Supplementary Methods

CYT score

The cytolytic (CYT) score was calculated as previously described as the geometric mean of granzyme (GZMA) and perforin (PRF1) expression.[1]

Evaluation of TILs

We evaluated tumor-infiltrating lymphocytes (TILs) on H&E slides according to the International TIL Working Group guidelines.[2] According to the guidelines, TILs are defined as mononuclear immune cells that infiltrate the tumor region (including lymphocytes and plasma cells, excluding polymorphonuclear leukocytes). The TILs were reported as the ratio of the area occupied by lymphocytes to the total area of the tumor region and recorded in 5% increments. Two experienced pathologists independently evaluated the histopathological results.

Pull-down assay

The ZNF689 and TRIM28 fusion proteins were purchased from Proteintech. The pull-down assays were performed as previously described.[3] Briefly, 2 μg of glutathione S-transferase (GST)-tagged ZNF689 and 2 μg of His-tagged TRIM28 recombinant protein were incubated with an anti-GST antibody in binding buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 0.05% NP-40, supplemented with a protease inhibitor cocktail) overnight at 4°C and then mixed with Dynabeads Protein A beads (Invitrogen) for 1 h. The beads were washed 5 times and eluted with SDS loading buffer by boiling for 5 min before electrophoresis. For His pull-down, 2 μg of His-tagged TRIM28 and 2 μg of

GST-tagged ZNF689 recombinant protein were incubated with Ni-NTA beads in binding buffer overnight at 4°C. After centrifugation, the beads were washed 5 times and boiled in 1 × SDS gel loading buffer before electrophoresis.

ATAC-seq and data analysis

ATAC-seq was performed as previously described with modifications.[4] For nuclear preparation, 100,000 live cells were lysed in cold lysis buffer (10 mM Tris-Cl, pH 7.4, 10 mM NaCl, 3 mM MgCl2 and 0.1% IGEPAL CA-630) before being washed at 500 × g for 10 min. For transposition, transposase reaction mix (25 μl 2× TD buffer, 2.5 μl transposase (APExBIO) and 22.5 μl of nuclease free water) was used in the transposase reaction carried out for 30 min at 37°C. After DNA purification, the library was amplified for 12 cycles and sequenced using Illumina NextSeq 500 with paired-end 2 × 150 as the sequencing mode.

Sequencing reads were trimmed using Fastp (v0.20.0) with the parameter 'length_required 80'. Subsequently, the processed reads were aligned to the human genome (hg19) using Bowtie2, employing the parameters 'very sensitive X 2000'. Reads mapped to the mitochondria were subsequently excluded. PCR duplicates were eliminated using the Picard tool (https://broadinstitute.github.io/picard/). Peak calling was executed with MACS2 (v2.2.7.1), using the parameters 'nomodel shift 100 extsize 200 keep dup all'. For subsequent analyses, read counts were normalized using the RPKM method. ATAC signal profiles were illustrated using deepTools. For the LINE-1 analysis, the UCSC hg19 r1_repeat_rmsk dataset specific to LINE-1 was employed to assess LINE-1 enrichment. Visualization of ATAC-seq signals was achieved with the Integrative Genomics Viewer (IGV), and differential peak

analysis was conducted using DiffBind.

Dual-luciferase reporter assay

Cells were seeded in 24-well plates and transfected with 0.5 μg/well luciferase reporter plasmids. To normalize the transfection efficiency, the cells were cotransfected with 10 ng of pRL-CMV (Renilla luciferase). Forty-eight hours after transfection, luciferase activity was detected using the Dual-Luciferase Reporter Assay System Kit (Promega) according to the manufacturer's instructions.

Metaphase chromosome spreading

Cells at 70% confluence were treated for 2 h with 1 mg/ml colcemid, harvested, and resuspended for 30 min in 1 ml of 75 mmol/l KCl at 37°C. After centrifugation, the cells were fixed with cold methanol/acetic acid (3:1) buffer and incubated for 15 min at room temperature. Metaphase spreads were made by dropping cells onto the slide, and the specimens were air-dried, stained with DAPI, and visualized under a Zeiss AxioImager microscope.

CIN score calculation

CIN score calculation was performed as previously described.[5] In brief, the average of the normalized gene expression data of the 70 genes that constitute the signature was used to calculate the CIN score.

Evaluation of chromosome missegregation errors in H&E samples

Diagnostic H&E-stained TNBC samples in the FUSCC cohort were evaluated

for anaphase chromosome missegregation events using a 100× objective light microscope. Only H&E sections with ≥10 cells undergoing anaphase were considered.

Aneuploidy score calculation

The aneuploidy score was defined as the total number of arm-level SCNAs for a tumor, adjusted for ploidy and was calculated using a total copy number-based method as previously described.[6] Specifically, the segment file with ploidy information generated by ASCAT (v.2.5.2) was used as input for the "get_Aneuploidy_score()" function of the sigminer package to assign aneuploidy scores to each sample.[7]

Apoptosis assay

Apoptosis analysis was performed with Annexin V-Alexa Fluor 647/PI Apoptosis Detection Kit (Yeasen). Briefly, cells and osteoclasts were seeded in 6-well plates (2×10^5 cells). After 24 h of incubation, the cells were treated with EFV for 24 h. The floating and adherent cells were harvested and doubly stained with Annexin V-Alexa Fluor 647 and PI based on the manufacturer's instructions, and then analyzed using CytoFLEX S flow cytometer (Beckman Coulter).

Splenocyte isolation and $CD8^+$ T-cell isolation

OT-I mice were killed by cervical dislocation, and spleens were collected into 50 ml conical tubes containing serum-free RPMI-1640 medium. Spleens were smashed and passed through sterile mesh filters using 10 ml of medium to wash the screens. The cells were spun down at 1,200 rpm for 5 min, and the

supernatant was removed and then resuspended in 10 ml ACK buffer (BioLegend) for red blood cell lysis. The cells were briefly vortexed, allowed to sit at room temperature for 1 min and then quenched with 5 ml of culture medium. Cells were then spun down and resuspended in culture medium, counted and plated in six-well plates at 2×10^6 cells/ml. $CD8^+$ T cells from the OT-I splenocytes obtained above were further isolated with a $CD8a^+$ T-Cell Isolation Kit (Miltenyi). Flow-through $CD8^+$ T cells were collected for future experiments.

PDTF cultures and analysis

The procedure has been delineated in prior work.[8] In brief, tissue samples designated for PDTF cultures were meticulously processed into 1-2 mm^3 fragments. These fragments were then embedded in a tailored artificial extracellular matrix combined with Matrigel, and allocated within a 96-well plate. After stabilization at 37°C, they received a tumor medium supplemented with either with EFV or anti-PD-1. After incubation for 48 h, the PDTFs were readied for flow cytometry. This procedure aimed to evaluate T-cell activation using designated antibodies, including ICOS, CD137, and CD25. Subsequently, the PDTFs were isolated, subjected to enzymatic digestion, and transformed into single-cell suspensions. After a blocking phase, these cells were stained and analyzed. Moreover, 48 h after culture, supernatants from the PDTF cultures underwent analysis to determine IFN-γ levels, using the Human IFN-γ ELISA Kit (Lianke) following the manufacturer's guidelines.

References

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