SUPPLEMENTARY MATERIALS AND METHODS

Cell culture

Human monocytic cell line THP-1 and human lymphoma cell line Raji are obtained from the American Type Culture Collection and cultured according to standard protocols.

Flow cytometry

For evaluating the phagocytosis of tumor cell DNA by macrophages, the macrophages were labeled with CellTracker Deep Red (CTDR, 0.25 μM) for 25 min at 37°C and then cocultured with CD19 CAR-T cells and Raji cells that were labeled with EdU for 24 h. The macrophages were washed and resuspended in phosphate buffered saline (PBS) and then labeled with EdU Flow Cytometry Kit-488 according to the manufactory's instruction. After washing with PBS twice, the labeled cells were analyzed on a FACScan flow cytometer using CellQuest software (BD Biosciences). $CTDR^+$ 488 $Fluorophore^+$ cells represent the macrophages phagocytosing tumor cell DNA. The experiments were repeated at least twice.

Quantitative real-time PCR (qRT-PCR)

To determine the IL-1β mRNA expression in macrophages or Raji cells, and CAR-T cells, total RNAs were prepared using Trizol reagent (Invitrogen) from each sample and reverse transcribed using One-Step gDNA Removal and cDNA Synthesis Kit (TransGen Biotech). The levels of IL-1β mRNA were detected by qRT-PCR following the manufacturer's instruction (TransGen Biotech) with the primers 5'-ATGATGGCTTATTACAGTGGCAA-3' (sense) and

5'-GTCGGAGATTCGTAGCTGGA-3' (antisense). The results were analyzed using the comparative threshold cycle method with GAPDH as an internal control with the primers 5'-CAAGGTCATCCATGACAACTTTG-3' (sense) and 5'-GTCCACCACCCTGTTGCTGTAG-3' (antisense). The experiments were performed three times independently.

ELISA

The level of IL-1β in the supernatants of the co-culture system was detected using the ELISA kits (R&D Systems) following the manufacturer's instructions. The experiments were performed three times independently.

Western Blot

For analysis of cleaved caspase-1 and cleaved IL-1β, the supernatants of the co-culture system were collected. For analysis the expression of pro-IL-1β, pro-caspase-1, AIM2, NLPR3, NLRC4, and NALP1 in the macrophages from the coculture, the total cell lysates of the macrophages were prepared. In order to determine the induction of PD-L1 and IDO in the macrophages upon IL-1β stimulation or in the coculture, total cell lysates of the macrophages were subjected to SDS-PAGE and transferred onto the polyvinylidene difluoride membranes. The membranes were incubated with the antibodies against cleaved IL-1β, cleaved caspase-1, pro-IL-1β, pro-caspase-1, AIM2, NLPR3, NLRC4, NALP1, PD-L1, IDO, β-actin and GAPDH (Cell Signaling Technology). Peroxidase-conjugated anti-rabbit antibody or anti-mouse antibody (Cell Signaling Technology) was used as secondary antibody. The stained membranes were visualized by using enhanced

chemiluminescence (Perkin Elmer). The experiments were performed in duplicate.

Co-immunoprecipitation

The whole cell lysates of the macrophages were incubated with Protein A/G PLUS-Agarose (Santa Cruz) and rabbit anti-human AIM2 antibody (Cell Signaling Technology) for 4 h at 4°C. After washing, the agarose-antigen-antibody complex was resuspended in the sample buffer and cooked for 5 min. Then the supernatant was analyzed by SDS-PAGE and Western Blot.

AIM2 shRNA mediated silencing

The shRNAs targeting AIM2 (1#, 5'-CCCGCTGAACATTATCAGAAA-3' and 2#, 5'-GCCACTAAGTCAAGCTGAAAT-3') and control shRNA (5'-TTCTCCGAACGTGTCACGT-3') were obtained from Genechem Co., LTD. 5×10^5 THP-1 cells were transduced with the lentiviral vectors carrying the shRNAs (MOI = 30) for 24 h at 37°C with HiTransG A (Genechem).

Immunofluorescence

2.5×10^5 THP-1 cells were seeded in 35 mm dishes and then polarized into M2 macrophages. For evaluating the phagocytosis of tumor cell DNA by macrophages, the macrophages were labeled with CTDR and then cocultured with CAR-T cells and Raji cells that were labeled with EdU for 24 h. The dishes were washed twice and the adherent cells were fixed with 4% paraformaldehyde at 4°C for 10 min followed by washing with PBS. After blocking with 3% BSA in PBS for 45 min at room temperature, the cells were incubated with the anti-AIM2 antibody (Cell Signaling Technology) for 12 h at 4°C. After washing with PBS twice, the cells were incubated

with Alexa Fluor 555-labeled secondary antibody (Invitrogen) and BeyoClick™ EdU-488 kit (Beyotime) following the manufacture's instruction. The cells were then stained with DAPI (5 μg/ml) in PBS for 15 min and observed under a laser scanning confocal microscope (Zeiss LSM880). Digital images were taken.

For evaluating the PD-L1 and IDO expression, the cells were incubated with the anti-PD-L1 and anti-IDO antibodies (Cell Signaling Technology), followed by washing. Then the cells were stained with indicated secondary antibodies (Invitrogen).

Cytotoxicity of tumor-specific CAR-T cells

1×10^6 PBMCs/well were seeded into the 6-well plate and polarized into M2 macrophages. The M2 macrophages were cocultured with CAR-T cells and Raji cells for 24 h.

To evaluate the effects of the interaction between CAR-T cells, tumor cells and macrophages on the cytotoxicity of CAR-T cells, 5×10^5 Raji/Luc cells were added into the coculture system mentioned above. To minimize the deference between different groups in the contents of cultural supernatants, the medium of the coculture were changed before the addition of the Raji cells expressing luciferase stably (Raji/Luc). 48 h later, D-Luciferin (Sigma) was added into the plate and the cells were harvested for quantification of luciferase activity. The number of viable target cells (Raji/Luc) was determined using a standard curve generated by serial dilution of Raji/Luc cells. The formula used to calculate the percentage of cytotoxicity is as follows: (Cell number in untreated well – Cell number in assay well)/(Cell number in

untreated well).

Another strategy was employed to investigate whether the phenotype of the macrophages is altered after contacting with CAR-T and tumor cells. After the coculture, the CAR-T cells, Raji cells, and the supernatants were discarded. The plates were washed with PBS, and then the new CD19 CAR-T cells were added and coincubated with the macrophages at a ratio of 2:1 for 36 h. The CAR-T cells were then isolated and incubated with the Raji/Luc cells at a CAR-T/target cell ratio of 2.5:1.