CD122-targeted interleukin-2 and αPD-L1 treat bladder cancer and melanoma via distinct mechanisms, including CD122-driven natural killer cell maturation

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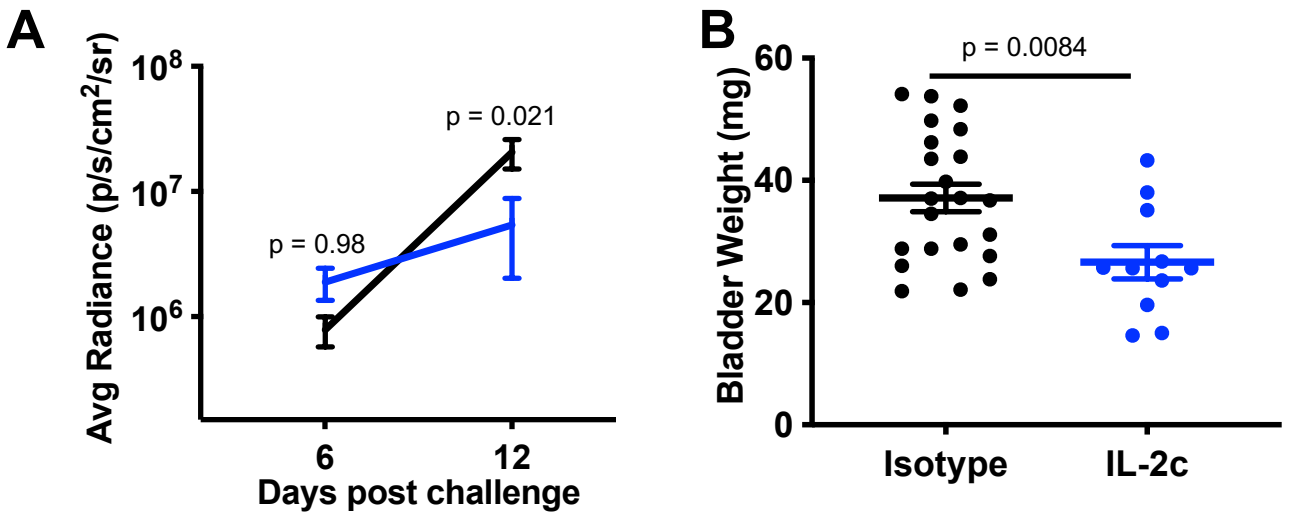
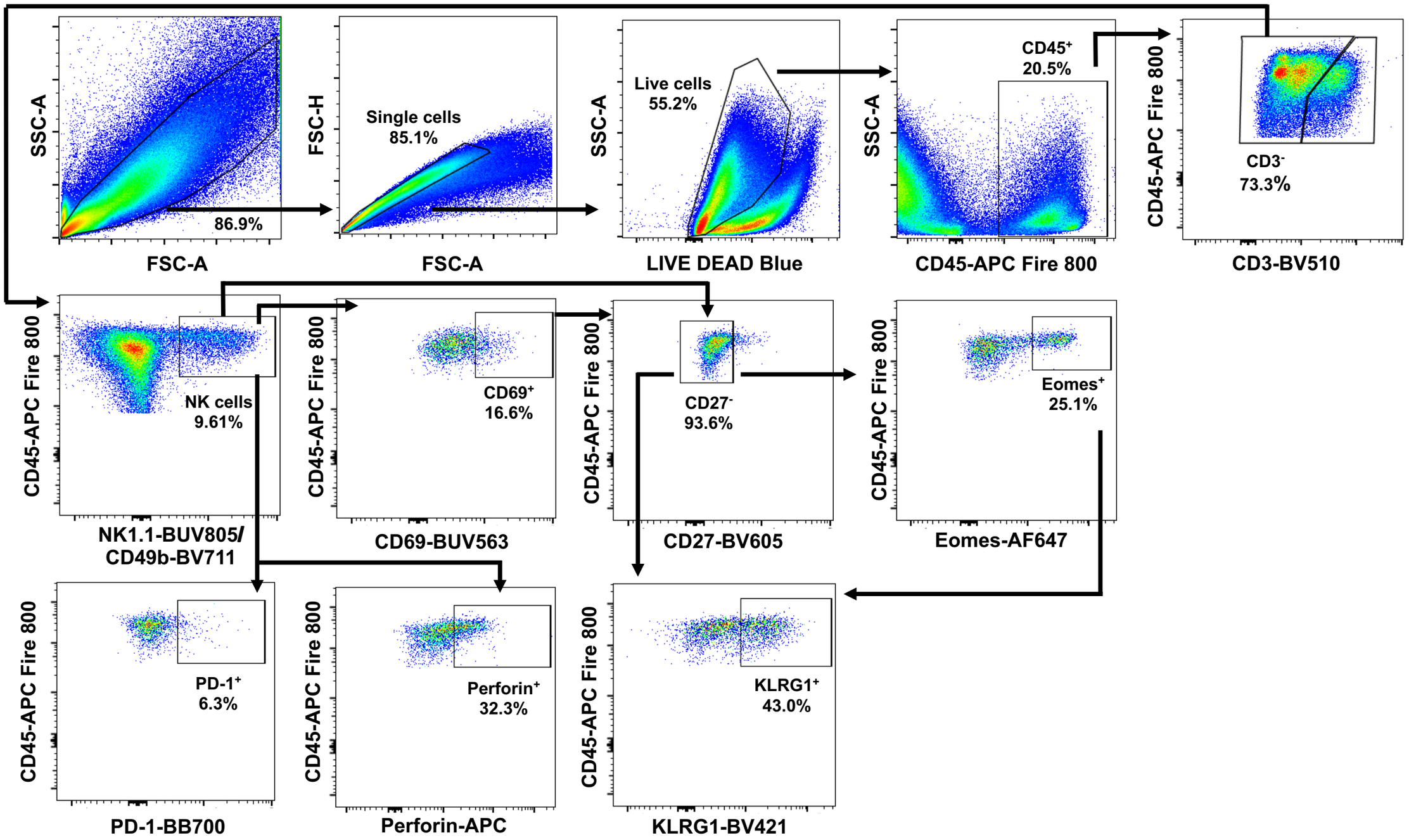
Supplementary Figures

Supplementary Fig 1



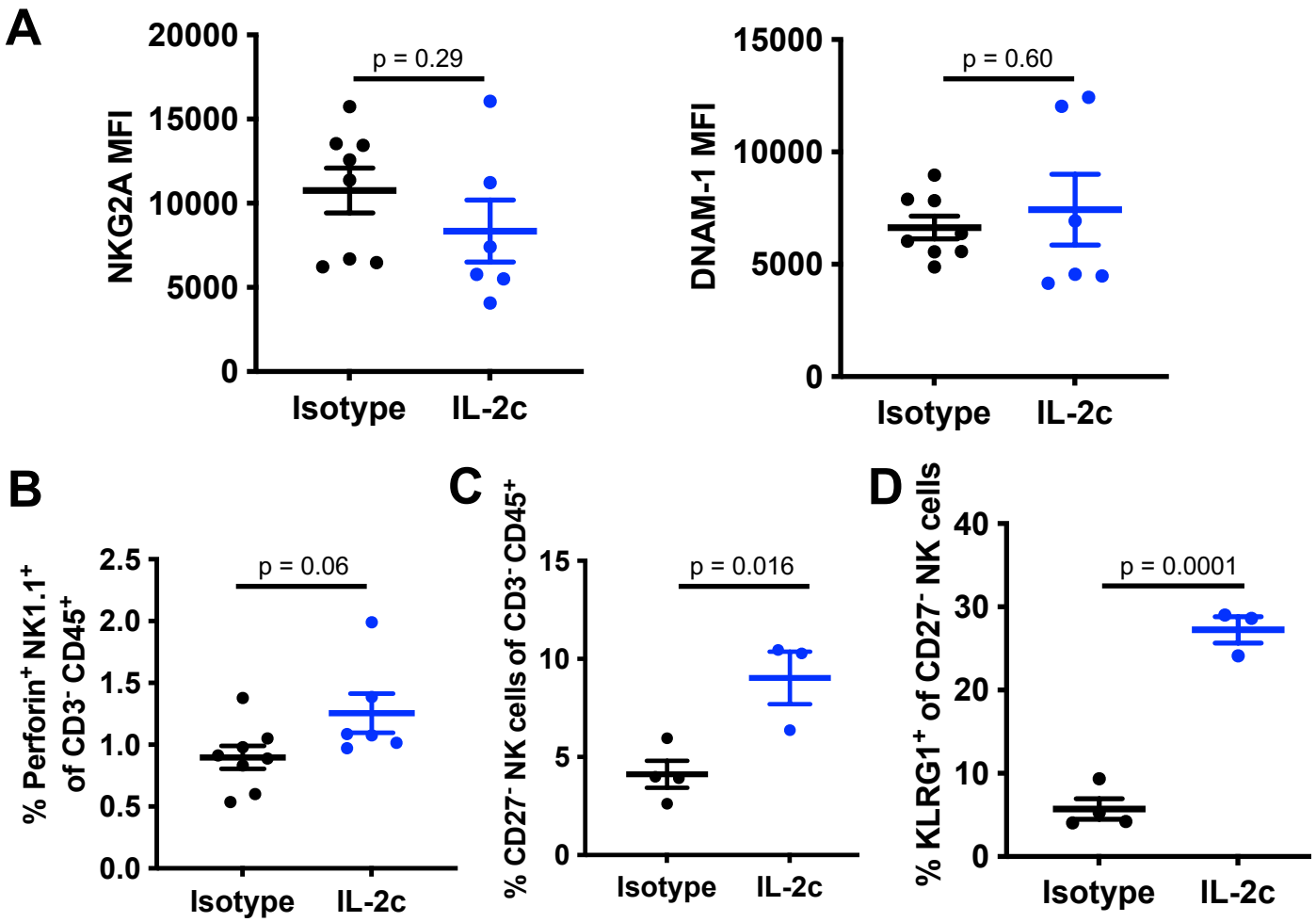
Supplementary Fig 1 IL-2c treats orthotopic MBT-2 and promotes NK cell maturation. Wild-type female mice were challenged orthotopically with (A,B,D-F) 1×10^6 MBT-2 or (C) 8×10^4 MB49 cells and treated with IL-2c on days 6, 8, 10 and 12, or isotype control. (A) Tumor bioluminescence corresponding with pre-treatment and post-treatment timepoints. N=11-22/group p value, two-way ANOVA of day 6 and 12 signal. (B) Mice from (A) sacrificed on day 13. N=6-8 pooled bladders/group. p value, unpaired t test. (IL-2c, interleukin-2 complex.

Supplementary Fig 2



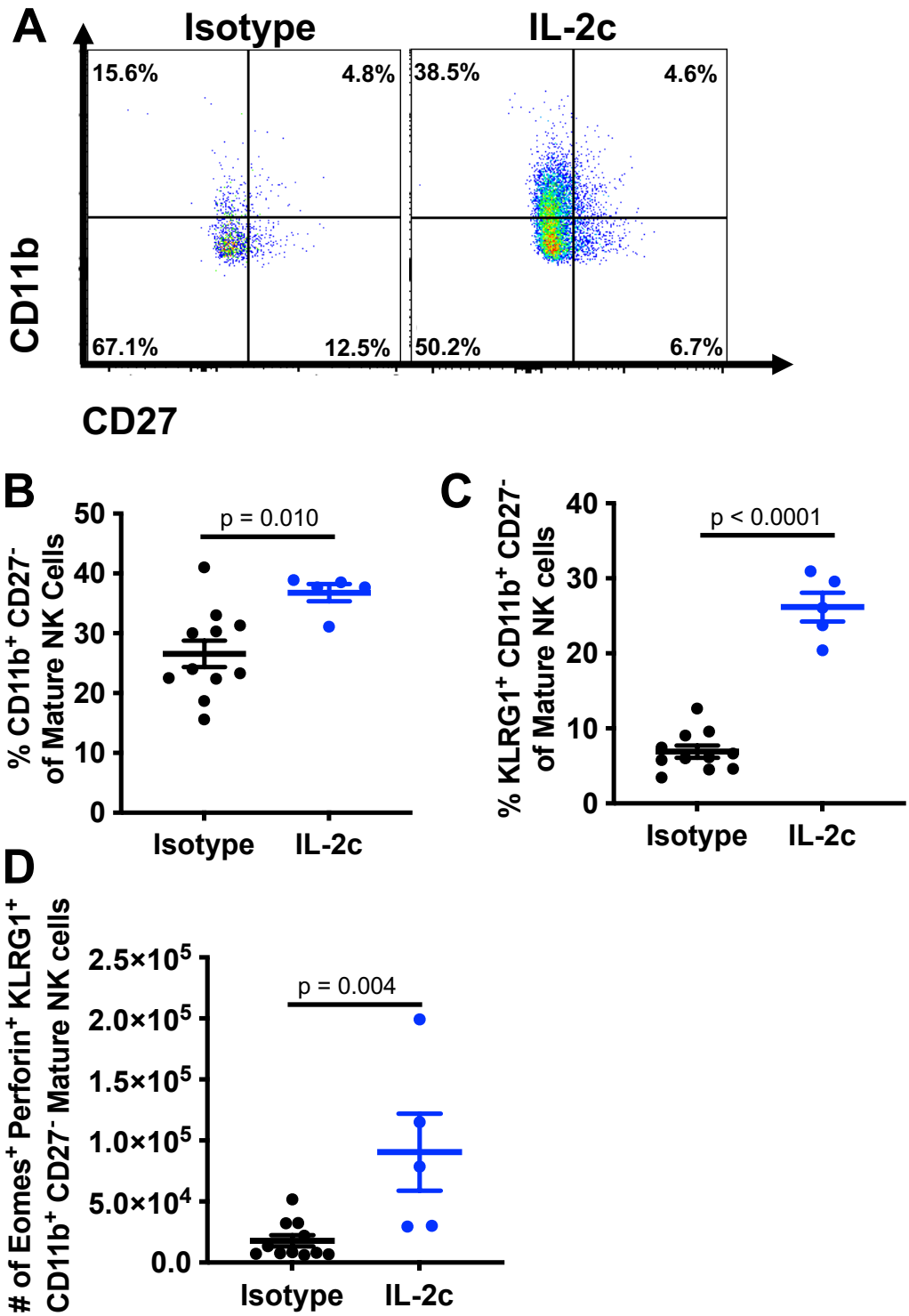
Supplementary Fig 2 Representative flow cytometry gating strategy of NK cells from the bladder of MB49 or MBT-2 tumor bearing mice (Fig. 1,2,S1,3,4). NK cells were defined as either $CD45^+$ $CD3^-$ $NK1.1^+$ (MB49; C57Bl6) or $CD45^+$ $CD3^-$ $CD49b^+$ (MBT-2; C3H) cells with additional gating as shown. Eomes, Eomesodermin. NK, Natural Killer. KLRG1, Killer Cell Lectin Like Receptor G1. PD-1, Programmed Death Receptor 1.

Supplementary Fig 3



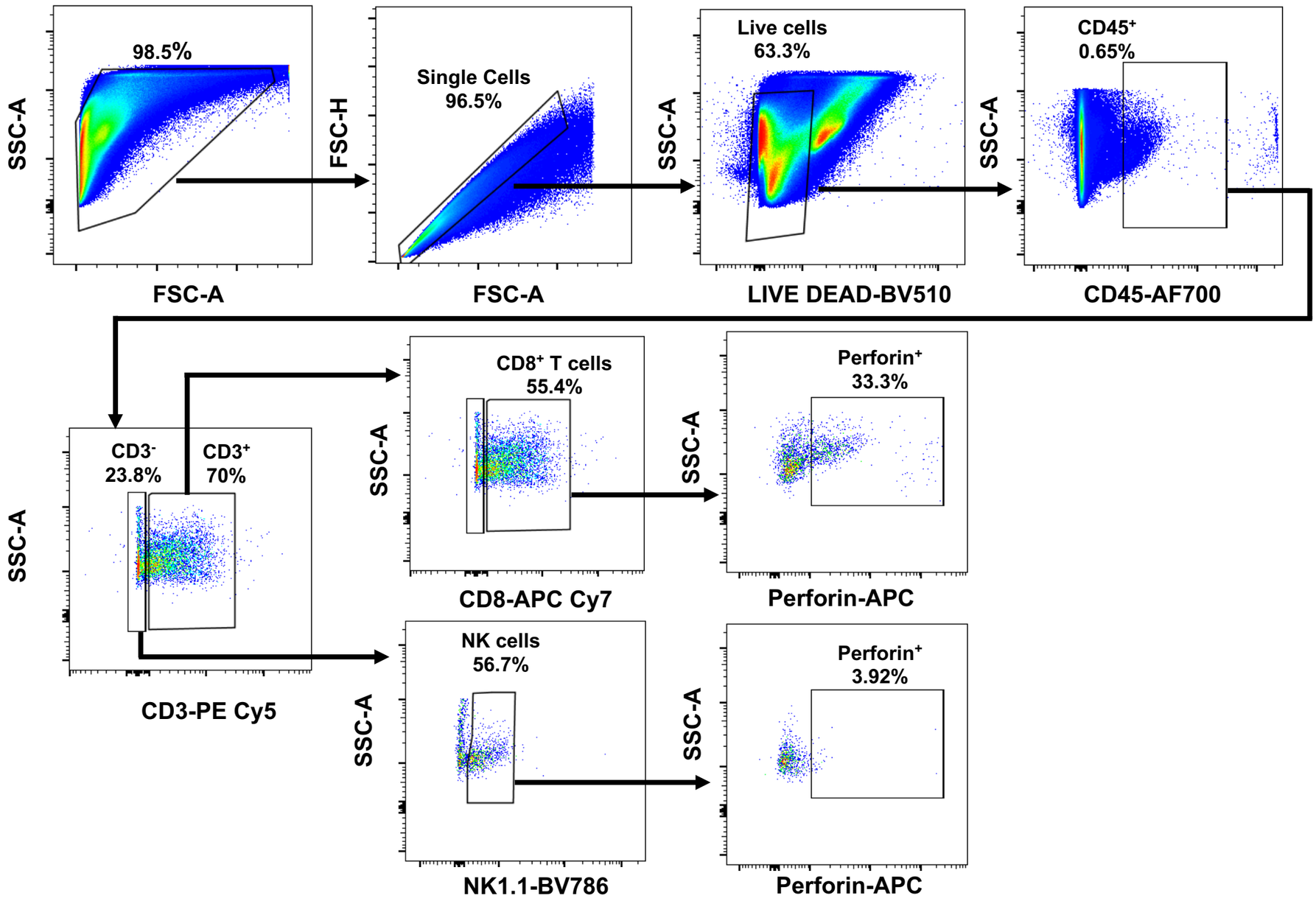
Supplementary Fig 3 IL-2c promotes NK cell maturation. Wild-type female mice were challenged orthotopically with (A,B) 8 x 10^4 MB49 or (C,D) 1×10^6 MBT-2 cells and treated with IL-2c on days 6, 8, 10 and 12, or isotype control. (A,B) Flow cytometric analysis of intratumoral NK cell inhibitory (NKG2A) and activating (DNAM-1) receptor expression (A) and perforin production (B) in MB49 bladder tumors. N=6-8 pooled bladders/group. p value, unpaired t test. (C,D) Flow cytometric analysis of intratumoral NK cell (C) maturation (i.e., $CD27^-$) , and (D) KLRG1 expression by $CD27^-$ NK cells in MBT-2 bladder tumors. Mice were sacrificed on day 13 post challenge. N=3-4 pooled bladders/sample. N=3-4 samples/group. p value, unpaired t test. ANOVA, analysis of variance. IL-2c, interleukin-2 complex. KLRG1, Killer Cell Lectin Like Receptor G1. NK, Natural Killer.

Supplementary Fig 4



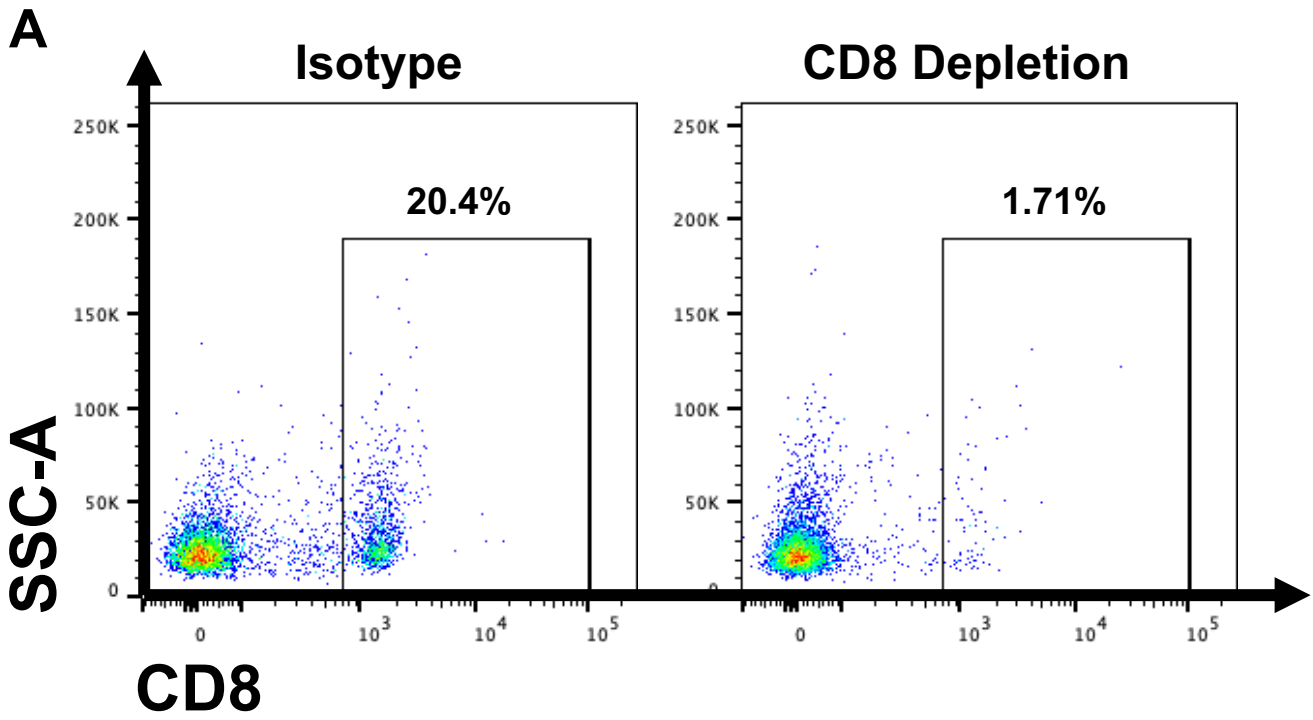
Supplementary Fig 4 TCRδ^{KO} female mice were challenged orthotopically with 8 x 10^4 MB49 cells and treated with IL-2c on days 9, 11, 13, and 15 or isotype control. Mice were sacrificed on day 17 for flow cytometric analysis. (A) Representative flow plot of CD11b and CD27 expression on mature NK cells (CD45$^+$ CD3$^-$ CD49b$^+$) isolated from bladders of MB49 tumor-bearing mice. CD11b$^+$ CD27$^-$ NK cell frequency (B) and their expression of KLRG1 (C), and Eomes and perforin (D). N=9-11 mice/group. Tumors were pooled to achieve 5 x 10^6 live cells per sample. p, unpaired t test. Eomes, Eomesodermin. IL-2c, interleukin-2 complex. KLRG1, Killer Cell Lectin Like Receptor G1. NK, Natural Killer. PD-1, Programmed Death Receptor 1.

Supplementary Fig 5



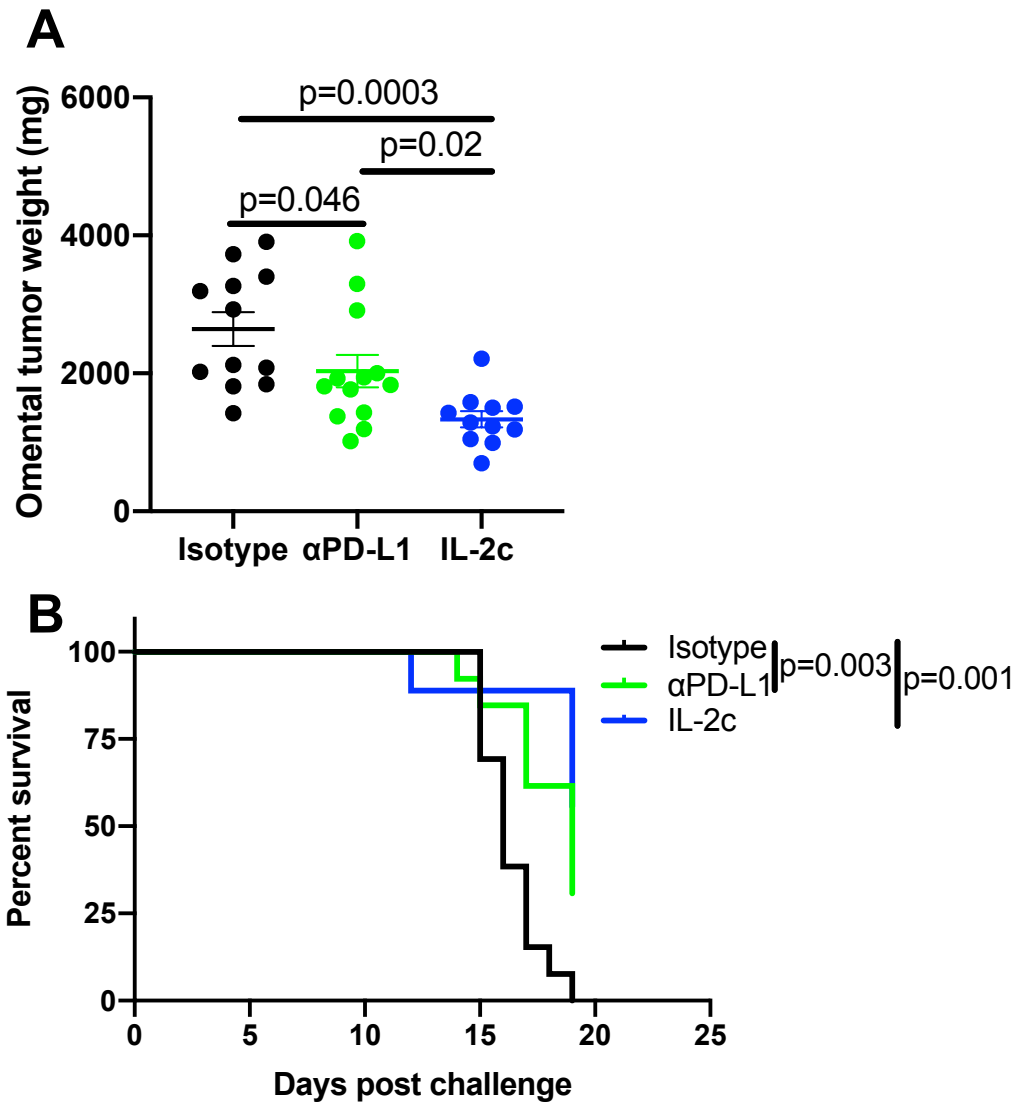
Supplementary Fig 5 Representative flow cytometry gating strategy of $CD8^+$ T and NK cells from subcutaneous B16 tumors. $CD8^+$ T cells were defined as $CD45^+$ $CD3^+$ $CD8^+$ cells and NK cells were defined as $CD45^+$ $CD3^-$ $NK1.1^+$ with additional gating as shown. Eomes, Eomesodermin. NK, Natural Killer.

Supplementary Fig 6



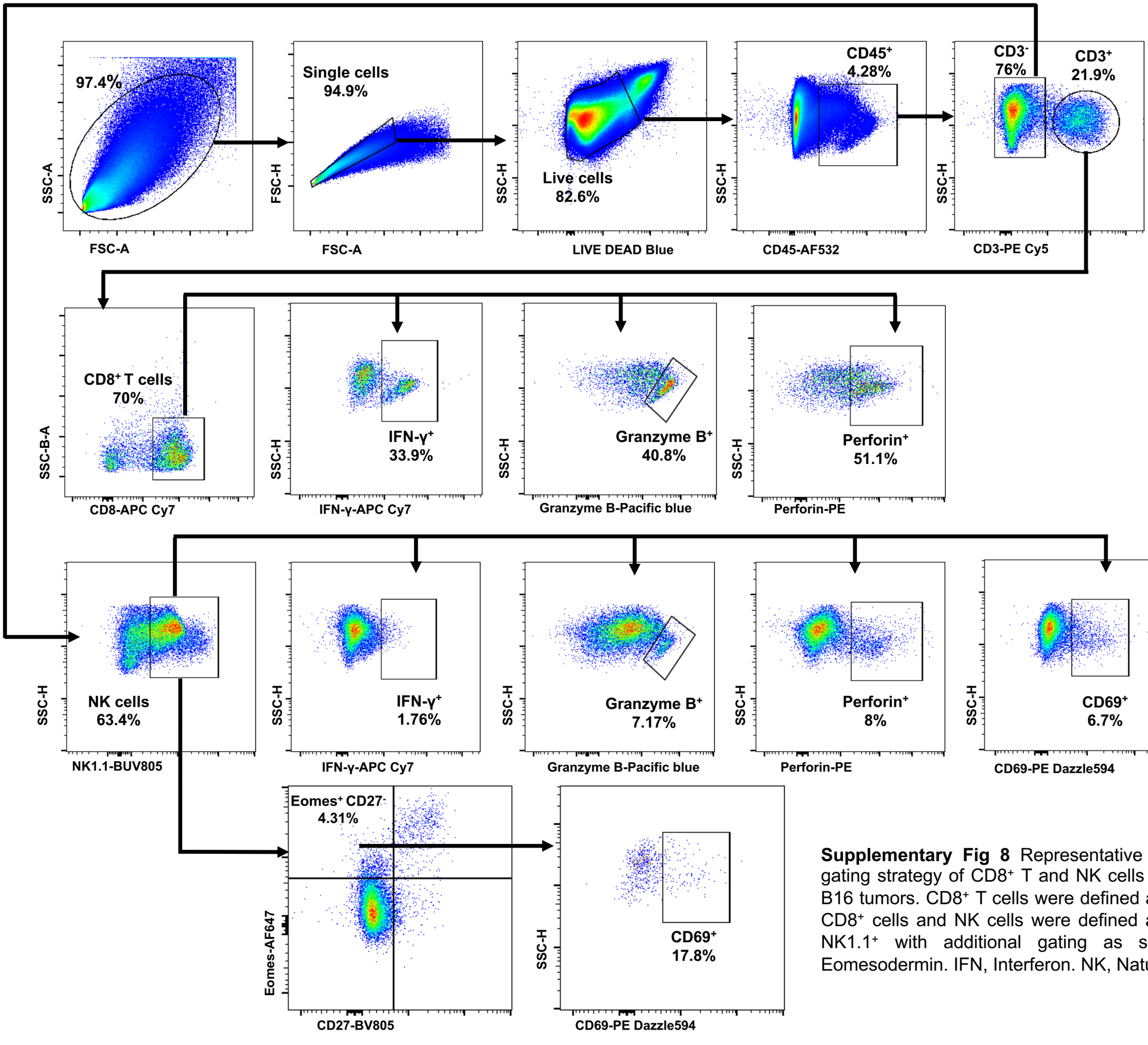
Supplementary Fig 6 $CD8^+$ T cell depletion efficiency in subcutaneous B16 melanoma. (A) Depletion efficiency of $CD8^+$ T cells using 250 ug αCD8, one day after intraperitoneal injection, as gated on live $CD45^+$ cells in spleen. SSC-A, side scatter area.

Supplementary Fig 7



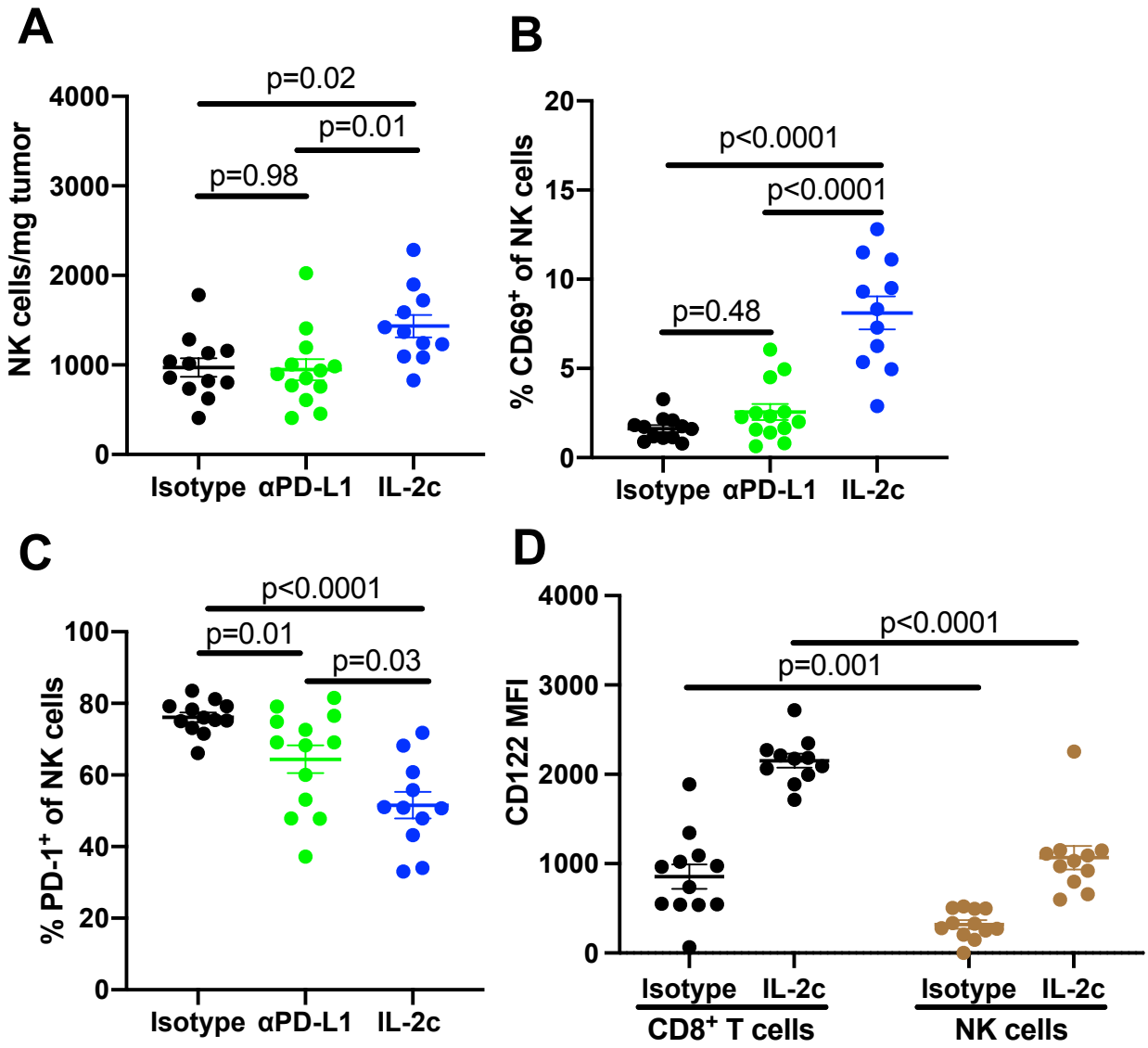
Supplementary Fig 7 Single agent IL-2c or αPD-L1 treats peritoneal B16 melanoma. Wild type male mice were challenged peritoneally with 4 x 10^5 B16 cells and treated with 100 µg αPD-L1 on days 7, 12, 17 or IL-2c on days 7, 9, 11, 13. (A) Omental tumor weight of mice sacrificed on day 15. N=11-13 mice/group, p value, one-way ANOVA. (B) Survival of mice bearing peritoneal B16 treated with αPD-L1 or IL-2c (as above). N=11-13 mice/group. p, log-rank. ANOVA, analysis of variance. IL-2c, interleukin-2 complex. PD-L1, Programmed Death Ligand 1.

Supplementary Fig 8



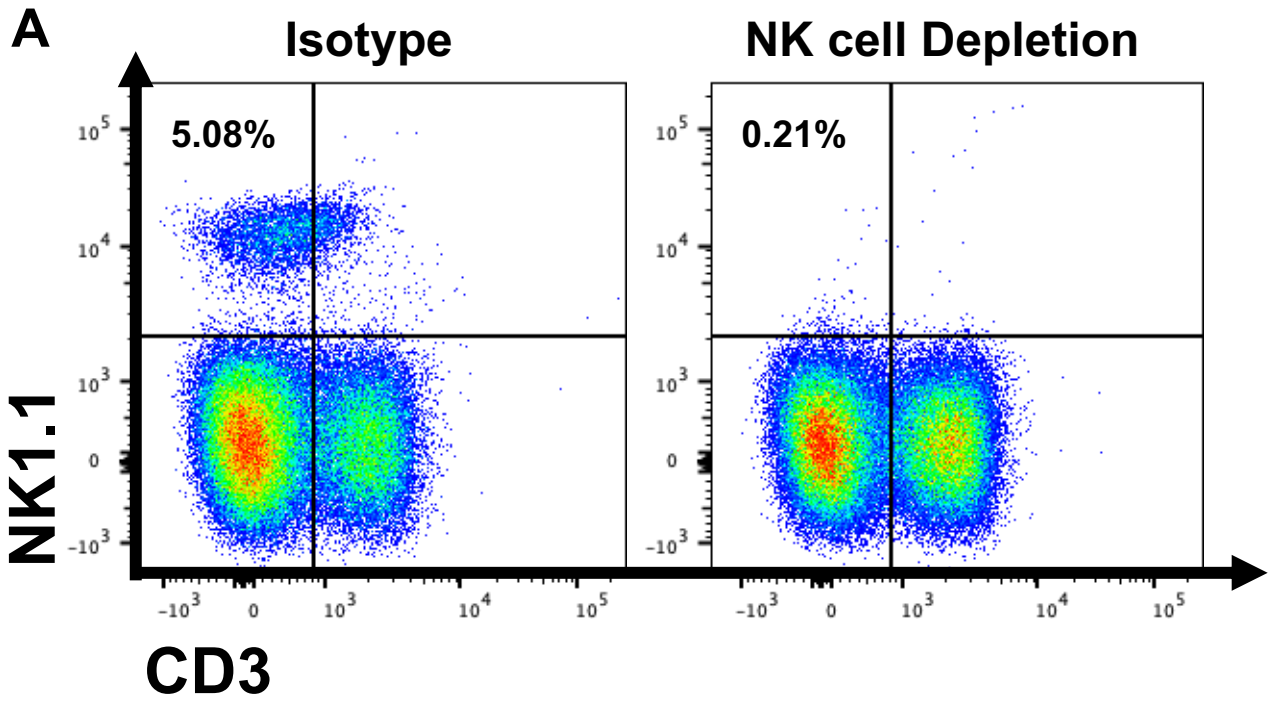
Supplementary Fig 8 Representative flow cytometry gating strategy of $CD8^+$ T and NK cells from peritoneal B16 tumors. $CD8^+$ T cells were defined as $CD45^+$ $CD3^+$ $CD8^+$ cells and NK cells were defined as $CD45^+$ $CD3^-$ $NK1.1^+$ with additional gating as shown. Eomes, Eomesodermin. IFN, Interferon. NK, Natural Killer.

Supplementary Fig 9



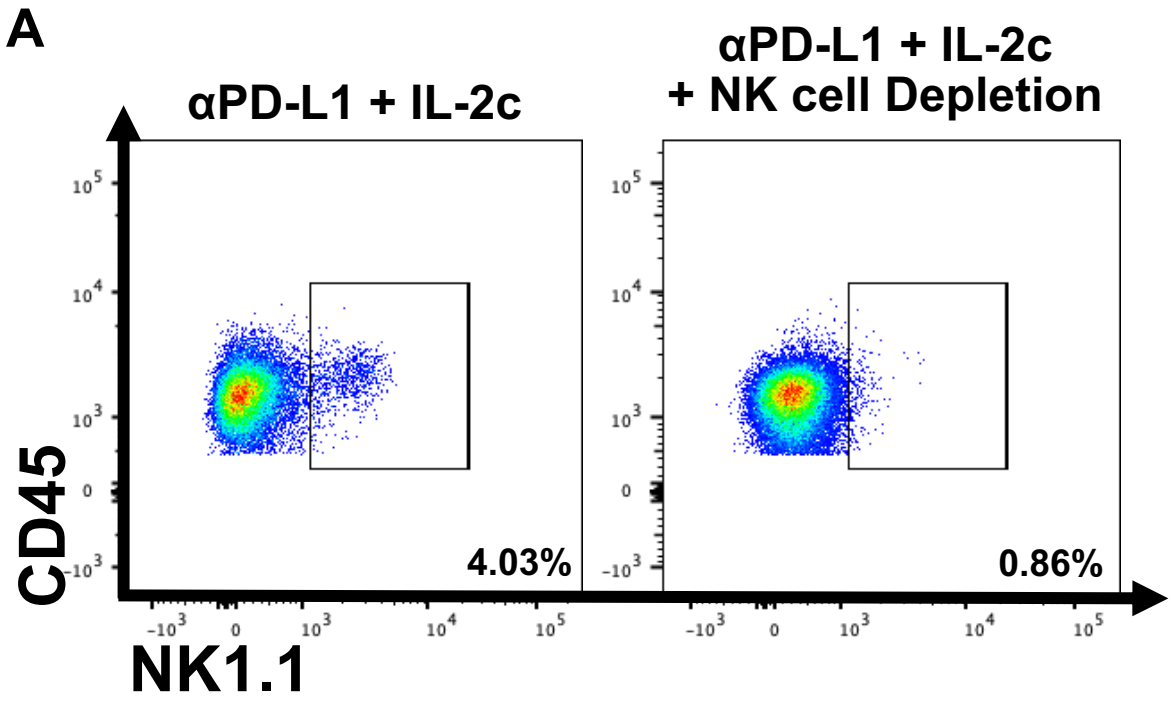
Supplementary Fig 9 IL-2c promotes NK cell effector function in peritoneal B16. Wild type male mice were challenged peritoneally with 4 x 10^5 B16 and treated with 100 μg αPD-L1 on days 7, 12, 17 or IL-2c on days 7, 9, 11, 13, or an isotype control. Mice were sacrificed on day 15 for flow cytometric analysis of intratumoral NK cell number normalized to tumor weight (A), CD69 expression (B), and PD-1 expression. (D) CD122 expression of $CD8^+$ T and NK cells in isotype and IL-2c treatment. N=11-12/group. p, one-way ANOVA. MFI, Mean Fluorescence Intensity. NK, Natural Killer. PD-L1, Programmed Death Ligand 1. PD-1, Programmed Death Receptor 1.

Supplementary Fig 10



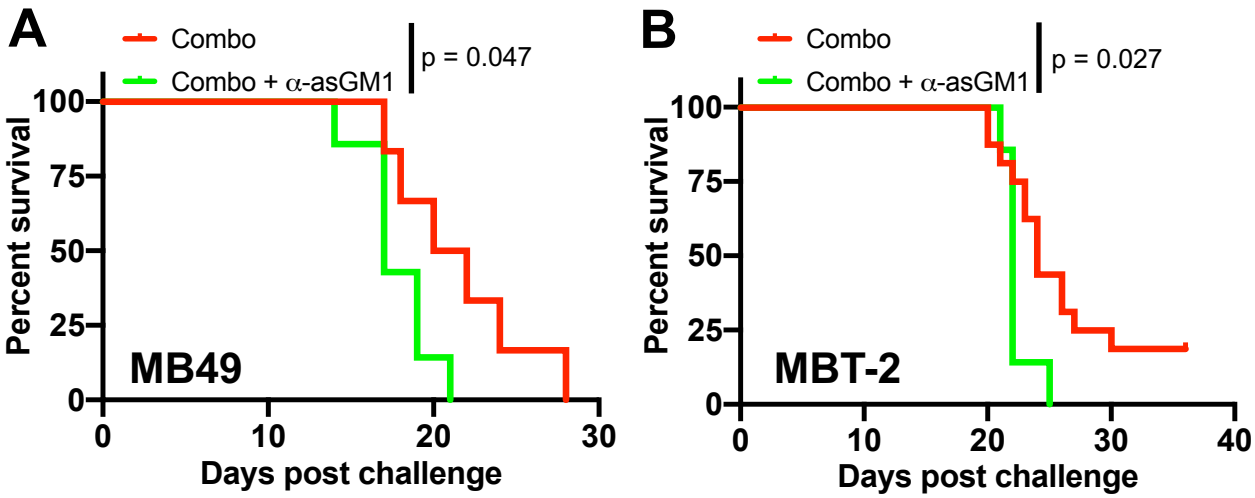
Supplementary Fig 10 NK cell depletion efficiency in B16 lung metastases. (A) Flow cytometric analysis showing NK cell frequency one day after intraperitoneal injection of 250 ug αNK1.1 in lung B16 tumor-bearing mice, as gated on live $CD45^+$ cells in spleen.

Supplementary Fig 11



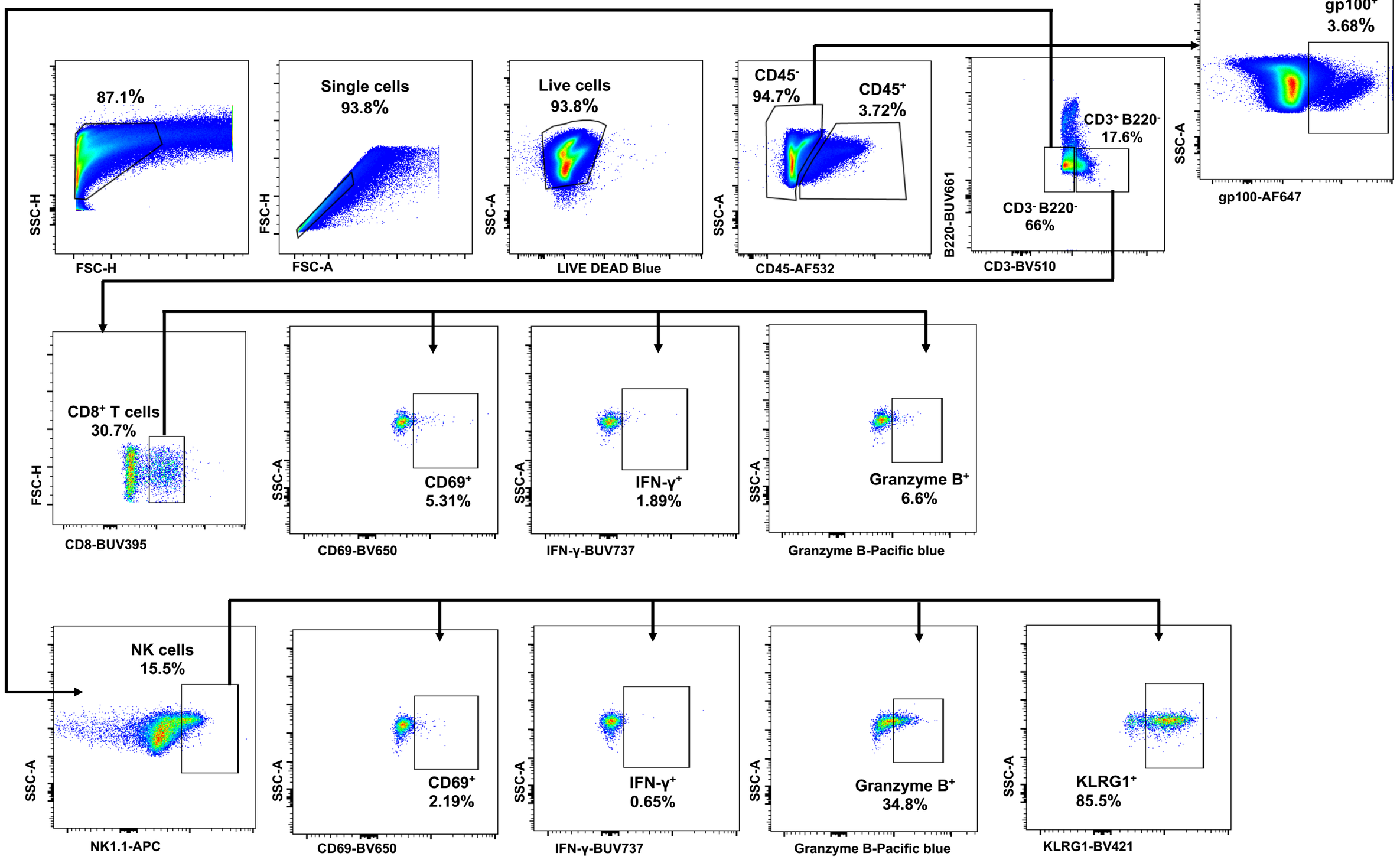
Supplementary Fig 11 NK cell depletion efficiency in MB49 lung tumors. (A) Flow cytometric analysis showing NK cell frequency one day after intraperitoneal injection of 200 μL α-asialo GM1 in lung MB49 tumor-bearing mice, as gated on live $CD45^{+}$ $CD3^{-}$ cells in lungs. PD-L1, Programmed Death Ligand 1.

Supplementary Fig 12



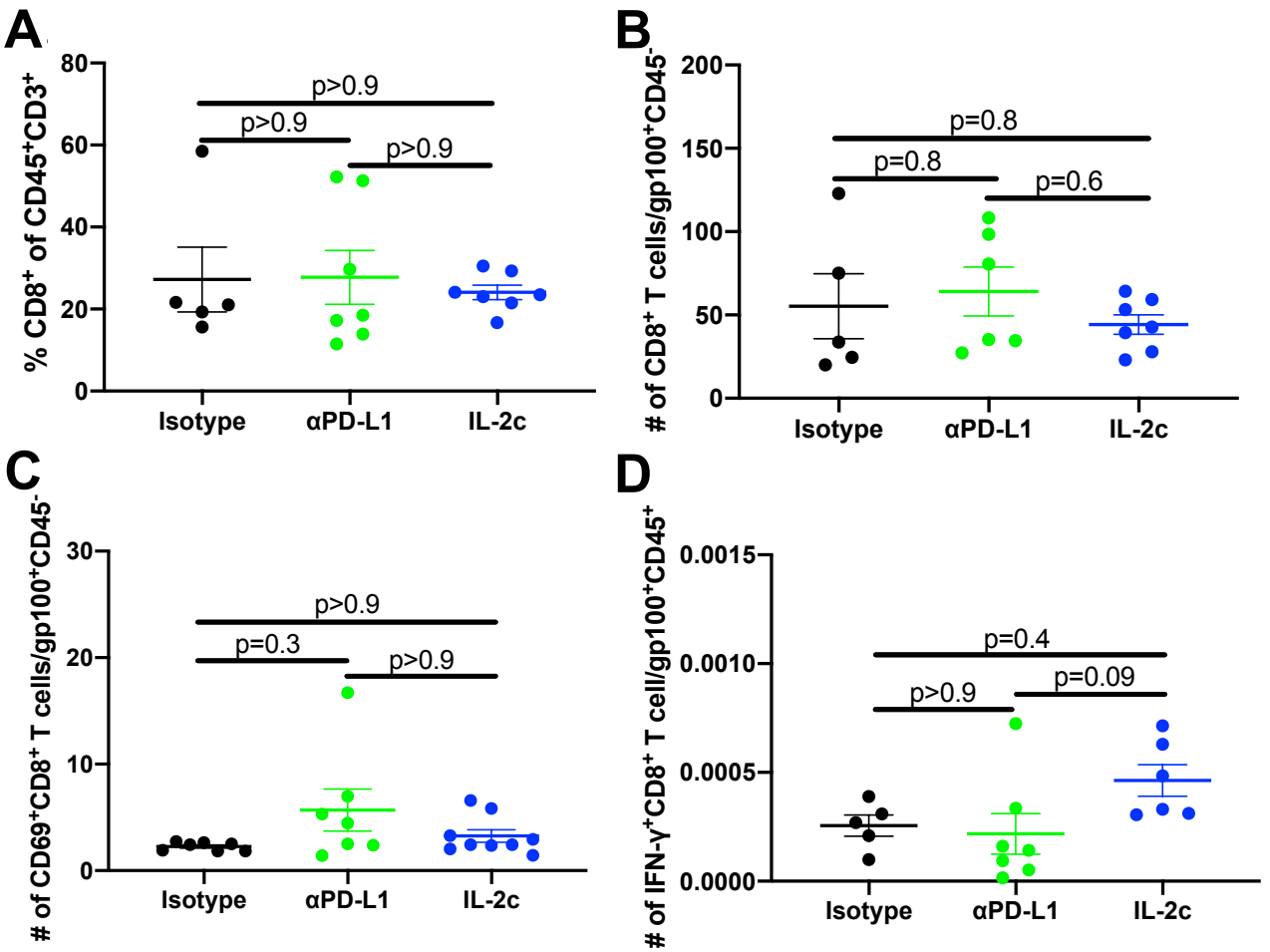
Supplementary Fig 12 NK cells are a critical mediator of IL-2c + αPD-L1 efficacy in BC lung metastasis. Wild type male mice were challenged intravenously with (A) 7 x 10^5 MB49, or (B) or 2.5 x 10^5 MBT-2 cells. Mice treated with IL-2c on days 8, 10, 12, 14 + 100 µg αPD-L1 on days 9, 14, 19 ± 200 µL α-asialo GM1 on days 7, 10, 13, 16, 19. (A) Mouse survival. N=6-7 mice/group. p, log-rank. (B) Mouse survival. N=7-16 mice/group. p, log-rank. PD-L1, Programmed Death Ligand 1

Supplementary Fig 13



Supplementary Fig 13 Representative flow cytometry gating strategy of $CD8^+$ T and NK cells from the lungs of mice challenged with intravenous B16 tumors. $CD8^+$ T cells were defined as $CD45^+$ $CD3^+$ $B220^-$ $CD8^+$ cells and NK cells were defined as $CD45^+$ $CD3^-$ $B220^-$ $NK1.1^+$ with additional gating as shown. Eomes, Eomesodermin. IFN, Interferon. KLRG1, Killer Cell Lectin Like Receptor G1. NK, Natural Killer.

Supplementary Fig 14



Supplementary Fig 14 IL-2c and αPD-L1 have no effect on lung $CD8^+$ T cell activation in B16 lung metastasis. Wild type male mice were challenged intravenously with 3 x 10^5 B16 cells and treated with 100 μg αPD-L1 on days 14, 19, or IL-2c on days 14, 16, 18, or isotype control. (A) $CD8^+$ T cell frequency. (B) $CD8^+$ T cell number normalized to $gp100^+$ $CD45^-$ cell number. (C) $CD69^+$ $CD8^+$ T cell number normalized to $gp100^+CD45^-$ cell number. (D) IFN-γ^+CD8^+ T cell number normalized to $gp100^+$ $CD45^-$ cell number. N=5-9 mice/group. p value, one-way ANOVA. ANOVA, analysis of variance. IFN-γ, interferon-gamma. IL-2c, interleukin-2 complex. PD-L1, Programmed Death Ligand 1.