Supplemental information

Combined CD44- and CD25-targeted Near-Infrared Photoimmunotherapy Selectively Kills Cancer and Regulatory T cells in Syngeneic Mouse Cancer Models

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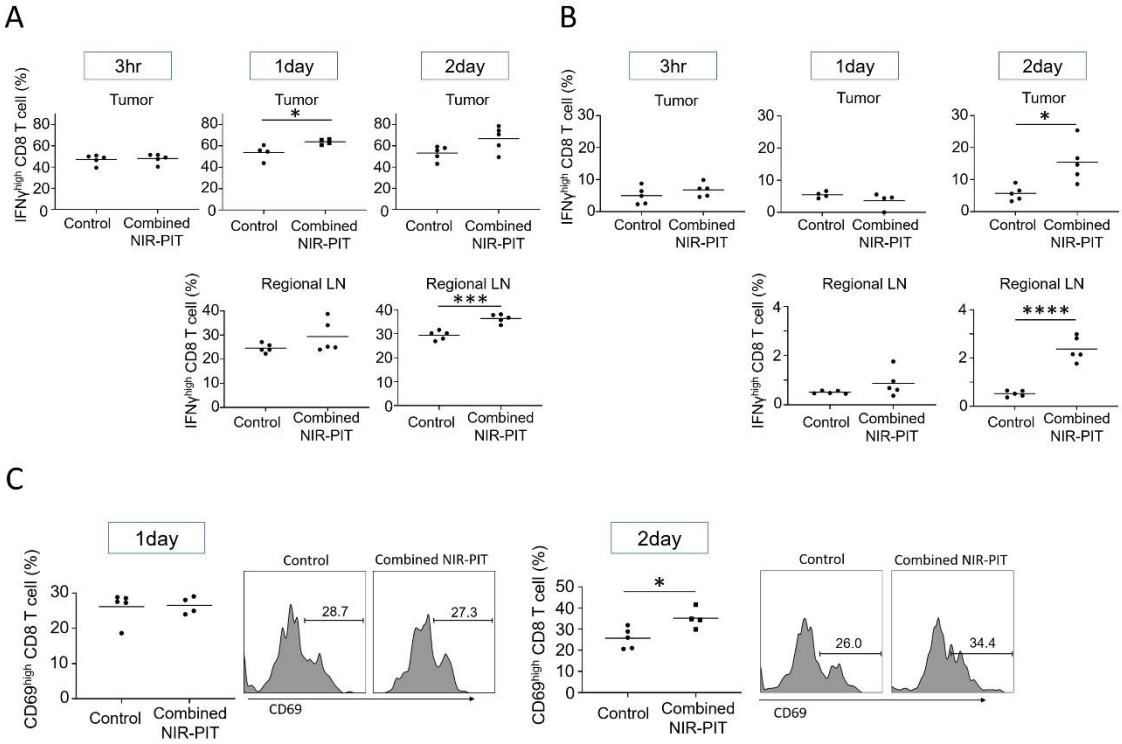
Supplementary Figure 1

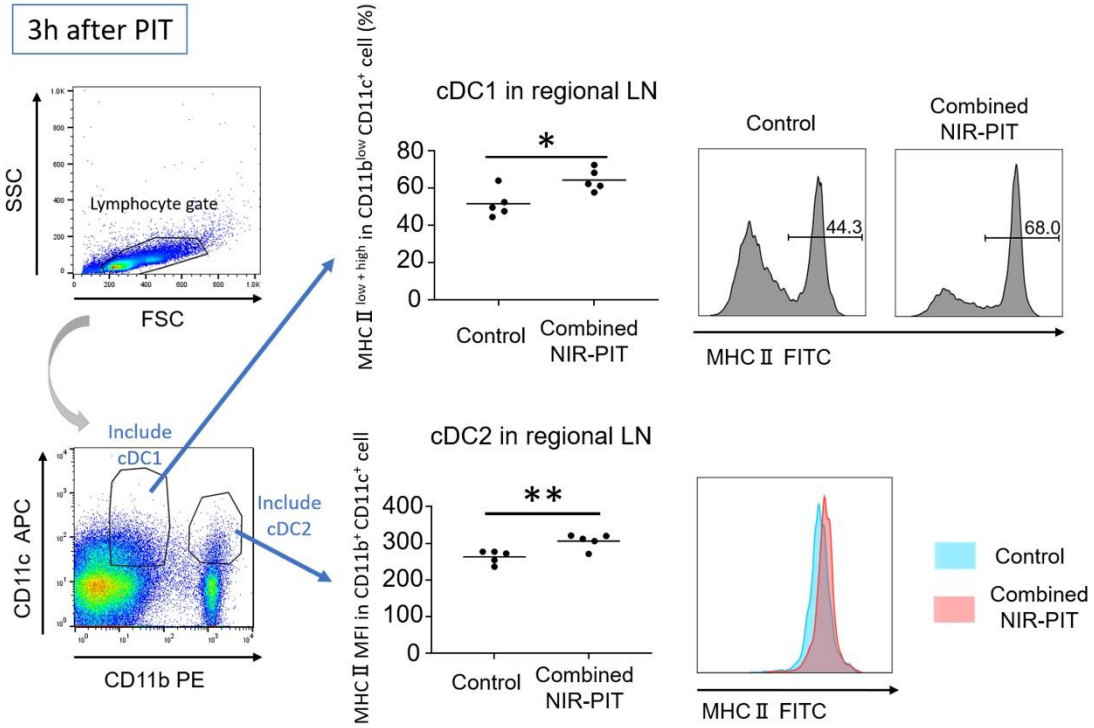


Supplementary Figure 1.

Cytotoxicity and activation of intratumoral CD8 T cells in acute phase after combined CD44- and CD25-targeted NIR-PIT.

A. Cytotoxic action of CD8 T cells (CD3+CD8+ cells) infiltrating into tumors or regional lymph nodes (LNs) in mice bearing MC38-luc tumors with or without combined CD44- and CD25-targeted NIR-PIT was examined by flow cytometry under IFN-γ stimulation. The tumors 1 day after the combined NIR-PIT showed significantly higher level of IFN-γ production in CD8 T cells (n = 4-5/group, *p < 0.05, Mann-Whitney U Test). The regional LNs 2 days after the combined NIR-PIT showed significantly higher level of IFN-γ production in CD8 T cells compared to control tumors (n = 5/group, ***p < 0.001, Mann-Whitney U Test). B. Cytotoxic action of CD8 T cells infiltrating into tumors or regional LNs in mice bearing MC38-luc tumors with or without combined CD44- and CD25-targeted NIR-PIT was examined by flow cytometry without IFN-γ stimulation. The tumors and regional LNs 2 days after the combined NIR-PIT showed significantly higher level of IFN-γ production in CD8 T cells compared to control tumors (n = 4-5/group, *p < 0.05, ****p < 0.0001, Mann-Whitney U Test). C. Two days after combined CD44- and CD25-targeted NIR-PIT, up-regulation of activation markers, CD69 in intratumoral CD8 T cells was observed (n = 4-5, *p < 0.05, Mann-Whitney test).

Supplementary Figure S2



Supplementary Figure 2.

Dendritic cell maturation shortly after combined CD44- and CD25-targeted NIR-PIT. Dendritic cell maturation in regional lymph nodes (LNs) in mice bearing MC38-luc tumors with or without combined CD44- and CD25-targeted NIR-PIT was examined by flow cytometry. The regional LNs 3 hours after the combined NIR-PIT showed significantly higher level of conventional type 1 dendritic cell (cDC1) compared to control LNs ($n = 5$, $*p < 0.05$, Mann-Whitney test). Mean fluorescence intensity (MFI) based on MHC class Ⅱ expression in CD11b+CD11c+ cells was significantly higher in regional LNs 3 hours after the combined NIR-PIT than in control LNs ($n = 5$, $**p < 0.01$, Mann-Whitney test), which suggests higher level of conventional type 2 dendritic cell (cDC2) in the combined NIR-PIT group.