**Single-cell transcriptome analysis**

Single-cell RNA-seq data was pre-processed with the scater (1) and normalized by scran (2). Data integration, unsupervised cell clustering, and differential expression analysis were carried out by the Seurat v3.0 (3). Reference-based cell type annotation was generated by SingleR (4).

Cells with more than 3 median absolute deviation were removed as outliers. Cells with less than 400 genes or 1000 UMIs or more than 15% of mitochondria genes were filtered out from the analysis. Altogether, the filtered data contained 27,998 cells and 24,421 genes from 6 samples. Cell-specific biases were normalized with pool-based size factors. The top 3000 highly variable genes were selected using the expression and dispersion (variance/mean) of genes, followed by a canonical correlation analysis (CCA) to identify common sources of variation between the patient and normal datasets. The first 105 CCA results were chosen to generate dimensional t-Distributed Stochastic Neighbor Embedding (tSNE) plots, Uniform Manifold Approximation and Projection (UMAP) plots, and cell clustering by a shared nearest neighbor (SNN) modularity optimization based clustering algorithm.

Cell types were manually identified by marker genes (5), and confirmed by SingleR (Single-cell Recognition) package using 358 mouse RNA-seq (4). Differential expression analysis was performed based on the MAST (Model-based Analysis of Single Cell Transcriptomics) (6).

**Code availability.** The scripts used for analysis and figure generation are available at https://github.com/nyuhuyang/scRNAseq-BladderCancer

1. McCarthy DJ, Campbell KR, Lun AT, Wills QF. Scater: pre-processing, quality control, normalization and visualization of single-cell RNA-seq data in R. Bioinformatics (Oxford, England). 2017;33(8):1179-86.

2. Lun A, McCarthy D, Marioni J. A step-by-step workflow for low-level analysis of single-cell RNA-seq data with Bioconductor [version 2; peer review: 3 approved, 2 approved with reservations]. F1000Research. 2016;5(2122).

3. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM, 3rd, et al. Comprehensive Integration of Single-Cell Data. Cell. 2019;177(7):1888-902 e21.

4. Aran D, Looney AP, Liu L, Wu E, Fong V, Hsu A, et al. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. Nature immunology. 2019;20(2):163-72.

5. Robertson AG, Kim J, Al-Ahmadie H, Bellmunt J, Guo G, Cherniack AD, et al. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. Cell. 2017;171(3):540-56 e25.

6. Finak G, McDavid A, Yajima M, Deng J, Gersuk V, Shalek AK, et al. MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. Genome biology. 2015;16(1):278.