Electronic Supplementary Information for:

Magnetic nanoclusters mediated photothermal effect and macrophage modulation for synergistically photothermal immunotherapy of cancer

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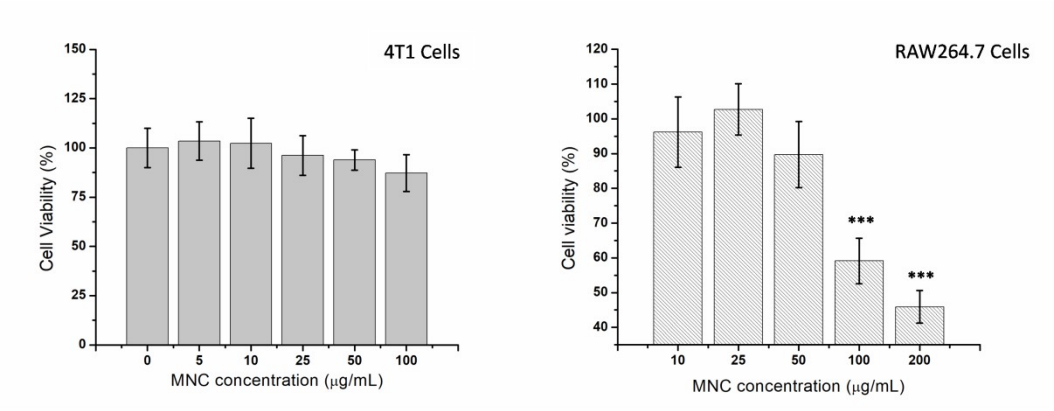

Fig. S1. 4T1 and RAW264.7 cell viabilities after incubation with MNCs for 24 h as a function of MNC concentrations. p <0.001 was denoted as ***.

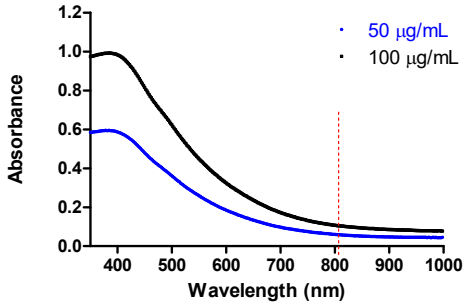

Fig. S2. UV-vis spectra of MNCs aqueous suspensions with different concentrations.

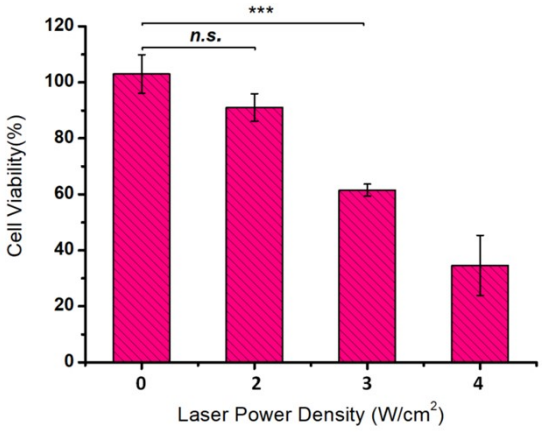

Fig. S3. Cytotoxicity results of RAW264.7 cells treated with MNCs (50 μg/mL) after 5 minutes of laser exposure with different laser power densities (n=3). p < 0.001 and not significant are denoted as *** and n.s., respectively.

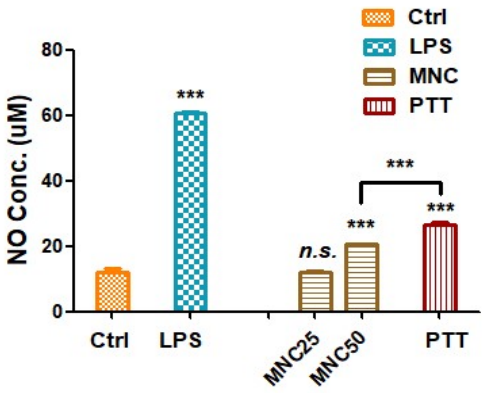

Fig. S4. NO concentration in RAW264.7 cell culture medium after 24 h incubation with different concentrations of MNCs (25 and 50 μg/mL) and PTT treatment (50 μg/mL+laser). $p < 0.001$ and not significant were denoted as *** and n.s., respectively. All results were analyzed using data of Ctrl group as a reference, if not specifically indicated.

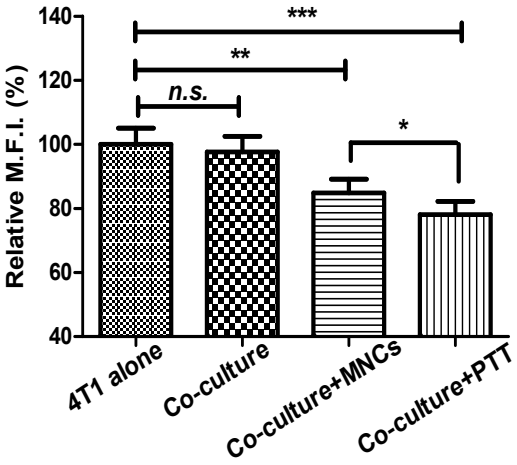

Fig. S5. Quantitative data of Fig 4C.

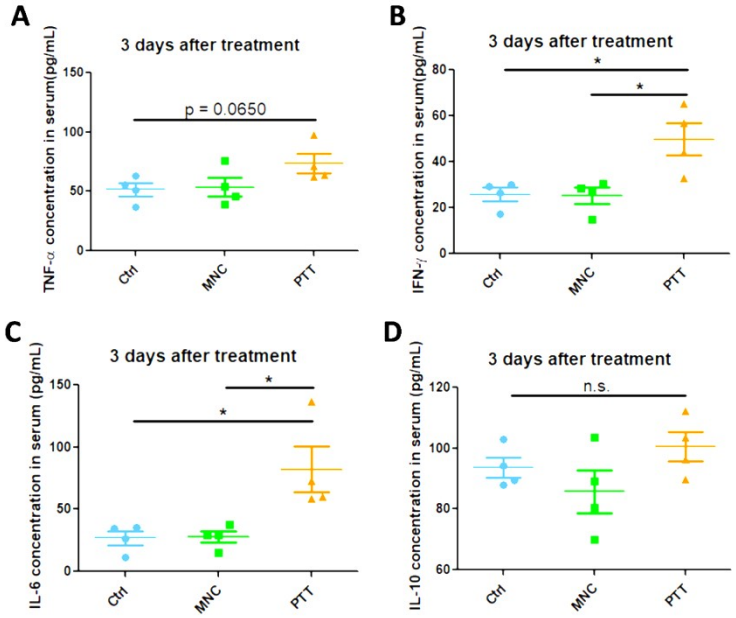

Fig. S6. (A-D) Serum cytokine levels of TNF-α (A), IFN-γ (B), IL-6 (C) and IL-10 (D) from mice 3 days after different treatments.p <0.05 and not significant were denoted as * and n.s., respectively.

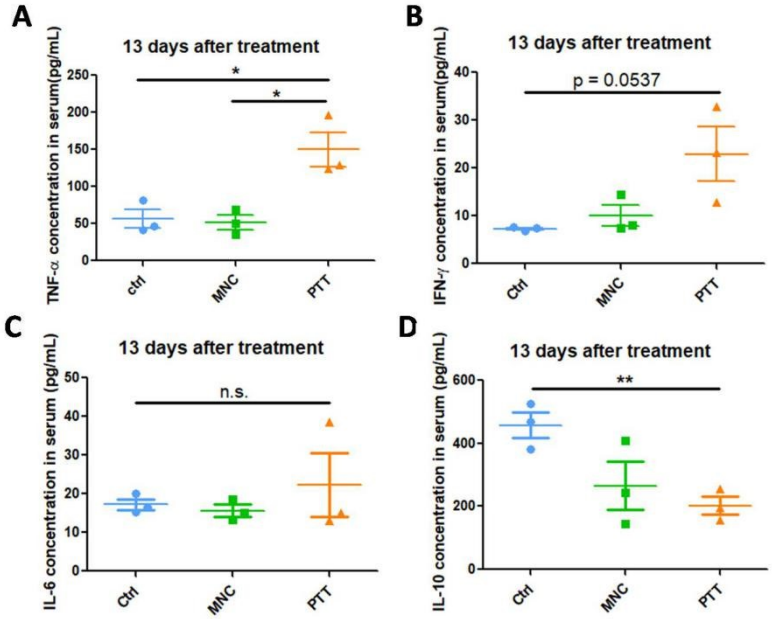

Fig. S7. (A-D) Serum cytokine levels of TNF-α (A), IFN-γ (B), IL-6 (C) and IL-10 (D) from mice 13 days after different treatments.p <0.05, p <0.01 and not significant were denoted as *, **, and n.s., respectively.

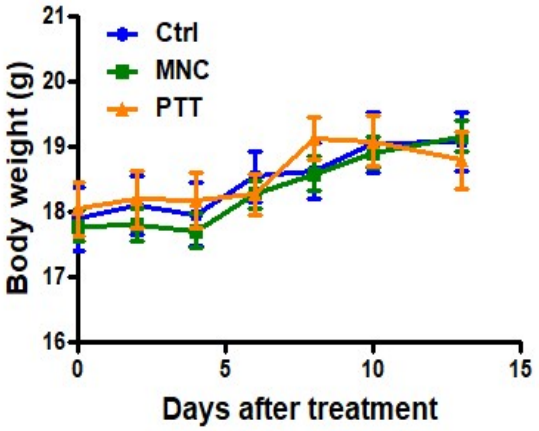

Fig. S8.Plot of average mice body weight of different treatment groups.

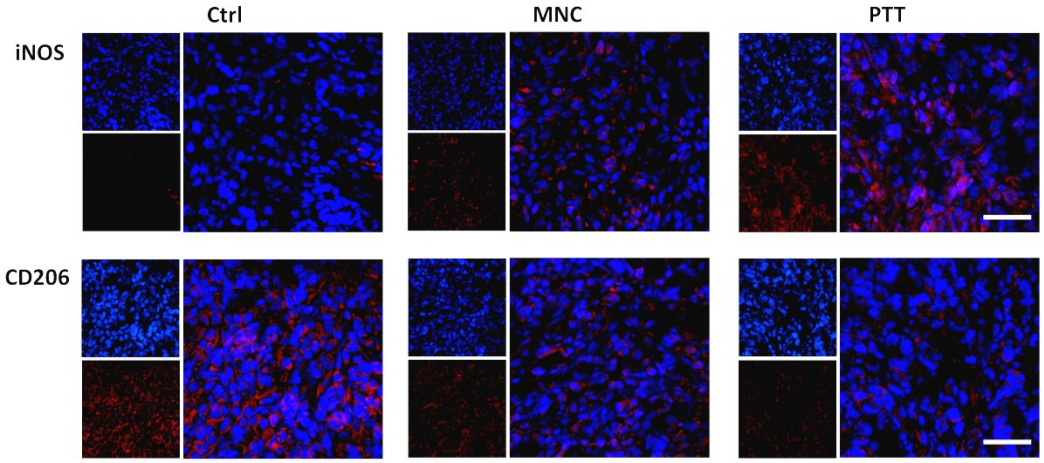

Fig. S9. Representative immunofluorescence images with iNOSstaining (red, upper panel) and CD206 (red, lower panel)showing increased expression of iNOS and decreased expression of CD206 were observed in tumor tissues after different treatments, confirming the macrophages re-polarization capabilities of MNCs and its PTT effect. Nuclei were stained with DAPI. The scale bar was 50 μm.

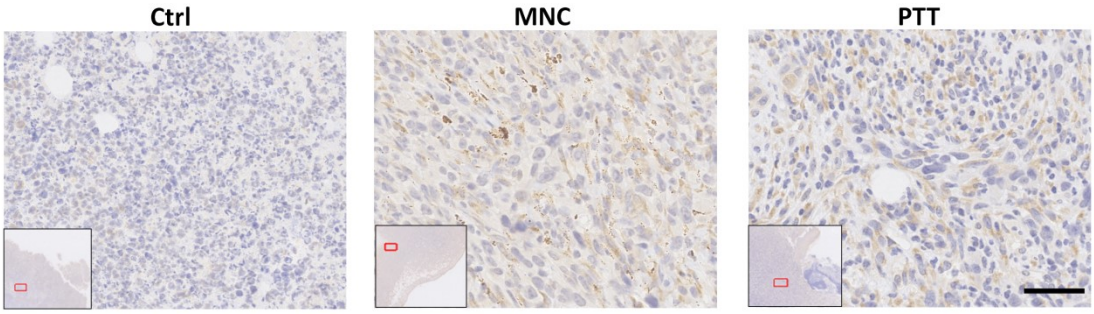

Fig. S10. Representative immunohistochemical analysis photos of tumor tissue stained with CD8 showing increased CD8+ T cells infiltrated into the tumor site after different treatments. The scale bar was 50 μm.

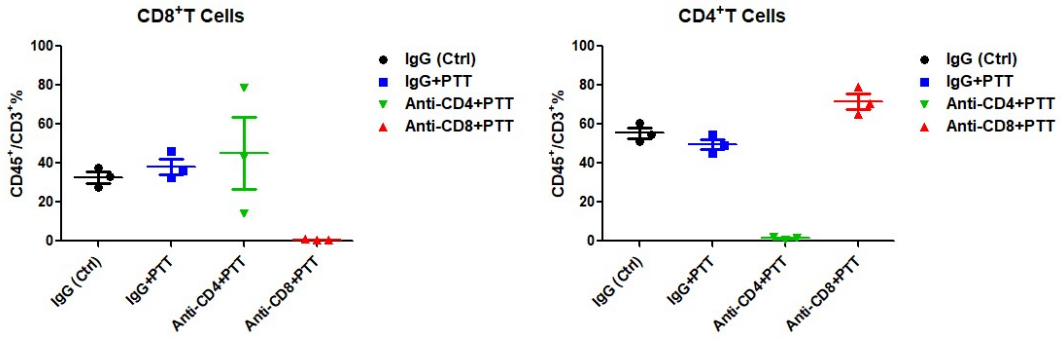

Fig. S11. Confirmation of T cell depletion assay. FCM quantification of $CD4^+$ cells or $CD8^+$ cells gating on $CD3^+$ cells in primary tumors in mice with or without $CD8^+/CD4^+$ T cells depletion.

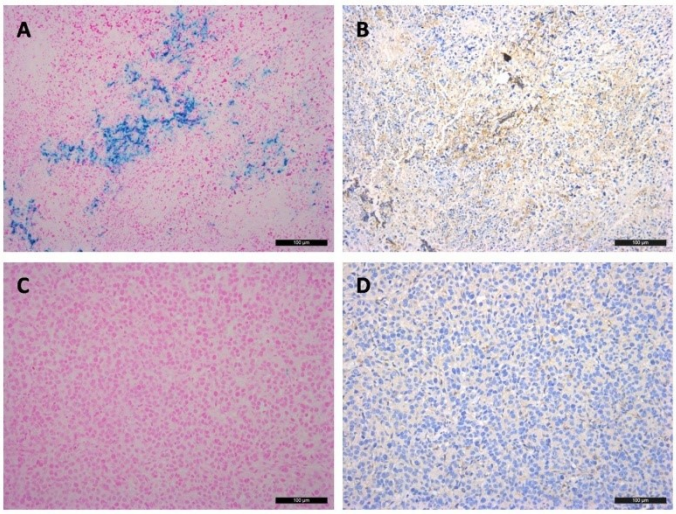

Fig. S12. Prussian blue staining of tumor tissues from MNCs injected group (A) and control group (C). MNCs are indicated by blue color after staining. F4/80 immunohistochemical staining with H&E staining of adjacent tumor tissues from MNCs injected group (B) and control group (D). Together, these results demonstrate a good spatial correlation between distribution of TAMs and MNCs in the tumor site.