Supplementary Materials

Supplementary Methods

Cell authentication

Cells were purchased from Jennio Biotech Co., Ltd. (Guangzhou, China). To authenticate the identities of the human tumor cell lines, genomic DNA of those tumor cells was extracted, and short tandem repeat (STR) loci were amplified by PCR. The STR profiles were further compared with the STR profiles of the reference cell lines in the DSMZ STR database (Date: 2019-12-06 for Jurkat; 2017-08-17 for DLD1).

Assessment of the MSI status

Organoid cells and matched PBMCs of dMMR1 and dMMR2 were used to evaluate the MSI-H status of tumor organoids. DNA from tumors and PBMCs was detected using a panel of 5 mononucleotide repeats (D5S346, BAT26, BAT25, D17S250, and D2S123) to determine MSI status. DNA was extracted using a DNA extraction kit according to the manufacturer's protocol (Qiagen). The DNA concentration was measured using an ND-1000 spectrophotometer (Thermo). MSI testing was performed by PCR using ABI PRISM True Allele PCR Premix (Applied Biosystems) according to the manufacturer's protocol, and fluorescently labelled primers were used to co-amplify the 5 mononucleotide-repeat markers. Electrophoresis of PCR products was conducted using an ABI 3500XL Gene Analyzer (Applied Biosystems), and MSI detection data were analyzed by GeneMapper software 4.1 (Thermo). Identical fragment profiles between tumor cells and matched PBMCs for all 5 mononucleotide loci were considered microsatellite stable; discordance in one mononucleotide locus was considered MSI low.

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Discordance in 2 or more mononucleotide loci was considered MSI-H.

Antibodies for flow cytometry

The following antibodies were used for flow cytometry analysis: CD3e-PC5.5 (551163, BD Bioscience), mouse CD8a-FITC (553030, BD Bioscience), mouse CD4-FITC (557307, BD Bioscience), mouse CD279 (PD-1)-APC (562671, BD Bioscience), mouse granzyme B-PE (12-9392-82, eBioscience), mouse IFN-γ-APC (562018, BD Bioscience), mouse perforin1-PE (12-9392-82, eBioscience), human CD3/CD4/CD8/CD45 (340499, BD Bioscience), human CD8-PE (557086, BD Bioscience), human CD107-PE (565306, BD Bioscience), human CD69-FITC (557049, BD Bioscience), human granzyme B-Alexa700 (561016, BD Bioscience), human IFN-γ-PC7 (561036, BD Bioscience), human CD279-BV421 (562516, BD Bioscience), human perforin1-PC5.5 (563762, BD Bioscience) and LEF1 (ab215999, Abcam). For LEF1 staining, goat anti-rabbit IgG (ab150081, Abcam) was used as the secondary antibody.

Antibodies for western blotting

Primary antibodies against mouse DKK1 (AF1096, R&D Systems), human PDCD1 (ab52587, Abcam), human GSK3β (ab2602, Abcam), human pGSK3β (ab75814, Abcam), human LRP6 (ab75358, Abcam), and human Lamin B1 (ab16048, Abcam) were applied.

Antibodies for IHC

Primary antibodies against DKK1 (1:200, ab109416, Abcam), CD3 (1:250, clone LN10, ORIGENE), CD4 (1:50, clone IF6, ORIGENE), CD8 (1:80, clone SP16, ORIGENE),

FOXP3 (1:100, ab20034, Abcam), PD-1 (1:100, UMAB199, ZSGB-BIO), MLH1 (clone ES05, ZSGB-BIO), MSH2 (clone RED2, ZSGB-BIO), MSH6 (clone EP49, ZSGB-BIO), and PMS2 (clone EP51, ZSGB-BIO) were used. The optimum dilutions for each antibody and the time for DAB staining were determined by IHC staining using positive controls according to the manufacturer's instructions (DKK1: liver sections; CD3, CD4, CD8, FOXP3 and PD-1: tonsil sections; MLH1, MSH2, MSH6 and PMS2: normal colorectal sections). To ensure reproducibility across runs, the optimal staining protocol was further validated using positive control sections with 3 replicates before the experiment.

Supplementary Figure Legends

Supplementary Fig S1. (A) In the GEO dataset GSE39582, the overall survival of CRC patients with high and low DKK1 expression was compared. (B) Typical images of each IHC score for DKK1 are shown (100X). (C) OS of dMMR/MSI CRC patients was compared between the high (*N*=45) and low (*N*=35) DKK1 expression groups. (D) Comparison of the DKK1 IHC scores between sporadic and Lynch-associated dMMR/MSI CRCs. (E) Progression-free survival after anti-PD-1 therapy in dMMR/MSI patients with high and low serum DKK1. (F) Spearman rank correlation test between T cells and DKK1 IHC scores. CN: cancer nest; TS: tumor stroma; *: $0.01 \leq P < 0.05$.

Supplementary Fig S2. (A and B) Typical images of each IHC score for DKK1 in stage II/III CRCs are shown (100X) (A), and DFS was compared between the high (*N*=40) and low (*N*=38) DKK1 expression groups (B). (C-E) Typical images of IHC staining for PD-1 and DKK1 in consecutive slices are shown (100X) (C), and the PD-1-positive ratio in total CD8+ TILs was compared between the high (*N*=41) and low (*N*=32) DKK1 expression groups (D). Spearman rank correlation test between the PD-1-positive ratio in total CD8+ TILs and DKK1 IHC scores is shown (E). *: $0.01 \leq P < 0.05$.

Supplementary Fig S3. (A) *DKK1* knockdown (sh1 and sh2) and control (Ctrl) CT26 cells were cultured with fresh medium for 48 hours, and then both the supernatant and cells were collected for western blotting to evaluate DKK1 levels. (B) PBMCs of tumor-grafted mice (*N*=6 for each group) were assessed by flow cytometry to evaluate the proportion of CD8+ T cells. (C-D) The ratio of IFN-γ (IFNG) + cells in CD8+ TILs (C) and CD8+ T cells co-cultured with CT26 cells (D) was measured using flow cytometry. (E) Lymphocytes were derived from mouse spleens, pre-stimulated and cultured in the presence (DKK1) or absence (Ctrl) of DKK1. Immune activation markers, including IFNG, granzyme-B (GZMB), and perforin-1 (PRF1), in CD8+ T cells were measured using flow cytometry. *: $0.01 \leq P < 0.05$; **: $0.001 \leq P < 0.01$; ***: $0.0001 \leq P < 0.001$; ****: $P < 0.0001$.

Supplementary Fig S4. (A) Images of IHC staining of the MMR proteins MLH1, MSH2, MHS6, and PMS2 in 2 dMMR CRC slices (200X). (B) the MSI status of organoids was determined. Discordances in D5S346 (blue at the front), BAT26 (green at the front), BAT25 (black), D17S250 (blue at the back), and D2S123 (green at the back) between organoid tumor cells and matched PBMCs were evaluated using PCR. *: detected discordance.

Supplementary Fig S5. (A) Typical bright field image (400X) and HE staining of dMMR2 organoids. (B-C) Organoids were cultured in 24-well plates with mock and IgG (Ctrl), 100 ng/ml DKK1 and IgG (DKK1), and mock and DKK1-neutralizing antibody (α-DKK1) in the presence of Matrigel for 24 hours (Day 0). Then, pre-treated

T cells were added to the medium and co-cultured for another 3 days (Day 3). Typical images on day 0 and day 3 were captured (100X), and the Matrigel was collected for HE staining (100X) (B). In addition, organoids co-cultured with T cells for 3 days were derived and digested into single cells, and the number of viable living cells was counted per well (C). (D) Cell apoptosis assays of organoids co-cultured with lymphocytes in the absence of Matrigel. (E) T cells were pre-stimulated and treated with 100 ng/ml DKK1 plus IgG (DKK1), DKK1 neutralizing antibody with mock (α-DKK1), and IgG with mock (Ctrl) for 24 hours. The MFIs of IFNG, GZMB, and PRF1 in CD8+ T cells were measured by flow cytometry. (F) CD8+ cells were derived from pre-treated T cells. The Transwell assay was conducted with the supernatant of PDOs. Typical images (100X) are shown. *: $0.01 \leq P < 0.05$; **: $0.001 \leq P < 0.01$; ****: $P < 0.0001$.

Supplementary Fig S6. (A) The expression of naïve markers, inhibitory receptors, effector molecules, co-stimulatory molecules, transcription factors and Treg markers was compared between mock- and DKK1-treated T cells from dMMR1. Genes with changes were marked using asterisks. (B) Immune activation markers in dMMR2 CD8+ T cells was measured by flow cytometry after culturing in the presence or absence of DKK1. (C) dMMR2 organoids were co-cultured with CD8+ T cells pre-treated with mock (Ctrl) or DKK1 (DKK1), and then tumor cells were collected for the cell apoptosis assay. (D) Immune activation markers in dMMR2 CD8+ T cells were measured by flow cytometry after PD-1 blockade in the presence or absence of DKK1. (E) dMMR2 organoids were co-cultured with CD8+ T cells pre-treated with PD-1 blockade (Ctrl+α-PD-1) or PD-1 blockade plus DKK1 (DKK1+α-PD-1), and then tumor cells were collected for the cell apoptosis assay. (F) A violin plot of *LRP6* expression in each subpopulation of CD8+ TILs was generated on http://crctcell.cancer-pku.cn/. CD8_C01-LEF1: Naïve CD8+ T cells; CD8_C02-GPR183: Central memory CD8+ T cells; CD8_C03-CX3CR1: Effector memory CD8+ T cells re-expresses CD45RA/Effector CD8+ T cells; CD8_C04-GZMK: Effector memory CD8+ T cells; CD8_C05-CD6: Tissue resident memory CD8+ T cells; CD8_C06-CD160: CD8+ intraepithelial lymphocyte; CD8_C07-LAYN: Exhausted CD8+ T cells; CD8_C08-SLC4A10: Mucosal-associated invariant T cells. (G) The ratio of LEF1+ cells in CD8+ T cells treated with mock or DKK1 was measured using flow cytometry. (H) The infiltration levels of CD8+ naïve cells, CD8+ effector memory cells and CD8+ cytotoxic cells were compared between the DKK1 high and low expression groups of MSI CRC cases from TCGA using ImmuCellAI. *: $0.01 \leq P < 0.05$; **: $0.001 \leq P < 0.01$; ***: $0.0001 \leq P < 0.001$.

Supplementary Fig S7. (A) Gene-set enrichment analysis (GSEA) concerning epithelial-mesenchymal transition (EMT) was conducted based on the RNA sequencing of CD8+ T cells. (B) Expression of Tc2, Tc9, and Tc17 products was compared between mock- and DKK1-treated CD8+ T cells from dMMR1 and dMMR2. (C-D) Western blot and coimmunoprecipitation assays were conducted on mock- (Ctrl) and DKK1-treated (DKK1) CD8+ T cells of dMMR2 to measure the GSK3β phosphorylation level as well as its interaction with LRP6 (C). Subcellular fractionation was performed on

mock- (Ctrl) and DKK1-treated (DKK1) CD8+ T cells of dMMR2 to measure the nuclear and cytoplasmic localization of GSK3β (D). (E) Downstream effectors of *TBX21* in the mock- (Ctrl) and DKK1-treated (DKK1) CD8+ T cells of dMMR2 were evaluated by qPCR. ***: $0.0001 \leq P < 0.001$; ****: $P < 0.0001$.

Supplementary Fig S8. DKK1-neutralized (α-DKK1) and IgG-treated (Ctrl) dMMR2 organoids were co-cultured for 6 hours with PD-1-neutralized (α-PD-1) or IgG-treated (IgG) T cells, respectively, and a cell apoptosis assay was conducted. *: $0.01 \leq P < 0.05$; **: $0.001 \leq P < 0.01$; ***: $0.0001 \leq P < 0.001$.