Electronic Supporting Information

Adoptive Cellular Immunotherapy of Tumors *via* Effective CPG Delivery to Dendritic Cells Using Dendrimer-Entrapped Gold Nanoparticles as a Gene Vector

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Experimental

Materials

G5 PAMAM dendrimers were purchased from Dendritech, Inc. (Midland, United States). *m*PEG with a carboxyl group at the other end (*m*PEG-COOH, Mw = 2000) was provided by Shanghai Yanyi Biotechnology Corporation (Shanghai, China). The Primary Amino Nitrogen (PANOPA) Assay Kit was obtained from Megazyme (Wicklow, Ireland). Agarose was from Gene Tech (Shanghai, China). Ethidium bromide (95%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), dimethyl sulfoxide (DMSO), N-hydroxy-succinimide (NHS), sodium borohydride ($NaBH_4$), were from Aldrich (St. Louis, MO). MTT and CCK-8 assay kit were acquired from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). RPMI 1640, fetal bovine serum (FBS), penicillin, and streptomycin was from Lingfeng Chemical Reagent Co.,Ltd. (Shanghai, China). B16 cells were ob-tained from the Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences. Single-stranded CpG (5'-

TCCATGACGTTCCTGACGTT-3') was purchased from Sangon Biotech (Shanghai, China) Co., Ltd. All the antibodies were purchased from ebioscience (USA). Cytokines IL-4 and GM-CSF were purchased from BBI (USA). Nylon wool was purchased from Kisker, Germany. C57BL/6 mice of 6-8 weeks old were obtained from Shanghai Slake Animal Co., Ltd. (Shanghai, China).

Extraction and culture of mouse bone marrow-derived dendritic cells

C57BL/6 mice were killed by cervical dislocation. The femoral and tibial epiphyses were clean off in PBS, and the bone marrow cavity was repeatedly washed by RPMI1640 medium until the bones became white. The Red Blood Cell Lysis Buffer was used to lyse red blood cells. RPMI 1640 medium was used to resuspend the cells and adjust its concentration to 1×10^6/mL. The extracted cells were seeded into 6-well plate, and at the same time, IL-4 and GM-CSF were added for induction at the concentration of 10 ng/mL and 20 ng/ml separately. The whole medium was changed at the first 2 days, then half of the medium was changed at the following 4 days. The immature dendritic cells (imBMDCs) were obtained at 7 day. To confirm the successful induction from bone marrow stem cells to BMDCs, the morphology of the BMDCs was observed at 1, 3, 5, and 7 days through the inverted microscope.

The extract and culture of T cells

C57BL/6 mice were killed by cervical dislocation and immersed in 75% alcohol for 2-5 min. The spleen was obtained in sterile conditions and it was removed to PBS. The spleen was grinded in 400 mesh screen, and the lymphocytes in the spleen were separated using lymphocyte separation buffer. Then, the obtained lymphocytes were filtered by the nylon wool column for 6-8 times to obtain T cells.

MTT assay for allogeneic mixed lymphocyte reaction (MLR)

Mitomycin-C (25 μg/mL) was incubated with mature dendritic cells (mDCs) for 45 min, then

was washed away with PBS. Then T cells were cultured in 96-well plate with the above treated BMDCs at the serious ratio of BMDCs: T cells (1: 10, 1: 20, 1: 50, 1: 100, 1:200 separately). The proliferation of T cells was measured by MTT assay. Five parallel samples were set up for each group, and T cells without any BMDCs were used as control. The calculation of stimulation index was carried out by the follow formula: stimulation index (SI) = (A value of the sample to be tested - A value of the culture control group) / (A value of the negative control group - A value of the culture control group).

Cell Culture

B16 cells (mouse melanoma cells) were obtained from Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences (Shanghai, China). The cells were cultured with RPMI 1640 medium with 10% FBS, 1% penicillin-streptomycin, and incubated at 37 °C in a Thermo Scientific cell incubator (Waltham, MA) with 5% CO_2.

Statistical Analysis

Data were presented as the means ± standard deviations. One-way ANOVA statistical method was adopted to analyze the experimental results. A p value of 0.05 was selected as the significance level, and the data were marked with (*) for $p < 0.05$, (**) for $p < 0.01$, and (***) for $p < 0.001$, respectively.

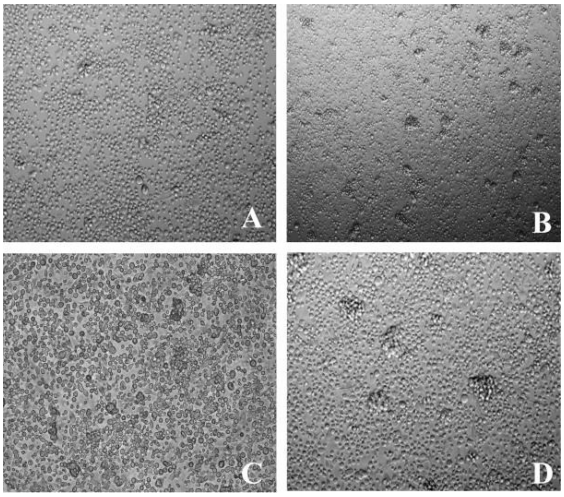

Figure S1. The inverted microscope images of mouse bone marrow-derived dendritic cells after induction for 1 (A), 3 (B), 5 (C), and 7 (D) days.

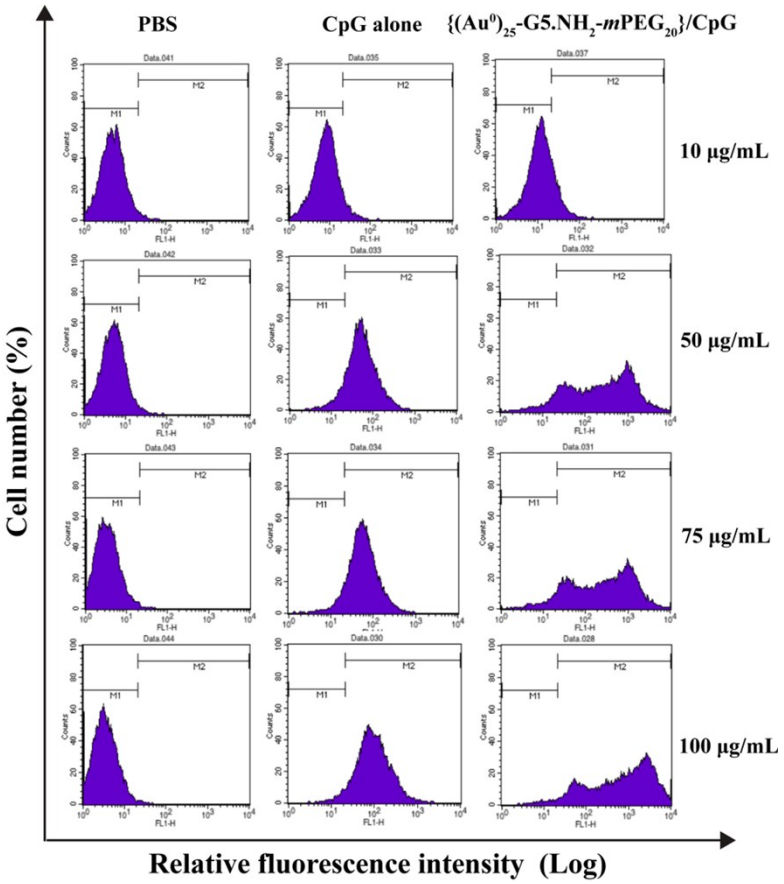

Figure S2. Cellular uptake results of BMDCs detected by flow cytometry. CpG was labeled with FAM (F1 channel).

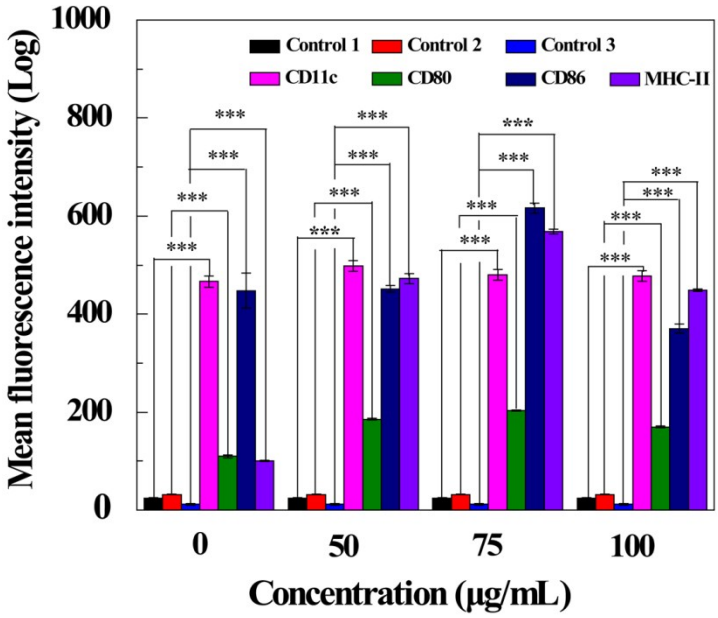

Figure S3. The expression of surfaces antibodies (CD11c, CD80, CD86 and MHC-II) of BMDCs after incubation with different concentrations of $\{(Au^0)_{25}$-G5.NH_2-*m*$PEG_{20}\}$/CpG polyplexes after 24 h.

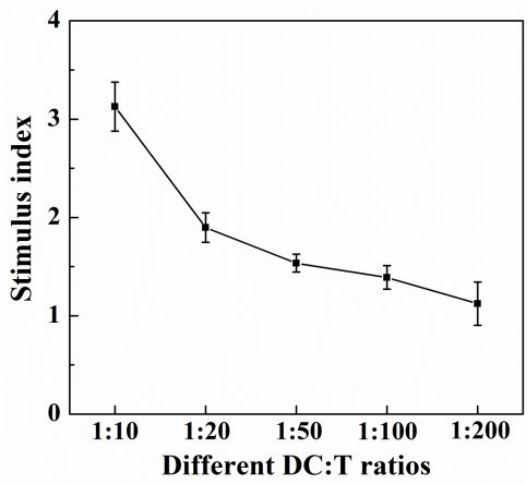

Figure S4. The proliferation result of T cells after co-culture with different ratio of mature BMDCs for 3 days by MTT assay.

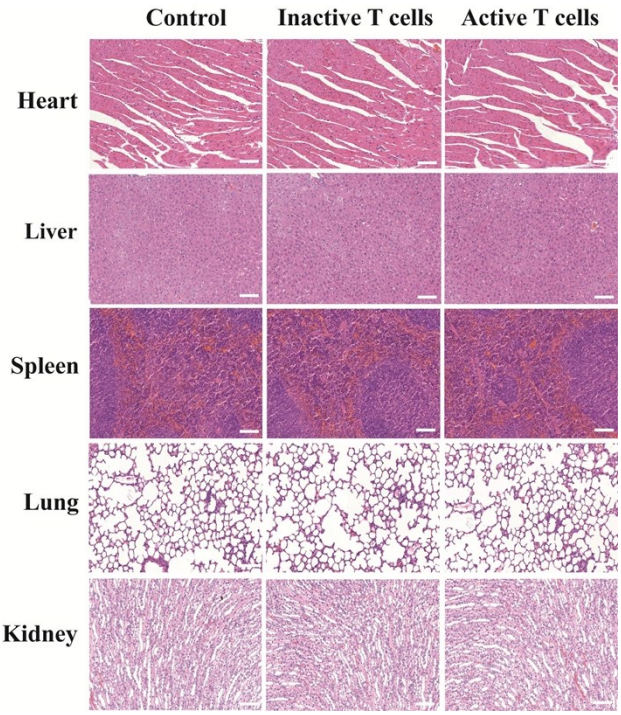

Figure S5. H&E staining images of different organs after intratumoral injection of the activated T cells. The scale bar is 100 μm.

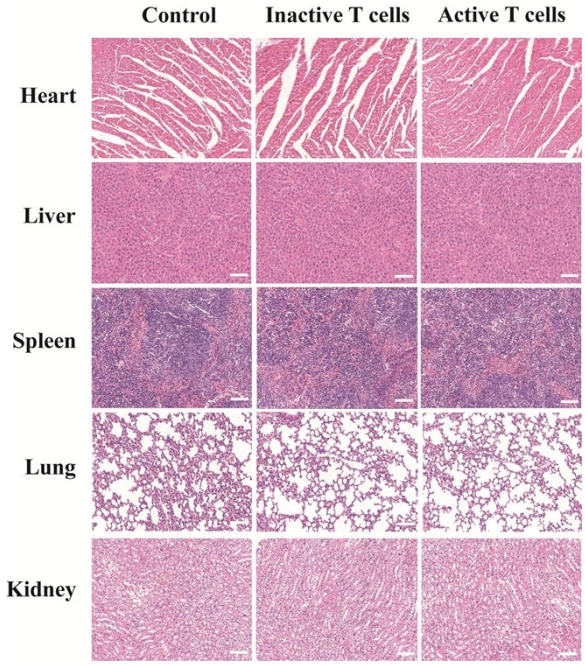

Figure S6. H&E staining images of different organs after intravenous injection of the activated T cells. The scale bar is 100 μm.

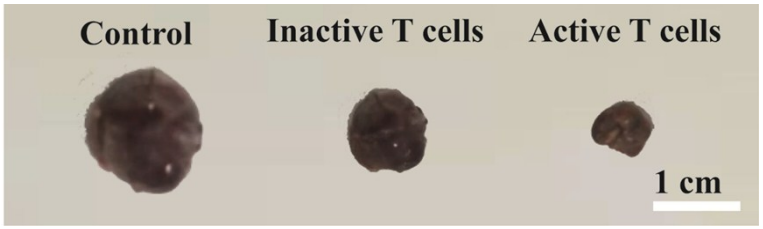

Figure S7. Representative tumor images after the treatment of reinfusion of activated T cells by intratumoral injection.

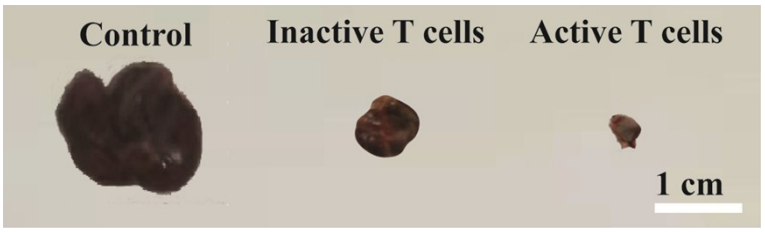

Figure S8. Representative tumor images after the treatment of reinfusion of activated T cells by intravenous injection.