$).

(D) GSEA of Hallmark interferon-γ, interferon-α and a validated interferon-related gene signature for DNA damage resistance[50] in macrophages from persistent tumor and tumor scar.

(E) Bar chart with normalized enrichment scores (NES) of Hallmark and a literature curated gene set[50] significantly enriched and depleted in SPP1/PARP14 TAMs. Two-sided Wilcoxon rank-sum test with Bonferroni FDR correction, $q < 0.05$.

(F) Heatmap of scaled normalized expression of curated genes within macrophage subpopulations.

Figure 4. Malignant urothelial cells and SPP1 TAMs define spatially restricted cellular neighborhoods in cisplatin resistant tumors.

(A) Heatmap with inferred cell-type specific interactions inferred by CellPhoneDB. Shown are receptor-ligand interactions between urothelial cells and macrophages across samples. Squares color indicates mean expression of receptor and ligand genes for each pair.

(B) Representative hematoxylin and eosin (H&E) and coarse spatial mapping of cell types per spot in the same bladder tumor using established lineage markers (as in Fig. 1C).

(C) Heatmap with cell type abundance across samples. Granular cell types were discerned in each spot using patient-matched single-nucleus references and cell-type specific gene markers. TAMs, Tumor-associated macrophages; CAF, cancer-associated fibroblasts; SMC, smooth muscle cells.

(D) Violin plot with TAMs single-nucleus gene signatures across spot-defined cell populations (**adjusted $p < 0.001$, two-sided Wilcoxon Rank Sum Test with Holm-Bonferroni FDR correction).

(E) Spatial domains with normalized expression of SpaGCN inferred signature defined SPP1-high TAM region (pink spots).

(F) Cell-cell interaction maps of CD44-SPP1 in cancer cells and TAMs identified using LIANA+. Single spots with higher co-expressed CD44-SPP1 interaction (yellow) in spatial contexts in 3 representative sections of persistent tumors.

Figure 5. PARP14 Inhibition combined with cisplatin reduces tumor growth in preclinical models of bladder cancer through downregulation of EMT programs.

(A) Bar chart with PARP14 expression in RAW264.7 mouse macrophages with increasing concentrations of cisplatin measured by flow cytometry (one-way ANOVA with Benjamini-Hochberg (FDR) correction (*$q < 0.05$; ****$q < 0.0001$)).

(B) Histogram (left) and bar chart (right) of PARP14 expression in RAW264.7 macrophages treated with DMSO, PARP14i, IFN-γ or PARP14i + IFN-γ (unpaired two-tailed t-test (*$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$)).

(C) Experimental design, treatment groups and measures of mouse xenografts.

(D) Tumor volume data for mice treated with vehicle, PARP14i, cisplatin or cisplatin + PARP14i.

(E) End-of experiment (day 34) tumor volumes for mice treated with vehicle, PARP14i, cisplatin, or cisplatin + PARP14i (unpaired two-tailed t test, **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

(F) Heatmap with correlation of bulk RNA-seq profiles of mouse tumors across treatment groups (5 mice per group).

(G) Box plot with normalized counts for the PARP14 target genes Cxcl11 and Tlk7 in mouse tumors across treatment groups (Krushkal-Wallis test, $p < 0.05$).

(H) Bar chart with normalized enrichment scores (NES) of MSigDB[62] gene sets significantly enriched and depleted in tumors from mice treated with cisplatin. Two-sided Wilcoxon rank-sum test with Bonferroni FDR correction, $q < 0.05$.

(I) GSEA of Hallmark EMT gene set in tumors treated with cisplatin or cisplatin + PARP14i.

Supplementary Fig. S1. Malignant and non-malignant cell nuclei captured across samples.

(A) UMAP of all nuclei captured across samples colored by cisplatin-based regimen, tumor stage and smoking status.

(B) Example of inferCNV analysis of urothelial, immune and endothelial cells in a tumor scar sample.

(C) InferCNV analysis of persistent tumor sample with inferred amplifications (red) and deletions (blue) identified in Cisplatin resistant bladder cancer[11].

Supplementary Fig. S2. Stability in selection of urothelial programs in consensus NMF and distribution across samples.

(A) Estimated stability (blue) and error (red) in cNMF solution learned with different number of programs for urothelial nuclei.

(B) Bar chart with the proportion of cNMF program in each sample.

(C) Violin plots with each cNMF program expression in persistent tumors and tumor scar (two-sided Wilcoxon rank sum test, $p < 0.05$).

Supplementary Fig. S3. Histopathological annotations and deconvolution across samples.

(A) Major cell types annotated by board certified pathology based on H&E morphology.
(B) Bar chart with the proportion of major cell type proportions per spot.
(C) Spatial mapping of the predicted cell subtypes after deconvolution across different anatomical regions.

Supplementary Fig. S4. PARP14 inhibition in macrophages and preclinical bladder cancer models.

(A) Baseline PARP14 expression in RAW264.7 mouse macrophages
(B) Representative H&E images of mouse tumors at the end of treatment (day 34).

Supplementary Table 1. Clinicopathologic characteristics of MIBC cohort.

Supplementary Table 2. Gene sets from MSigDB[63] enriched in each program (two-sided Wilcoxon rank-sum test with Bonferroni FDR correction $q < 0.05$).

Supplementary Table 3. GSEA of hallmark pathways in BBN tumors treated with vehicle, PARP14i, cisplatin, cisplatin + PARP14i.

Methods

Ethics and human patient specimens

All patient samples were collected under the Dana-Farber Cancer Institute IRB protocol #02-021. All patients in this study were consented without compensation at the time of their clinic appointments by a member of the surgery staff and asked if they were interested in participating in an excess tissue biobank protocol involving the collection of biological samples and clinical data. For inclusion in this protocol, patients had been diagnosed with localized bladder cancer and went surgical resection. The study was explained in detail to all patients, all questions were answered and informed consent signed to participate. Patients were specifically consented to (i) collect tissue, blood and urine; (ii) link clinical information to collected tissues; (iii) permission for the study staff to contact the patient in the future for potential studies they might be appropriate to

participate; (iv) use samples for the creation of cell lines and testing with available or under development sequencing techniques; (v) samples to be stored indefinitely until used. A copy of the consent was provided to the patient and the consent status recorded and kept updated in the database. Resected primary bladder tumors was examined by a board-certified pathologist to confirm neoplastic content and then excess tissue frozen in OCT and stored at –80°C for single nucleus RNA-sequencing. Matched FFPE and OCT blocks were used for Visium (10x Genomics) in 4 cases.

Single nucleus isolation from frozen samples

The following Tween-20- Salt-Tris (TST) nuclei isolation protocol was adapted from a previously described technique[64] and optimized by us for bladder tumors. All reagents were prepared and maintained on ice. A 2x stock ST was prepared to a final concentration of 292 mM NaCl (Thermo Fisher, cat. No. AM9759), 42 mM $MgCl_2$ (Sigma-Aldrich, cat. No. M1028), 20 mM Tris (Sigma-Aldrich, cat. No. P7949), and $CaCl_2$ (Sigma-Aldrich, cat. No. 10043-52-4). TST buffer was prepared containing 1zST buffer, 0.3% Tween-20 (Sigma-Aldrich, cat. No. P7949), 0.5% BSA (Miltenyi Biotec, cat. No. 130-091-376), and 1U/ml RNase inhibitor (Sigma-Aldrich, cat. No. 3335402001). 1X Lysis buffer was prepared on ice containing 1 % BSA (Miltenyi Biotec, cat. No. 130-091-376), 10 mM Tris HCL (pH 7.4, Sigma-Aldrich, cat. No. T2194), 0.01% Tween -20 (Sigma-Aldrich, cat. No. P7949), 0.01% Nonidet P40 Substitute (Sigma-Aldrich, cat. No. 74385), 3 mM $MgCl_2$ (Sigma-Aldrich, cat. No. M1028), 10 mM NaCl (Thermo Fisher, cat. No. AM9759), 0.001% digitonin (Thermo Fisher, cat. No. BN2006), 1 mM DTT (Sigma-Aldrich, cat. No. 646563), 1 U/μl. RNase inhibitor (Sigma-Aldrich, cat. No. 3335402001). Lysis Dilution buffer contained 1% BSA, 10 mM Tris-HC (pH7.4), 3mM MgCl2, 10 mMNaCl, 1mM DTT and RNase inhibitor. Wash buffer was prepared to a final concentration of 1% BSA, 10 mM Tris-HCL, 0.1% Tween-20, 3 mM $MgCl_2$, 10 mM NaCl, 1 mM DTT, 1 U/μl RNase inhibitor.

A 25-50 mg frozen tumor sample was transferred to a 1.5 mL tube (Eppendorf, cat no.022431021) on dry ice before processing. Tissue was chopped in TST buffer using Noyes Spring scissors (Fisher Scientitic cat No. N9019530) for 10 minutes on ice. The homogenized solution was filtered through a 30 μm MACS SmartStrainer (Miltenyi Biotec, cat. No. 130-098-458) into a 15-mL tube. An additional 1 mL of TST was used to rinse the

eppendorf and 3 mL of ST buffer to rinse the filter. The 5 ml homogenized volume was spun for 5 min at 500 g at 4°C. The supernatant was discarded, and the pellet was resuspended in 0.1X lysis buffer for 2 minutes on ice and then immediately rinsed with 1 ml wash buffer and spun for 5 minutes at 500 g at 4^0C. The supernant was discarded and the nuclei pellet was resuspended in buffer, containing 1x nuclei buffer (20X nuclei buffer 10x Genomics, PN200207), 1 mM DTT, and 1U/μl RNase inhibitor. Final nuclei resuspension concentration was aimed 3,230-8,060 nuclei/μl with the target recovery maximum aimed at 10,000 nuclei. Cell count was performed using AO/PI staining and quantified using LUNA-FL™ Dual Fluorescence Cell Counter.

Single nucleus RNA-sequencing

Transposition, GEM Generation and GEX libraires were prepared according the 10x Genomics Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Guide CG000338. Approximately 8,000-10,000 nuclei per sample were loaded into each channel of a Chromium single-cell J chip (10x Genomics) and partitioned into droplets using 10x Chromium Controller to form emulsions, after which nuclear lysis, barcoded reverse transcription of mRNA, generation and amplification of complementary DNA, and 5' adapter and sample index attachment were performed according to standard manufacturer's protocol. All single-cell libraries were sequenced using an Illumina NextSeq 2000 sequencer.

snRNA-seq data processing

CellRanger ARCv2.0 was used to demultiplex FASTQ reads, align them to the human reference genome (GRCh38) and extract the unique molecular identifiers (UMI) and nuclei barcodes. The output of this pipeline is a gene-barcode data matrix for each sample with the number of UMIs aligned to each gene. Cellbender [65] was used to remove ambient RNA and other technical artifacts from the count matrices. The parameters "expected-cells" and "total-droplets-included" were chosen for each dataset based on the total UMI per cell versus cell barcode curve in accordance with CellBender documentation. The false positive rate parameter was set to 0.01 by default. The false positive rate parameter "fpr" was set to 0.01.

Scrublet package (Python, version 0.2.3)[66] was used for detection and removal of multiplet artifacts due to droplet encapsulation of more than one cell. An expected doublet rate was 0.05 and manually selected doublet score thresholds used to distinguish singlets from doublets in the bimodal score distribution graph. A singlet-only and ambient RNA-free count matrix was created for downstream analysis. Further quality control, dimensionality reduction, unsupervised clustering, and differential gene expression analysis were performed using Scanpy toolkit (version 1.9.2).

Dimensionality reduction, clustering and cell-type annotation

Cells with fewer than 200 genes or greater than 5% counts representing mitochondrial genes were removed from each sample. Genes detected in fewer than three cells across all samples were also excluded. Raw counts data from responders and non-responder specimens were then normalized, scaled using default log-normalization parameters from Scanpy and aggregated into a single dataset. Linear dimensional reduction was performed on the integrated and normalized count matrix by performing principal components analysis (PCA) on the 5000 most highly variable genes in our dataset. The first 50 principal components were used for Leiden clustering of cells with a resolution parameter of 0.5. Uniform manifold approximation and projection (UMAP) was then performed using the same principal components with 100 nearest neighbors to visualize preliminary cell clusters from the nonintegrated dataset in two dimensions. To classify cells into broad cell types, differential expression analysis was performed comparing cells in each Leiden cluster to all other clusters using a two-sided Wilcoxon-rank sum test with Bonferroni FDR correction ($q<0.01$ considered significant). All samples were merged and integrated into a single data set using Harmony [22] to reduce technical variation and preserve biological diversity. The resulting integrated dataset included 59,006 nuclei and 12,169 detected genes across the 14 samples of the cohort.

Identification of malignant cells

Cancer cells were identified using transcriptome inferred CNV profiles and cluster-level marker genes from the same tumor. Each sample underwent Leiden clustering and view map projection to identify *PTPRC*$^{+}$ (CD45^{+}) and *PTPRC*$^{-}$ clusters. We then performed

inferCNV[67] within each sample using *PTPRC*$^{+}$ as a reference group and *PTPRC*$^{-}$ as observation groups. Malignant clusters were identified based on established copy number features of MIBC[11,12] and combined to identify "tumor" cells within our cohort.

Differential expression and gene set enrichment analysis.

Differential expression analysis comparing cells from response groups (persistent tumor vs tumor scar) was performed using a two-sided Wilcoxon rank -sum test with Bonferroni FDR correction. We used fgsea v1.32.2 [62] for gene set enrichment analysis (GSEA). The $\log_2$(fold change) values for each gene were as ranks for pre-ranked for GSEA that was performed using as input all Hallmark gene sets from version 6.2 of the MSigDB [63] or select gene sets curated from literature[50]. Gene sets with $p < 0.05$ and FDR < 0.25 were considered significant. Single-cell signature scoring was performed using VISION R package (version 3.0.0)[68]. Previously described macrophage, monocytes and DC markers described in the pan-cancer tumor infiltrating myeloid cell atlas[23] were used to score bladder myeloid compartment (Fig. 3).

Consensus non-negative matrix factorization

We utilized consensus non-negative matrix factorization (cNMF) to infer gene expression from programs from single-cell RNAseq data from bladder tumors [28]. Gene expression matrices from both responders and non-responder samples were used as input. We performed 20 iteration per cell-type category and computed a set of consensus programs by aggregating results from all 20 runs and computed a stability and reconstruction error. Each program was annotated utilizing known described markers in the literature.

Receptor-ligand interaction inference

CellPhoneDB [54] was used to infer receptor-ligand interactions. The algorithm was run on log-normalized expression values for malignant cells and tumor-associated macrophages.

Tissue processing for spatial transcriptomics

RNA was extracted from the sample blocks to assess its integrity by running a DV200 assay for FFPE samples or RIN (RNA integrity number) measurement for fresh-frozen

samples. For 10x Visium spatial transcriptomic assays, 5 µm FFPE or 10 µm fresh frozen sections were loaded on a Fisherbrand Superfrost Plus slide based on 10x Genomics Protocol CG000518. Subsequently, the slides were deparaffinized and stained for H&E. Tissue sections were scanned by a Nikon Eclipse Ti2 microscope for histological record then transferred to decrosslinking. Visium Human Transcriptome Probe Set was used and hybridized on a BioRad C1000 thermal cycler. RNA was then digested, probe ligated, released and extended. qPCR was used to quantify the eluted probes before running the sample index PCR and its cleanup. Libraries QC was performed on a Qubit 4 and Agilent Bioanalyzer 5200 and sequenced on the Illumina NovaSeq 6000 system or a Singular Genomics G4 with paired end reads according to the manufacturers guidelines.

Spatial transcriptomics data processing

All persistent tumor speciments profiled for spatial transcriptomics (10x Visium) had matching snRNA-seq and spatial data was deconvoluted based on single nucleus cell-type signatures. We mapped the expression of each cell type to individual spots to label cell-types within the tissue sections. High resolution tiff images of the samples were annotated by a board-certified pathologist using the Samui browser. We used Cell2Location[69] for single-cell reference-based spot deconvolution. We quantify a total of 11169 Visum spots each of which contain a median of 2718 unique genes after QC and decontamination with SpotClean. Subsequently, the Samui.json polygons with the pathologist the annotations (Suppl Fig. 4A) were used to subset spots using sf (1.0.14) and intersect that with the proportions, visualizing them with pheatmap (1.0.12). Visualizations for both single-cell and Visium data were created using ShinyCell (2.1.0) with the 10x Genomics VISIUM. Spatial domains to identify the co-existence and determine the proximity between SPP1/PARP14-Hi TAM and cancer urothelial cells was defined by SpaGCN[55]. Unsupervised iterative clustering (100 iterations per sample) that integrated spatially variable genes (SVGs), spatial location and histologic features defined a spatial domain overlaying the tissue area enriched in TAMs (Suppl. Fig 4).

Survival analysis of bulk RNA-seq data

Bulk RNA-seq data from two MIBC cohorts (GREEK, n=62; HM, n=55) treated with platinum chemotherapy (cisplatin and carboplatin) with OS annotated was obtained[10,38]. Patients who received carboplatin were excluded from this analysis, yielding a total of 66 cisplatin treated patients. Clinical and bulk RNA-seq data from TCGA was publicly available and used as a comparison group. To score malignant cNMF programs in each tumor, we summed the expression of the top 200 genes for each program and z-scored normalized the expression scores within GREEK/HM cohorts. Age, sex, and tumor stage were available for all patients. We used cNMF gene expression programs (usage 1, usage 2 etc) as aggregated programs to avoid model overfitting. Multivariable cox regression analysis was performed for OS in R 4.4.1.

Macrophage cell culture and PARP14 assay

RAW264.7 cells were purchased from ATCC and cultured in DMEM medium (Gibco; 11965092) containing 10% heat-inactivated fetal bovine serum (Corning) and Penicillin/Streptomycin (Gibco; 15140122). For PARP14i (RBN012759; MedChemExpress; HY-136979) *in vitro* experiments, RAW264.7 cells were treated with 0.1μM PARP14i for 24 hours *in vitro*. Where indicated, RAW264.7 cells were treated with 20ng/mL recombinant mouse IFNγ (Biolegend; 575302) for the final 18 hours of PARP14i treatment *in vitro*. For Cisplatin *in vitro* experiments, RAW264.7 cells were treated with cisplatin for 72 hours *in vitro* using the indicated concentrations. RAW264.7 cells were assessed for PARP14 protein levels using flow cytometry. RAW264.7 cells were harvested from culture plates using TrypLE Express (Gibco; 12605036) and blocked for non-specific antibody binding using True-Stain Monocyte Blocker (Biolegend; 426101) and TruStain FcX Block (Biolegend; 101319) in FACS buffer (PBS + 1% FBS) for 10 minutes at room temperature. Cells were fixed and permeabilized using the Cytofix/Cytoperm Kit (BD; 554714) according to manufacturer's instructions and stained for PARP14 (Santa Cruz; sc-377150) for 1 hour at 4C. Cells were acquired on an Aurora Spectral Flow Cytometer (Cytek) and analyzed using OMIQ (Dotmatics).

Mouse experiments, treatments and tumor harvest.

All animal experiments were performed in accordance with Dana-Farber Cancer Institute (DFCI) IACUC guidelines at the DFCI Longwood Center Animal Resource Facility under an approved protocol. The BBN963 bladder cancer cell line was a kind gift from William Kim (University of North Carolina). The PARP14 inhibitor RBN012759 was purchased from MedChemExpress (MCE) and dissolved in 0.5% methylcellulose (MC) plus 0.2% Tween 80. Cisplatin was purchased from the DFCI Research Pharmacy. Seven-week-old C57BL6/J mice (Jackson Labs) were subcutaneously implanted with 4.5 million BBN963 bladder cancer cells suspended in 75 ul PBS mixed 1:1 with Matrigel (Corning, 345230). Tumor volumes were monitored with digital calipers. When the tumor volume reached approximately 100 mm^3, mice were randomized to one of four groups (9 mice per group) and treated with: (1) vehicle control (150 uL PBS injected intraperitoneally (i.p.) three times per week for 3 weeks and 250 µL of solvent (0.5% MC plus 0.2 tween 80) delivered via oral gavage (OG) twice per day for 3 weeks); (2) cisplatin alone (cisplatin 1 mg/kg in 150 uL injected i.p. three times per week for 3 weeks and 250 uL solvent (0.5% MC plus 0.2 tween 80) delivered via OG twice per day for 3 weeks); (3) PARP14 inhibitor alone (PARP14 inhibitor 500 mg/kg delivered via OG twice per day for 3 weeks and 150 uL PBS injected i.p., three time per week); and (4) cisplatin plus PARP14 inhibitor. Tumor volumes were measured with digital calipers three times per week and mouse weights were measured twice per week during treatment and for an additional 11 days. At the end of the experiment, tumors were fixed in formalin and embedded in paraffin. Half of each tumor was sectioned for H&E staining and the other half was processed for bulk RNA sequencing.

Mouse bulk RNA-seq data

Tumors were sent to Novogene (Durham, NC) for RNA extraction and sequencing (20 million reads per sample). The Nextflow RNA-seq workflow (nf-core/rnaseq; version 3.9) [70] was used to perform quality control, and STAR aligner and Salmon were used for alignment and reads quantification[71]. Counts were normalized using the median of ratios method, differential expression analysis was performed with DESeq2[72] and GSEA using MiSgDB[62].

Data and code availability

Code and files used to generate all figures can be found at https://github.com/CarvalhoFilipeL/MIBC_tumor_myeloid and bulk RNA-seq cohorts used for single-nucleus RNA-seq tumor-intrinsic gene programs (Fig. 2D&E) in https://github.com/CarvalhoFilipeL/bladder_cancer_B_cell_sigs. Raw single-nucleus RNA-seq, spatial transcriptomic and bulk RNA-seq data will be deposited to dbGaP at the time of publication.

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Declaration of interests

E.M.V.A. reports advisory/consulting with Enara Bio, Manifold Bio, Monte Rosa, Novartis Institute for Biomedical Research, Serinus Bio, TracerDx; research support from Novartis, BMS, Sanofi, NextPoint; Equity in Tango Therapeutics, Genome Medical, Genomic Life, Enara Bio, Manifold Bio, Microsoft, Monte Rosa, Riva Therapeutics, Serinus Bio, Syapse, TracerDx; Editorial Boards in Science Advances; institutional patents filed on chromatin mutations and immunotherapy response, and methods for clinical interpretation; intermittent legal consulting on patents for Foaley & Hoag. A.S.K. reports consulting with Janssen, Merck, Pfizer,Tolmar, Bristol Myers Squibb, Profound. K.W.M. reports Advisory/Consulting with EMD Serono, Pfizer, UroGen, Riva Therapeutics, Natera; Research Support from Pfizer, Novo Ventures; Equity in Riva Therapeutics, Illudent Therapeutics; writing/editor fees from UpToDate; Speaking fees from OncLive; Institutional patent filed on mutational signatures of DNA repair deficiency; editorial boards in Science Advances. J.L.G. reports consultanting for BD Biosciences, Glaxo-Smith Kline, Laverock, Voro, LTZ, iTEOS, Synkine, Duke Street Bio., Array BioPharma;

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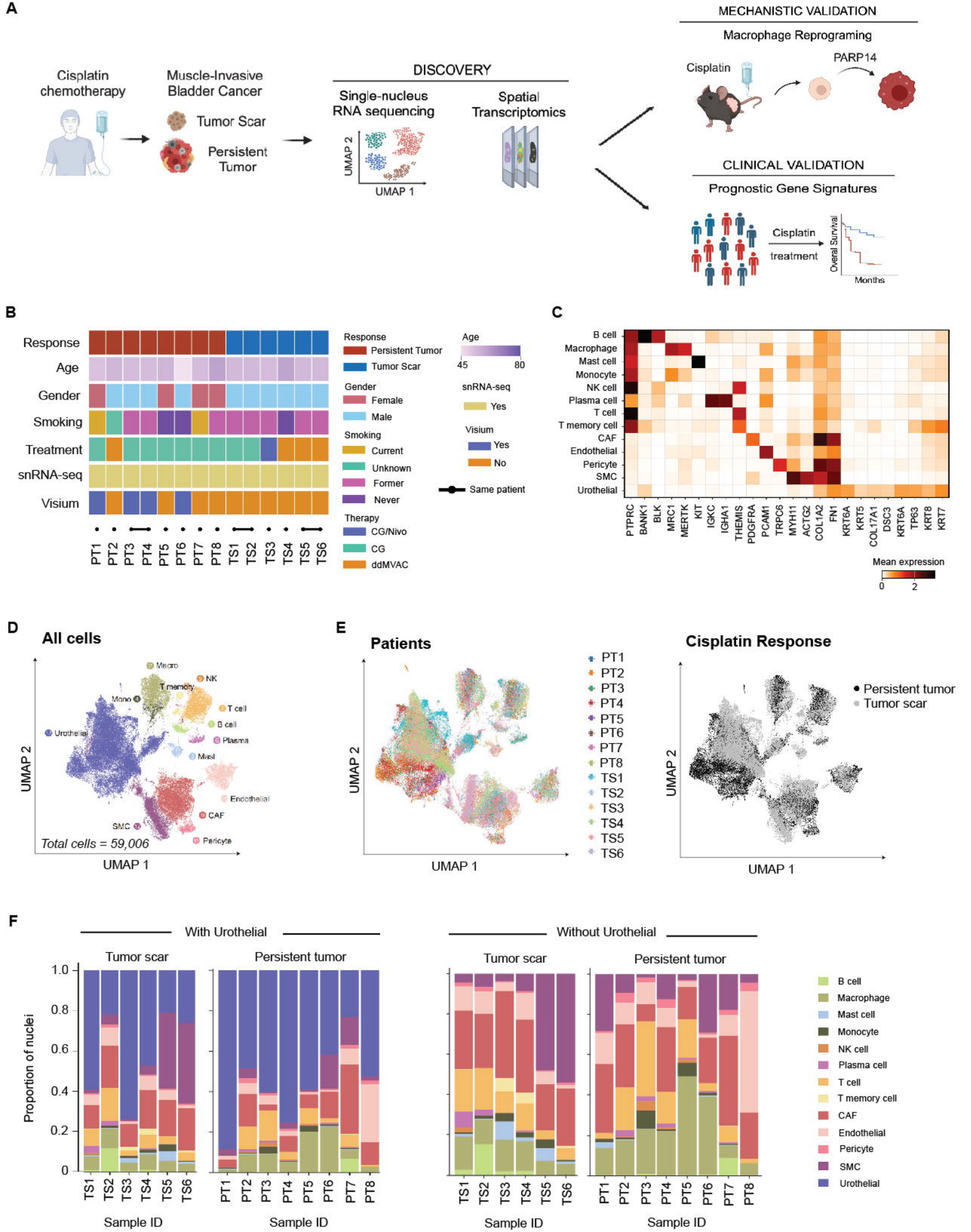
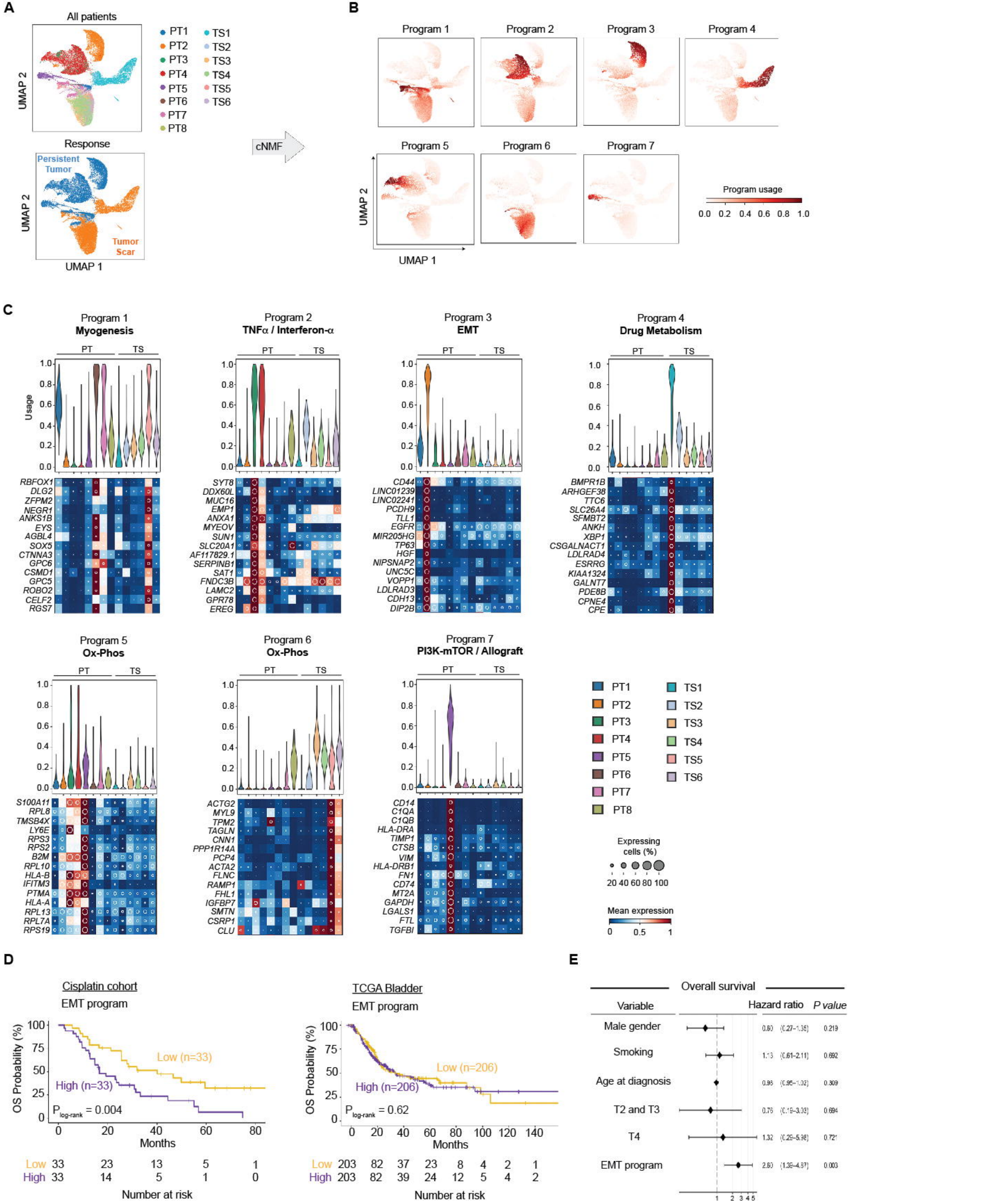
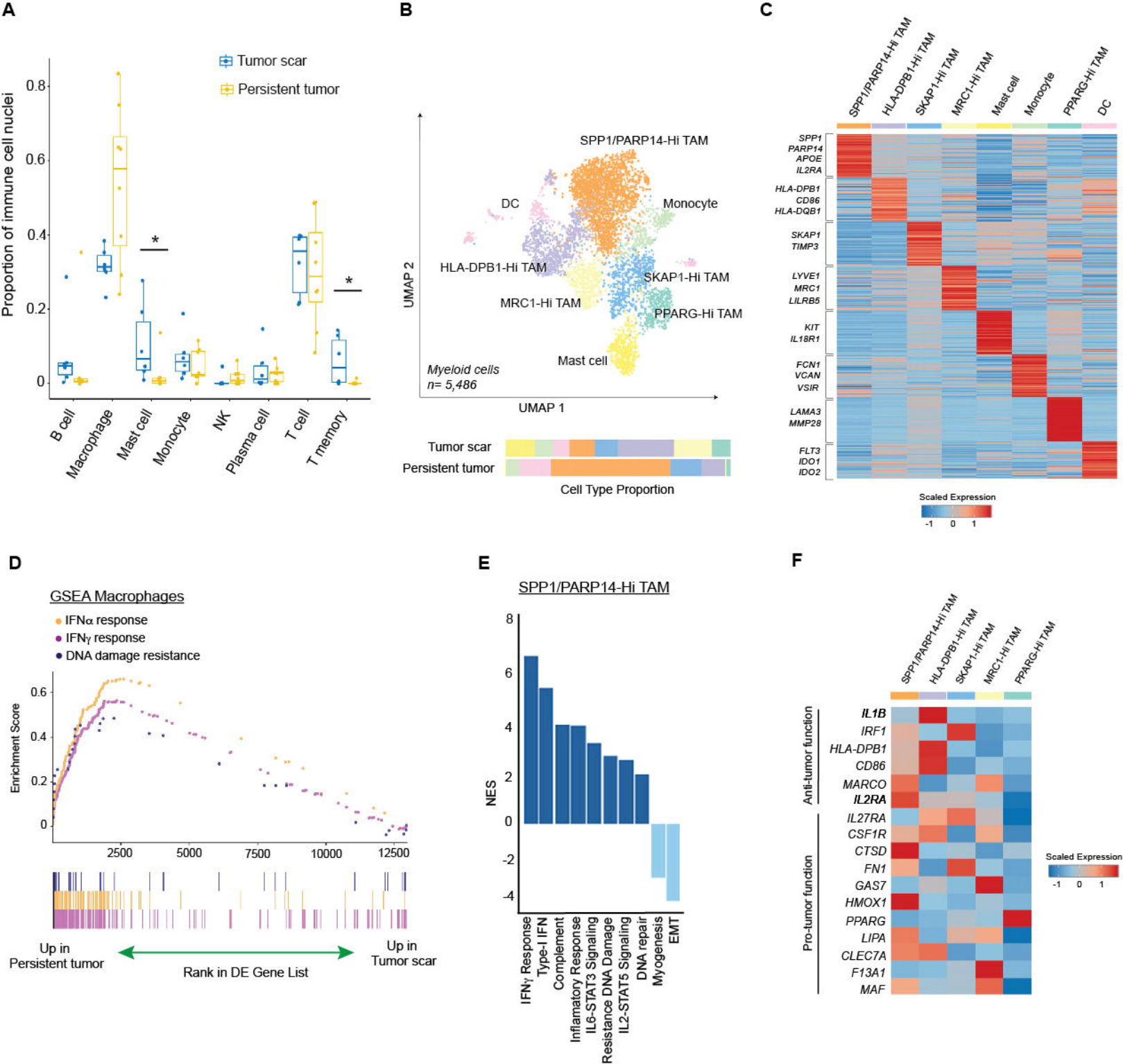
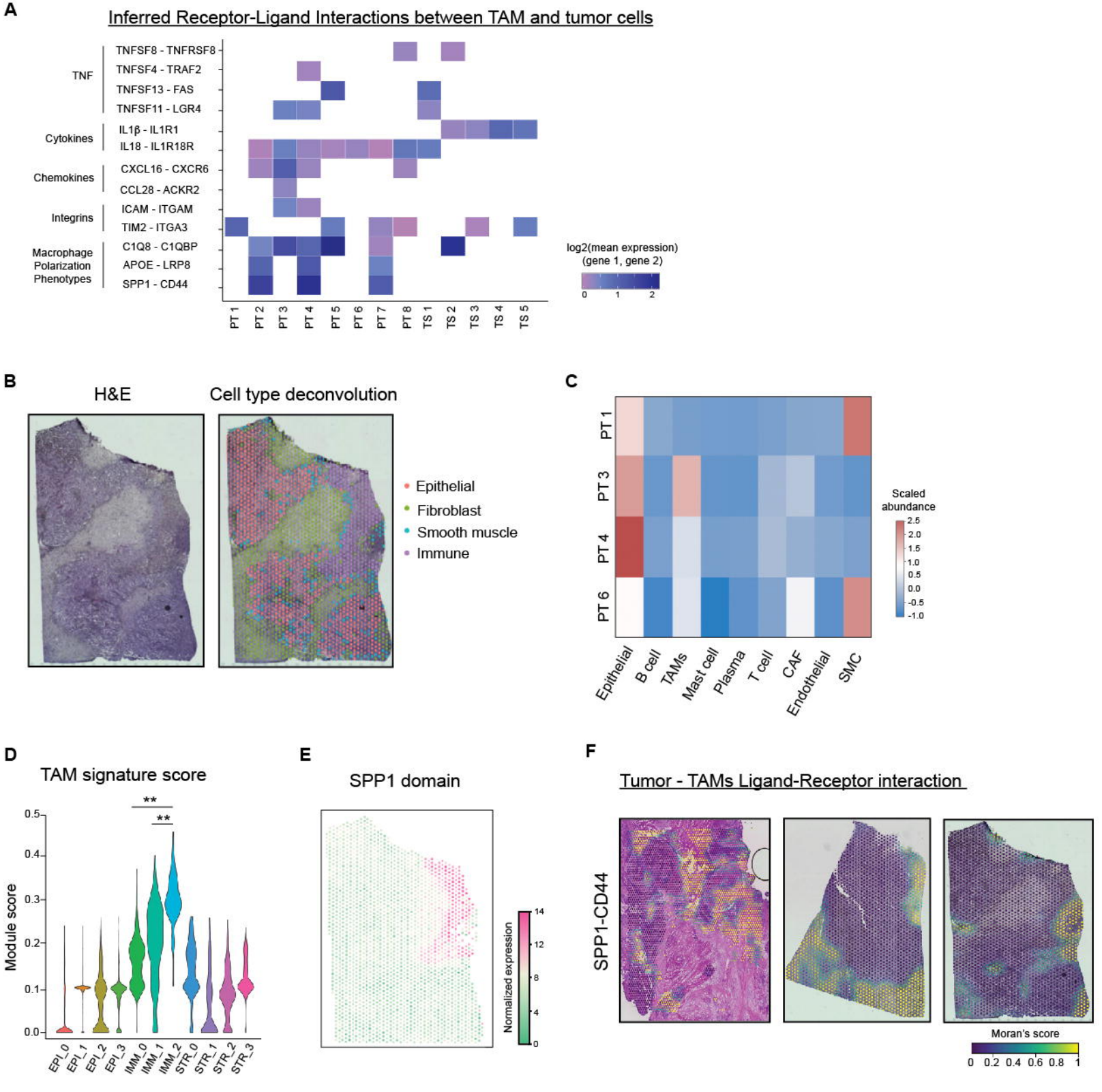
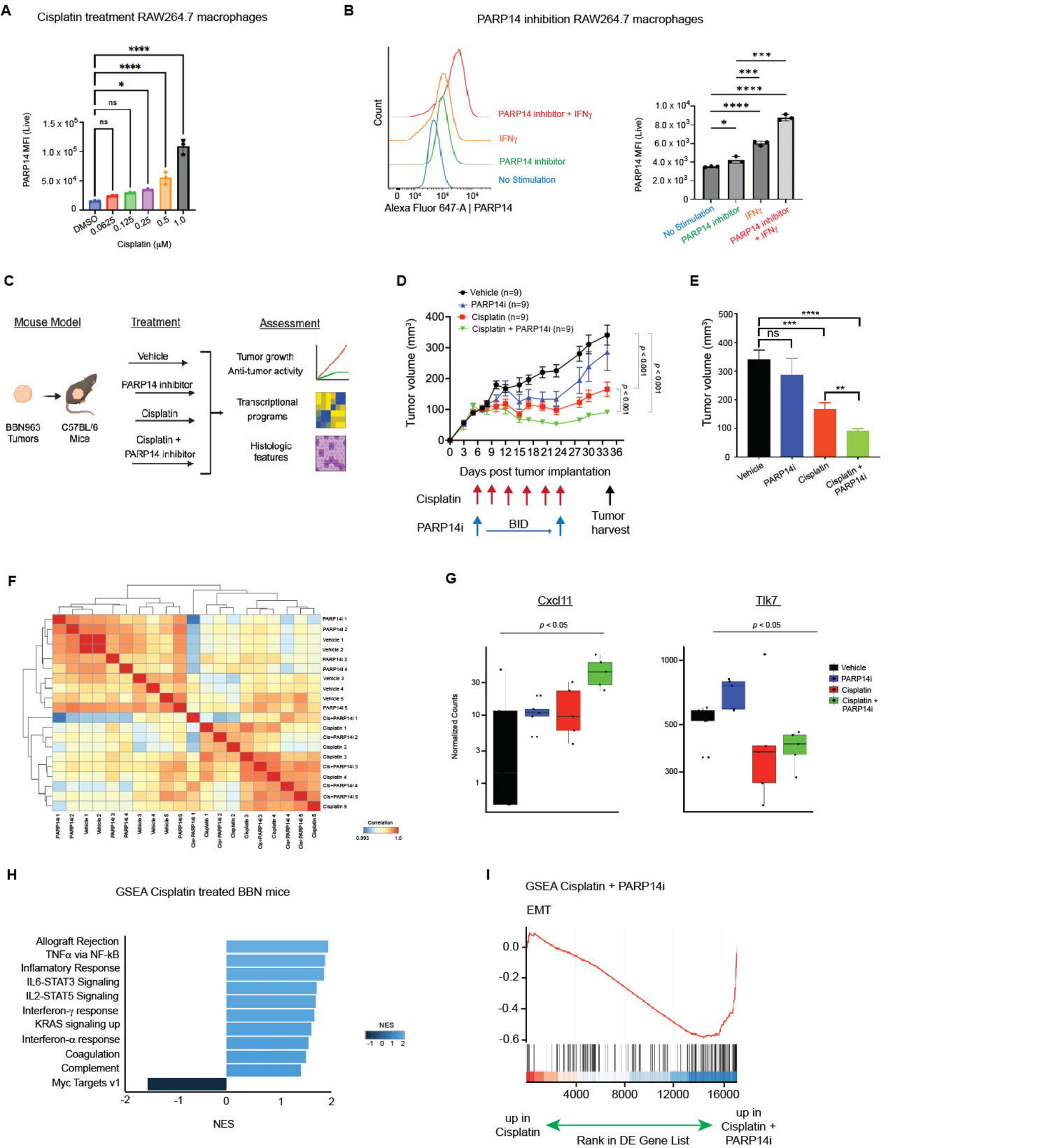

A
Cisplatin chemotherapy
Muscle-Invasive Bladder Cancer
Tumor Scar
Persistent Tumor
DISCOVERY
Single-nucleus RNA sequencing
Spatial Transcriptomics
UMAP 2
UMAP 1
MECHANISTIC VALIDATION
Macrophage Reprograming
Cisplatin
PARP14
CLINICAL VALIDATION
Prognostic Gene Signatures
Cisplatin treatment
Overal Survival
Months
B
Response
Age
Gender
Smoking
Treatment
snRNA-seq
Visium
PT1 PT2 PT3 PT4 PT5 PT6 PT7 PT8 TS1 TS2 TS3 TS4 TS5 TS6
Response
Persistent Tumor
Tumor Scar
Age
45
80
Gender
Female
Male
snRNA-seq
Yes
Smoking
Current
Unknown
Former
Never
Visium
Yes
No
Therapy
CG/Nivo
CG
ddMVAC
Same patient
C
B cell
Macrophage
Mast cell
Monocyte
NK cell
Plasma cell
T cell
T memory cell
CAF
Endothelial
Pericyte
SMC
Urothelial
PTPRC BANK1 BLK MRC1 MERTK KIT IGKC IGHA1 THEMIS PDGFRA PCAM1 TRPC6 MYH11 ACTG2 COL1A2 FN1 KRT6A KRT5 COL17A1 DSC3 KRT6A TP63 KRT8 KRT7
Mean expression
0
2
D
All cells
Macro
NK
T memory
Mono
T cell
B cell
Urothelial
Plasma
Mast
Endothelial
CAF
SMC
Pericyte
Total cells = 59,006
UMAP 2
UMAP 1
E
Patients
PT1
PT2
PT3
PT4
PT5
PT6
PT7
PT8
TS1
TS2
TS3
TS4
TS5
TS6
UMAP 2
UMAP 1
Cisplatin Response
Persistent tumor
Tumor scar
UMAP 2
UMAP 1
F
With Urothelial
Without Urothelial
Tumor scar
Persistent tumor
Proportion of nuclei
1.0
0.8
0.6
0.4
0.2
0
TS1 TS2 TS3 TS4 TS5 TS6
PT1 PT2 PT3 PT4 PT5 PT6 PT7 PT8
Sample ID
B cell
Macrophage
Mast cell
Monocyte
NK cell
Plasma cell
T cell
T memory cell
CAF
Endothelial
Pericyte
SMC
Urothelial


A
All patients
UMAP 2
PT1
PT2
PT3
PT4
PT5
PT6
PT7
PT8
TS1
TS2
TS3
TS4
TS5
TS6
Response
Persistent Tumor
Tumor Scar
UMAP 1
cNMF
B
Program 1
Program 2
Program 3
Program 4
Program 5
Program 6
Program 7
UMAP 2
UMAP 1
Program usage
0.0 0.2 0.4 0.6 0.8 1.0
C
Program 1
Myogenesis
Program 2
TNFα / Interferon-α
Program 3
EMT
Program 4
Drug Metabolism
PT
TS
Usage
RBFOX1
DLG2
ZFPM2
NEGR1
ANKS1B
EYS
AGBL4
SOX5
CTNNA3
GPC6
CSMD1
GPC5
ROBO2
CELF2
RGS7
SYT8
DDX60L
MUC16
EMP1
ANXA1
MYEOV
SUN1
SLC20A1
AF117829.1
SERPINB1
SAT1
FNDC3B
LAMC2
GPR78
EREG
CD44
LINC01239
LINC02241
PCDH9
TLL1
EGFR
MIR205HG
TP63
HGF
NIPSNAP2
UNC5C
VOPP1
LDLRAD3
CDH13
DIP2B
BMPR1B
ARHGEF38
TTC6
SLC26A4
SFMBT2
ANKH
XBP1
CSGALNACT1
LDLRAD4
ESRRG
KIAA1324
GALNT7
PDE8B
CPNE4
CPE
Program 5
Ox-Phos
Program 6
Ox-Phos
Program 7
PI3K-mTOR / Allograft
S100A11
RPL8
TMSB4X
LY6E
RPS3
RPS2
B2M
RPL10
HLA-B
IFITM3
PTMA
HLA-A
RPL13
RPL7A
RPS19
ACTG2
MYL9
TPM2
TAGLN
CNN1
PPP1R14A
PCP4
ACTA2
FLNC
RAMP1
FHL1
IGFBP7
SMTN
CSRP1
CLU
CD14
C1QA
C1QB
HLA-DRA
TIMP1
CTSB
VIM
HLA-DRB1
FN1
CD74
MT2A
GAPDH
LGALS1
FTL
TGFBI
Expressing cells (%)
20 40 60 80 100
Mean expression
0 0.5 1
D
Cisplatin cohort
EMT program
OS Probability (%)
Low (n=33)
High (n=33)
$P_{log\text{-}rank}$ = 0.004
Months
Low 33 23 13 5 1
High 33 14 5 1 0
Number at risk
TCGA Bladder
EMT program
Low (n=206)
High (n=206)
$P_{log\text{-}rank}$ = 0.62
Low 203 82 37 23 8 4 2 1
High 203 82 39 24 12 5 4 2
E
Overall survival
Variable
Hazard ratio
P value
Male gender
Smoking
Age at diagnosis
T2 and T3
T4
EMT program


A
Tumor scar
Persistent tumor
Proportion of immune cell nuclei
0.8
0.6
0.4
0.2
0
*
*
B cell
Macrophage
Mast cell
Monocyte
NK
Plasma cell
T cell
T memory
B
SPP1/PARP14-Hi TAM
DC
Monocyte
HLA-DPB1-Hi TAM
SKAP1-Hi TAM
MRC1-Hi TAM
PPARG-Hi TAM
Mast cell
UMAP 2
UMAP 1
Myeloid cells
n= 5,486
Tumor scar
Persistent tumor
Cell Type Proportion
C
SPP1/PARP14-Hi TAM
HLA-DPB1-Hi TAM
SKAP1-Hi TAM
MRC1-Hi TAM
Mast cell
Monocyte
PPARG-Hi TAM
DC
SPP1
PARP14
APOE
IL2RA
HLA-DPB1
CD86
HLA-DQB1
SKAP1
TIMP3
LYVE1
MRC1
LILRB5
KIT
IL18R1
FCN1
VCAN
VSIR
LAMA3
MMP28
FLT3
IDO1
IDO2
Scaled Expression
-1
0
1
D
GSEA Macrophages
IFNα response
IFNγ response
DNA damage resistance
Enrichment Score
0.6
0.4
0.2
0
2500
5000
7500
10000
12500
Up in
Persistent tumor
Rank in DE Gene List
Up in
Tumor scar
E
SPP1/PARP14-Hi TAM
NES
8
6
4
2
0
-2
-4
IFNγ Response
Type-I IFN
Complement
Inflamatory Response
IL6-STAT3 Signaling
Resistance DNA Damage
IL2-STAT5 Signaling
DNA repair
Myogenesis
EMT
F
SPP1/PARP14-Hi TAM
HLA-DPB1-Hi TAM
SKAP1-Hi TAM
MRC1-Hi TAM
PPARG-Hi TAM
Anti-tumor function
Pro-tumor function
IL1B
IRF1
HLA-DPB1
CD86
MARCO
IL2RA
IL27RA
CSF1R
CTSD
FN1
GAS7
HMOX1
PPARG
LIPA
CLEC7A
F13A1
MAF
Scaled Expression
-1
0
1


A
Inferred Receptor-Ligand Interactions between TAM and tumor cells
TNF
TNFSF8 - TNFRSF8
TNFSF4 - TRAF2
TNFSF13 - FAS
TNFSF11 - LGR4
Cytokines
IL1β - IL1R1
IL18 - IL1R18R
Chemokines
CXCL16 - CXCR6
CCL28 - ACKR2
Integrins
ICAM - ITGAM
TIM2 - ITGA3
Macrophage Polarization Phenotypes
C1Q8 - C1QBP
APOE - LRP8
SPP1 - CD44
PT 1
PT 2
PT 3
PT 4
PT 5
PT 6
PT 7
PT 8
TS 1
TS 2
TS 3
TS 4
TS 5
log2(mean expression) (gene 1, gene 2)
0
1
2
B
H&E
Cell type deconvolution
Epithelial
Fibroblast
Smooth muscle
Immune
C
PT 1
PT 3
PT 4
PT 6
Epithelial
B cell
TAMs
Mast cell
Plasma
T cell
CAF
Endothelial
SMC
Scaled abundance
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
D
TAM signature score
Module score
0.5
0.4
0.3
0.2
0.1
0.0
**
**
EPI_0
EPI_1
EPI_2
EPI_3
IMM_0
IMM_1
IMM_2
STR_0
STR_1
STR_2
STR_3
E
SPP1 domain
Normalized expression
14
12
8
4
0
F
Tumor - TAMs Ligand-Receptor interaction
SPP1-CD44
Moran's score
0
0.2
0.4
0.6
0.8
1


A
Cisplatin treatment RAW264.7 macrophages
PARP14 MFI (Live)
Cisplatin (μM)
DMSO
0.0625
0.125
0.25
0.5
1.0
ns
B
PARP14 inhibition RAW264.7 macrophages
Count
Alexa Fluor 647-A | PARP14
PARP14 inhibitor + IFNγ
IFNγ
PARP14 inhibitor
No Stimulation
PARP14 MFI (Live)
C
Mouse Model
Treatment
Assessment
BBN963 Tumors
C57BL/6 Mice
Vehicle
PARP14 inhibitor
Cisplatin
Cisplatin + PARP14 inhibitor
Tumor growth
Anti-tumor activity
Transcriptional programs
Histologic features
D
Vehicle (n=9)
PARP14i (n=9)
Cisplatin (n=9)
Cisplatin + PARP14i (n=9)
Tumor volume (mm³)
Days post tumor implantation
Cisplatin
PARP14i
BID
Tumor harvest
E
Tumor volume (mm³)
Vehicle
PARP14i
Cisplatin
Cisplatin + PARP14i
F
Correlation
G
Cxcl11
Tlk7
Normalized Counts
Vehicle
PARP14i
Cisplatin
Cisplatin + PARP14i
H
GSEA Cisplatin treated BBN mice
Allograft Rejection
TNFα via NF-kB
Inflamatory Response
IL6-STAT3 Signaling
IL2-STAT5 Signaling
Interferon-γ response
KRAS signaling up
Interferon-α response
Coagulation
Complement
Myc Targets v1
NES
I
GSEA Cisplatin + PARP14i
EMT
up in Cisplatin
Rank in DE Gene List
up in Cisplatin + PARP14i