Supplemental Materials and Methods

Western blotting

The cells were lysed on ice with RIPA Lysis Solution (P0013B, Beyotime, China) containing phenylmethylsulfonyl fluoride and phosphatase inhibitor. The protein concentration of the samples was determined using a BCA protein quantification kit (P0010, Beyotime, China). Total protein (20 μg) was aspirated and separated by SDS–PAGE. Proteins in the gels were transferred to polyvinylidene fluoride (PVDF) membranes by the wet transfer method. PVDF membranes were soaked in 5% skimmed milk and blocked for 1.5 h. Proteins were separated using PER2, PD-L1, IKKα, IKKβ, p-IKKα/β, IKKγ, IκBα, p-IκBα, p65, p-p65, HSP90, and Flag primary antibodies with incubation overnight at 4 °C. The details of the antibodies are shown in Supplemental Table S4. This was followed by incubation with horseradish peroxidase (HRP)-labeled secondary antibodies for 60 min at 37 °C. Western protein detection fluorescent reagent (34577, Thermo Scientific, USA) was used for visualization by with a Chemi Scope S6 (CLINX, China). β-actin was used as a loading control, and the corresponding total protein was used as the loading control for phosphorylated proteins. The assay was performed on the same membrane each time. Each experiment was repeated three times. Protein bands were analyzed using Image J 5.0 software for gray values.

Nuclear and cytoplasmic protein extraction
Nuclear and cytoplasmic proteins were extracted according to the instructions of the P0028 kit (Beyotime, China), and western blotting for protein detection was performed as above. PCNA was used as the loading control for nuclear proteins, and β-actin was used as the loading control for cytoplasmic proteins.

**Western blot and strip assays**
Western blotting was performed to detect the expression levels of IKKα and IKKβ in protein samples fished with IKKα and IKKβ as decoys. The PVDF membranes were then immersed in weakly basic western antibody removal solution (P0025B, Beyotime) to remove the original antibodies and blocked with 5% skimmed milk for 1.5 h. The membranes were then placed in contact with ubiquitinated protein primary antibody ubiquitin (P4D1; CST, USA) and incubated overnight at 4 °C. The details of the antibodies are shown in Supplemental Table S4. Then, the PVDF membranes were incubated with HRP-labeled secondary antibody for 1 h at 37 °C and visualized using a Chemi Scope S6 with western protein detection fluorescent reagent. The assay was performed on the same membrane each time. Each experiment was repeated three times. The protein bands were analyzed for gray values using ImageJ 5.0 software.

**Flow cytometry**
For the detection of membrane proteins, 1×10^6 cells per well were inoculated into six-well plates, incubated until cell density reached 80% or more, digested with 0.25% trypsin, washed twice by centrifugation in PBS, resuspended in a 100-μl dilution of
PD-L1 primary antibody (dilution ratio: 1:500), incubated for 1 h at room temperature, and washed twice again by centrifugation in PBS. Then, cells were resuspended in a 100-μl dilution (dilution ratio: 1:500) of Dylight 649-coupled secondary antibody, incubated for 30 min at room temperature and protected from light, and washed twice by centrifugation in PBS. Details of the antibodies used are shown in Supplemental Table S4. Last, cells were resuspended in 200 μl PBS, and the cell surface PD-L1 positive expression rate was detected by flow cytometry (FACSVantage, BD, USA) with blank control cells incubated with PBS instead of antibody used as the background signal.

For the detection of tumor-infiltrating CD8⁺ T cells, samples were mechanically separated into small fragments using scissors and scalpel, placed in DMEM containing 2% fetal bovine serum, collagenase IV (1 mg/ml; 17104019, Thermo Fisher Science), DNAase (10 μg/ml; D5025, Sigma-Aldrich), Dispase (0.6 mg/ml; 17105041, Gibco), and CaCl₂ (3 Mm; 21115, Sigma-Aldrich) and incubated at 37 °C with shaking at 200 rpm for 40-60 min. RPMI containing 10% fetal bovine serum was added to terminate the digestion, and the cells were filtered through a 40-μm cell filter (08-771-1, Thermo Fisher Science), followed by washing in PBS. Then, the cells were resuspended in 100 μl of diluted CD8 primary antibody, incubated for 1 h at room temperature, protected from light, washed twice by centrifugation in PBS, and resuspended in 200 μl of PBS. Antibody details are shown in Supplemental Table S4. Finally, tumor-infiltrating CD8⁺T cells were detected by flow cytometry (FACSVantage, BD, USA).
**T-cell-mediated tumor cell killing assay**

T cells from total human PBMCs were pre-activated with CD3 antibody (100 ng/ml) and IL-2 (10 ng/ml); 100 μl of tumor cell suspension at a concentration of $1 \times 10^5$ cells/ml was added to each well of a 96-well plate, with three replicate wells set up for each group; and the plates were placed in an incubator (37 °C, 5% CO$_2$) overnight. After the tumor cells had adhered to the wall, pre-activated T cells were added to each well at a tumor cells/T cells ratio of 1:5 and co-cultured with the tumor cells for 24 h. Then, the tumor cell survival rate was detected using Cell Counting Kit-8 assay (CK04, Dojindo, China) after T cell removal. The experiment was repeated three times.

**CHX chase assay**

Cells from each group were inoculated into six different 6-cm dishes. After the cells had adhered to the wall, the medium was aspirated, and 2 ml of DMEM containing 100 μg/ml actinomycin (CHX; Sigma-Aldrich, USA) was added to each dish. Total protein was extracted at the 0 h, 6 h, 12 h, 18 h, 24 h, and 30 h time points and assayed by western blotting.