Supplementary Materials and Methods

Cancer cell supernatants preparation

Once the T24 or MB49 cells reached 90% confluence, the medium was removed, and the flasks were washed twice with PBS. The cells were replenished with fresh complete RPMI 1640 or DMEM. The supernatant was collected after 72 h and centrifuged at 700 ×*g* for 10 min and 3220 ×*g* for 15 min at 4°C before being stored at −80°C.

Preparation and culture of mouse lymphocytes

To obtain mouse lymphocyte suspensions, 6-to-8-week-old C57BL/6 mice were euthanized by CO_2 asphyxiation followed by cervical dislocation and immersed in 75% ethanol for 5 min, then transferred to the biosafety cabinet. The spleen was collected and placed in sterile 6-well plates (Corning Costar, cat# 07-200-83) with 3 mL 1× phosphate-buffered saline (PBS; cat# 10010, ThermoFisher) on ice. Spleen tissues were minced with a razor (cat# G535010, ThermoFisher) followed by digestion with 1× PBS solution supplemented with 1 mg/mL collagenase II, 1 mg/mL DNase, and 5% heat-inactivated FBS for 20 min at 37°C with 5% CO_2. The digested tissues were then passed through 70-mm cell strainers (cat# BS-70-CS, Biosharp) using mechanical force with the rubber end of a 5-mL syringe. Cell suspensions were then treated with 0.1 M EDTA (cat# 15575, ThermoFisher) for 5 min at 37°C and washed two times with PBS. After the lysis of red blood cells with RBC lysis buffer (cat# CW0613S, CWBio), cells were collected by centrifugation at 450 ×*g* for 10 min at 4°C.

In Vitro lymphocyte co-culture assay

For the lymphocyte co-culture assay, 3×10^5 cancer cells or 5×10^5 stimulated macrophages were seeded into each well of a 24-well plate. On the next day, 5×10^5 lymphocytes were added to each well. After 48 h, the cell suspension was collected, and the flow cytometry staining was performed to evaluate the granzyme B level of the T cells.

Generation of the SYSMH cohort

This study includes 34 patients with BCa who underwent NGS testing from Sun Yat-sen Memorial Hospital. Patients with incomplete clinical information, or missing pathological tissue sections were excluded from this study. 24 patients were identified as WT, while 10 patients were identified as a*FGFR3*. Clinicopathological data, including gender, age and tumor characteristics were collected. This retrospective analysis was approved by the ethics committee of Sun Yat-sen Memorial Hospital (approval number SYSKY-2023-422-01), and written informed consent was obtained from each enrolled patient.

IHC details

Tissue sections were deparaffinized and subjected to heat-mediated antigen retrieval with Tris-EDTA (pH 9.0) in a Cuisinart high-pressure cooker. Bovine serum albumin (cat# ccs30014.01, MRC) was used to block non-specific protein-binding sites. The slides were incubated with primary antibodies followed by corresponding HRP-conjugated secondary antibodies (cat# MP-7451, cat# MP-7444, and cat# MP-7405, Vector). DAB staining (cat# K5007, DAKO) was performed, and the cells were counterstained with hematoxylin. The number of positively stained cells was manually counted in three high-power fields (400×).

Tumor-infiltrating lymphocyte classification

For tumor-infiltrating lymphocyte classification, tumors were assessed by two pathologists and categorized into three groups: (1) "immune-desert" if tumors were with rare isolated lymphocytes in any tumor compartment; (2) "immune-excluded" if tumors were with lymphocytes only found at the invasive margin or within stroma, and with rare isolated lymphocytes in the intratumoral compartment; (3) "inflamed" if tumors were with lymphocytes present in stroma and infiltrating the tumor parenchyma, with direct contact with tumor cells.

Evaluation of PSAT1 IHC scores

Two pathologists, who were blinded to the clinicopathological data, independently examined the tissue sections. The expression levels of PSAT1 were graded on a scale of 0 to 5: 0 indicated no expression, 1 indicated weak expression in

partial cells, 2 indicated moderate expression in partial cells, 3 indicated moderate expression in most cells, 4 indicated strong expression in most cells, and 5 indicated very strong expression in most cells (Supplementary Figure 4C). The scoring was performed on three representative high-power fields for each specimen (magnification ×400) and the average score was calculated.

Cell viability assay

To measure the cell viability of cancer cells, we used a Cell Counting Kit 8 (CCK-8, cat# G4103, Servicebio) according to the manufacturer's instructions.