Supporting Materials for

Man-HA-MnO_2 Nanoparticles Enhance Chemotherapy Response by Priming Tumor-Associated Macrophages toward M1-like Macrophages and Attenuating Tumor Hypoxia

Manli Song[1,#], Ting Liu[1,#,*], Changrong Shi[1], Xiangzhong Zhang [1,*], Xiaoyuan Chen[2,*]

[1] State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics &Center for Molecular Imaging and Translational Medicine, School of Public Health, Xiamen University, Xiamen, 361102, China.

[2] Laboratory of Molecular Imaging and Nanomedicine, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD 20892

* Address correspondence to tingliu20072008@yahoo.com (Ting Liu), zhangxzh@xmu.edu.cn (Xiangzhong Zhang), shawn.chen@nih.gov (Xiaoyuan Chen)

[#] These authors contributed equally on this work.

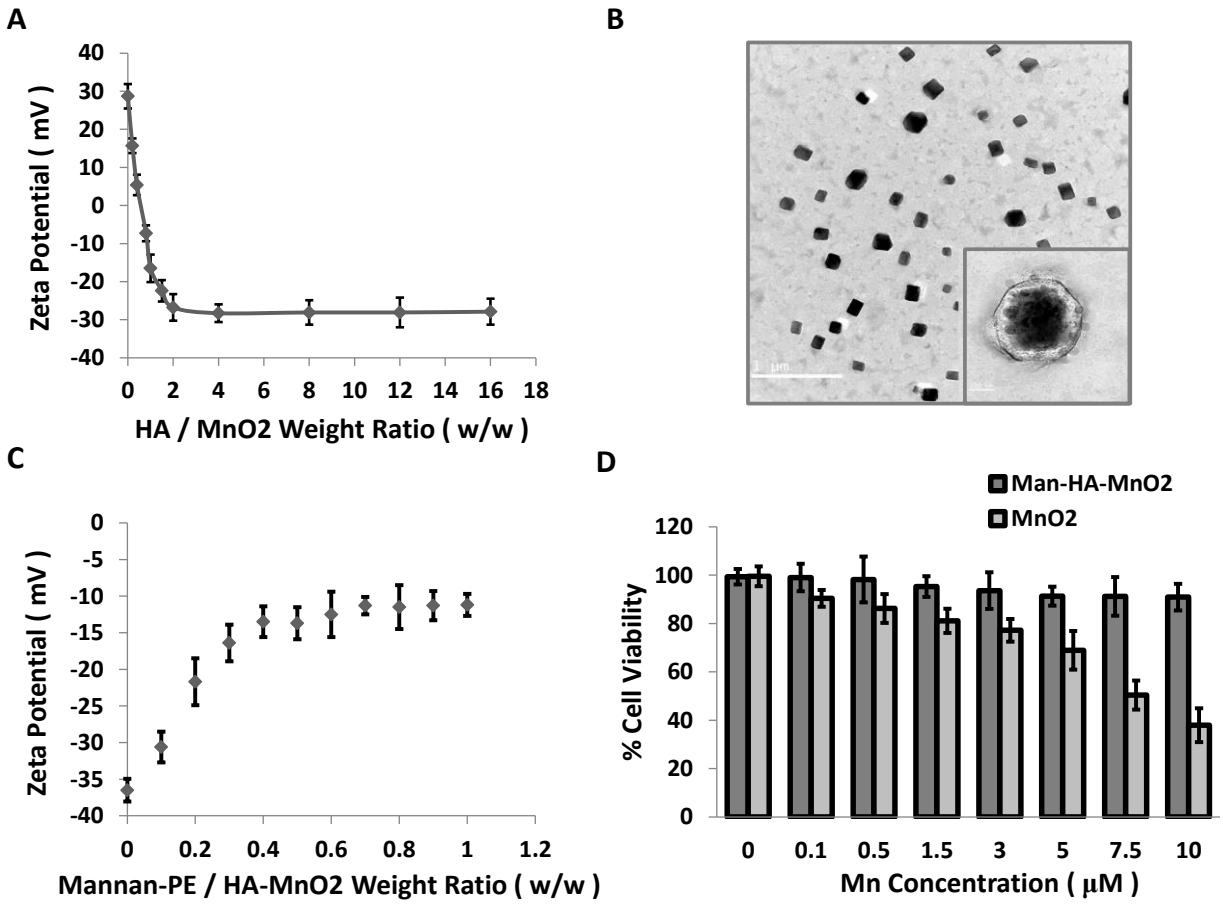

Figure S1. Characterization of Man-HA-MnO_2 NPs: (A) Effect of HA conjugation on zeta potential of the NPs at various HA/MnO_2 ratios (w/w). (B) TEM images of Man-HA-MnO_2 NPs. (C) Effect of mannan modification on zeta potential of the NPs at various Mannan-PEs to HA-MnO_2 weight ratios (w/w). (D) Viability of macrophages exposed to various concentrations of Man-HA-MnO_2 NPs and MnO_2 NPs for 4 h (n=3).

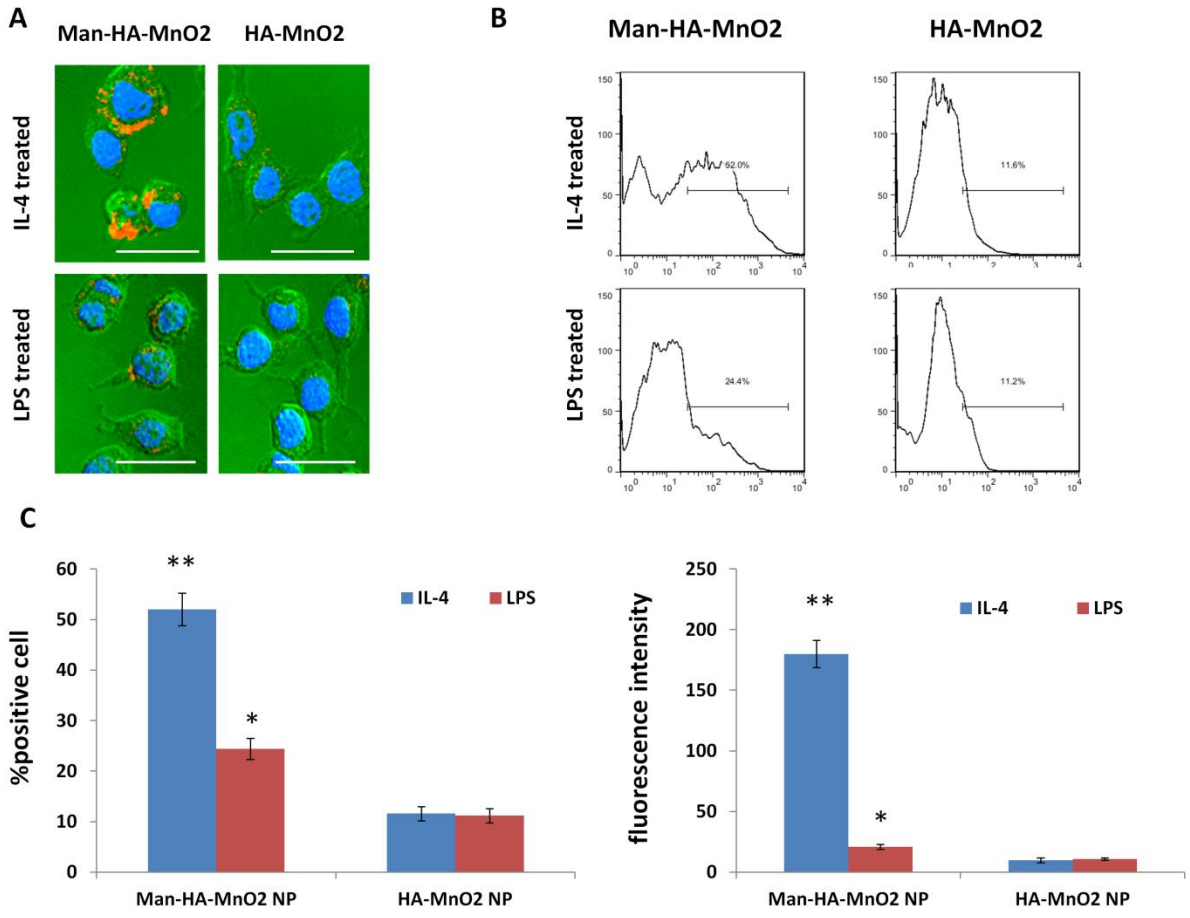

Figure S2. Cell binding of Man-HA-MnO_2 NPs: (A) Fluorescence images of cell binding of Man-HA-MnO_2 NPs (red) at 4 ºC by M2 macrophages following 1 h incubation with NPs. Scale bar, 100 µm. (B), (C) Flow cytometric analysis of cell binding of Man-HA-MnO_2 NPs at 4 ºC by M2 macrophages following 1 h incubation with NPs (n=3). ** p = 0.002 as compared to HA-MnO_2 NPs.

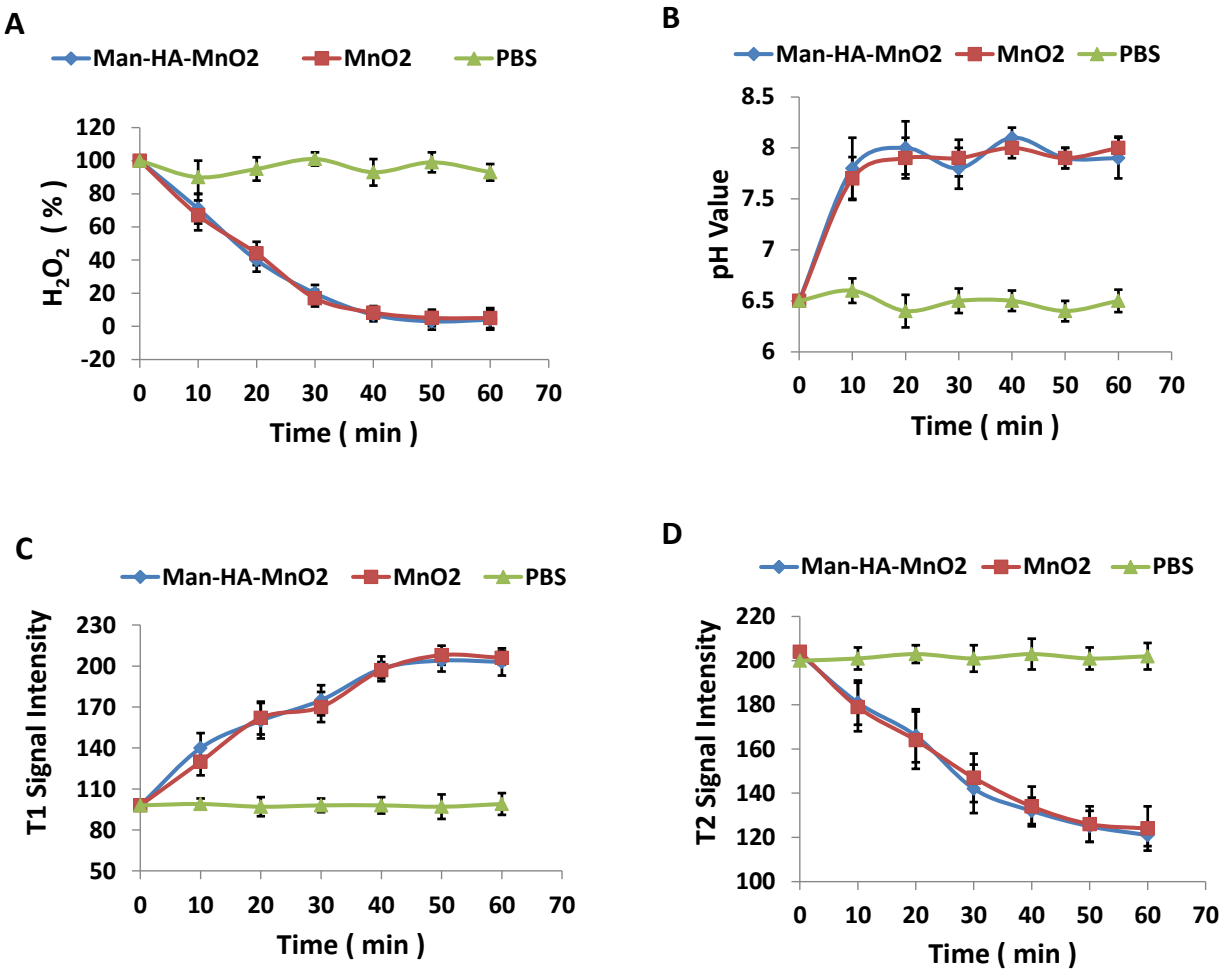

Figure S3. Reaction of Man-HA-MnO_2 NPs toward H_2O_2: (A) Quenching of H_2O_2 (300 μM) by Man-HA-MnO_2 NPs (50 μM). (B) pH increase over time by the Man-HA-MnO_2 NPs. (C), (D) Enhancement in T_1-, T_2-weighted MR signal *vs.* time by the Man-HA-MnO_2 NPs (n=3).

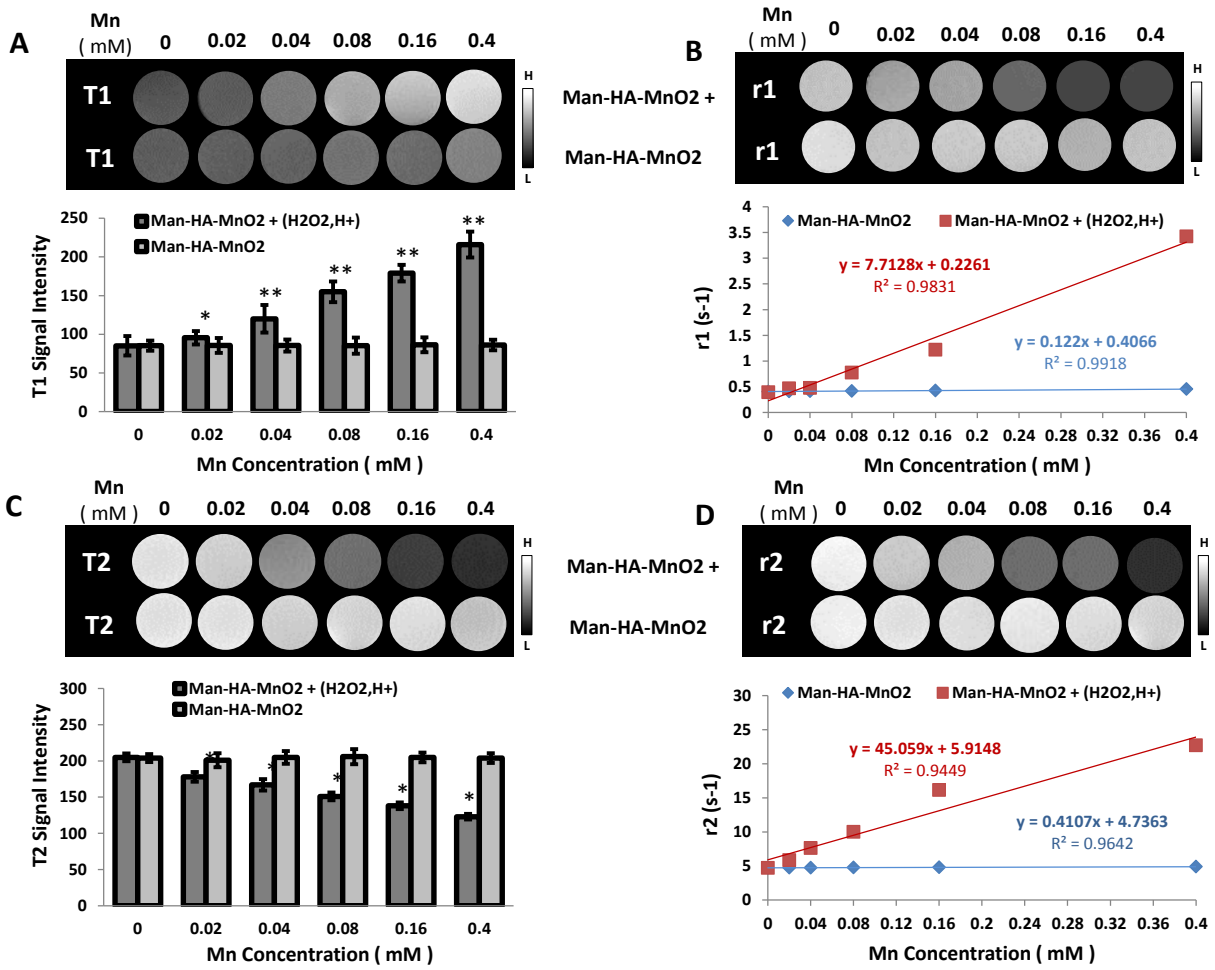

Figure S4. The T_1- (A) and T_2- (C) weighted MR signals, and r_1 (B) and r_2 (D) relaxivities *vs.* Mn concentration for Man-HA-MnO_2 NP solution (blue lines) and Man-HA-MnO_2 NP solution treated with H_2O_2 (red lines).

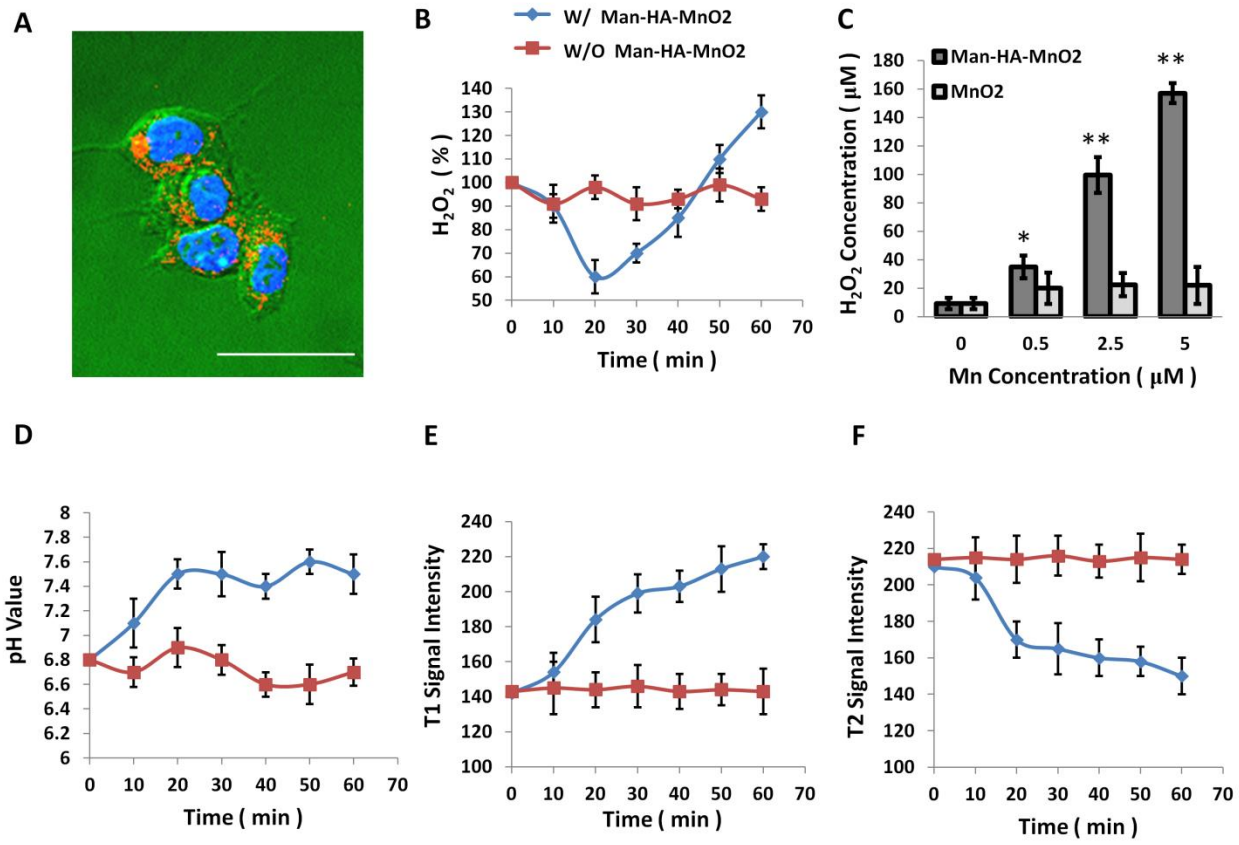

Figure S5. Cellular uptake and *in vitro* reactivity of Man-HA-MnO_2 NPs toward H_2O_2: (A) Fluorescence image of cellular uptake of Man-HA-MnO_2 NPs (red) at 37 ºC by M2 macrophages following 1 h incubation with NPs. Scale bar, 100 μm. (B) Change of cellular H_2O_2 level over time by Man-HA-MnO_2 NPs incubated with M2 macrophages at pH 6.8 (n = 3). (C) Effect of Man-HA-MnO_2 NPs and MnO_2 NPs on H_2O_2 release by M2 macrophages after 1 h incubation. (D) Increase in medium pH over time by Man-HA-MnO_2 NPs incubated with M2 macrophages at pH 6.8 (n=3). (E, F) Enhancement in cellular T_1-, T_2-weighted MR signals over time by the Man-HA-MnO_2 NPs incubated with M2 macrophages at pH 6.8 (n=3). *$p < 0.05$, **$p < 0.005$ in H_2O_2 level as compared to saline control.

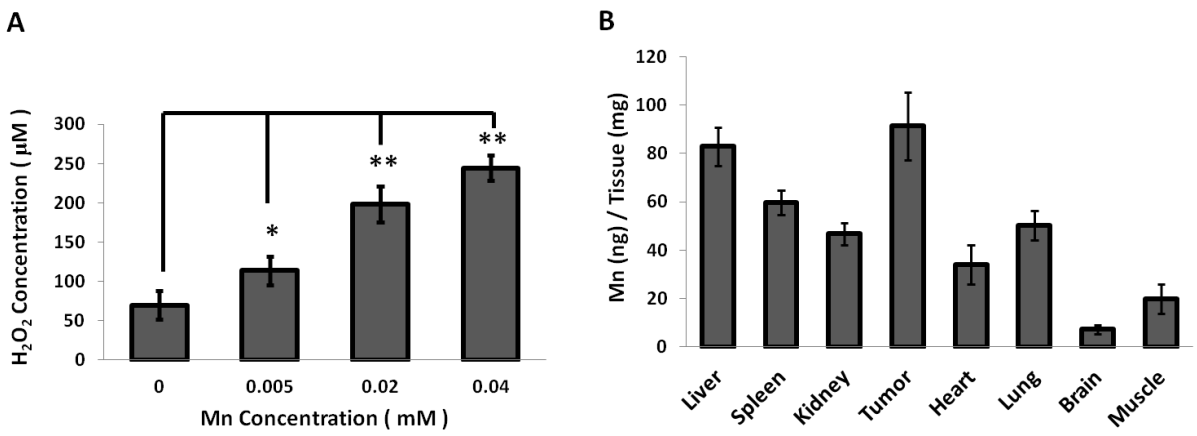

Figure S6 (A) Effect of Man-HA-MnO_2 NPs on H_2O_2 level of tumors (n=5/group). Error bars are standard error of the mean. *$p < 0.05$, **$p < 0.005$ in H_2O_2 level as compared to saline control. (B) The biodisctribution of Man-HA-MnO_2 NPs. ICP-MS was performed to detect Mn^{2+} in the major organs. (n=3/group) Error bars are standard error of the mean.

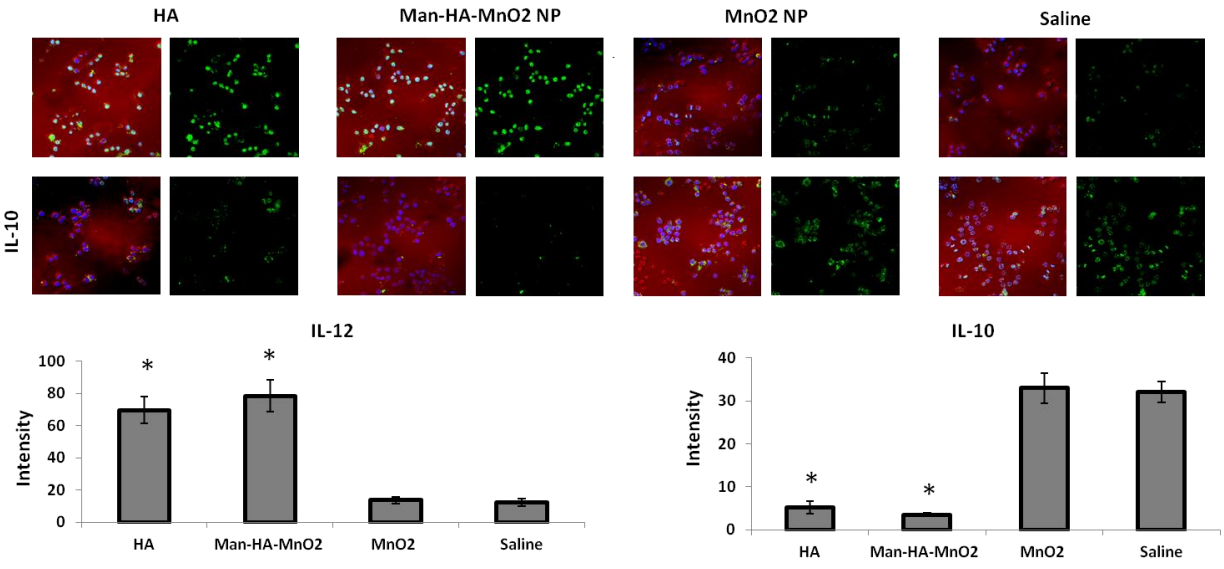

Figure S7. Confocal laser scanning microscopy (CLSM) images of M2 macrophages incubated with Man-HA-MnO_2 NPs, MnO_2 NPs or HA. Immunofluorescense staining with IL-10 and IL-12 antibodies was performed to indentify the cytokine secretion (green) after M2 macrophages incubated with Man-HA-MnO_2 NPs, MnO_2 NPs or HA. Man-HA-MnO_2 NPs altered the cytokine secretion of M2 macrophages after 1 h incubation (n=3/group). Error bars are standard error of the mean. * $p < 0.05$ as compared to saline control.

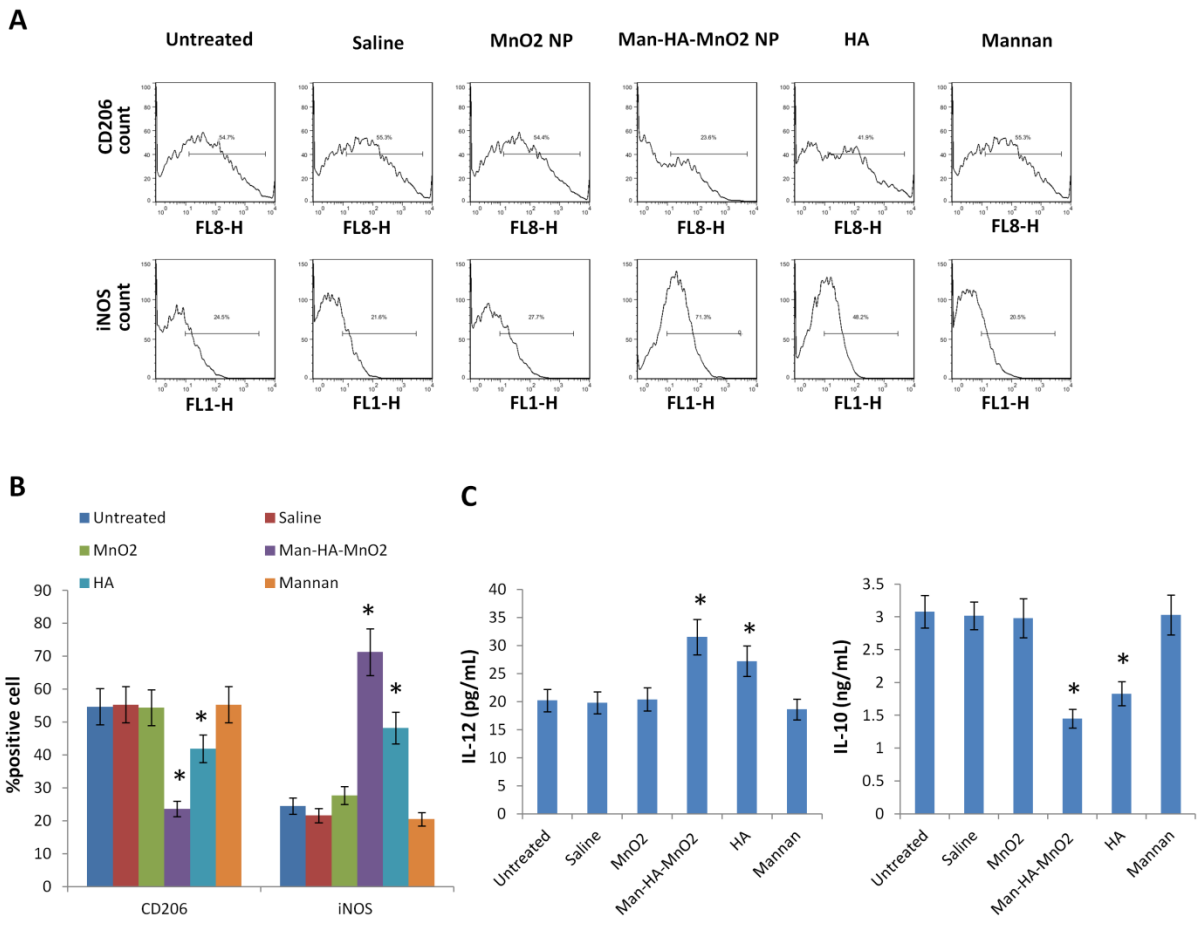

Figure S8. Man-HA-MnO_2 NPs prime M2 TAMs towards M1 macrophages: (A, B) Flow cytometric analysis of expression of iNOS (M1 macrophage marker) and CD206 (M2 macrophage marker) after administration of Man-HA-MnO_2 NPs (5 μM Mn, 15 μM HA), MnO_2 NPs (5 μM Mn), HA (15 μM) or mannan (3 μM). Man-HA-MnO_2 NPs increased expression of iNOS and decreased expression of CD206. (C) Man-HA-MnO_2 NPs altered the cytokine secretion of M2 TAMs measured by ELISA (n=3/group). Error bars are standard error of the mean. * $p < 0.05$ as compared to saline control.

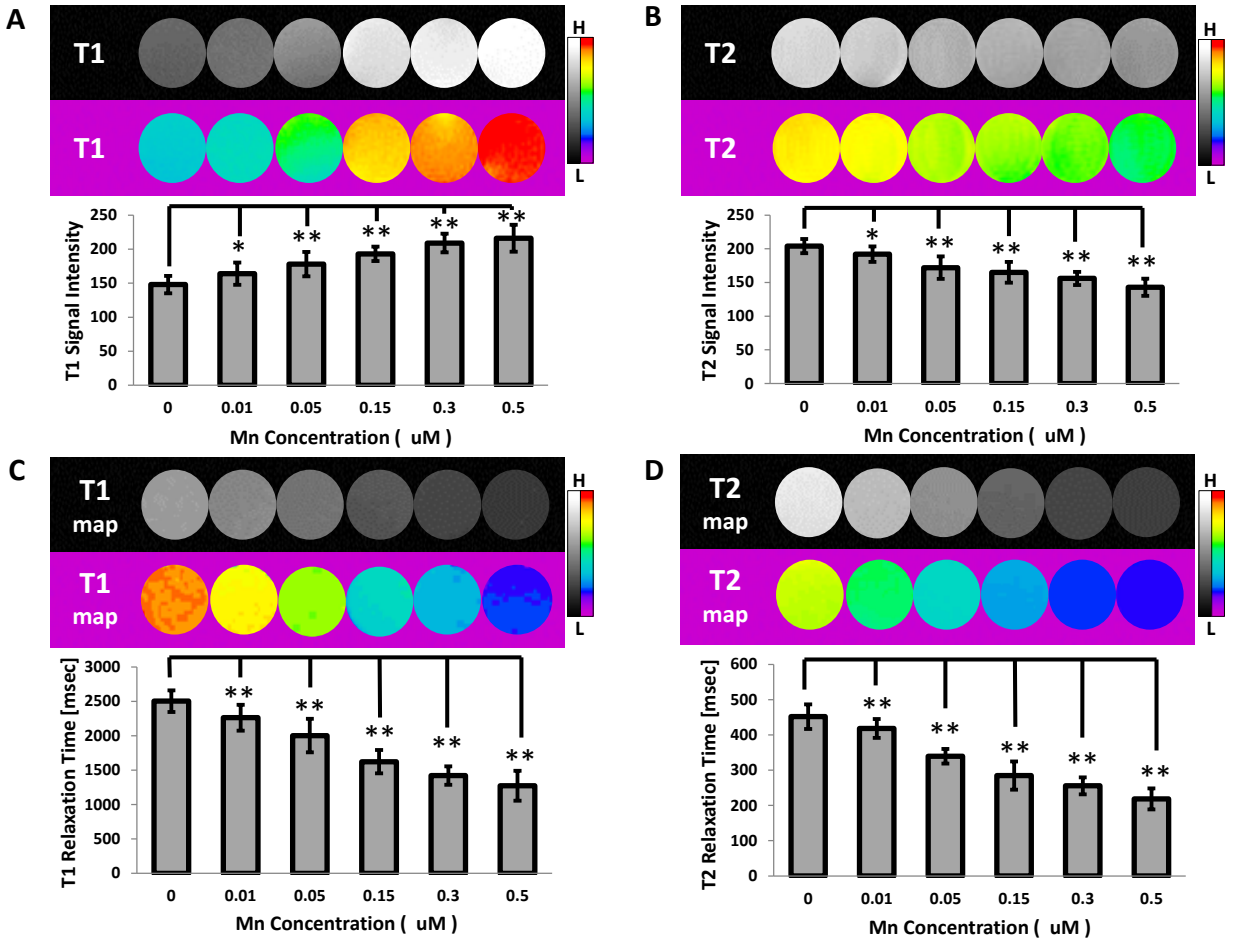

Figure S9. The T_1- and T_2- weighted MR signal, and T_1 and T_2 relaxation *vs.* Mn concentration for M2 macrophages incubated with Man-HA-MnO_2 NPs. *$p < 0.05$, **$p < 0.005$ as compared to saline control.

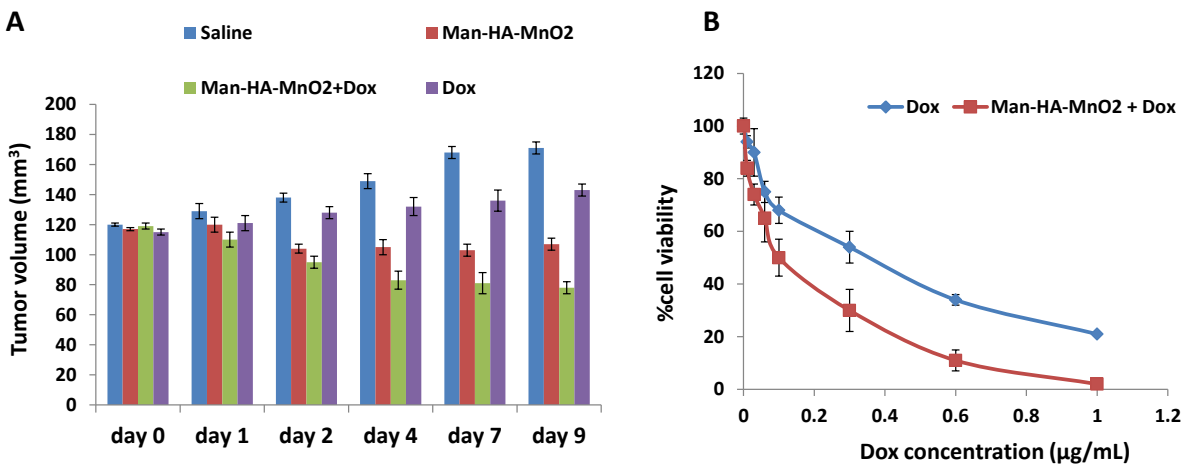

Figure S10. Effect on tumor growth and cell viability after treatment with Dox and Man-HA-MnO_2 NPs: (A) Tumor volume measured over time after treatment. (B) Cell viability exposed to various concentrations of Man-HA-MnO_2 NP+Dox or Dox for 24 h (n=3).