Supplementary Methods

Procedure of double IHC

Tissue microarrays (TMA) were deparaffinized and hydrated, heated with an autoclave in sodium citrate buffer (pH=6) for antigen retrieval. Next, TMAs were placed in Horseradish Peroxidase (HRP) enzyme and alkali-phosphatase (AP) blocking solution. Then TMAs were incubated in PBS buffer containing 1% BSA for antigen blocking. Incubation with the mixture of two first primary antibodies was carried out at 37 °C for 2 h, followed by HRP-labeled secondary antibody incubation and visualization using DAB reagent. Next, TMAs were incubated with an AP-labeled secondary antibody and visualized by Vector Blue staining.

Bioinformatics analysis

The scRNA-seq data of 2469 $CD8^+$ T cells from ccRCC patients were downloaded from https://science.sciencemag.org/content/361/6402/594/tabfigures-data [1]. We use “Seurat”, “Singler”, “outliers” and “GSVA” package to analysis.

The RNA (mRNA) sequencing data of 530 ccRCC patients from the Cancer Genome Atlas (TCGA) were downloaded using TCGA-Assembler 2.0.5 at May.14th, 2019. We use the average of the ENTPD1 and CD8A as the CD39+CD8+ T cell score. The related gene sets were obtained from Molecular Signatures Database v7.0 (MSigDB) and the gene signature score [2, 3] (supplementary table 5) was defined using Gene Set Variation Analysis (GSVA) package.

Reference:

1. Young, M.D., et al., Single-cell transcriptomes from human kidneys reveal the cellular identity of renal tumors. Science, 2018. 361(6402): p. 594-599.
2. Charoentong,P.,et al., Pan-cancer Immunogenomic Analyses Reveal Genotype-Relationships and Predictors of Response to Checkpoint Immunophenotype Blockade. Cell Rep, 2017. 18(1): p. 248-262.
3. Xiao, Y., et al., Multi-Omics Profiling Reveals Distinct Microenvironment Characterization

and Suggests Immune Escape Mechanisms of Triple-Negative Breast Cancer. Clin Cancer Res, 2019. 25(16): p. 5002-5014.