**Single-cell transcriptome analysis**

Single-cell RNA-seq data is pre-processed with the scater R package (1). Data normalization, unsupervised cell clustering, and differential expression analysis were carried out by the Seurat R package (2). Reference-based cell type annotation were carried out using the SingleR R package (3).

Cells with less than 800 genes or 1500 UMIs or more than 15% of mitochondria genes were excluded from the analysis. Gene expression raw counts were normalized following a global-scaling normalization method with a scale factor of 10,000 and natural log transformation, using the Seurat NormalizeData function. The top 2000 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 20 CCA results were chosen to generate dimensional t-Distributed Stochastic Neighbor Embedding (tSNE) plots, and cell clustering by a shared nearest neighbor (SNN) modularity optimization based clustering algorithm.

Cell types were manually identified by marker genes (4), and confirmed by SingleR (Single-cell Recognition) package (3). Differential expression analysis was performed based on the MAST (Model-based Analysis of Single Cell Transcriptomics) (5). Gene Set Analysis and density plots are generated using gplots and ggplot2 R package.

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

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