Supporting Information for

ORIGINAL ARICLE

Tetrahedral DNA nanostructures synergize with MnO_2 to enhance antitumor immunity *via* promoting STING activation and M1 polarization

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Supporting Figures S1–S6

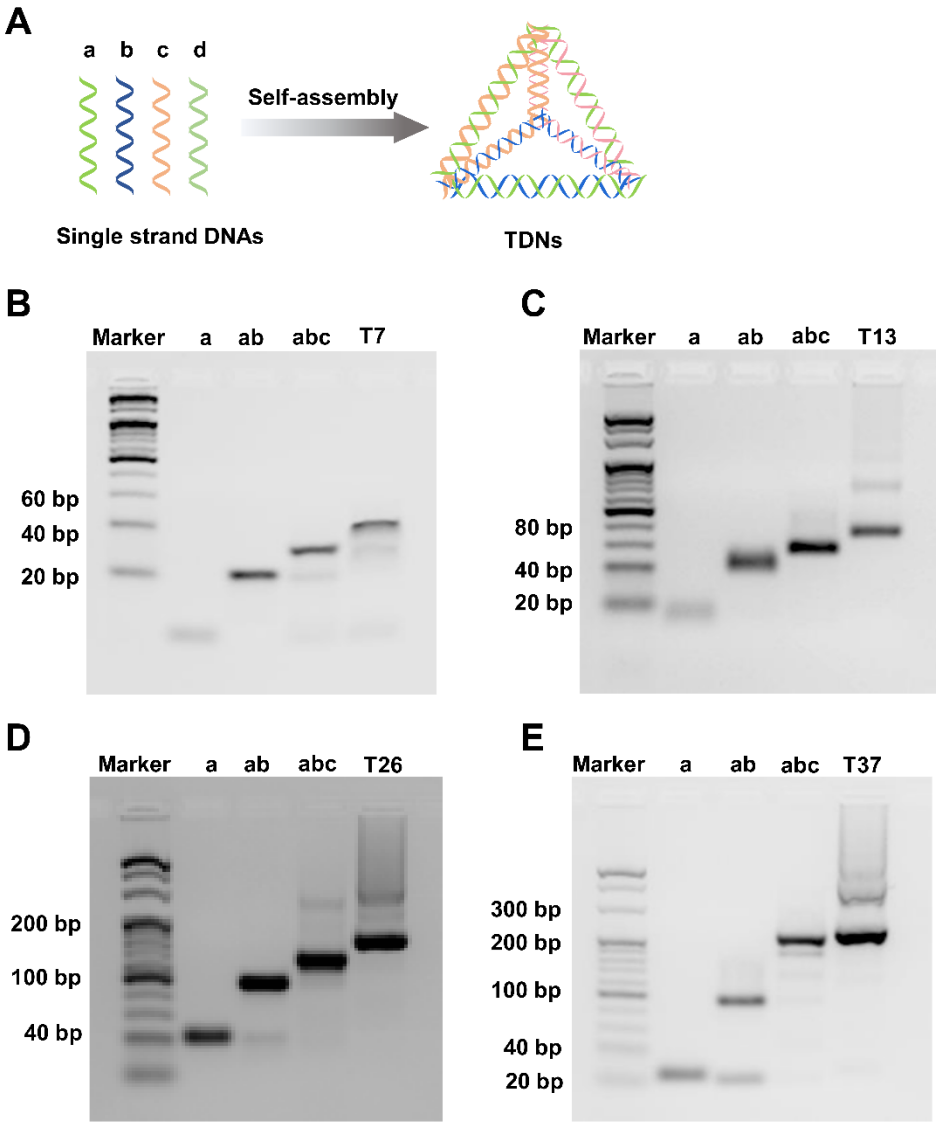

Figure S1 Synthesis of TDNs with different sizes. (A–E) The synthesis of TDNs with sizes of 7, 13, 26 and 37 bp was analyzed by agarose gel electrophoresis.

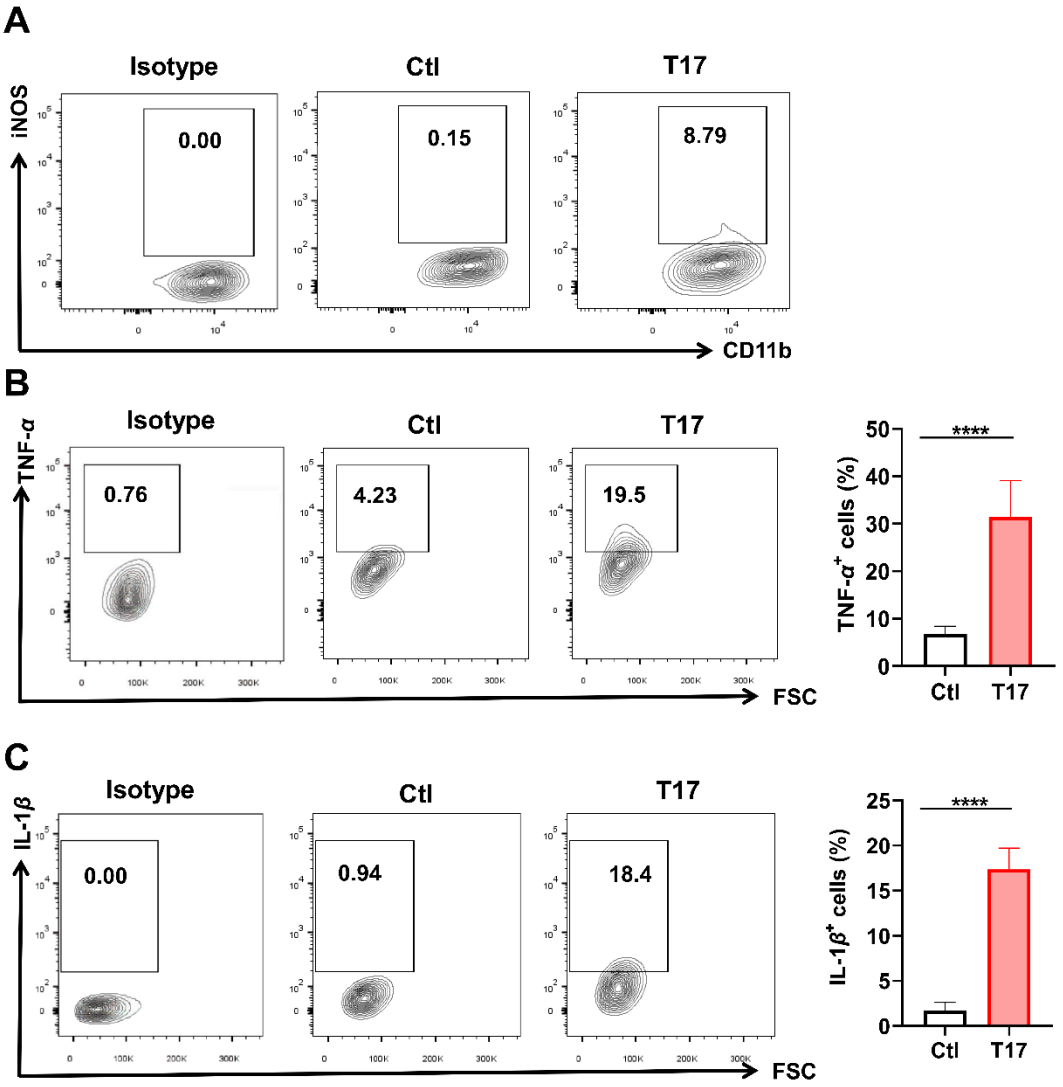

Figure S2 Inflammatory cytokines expression in macrophages after T17 treatment. After treatment of T17 (200 nmol/L), the expression of (A) INOS, (B) TNF-α, (C) IL-1β in BMDMs was detected by flow cytometry, and the representative figures were shown. Data are presented as mean±SD (n=3). $^{****}P$<0.0001.

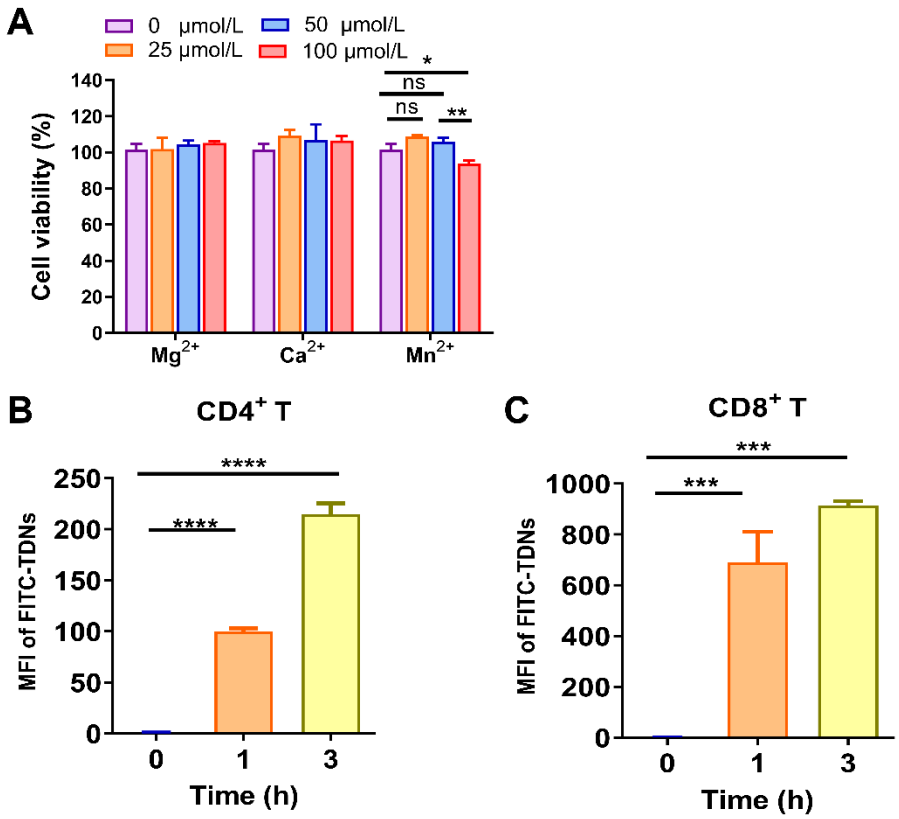

Figure S3 The cytotoxicity of divalent metal cations in macrophages and cellular uptake of T17 by T cells. (A) After treatment with different divalent metal cations including Mg^{2+}, Ca^{2+} and Mn^{2+} for 24 h, cell viability of BMDMs was detected by CCK-8 assay. Cellular uptake of T17-FITC (200 nmol/L) by (B) $CD4^+$ T cells and (C) $CD8^+$ T cells were detected by flow cytometry. Data are presented as mean ±SD (n=3). $^*P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001, $^{****}P$<0.0001, ns, not significant.

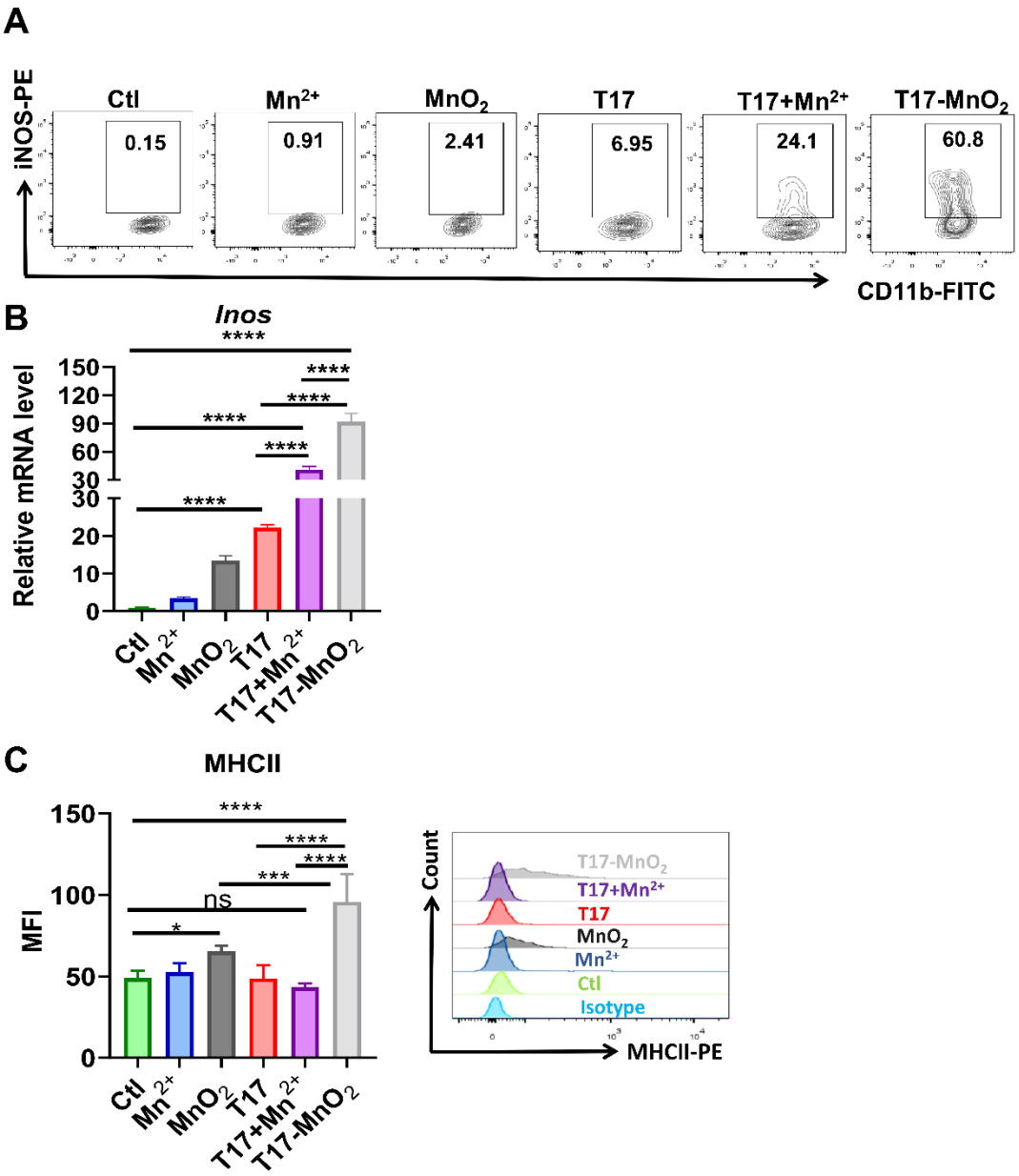

Figure S4 T17-MnO_2 promoted the M1 polarization and the antigen-presentation of BMDMs. After treatment of $MnCl_2$ (50 μmol/L), MnO_2 (50 μmol/L), T17 (200 nmol/L), T17+$MnCl_2$, and T17-MnO_2 for 24 h, the protein level (A) and mRNA level (B) of INOS was detected by flow cytometry and real-time PCR, and the protein level of MHCII (C) was detected by flow cytometry. Data are presented as mean ±SD (n=3). $^{*}P$<0.05, $^{***}P$<0.001, $^{****}P$<0.0001, ns, not significant.

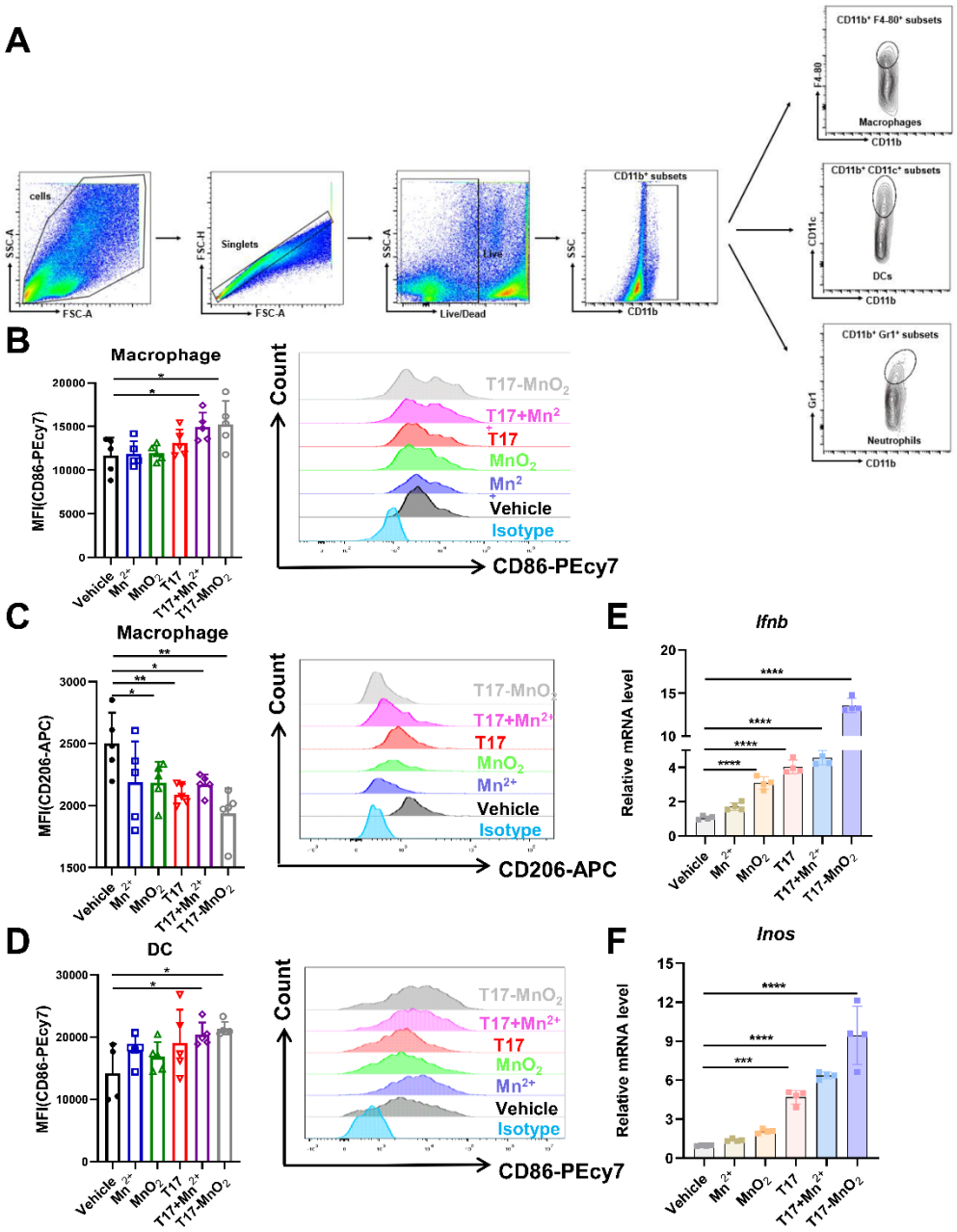

Figure S5 T17-MnO_2 promotes M1 polarization *in vivo*. Hepa1-6 growth and mice treated with vehicle control (0.9% NaCl), Mn^{2+} (50 mmol/L), MnO_2 (2 mmol/L), T17 (3 μmol/L), T17 plus Mn^{2+} or T17-MnO_2. Mice were sacrificed and tumor-infiltrating leukocytes were analyzed by flow cytometry at Day 20. (A) Representative gating strategies of myeloid cells. (B) Representative flow cytometry histogram figure (left) and quantification (right) of CD86 expression on macrophages, (C) CD206 expression on macrophages and (D) CD86 expression on DCs. The mRNA level of *Ifnb* (E) and *Inos* (F) was detected by real-time PCR. Data are presented as mean±SD (n=4). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

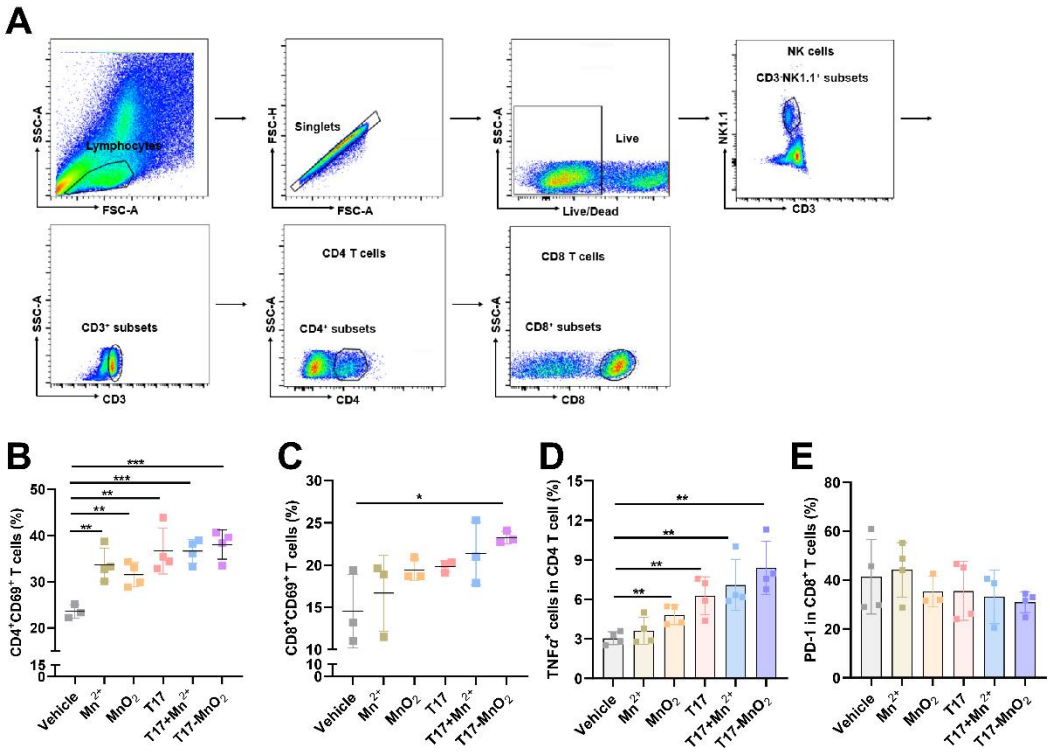

Figure S6 T17-MnO_2 stimulates $CD4^+$ and $CD8^+$ T cell activation. Mice were inoculated with Hepa1-6 cells on Day 0. On Day 8, 14, and 17 mice were treated with either vehicle control (0.9% NaCl), Mn^{2+} (50 mmol/L), MnO_2 (2 mmol/L), T17 (3 μmol/L), T17 plus Mn^{2+} or T17-MnO_2 by i.t. administration. On Day 20, mice were sacrificed and tumor infiltrated leukocytes were analyzed by flow cytometry. (A) Representative gating strategies of NK cells, $CD4^+$ T cells and $CD8^+$ T cells. (B, C) Quantification of tumor-infiltrating $CD4^+CD69^+$ T cells and $CD8^+CD69^+$ T cells. (D) TNF-α^+ in $CD4^+$ T cells. (E) Quantification of PD-1^+ cells in tumor-infiltrating $CD8^+$ T cells. Data are presented as mean ±SD (n=4). $^*P$<0.05, $^{**}P$<0.01 $^{***}P$<0.001.