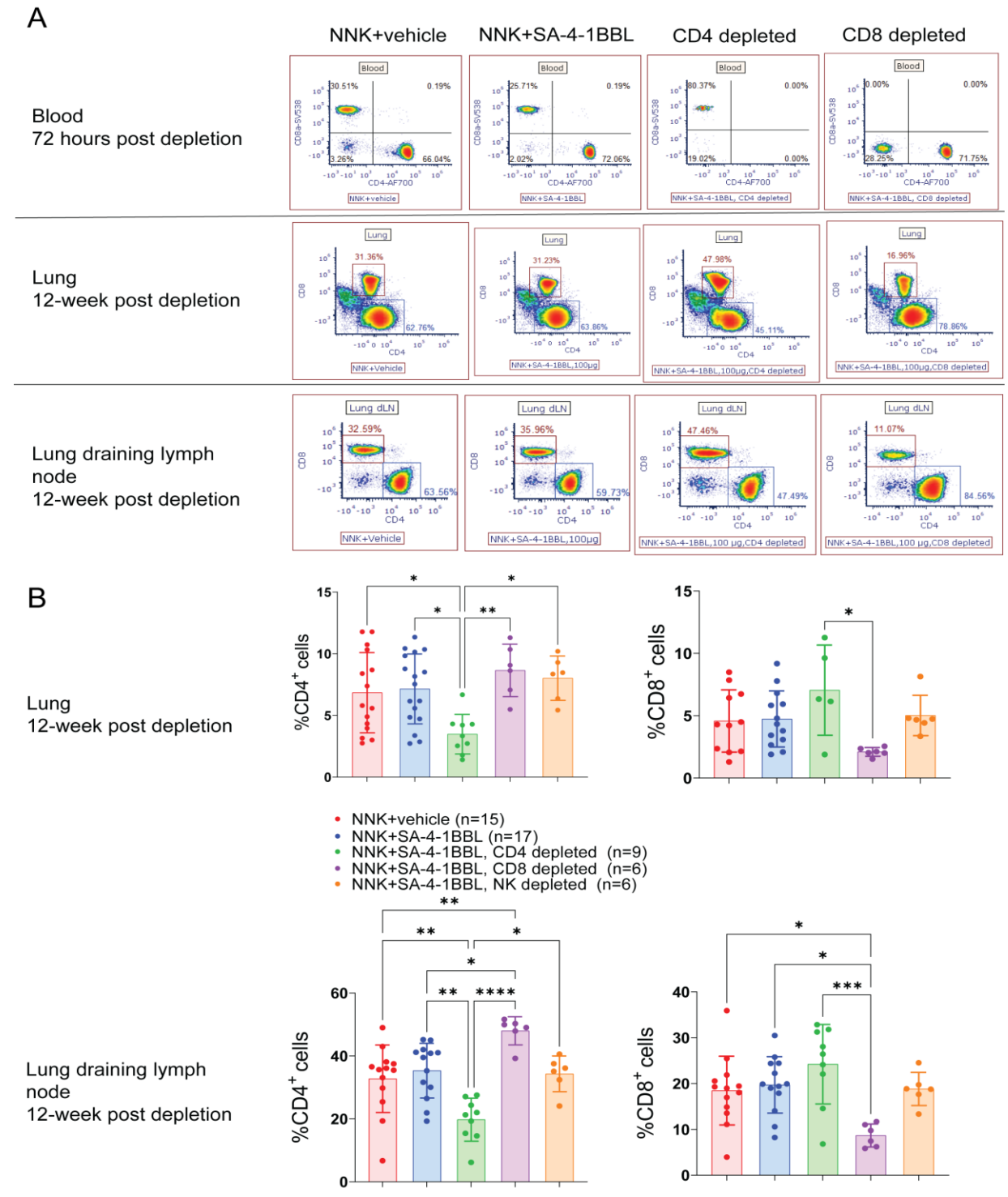
Supplementary Fig. S1



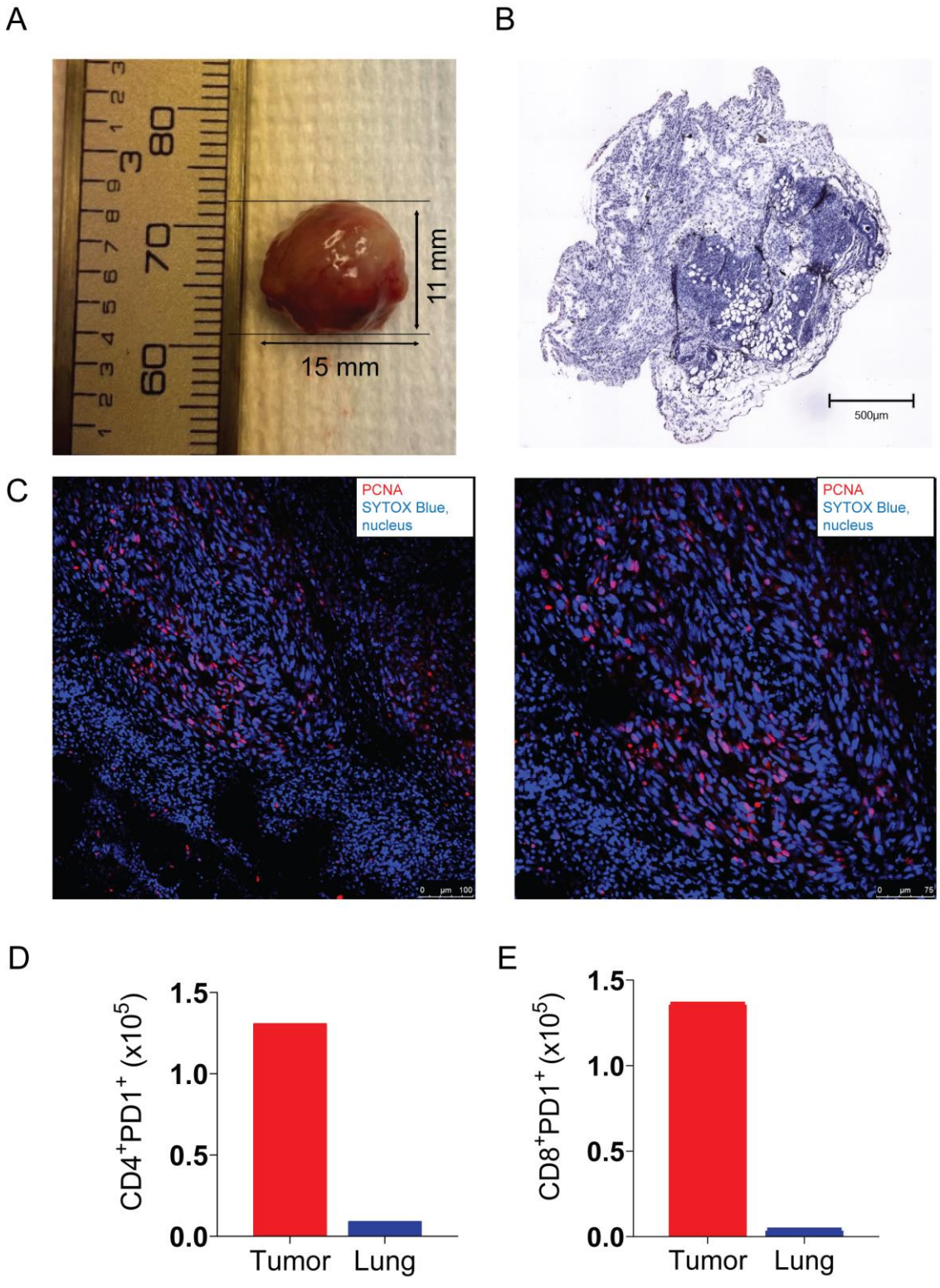
Supplementary Fig. S1. Flow analysis of CD4+ T, CD8+ T cells depletion at 72 hours and 12 weeks post depletion in the peripheral blood, lung, and lung draining lymph nodes

As previously described, female A/J mice received depletion antibodies intraperitoneally 24 hours after SA-4-1BBL treatment in the sixth and eighth weeks of NNK injections. **A.** Representative dot plots show the CD4+ T and CD8+ T cell depletion at 72 hours post-depletion (first row). Control animals (NNK+vehicle) and animals treated with NNK+SA-4-1BBL without antibody depletion served as controls. Recovery of CD4+ and CD8+ T cells following depletion was incomplete in the lung and lung draining lymph nodes 12 weeks post depletion (second and third rows). Using flow analysis to compare the peripheral blood of NK-depleted A/J mice to that of non-depleted A/J mice, we were unable to detect a distinct NK cell depletion. The same clone of NK-depleting antibody effectively depleted NK cells in C57BL/6 mice by our team[1] (Supplementary Fig. S5 in ref#1) and others [2] at the dose used for A/J mice, demonstrating antibody activity on NK cells. This phenotype was consistent with previously published findings that A/J rodents have significantly fewer NK cells in their circulation and spleen [3]. **B.** Tabulated CD4+ T and CD8+ T cell percentage in the lung and lung-draining lymph nodes 12 weeks post depletion. Lung and lung-draining lymph node immune cell compositions were compared using a one-way ANOVA test. The presented data were compiled from 3-4 repeated experiments and are represented as mean ± SD. A p-value ≤ 0.05 was considered significant

Reference List

1. Barsoumian, H.B., et al., *A Novel Form of 4-1BBL Prevents Cancer Development via Nonspecific Activation of CD4(+) T and Natural Killer Cells.* Cancer Res, 2019. **79**(4): p. 783-794.
2. Nilsson, N., et al., *Protective role of NK1.1+ cells in experimental Staphylococcus aureus arthritis.* Clin Exp Immunol, 1999. **117**(1): p. 63-9.
3. Whyte, A.L. and S.C. Miller, *Strain differences in natural killer cell-mediated immunity among mice: a possible mechanism for the low natural killer cell activity of A/J mice.* Immunobiology, 1998. **199**(1): p. 23-38.

Supplementary Fig. S2



Supplementary Fig. S2. A mass found in CD4 T cells depleted group.

In the 18-week follow-up, a mass was observed on the chin of one of the mice from the CD4+ T cell-depleted group. **A.** Morphological picture of the mass. The tumor had distinct borders and was easily removed. The tumor size was measured using a digital caliper. It was approximately 11 mm x 15 mm. **B.** In H&E staining, inside the adipose and fibrous tissue, a hyperplastic tumoral area was seen, with similar histopathological features of microscopic tumor nodules found in the lung tissues. Enlarged cuboidal cells, increased mitoses, and hyperchromatic cell nuclei characterized the tumoral focus. Magnification of H&E-stained sample image: 10X, scale bar shows 500 µm. **C**. Confocal photomicrographs showed positive PCNA staining. Nuclear proliferation marker PCNA antibody (red) and SYTOX blue nuclear stain (blue) were used to show increased proliferative capacity in the malignancy area. Zoom factors of confocal microscopy photomicrographs are 1 and 1.42. Scale bars are 100 µm (left) and 75 µm (right). At the endpoint of the experiment, deep immune phenotyping was also performed on this metastatic nodule. **D.** The metastatic nodule had fourteen-fold more CD4+PD-1+ T cells than the lung infiltrating CD4+ T cells. **E**. The metastatic nodule had a thirty-one-fold increase in CD8+PD-1+ T cell population compared to lung infiltrating CD8+ T cells. H&E (Hematoxylin and eosin), PCNA (Proliferating cell nuclear antigen).

Supplementary Table S1

Antibody	Clone	Company, Catalog no.
Anti-mouse CD3-V500	500A2	BD Horizon, 560771
Anti-mouse CD4-BUV496	GK1.5	BD Biosciences, 612952
Anti-mouseCD8-SparkViolet538	QA17A07	Biolegend, 155019
Anti-mouseCD11b-PerCP Cy 5.5	M1/70	BD Pharmakon, 550993
Anti-mouse CD11c-AF647	N418	Biolegend, 117312
Anti-mouse CD19-BV570	6D5	Biolegend, 115535
Anti-mouse CD25-PE/Cy5	PC61	Biolegend, 102010
Anti-mouse CD44-BB700	IM7	BD Biosciences, 566506
Anti-mouse CD45-APC Fire810	30-F11	Biolegend, 103174
Anti-mouse CD62L-BV711	MEL-14	Biolegend, 104445
Anti-mouse CD107a-PE/Cy7	1D4B	BD Biosciences, 560647
Anti-mouse PD-1 (CD279) APC	29F.1A12	Biolegend, 135210
Anti-Mouse CD335 (NKp46)- BUV737	29A1.4	BD Biosciences, 612805
Anti-mouse NK1.1 AF700	PK136	Biolegend, 108730
Anti-mouse Ly6C-BV605	HK1.4	Biolegend, 128036
Anti-mouse Ly6G-BUV395	1A8	BD Biosciences, 563978
Anti-mouse F4/80-Pacific Blue	BM8	Biolegend, 123124
LIVE/DEAD™ Fixable Blue Dead Cell	N/A	Fischer Scientific, L23105
Anti-mouse γδ T-Cell Receptor-BUV563	V65	BD Biosciences, Cat#749464
Anti-mouse CD366 (Tim-3)- PE	RMT-3-23	BioLegend, 119704

Supplementary Table S1. Fluorescent-conjugated antibodies panel used for flow cytometric immunophenotyping to evaluate the immune cell profiles in the lungs and lung-draining lymph nodes.