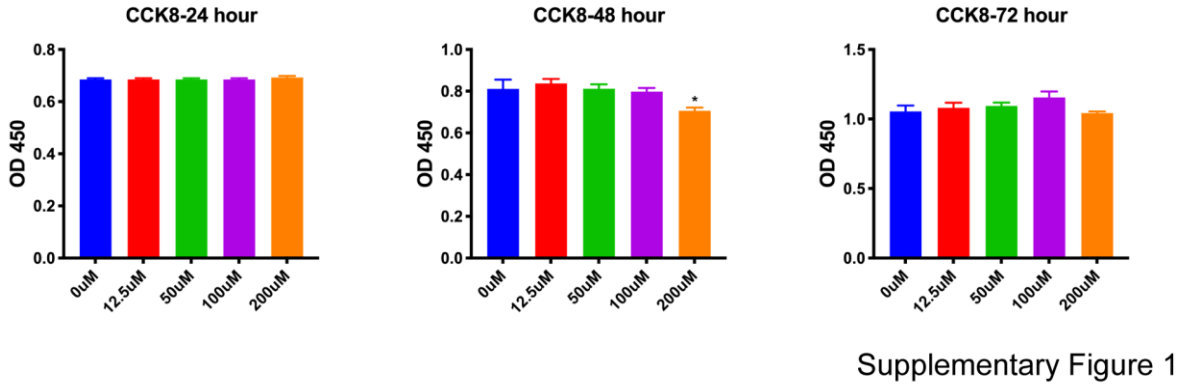
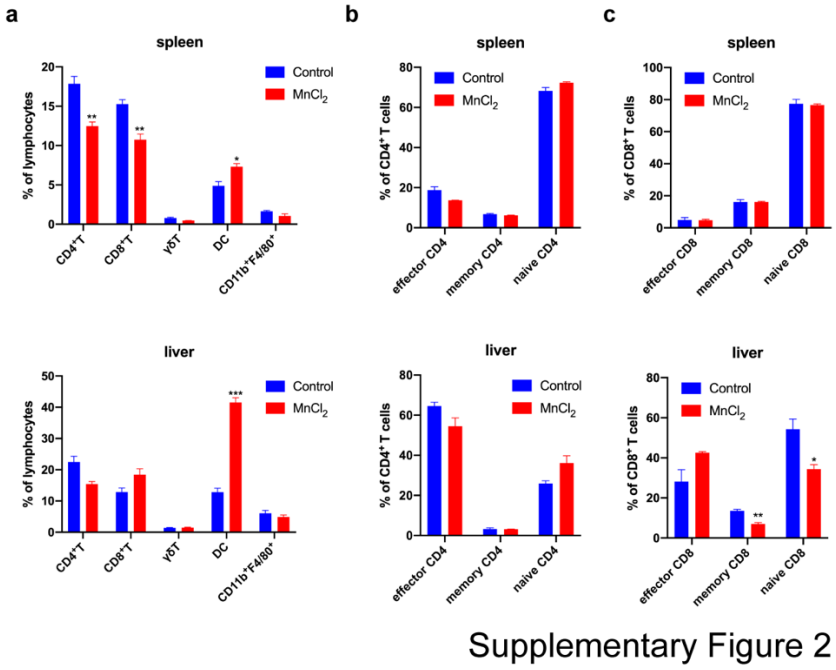
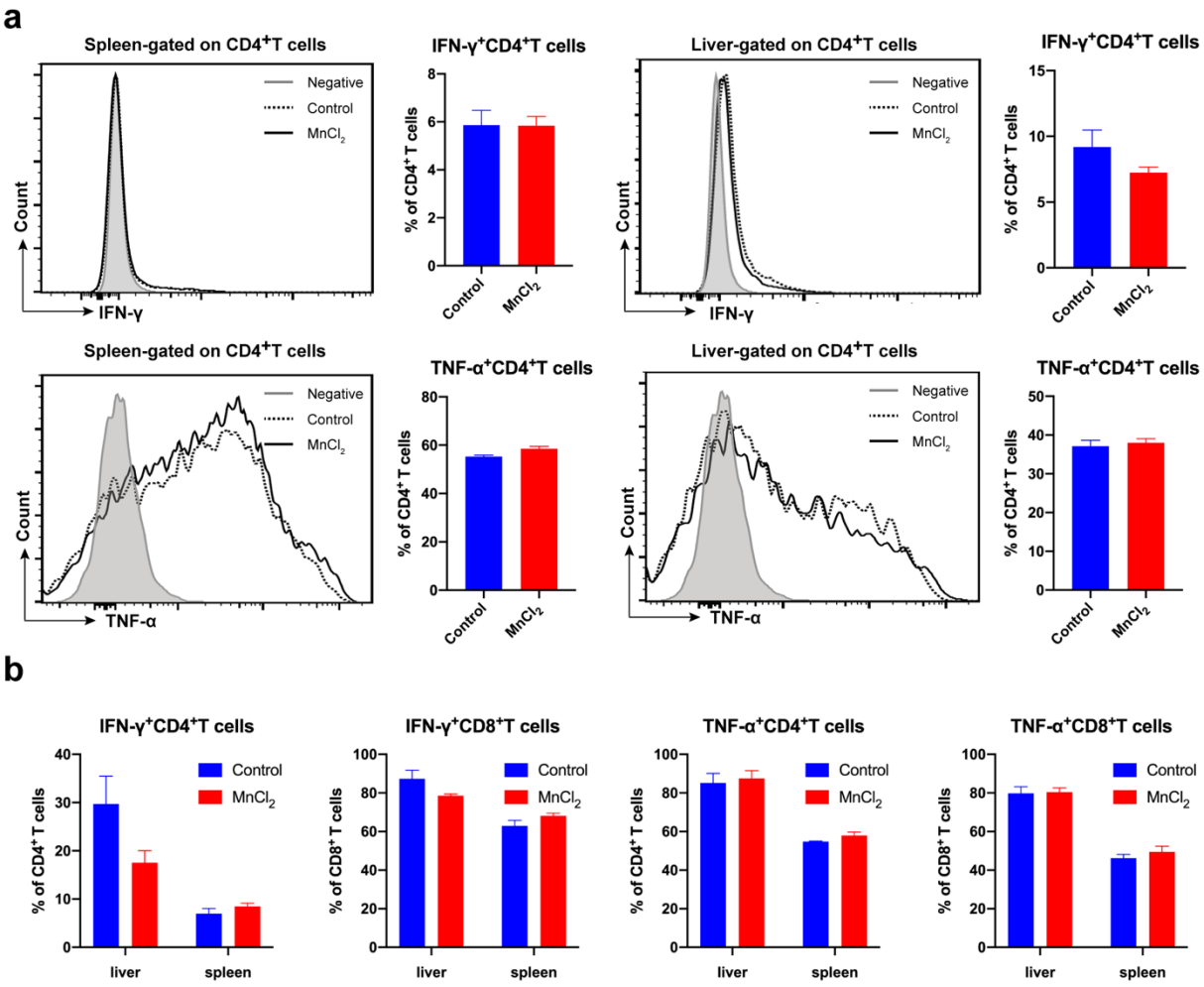


Supplementary Figure 1

Supplementary Figure 1 Mn^{2+} does not affect the tumor cell growth *in vitro*. Hepa1-6 cells were treated with or without $MnCl_2$ for 24, 48, or 72 hours *in vitro*. CCK-8 assay was performed to examine cell proliferation. Data shown are mean ± SEM. All data are representative of two independent experiments. One-way ANOVA followed by Dunnett's multiple comparisons test was used for statistical analysis. $*p < 0.05$.



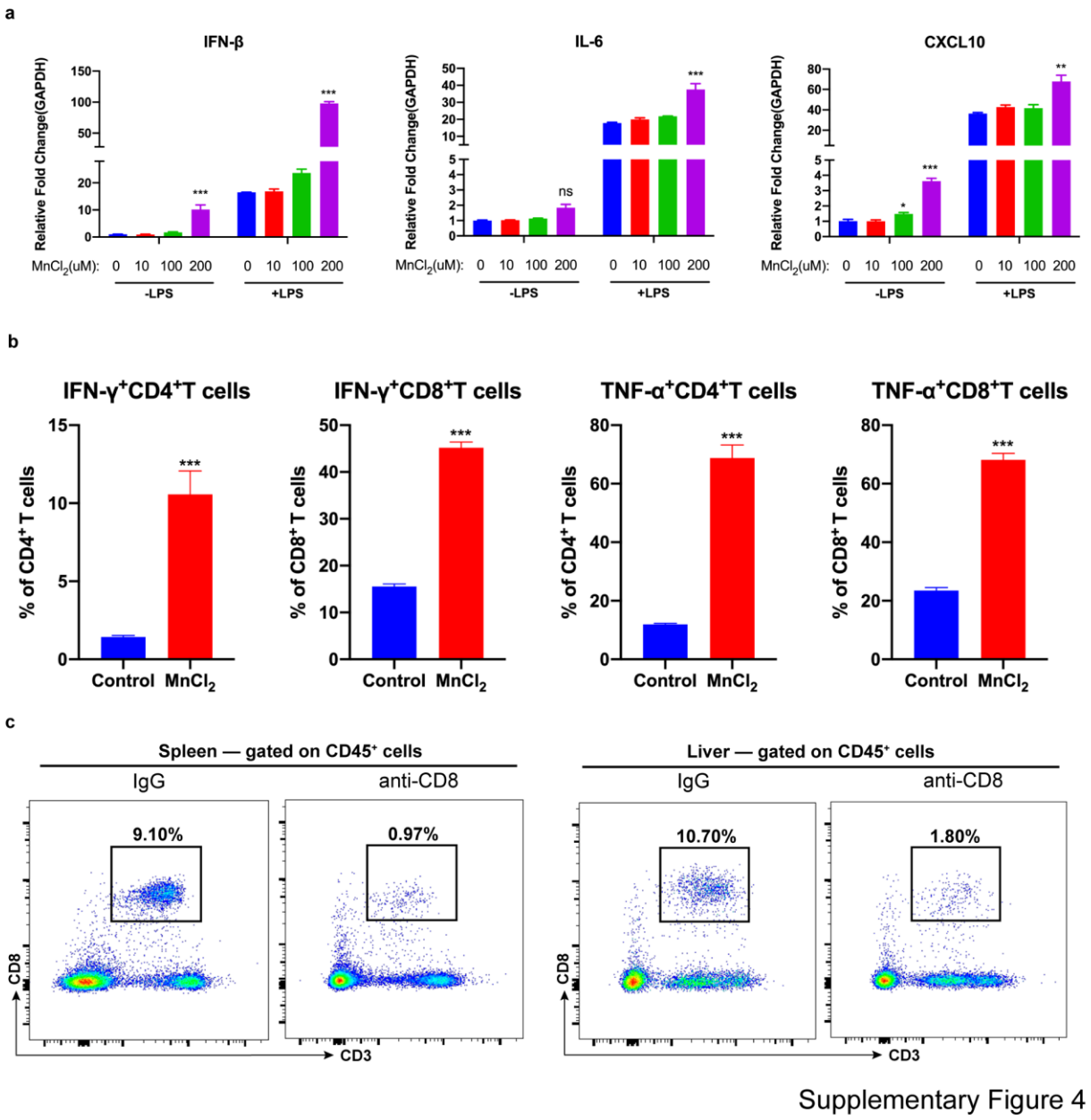
Supplementary Figure 2 Percentages of T cells and myeloid cells in the spleen and liver of tumor-bearing mice upon Mn^{2+} treatment. Murine orthotopic HCC model was established by hydrodynamic injection of hepa1-6 cells (1.5×10^6 cells/2ml/mouse, n=5 or 6 per group). $MnCl_2$ in 200μl PBS was administered to the tumor-bearing mice once every other day at 5mg/kg body weight intravenously from day 1. PBS was injected as controls. On day 7, the mice were euthanized, and the lymphocytes were isolated from the spleen and tumor-bearing liver. The immune cells subsets from the spleen and liver were detected by flow cytometry. a. The percentages of $CD4^+$T, $CD8^+$T, γδT, DC and macrophages in the spleen and tumor-bearing liver of HCC mice. b. The percentages of effector $CD4^+$T, memory $CD4^+$T and naïve $CD4^+$T cells in the spleen and tumor-bearing liver of HCC mice. c. The percentages of effector $CD8^+$T, memory $CD8^+$T and naïve $CD8^+$T cells in the spleen and tumor-bearing liver of HCC mice. Data shown are mean ± SEM. All data are representative of at least three independent experiments. Two-tail unpaired student's t-test was used for statistical analysis. $*p < 0.05$, $**p< 0.01$, $***p<0.001$.



Supplementary Figure 3

Supplementary Figure 3 Cytokine production by $CD4^+$ T cells in the spleen and liver of tumor-bearing mice and the direct effect of Mn^{2+} treatment in T cells. Murine orthotopic HCC model was established by hydrodynamic injection of hepa1-6 cells (1.5×10^6 cells/2ml/mouse, n=5 or 6 per group). $MnCl_2$ in 200ul PBS was administered to the tumor-bearing mice once every other day at 5mg/kg body weight intravenously from day 1. PBS was injected as controls. On day 7, the mice were euthanized, and the lymphocytes were isolated from the spleen and tumor-bearing liver. The immune cells subsets from the spleen and liver and cytokine production were detected by flow cytometry. a. The IFN-γ and TNF-α productions in the $CD4^+$T from the spleen and liver

of tumor-bearing mice. b. $CD3^+$T cells isolated from the spleen and liver of tumor-bearing mice were stimulated with or without $MnCl_2$ (5μM) *in vitro* in the presence of anti-mouse CD3 (2μg/ml) and anti-mouse CD28 (1μg/ml). 3 days later, the percentages of IFN-γ or TNF-α producing $CD4^+$T and $CD8^+$T cells were detected by flow cytometry (n=3 repeats per group). Data shown are mean ± SEM. All data are representative of at least three independent experiments. Two-tail unpaired student‘s t-test was used for statistical analysis.



Supplementary Figure 4

Supplementary Figure 4 The effect of Mn^{2+} treatment on macrophages and subsequent induction of cytokine production of T cells *in vitro*. J774A.1 cells were stimulated with different doses of $MnCl_2$ (0, 10, 100, 200 μM) in the presence or absence of LPS (1μg/ml). a. The expressions of IFN-β, IL-6, CXCL10 in J774A.1 cells were detected by qPCR. b. Sorted $CD3^+$T cells from naïve mice were cultured with the supernatants of J774A.1 cells stimulated with or without $MnCl_2$ and LPS. 3 days later, the productions of IFN-γ and TNF-α by T cells were

detected by flow cytometry. c. The depletion of $CD8^+$T cells *in vivo*. Murine orthotopic HCC model was established by hydrodynamic injection of hepa1-6 cells (1×10^6 cells/2ml/mouse, n=5 or 6 per group). $MnCl_2$ in 200μl PBS was administered to the tumor-bearing mice once every other day at 5mg/kg body weight intravenously from day 1 to day 20. PBS was injected as controls. Tumor-bearing mice treated with $MnCl_2$ or PBS were injected with anti-CD8 antibody intraperitoneally (300μg/200ul/mouse) once every week. IgG (300μg/200μl/mouse) was injected into the tumor-bearing mice as isotype controls. 3 weeks later, the percentages of $CD8^+$T cells was detected in the spleen (left panel) and liver (right panel) of tumor-bearing mice. Data shown are the representative of two independent experiments. Data shown are mean ± SEM. Data are representative of at least two independent experiments. Two-tail unpaired student's t-test (b) or one-way ANOVA followed by Dunnett's multiple comparisons test (a) was used for statistical analysis. $*p < 0.05$, $**p< 0.01$, $***p<0.001$.