Supporting Information

Coordination microparticles vaccine engineered from tumor cell templates

Xiaoli Wang‡[a], Jiayi Liang‡[a], Chuangnian Zhang[a], Guilei Ma,*[a] Chun Wang[b], Deling Kong[c]

a. Tianjin Key Laboratory of Biomaterial Research, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300192, China. * Corresponding Author, E-mail: bmemgl@126.com

b. Department of Biomedical Engineering, University of Minnesota, Minneapolis, Minnesota, USA

c. The Key Laboratory of Bioactive Materials of Ministry of Education, Institute of Molecular Biology, College of Life Science, Nankai University, Tianjin, 300071, China

‡ XL Wang and JY Liang contributed equally.

Materials

Aluminium chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich. Epigallocatechin-3-gallate (EGCG, 95%) was purchased from Melone Pharmaceutical Co. Ltd (Dalian, China). RPMI-1640, DMEM and fetal bovine serum (FBS). DAPI, red blood cell lysis and Dil were purchased from Solarbio Science &Technology Co. Ltd (Beijing, China). Cy7 NHS ester was purchased from ApexBio (USA). BCA Protein Assay Kit was

purchased from Beyotime (Tianjin, China). Recombinant mouse GM-CSF and IL-4 were purchased from Peprotech (Rocky, Hill, USA). High-purity water with a resistivity of 18.2 MΩ.cm was obtained from the Millipore Milli-Q purification system before use in all the experiments. All chemicals were used without further purification.

Cell lines and animals

The immortalized mouse dendritic cells line DC2.4 and murine melanoma cells line B16 were purchased from the Cell Bank of China Academy of Sciences, and cultured according to the manufacture's guidelines. Female C57BL/6 (6-8 weeks old) mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), and raised under SPF conditions. All procedures were in compliance with the regulations of the Tianjin Committee of Use and Care of Laboratory Animals and the overall project protocol was approved by the Animal Ethics Committee of the Chinese Academy of Medical Science.

Preparation of tumor cell lysates (TL)

Tumor lysates (TL) were obtained as previously reported in the literature.[1] Briefly, B16 cells were seeded in a cell culture dish with RPMI-1640, and the cells were detached from the dish when they were grown to 80% confluency. To acquire TL, tumor cells underwent five freeze-thaw cycles in PBS, and the total protein concentrations of TL were decided by BCA assay. TL was diluted to required concentration before use.

Encapsulation of tumor cells and preparation of TL@EGCG/Al_n

Tumor cells were detached from the dish, and single cell suspension was obtained (2×10^6 cells/mL). Then, EGCG (0.5 mL, 20 mg mL^{-1}) and $AlCl_3$ (0.5 mL, 5 mg mL^{-1}) aqueous solutions were added to 9 mL of the cell suspension. The cell suspension was vigorously mixed for 60 s immediately after the additions of EGCG and $AlCl_3$. Then the cells were centrifuged (200 g, 3 min) and washed with isotonic saline solution to remove excess EGCG and $AlCl_3$ after each layer deposition. This coating process was repeated two or three times, B16@EGCG/Al_n (n=1, 2, 3) were prepared, where n was the number of deposited layer. After coating, the cells were incubated in high-purity water for 30 min to kill the cells, and tumor lysates-containing microparticles (TL@EGCG/Al_n, n=1, 2, 3) were obtained. The whole process was performed at 4 ^oC to block energy-dependent internalization pathways. The loading capacity represented the ratio of protein amount encapsulated in the microparticles to the microparticles weight. SEM and TEM of TL@EGCG/Al_3 were performed.

Preparation of TL-FITC and TL-FITC@EGCG/Al_n

B16 cells were labeled with FITC according to the reported method.[2] Then, TL-FITC and TL-FITC@EGCG/Al_n were obtained with FITC-labeled B16 cells according to the preparation process of TL and TL@EGCG/Al_n. Firstly, FITC was dissolved in DMSO with a concentration of 1 mg mL^{-1} and the stock solution was stored at -20 °C in dark place. B16 cells were harvested and re-suspended in serum-free RPMI-1640, and diluted to a concentration of $2.5\text{-}5\times10^8$ cells/10ml, then 500 uL of FITC stock solution was added to make its final concentration of 50 μg ml^{-1}. After staining at 37 °C for 30 min, FITC-labeled B16 was obtained after centrifuging and washing for three times.

Finally, TL-FITC and TL-FITC@EGCG/Al_n were obtained with FITC-labeled B16 according to the preparation process of TL and TL @EGCG/Al_n as described above.

Generation of BMDCs and antigens uptake by dendritic cells

C57Bl/6 mice were sacrificed and bone marrow was flushed out of femur and tibia. After red blood cell lysis, cells were seeded in six-well plates (2×10^5 cells/ml) and incubated at 37 °C in 5% CO_2. Culture medium was RPMI-1640 supplemented with 10% FBS, 1% penicillin/streptomycin and 1% L-glutamine (Invitrogen), 10 ng/ml IL-4 and 20 ng/ml GM-CSF. On day 6 of culture, the non-adherent cells were harvested, and immature BMDC cells were obtained.

Immature BMDCs (5×10^5 cells/mL) were cultured with TL-FITC@EGCG/Al_3 for 1, 4 and 24 h. After the incubation, the cells were washed twice with PBS, stained with DAPI and Dil. The fluorescent images were recorded by CLSM (CarlZeiss LSM710).

Immature BMDCs were seeded in 24-well plate (1×10^6 cells/well) and cultured with TL-FITC@EGCG/Al_3 for 2, 4, 8, 16, 24, 48 h, the dosage of TL-FITC was 20 ug/well. Then, FACS was used to observe the change in cell population of BMDCs, TL-FITC@EGCG/Al and their mixture.

DC2.4 cells were seeded in 24-well plate (1×10^6 cells/well) and incubated with TL-FITC and TL-FITC@EGCG/Al_3 for 3, 6, 12, 24 and 48 h, the dosage of TL-FITC was 20 ug/well. After extensive washing, FACS was used to determine the antigen uptake by DC2.4.

Inhibitor assays

For the endocytosis inhibitor studies, cells were pre-treated with inhibitors including chlorpromazine (APExBIO, 15 μg mL^{-1}), Filipin III (APExBIO, 10 μg mL^{-1}), rottlerin (Sigma, 25 μg mL^{-1}) and cytochalasin D (APExBIO, 10 μg mL^{-1}) for 1 hour, and then incubated for 4 h with TL-FITC@EGCG/Al_3. Subsequently, cells were washed with PBS, fixed with 4% paraformaldehyde, and then FACS analysis was conducted.

Antitumor therapeutic of TL@EGCG/Al_3 in B16 model

Female 6-8 weeks C57BL/6 mice were s.c. injected in the right flank with 1×10^5 B16 cells suspended in 100 μL PBS at day 0, and randomly assigned into four groups (n=7 or 11): 1) Control group, 2) free TL group, 3) free TL+PIC, and 4) TL@EGCG/Al_3. Mice were monitored every other day for tumor volume and body weight. On day 4, 9 and 14, mice in each group were s.c. immunized with 100 μL the corresponding samples containing 200 ug of TL. Mice were considered as mortality when the tumor volume exceeded 1300 mm^3 or sacrificed at day 25 by cervical dislocation. Single cells suspension of spleen was prepared for FACS analysis of T cells subpopulations. Single cells suspension of spleen was plated in 96-well plate (4×10^6 cells/ml), stimulated with 20 ug mL^{-1} of TL for 48 h. The cells were stained with CD4, CD8 and Ki67. All animal operations were ethically approved by Center of Tianjin Animal Experiment ethics committee and authority for animal protection.

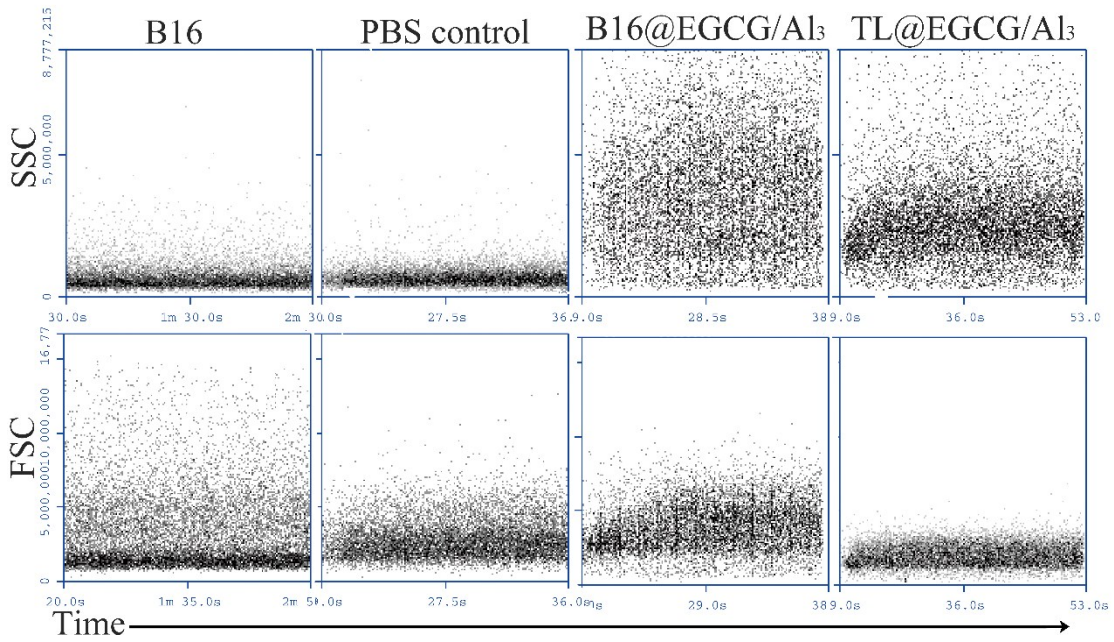

Figure S1. The forward (FSC), respectively side (SSC) scatter channels as function of time.

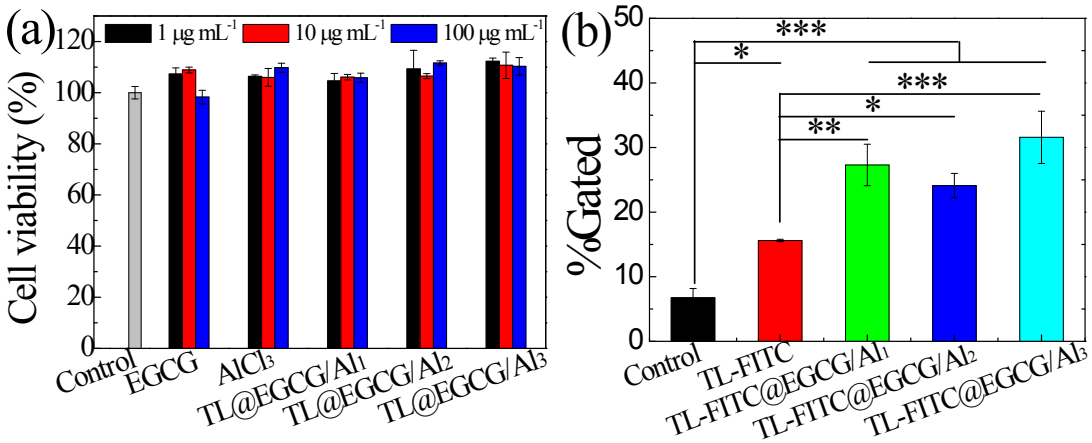

Figure S2. (a) Cell viability measured by MTT assay was performed on DC2.4 cells of the microparticles vaccine and their respective components for 24 h, all the data represent the mean ± SD (n = 6). (b) DC2.4 cells were incubated with TCL-FITC and the microparticles for 24 h, the percentage of positive cells (%Gated) of DC2.4 detected by flow cytometry (Accuri C6), all the data represent the mean ± SD (n = 3). The differences were analyzed by one way ANOVA with Bonferroni multiple comparison post-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

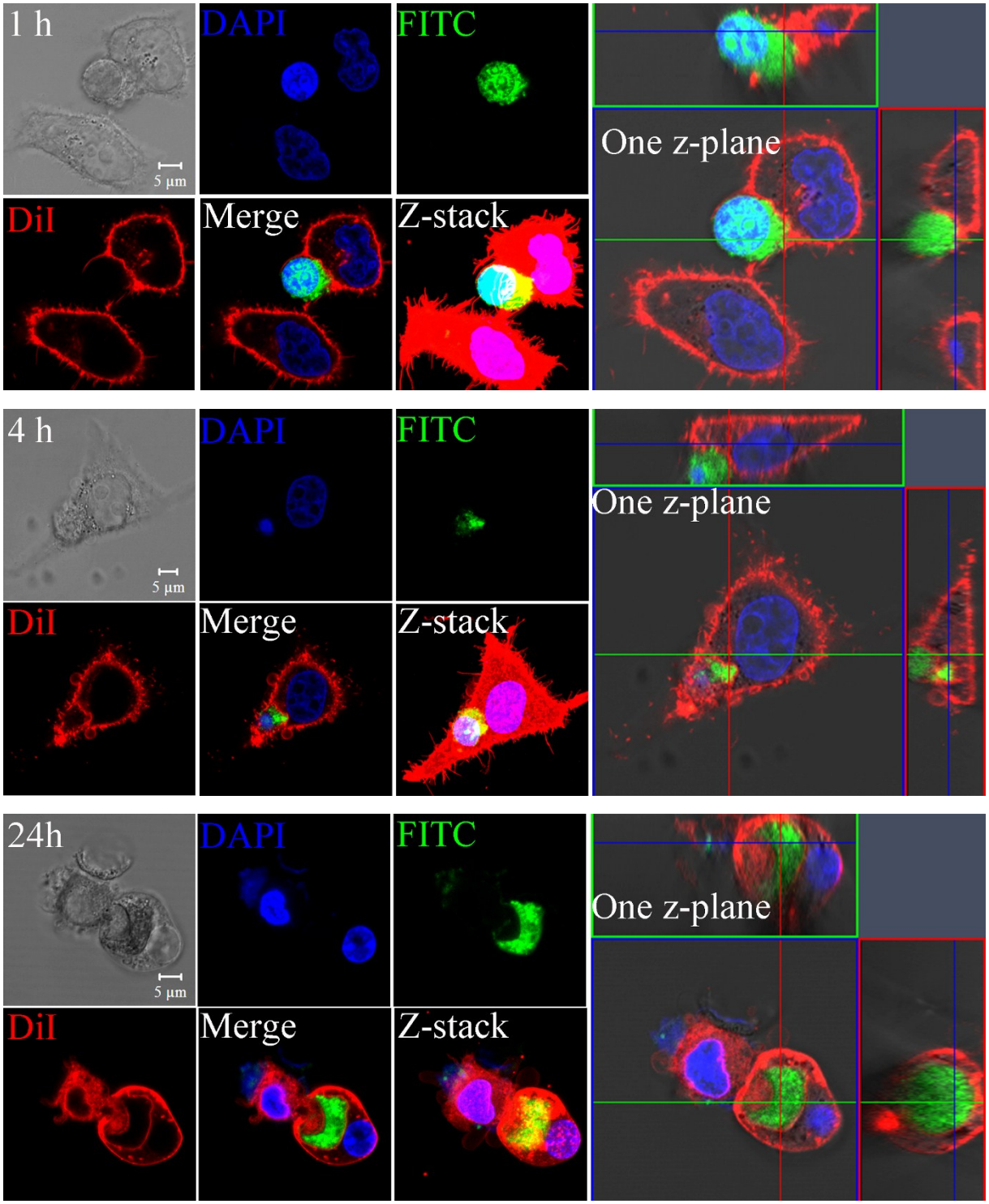

Figure S3. CLSM images of BMDC pulsed with TL-FITC@EGCG/Al_3 for 1 h, 4 h and 24 h. Serial optical sections were acquired at 0.5 μm Z-plane step. CLSM images showed the maximum intensity projection of the full z-stack and one z-plane plus corresponding cross sections.

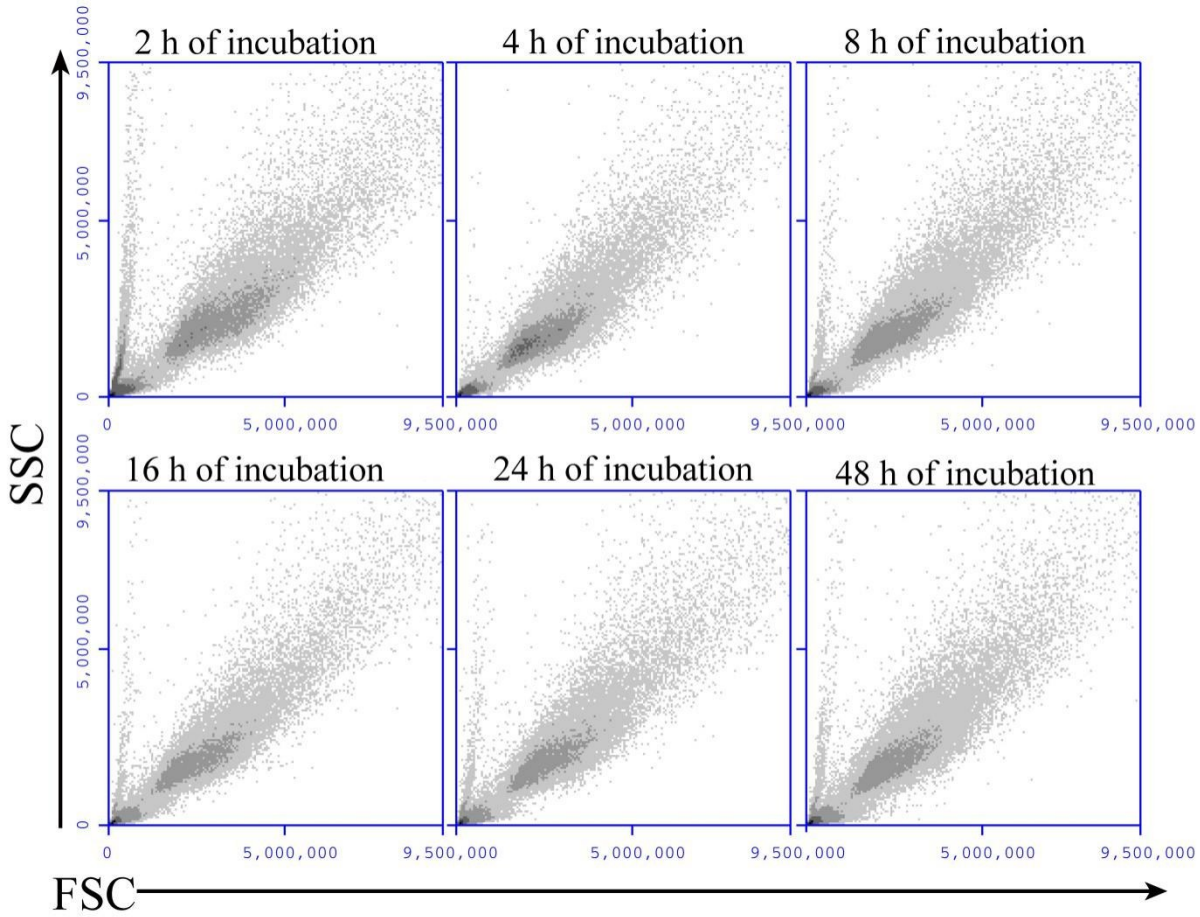

Figure S4. Flow cytometry scatter plot of TL@EGCG/Al_3 microparticles after incubation in medium for different time.

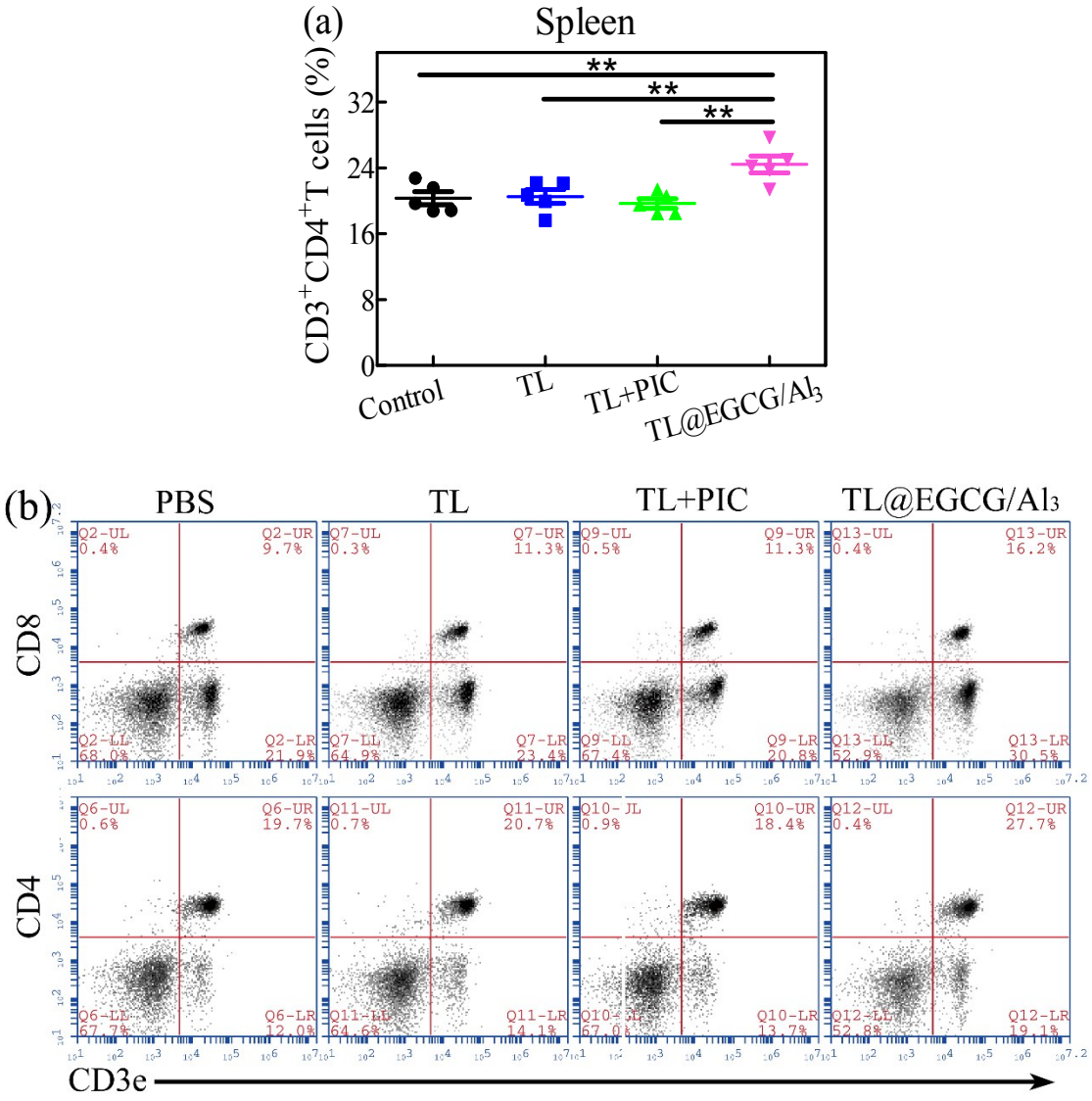

Figure S5. TL@EGCG/Al_3 induced therapeutic effect in B16 tumor model. (a) Phenotype analysis of $CD3^+CD4^+$ T cells in mice spleen 25 days after B16 tumor cells inoculation, (b) The representative FACS plots of spleen T cells subpopulations. Bars shown were mean ± SEM (n=5) and the differences were determined using unpaired student's t-test, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

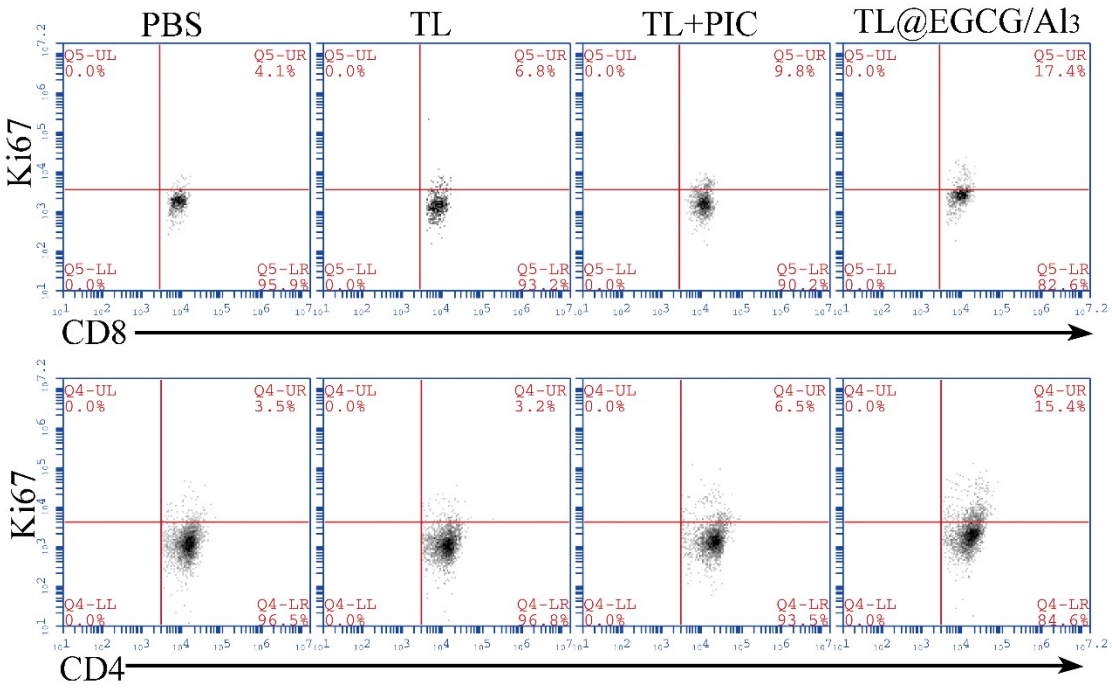

Figure S6. TL@EGCG/Al_3 induced therapeutic effect in B16 tumor model, the representative FACS plots of $Ki67^+$ cells among $CD8^+$ and $CD4^+$ T cells in mice spleen.

References

1. L. Alaniz, M. M. Rizzo and G. Mazzolini, *Pulsing Dendritic Cells with Whole Tumor Cell Lysates*, Springer New York, 2014.
2. E. C. Butcher and I. L. Weissman, *J. Immunol. Methods*, 1980, **37**, 97-108.