

Supplementary figure legends

Supplementary Figure 1. Schema of the gating strategy for determination of GD2⁺ tumor cells, NK1.1⁺ NK cells, CD4⁺ T-cells, CD8⁺ T-cells, and T regulatory cells.

Supplementary Figure 2. Rechallenge tumor growth curves. A) Individual tumor growth curves of mice treated with resection alone, or resection with or without RT and/or IT-IC and rechallenged in the contralateral flank with B78 melanoma at day 30 following the start of neoadjuvant immunotherapy, along with simultaneous implantation of B78 into naïve mice. B) Individual tumor growth curves for mice undergoing early or late resection following IT-IC and rechallenged in the contralateral flank with B78 melanoma at day 30. C) Individual tumor curves of mice undergoing early resection following IT-IC and rechallenged in the contralateral flank with B78 melanoma at day 8, 15 or 35 following the start of neoadjuvant IT-IC, along with simultaneous implantation of B78 on day 8 or 35 into naïve mice, and on day 8 into mice cured by resection alone.

Supplementary Figure 3. Neoadjuvant IT-IC inhibits establishment of GD2-expressing B16-GD2 lung metastasis. A) Mice bearing B78 melanoma tumors were untreated or treated with neoadjuvant RT, IT-IC, or RT + IT-IC and tumors were subsequently resected. Mice were challenged IV on day 80 with B16-GD2 cells on the day following surgery to induce experimental lung metastasis. B) Overall survival following challenge with IV B16-GD2 induced experimental lung metastasis. C) Representative IVIS images of mice from each group following challenge with IV B16-GD2 experimental lung metastasis.

Supplementary Figure 4. Confirmation of CD8 and CD4 T-cell depletion. A) Representative flow cytometry plots of whole blood from mice two days following treatment with anti-CD8 antibodies, anti-CD4 antibodies, or rat IgG. C) Quantification of CD8 and CD4 T-cell populations in whole blood two days following treatment with depleting with following treatment with anti-CD8 antibodies, anti-CD4 antibodies, or rat IgG. Each dot represents data from a separate mouse.