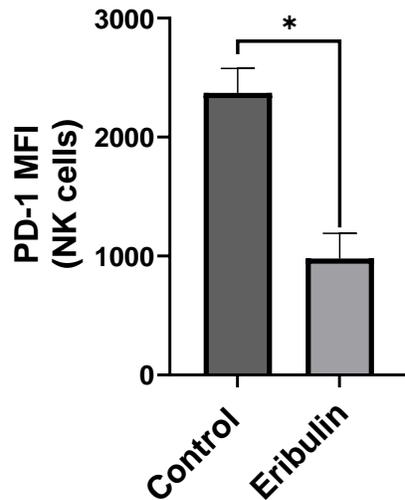


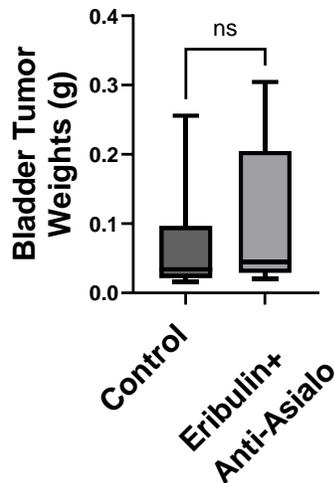
Supplementary Figures

Supplementary Figure 1. Eribulin treatment decreases the expression of exhaustion markers on NK cells *in vivo*



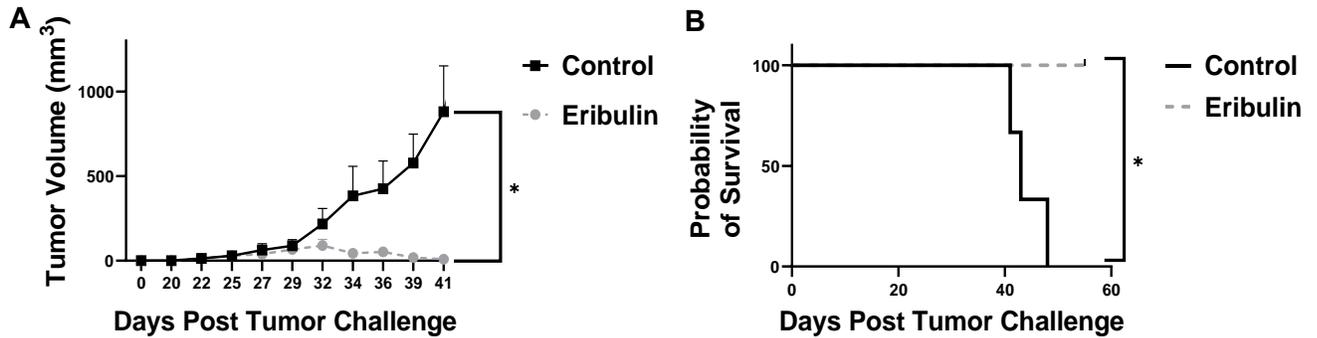
Supplementary Figure S1. Eribulin treatment decreases the expression of exhaustion markers on NK cells *in vivo* C57BL/6 mice were challenged by intravesical instillation of MB49 murine BCa cells and treated with 1.8 mg/kg of eribulin or 100 μ L of DPBS by intravesical instillation weekly for four weeks beginning on day 1. Shown is the expression of PD-1 on tumor infiltrating NK cells from one representative results of two independent experiments (n=5 per group).

Supplementary Figure S2. NK cell depletion abolishes the anti-tumor effects of eribulin treatment in bladder cancer



Supplementary Figure S2. NK cell depletion abolishes the anti-tumor effects of eribulin treatment in bladder cancer C57BL/6 mice were challenged by intravesical instillation of MB49 murine BCa cells and treated with 1.8 mg/kg of eribulin or 100 μ L of DPBS by intravesical instillation weekly for four weeks beginning on day 1. NK cells were depleted by intraperitoneal injection of anti-Asialo GM-1, weekly starting 3 days prior to orthotopic challenge. On day 20, bladders were excised, and the bladder weight was recorded prior to digestion (n=5 per group). Ns: not significant.

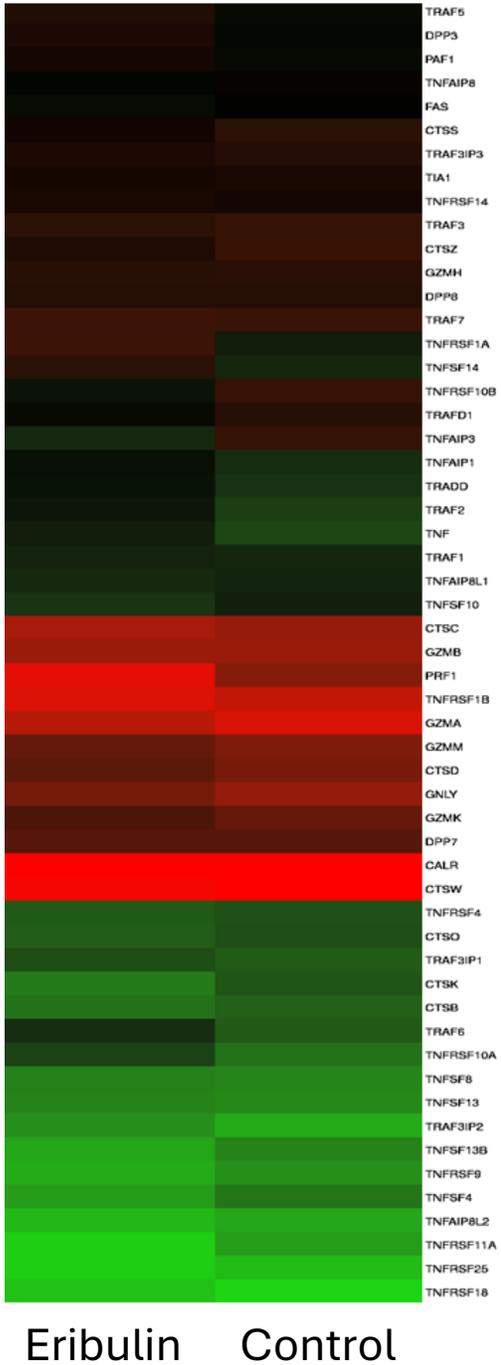
Supplementary Figure S3. Eribulin treatment reduces tumor burden in humanized murine BCa model



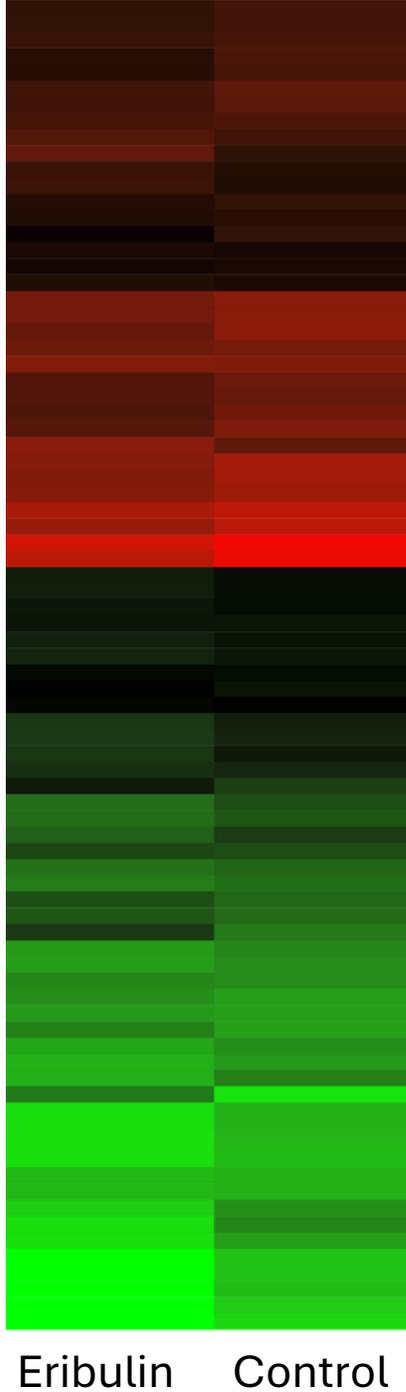
Supplementary Figure S3. Eribulin treatment reduces tumor burden in humanized murine BCa model NSG mice were injected subcutaneously with NK92.MI and UMUC3 BCa cells in a 3:1 ratio and treated with 1 mg/kg of eribulin by weekly intratumoral injection once tumors reached 100 mm³ (approximately day 20). Tumor volumes were measured three times a week and mice were euthanized once tumor volumes reached 1500 mm³ (**A**). *p<0.05 by a two-way ANOVA test. (**B**) Kaplan-Meier curve of NSG mice based on days post challenge until tumor volume reached 1500 mm³. *p<0.05 by log-rank (Mantel-cox) test.

Supplementary Figure S4. Eribulin treatment significantly alters the RNA expression of genes involved in NK cell functionality

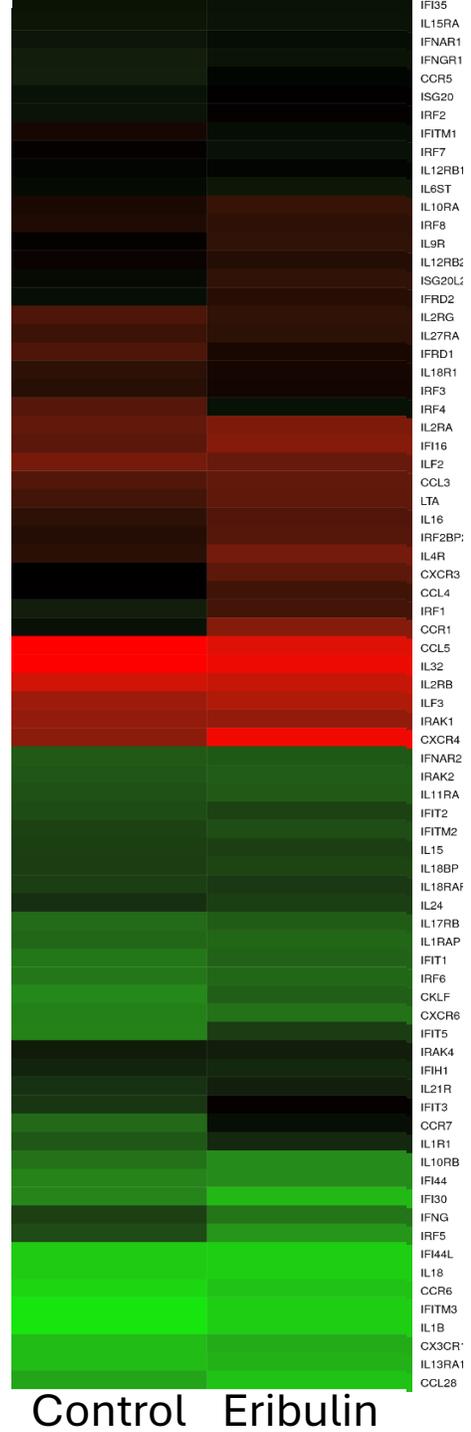
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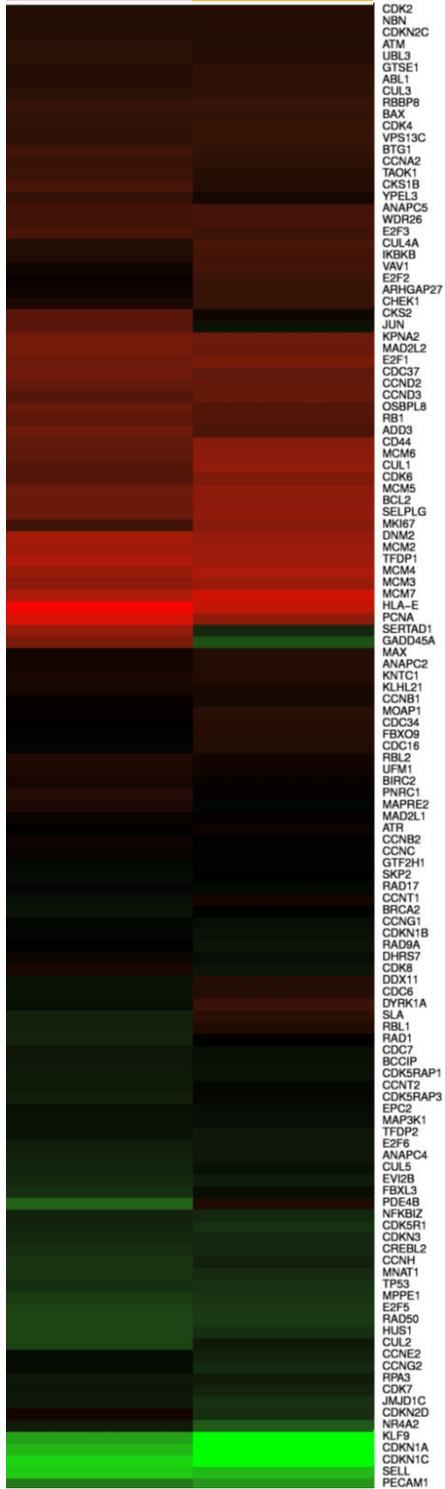
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C



D



Control Eribulin

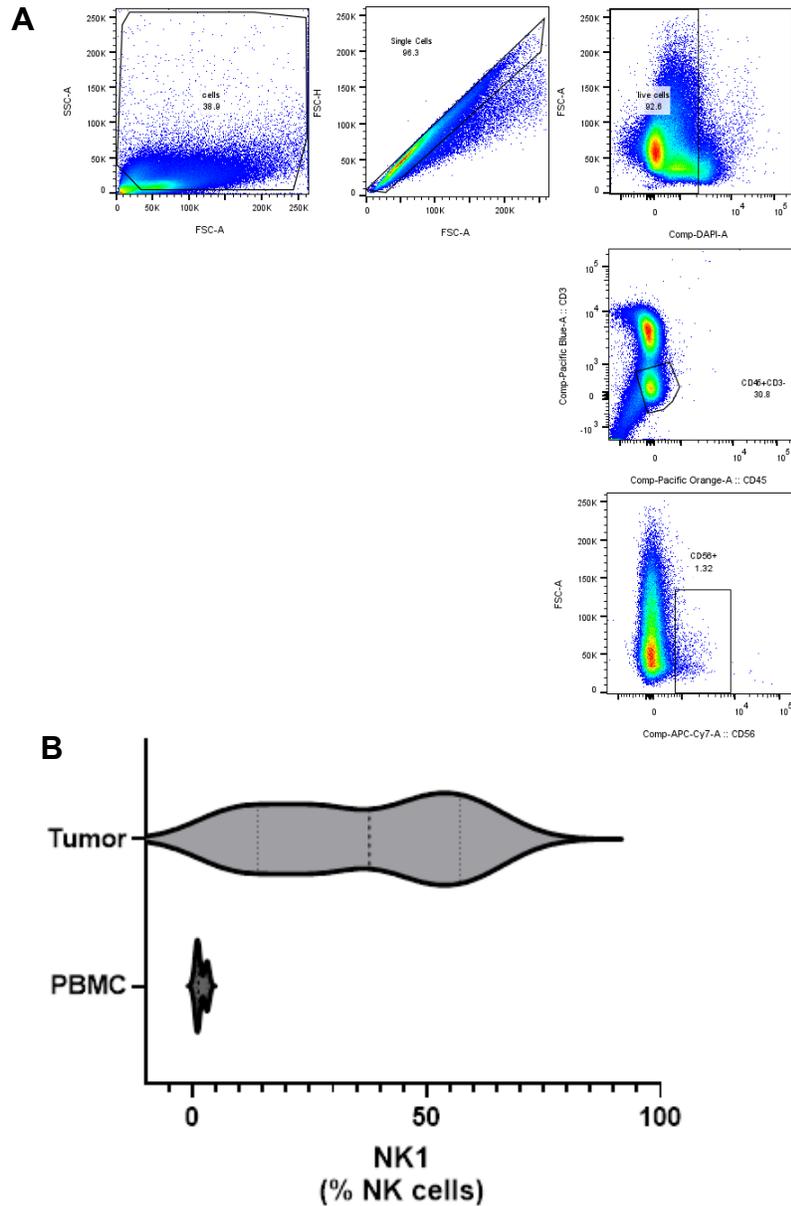
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Eribulin Control

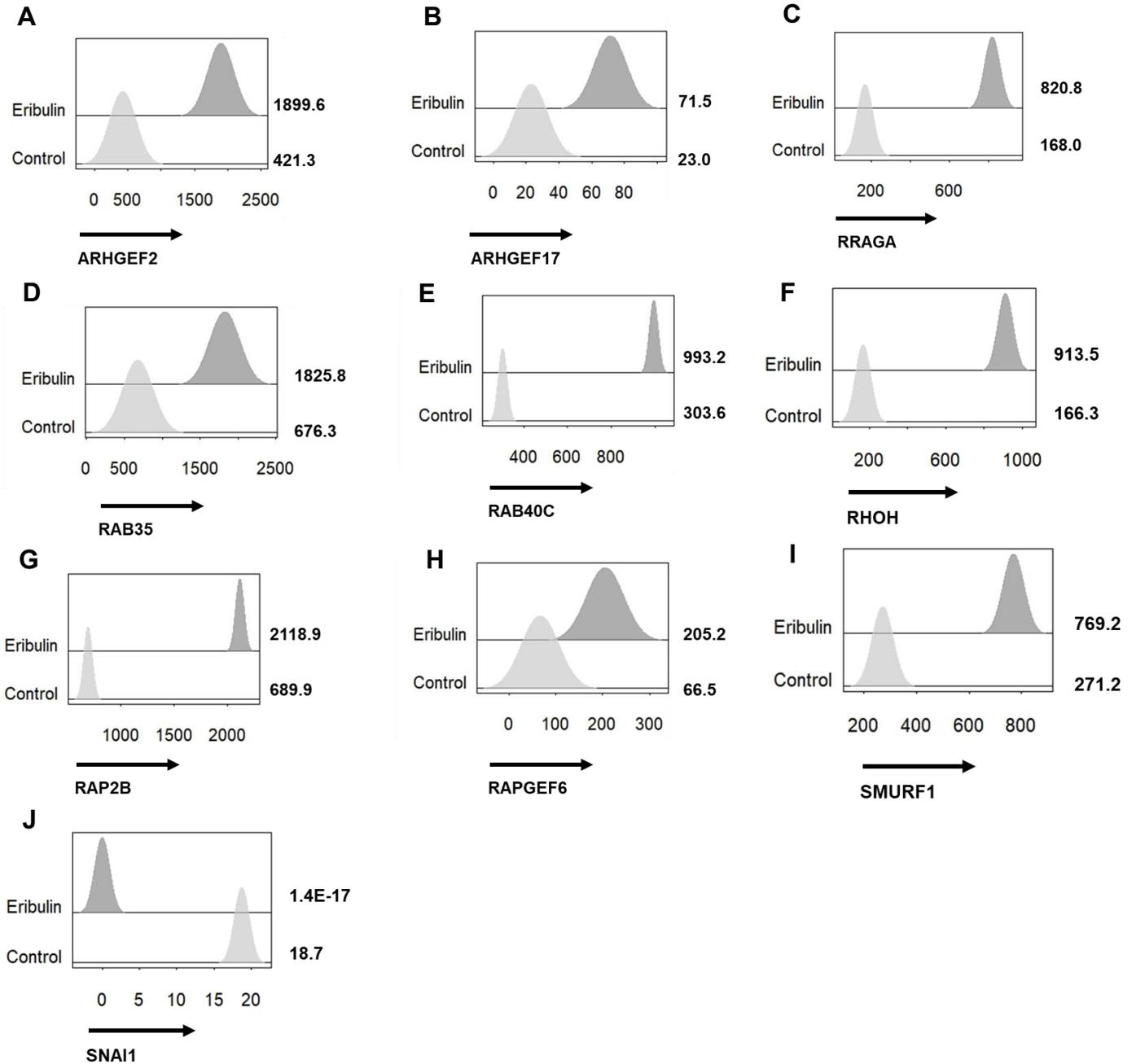
Supplementary Figure S4. Eribulin treatment significantly alters the RNA expression of genes involved in NK cell functionality Heatmap of bulk RNAseq data of NK92 cells treated with 100 nM of eribulin for 3 hours. Genes related to **(A)** cytotoxicity, **(B)** adhesion, **(C)** secreted cytokines, **(D)** cell cycle, **(E)** and quiescence.

Supplementary Figure S5. Subset of tumor infiltrating NK cells display a unique phenotype not seen in peripheral blood NK cells



Supplementary Figure S5. Subset of tumor infiltrating NK cells display a unique phenotype not seen in peripheral blood NK cells Human peripheral blood and tumor samples were stained and analyzed by flow cytometry. **(A)** A representative gating strategy used to identify NK cell subsets in human PBMC samples. The population of doublets was first excluded, followed by selecting living cells (FVD⁻). NK cells, defined as CD56⁺ cells, were gated from the total CD45⁺CD3⁻ population. **(B)** Shown is the proportion of peripheral or tumor-infiltrating NK cells that are CD49a⁺CD103⁺. Values were based on pooled data from 3 patients (PBMC) and 4 patients (Tumor).

Supplementary Figure S6. Eribulin treatment influences TGF β signaling related genes in NK cells



Supplementary Figure S6 Eribulin treatment influences TGF β signaling related genes in NK cells

For bulk RNA sequencing, NK92 cells were treated with 100 nM of eribulin for 3 hours before extraction of RNA. Shown are the FPKM of (A) *ARHGEF2*, (B) *ARHGEF17*, (C) *RRAGA*, (D) *RAB35*, (E) *RAB40C*, (F) *RHOH*, (G) *RAP2B*, (H) *RAPGEF6*, (I) *SMURF1*, and (J) *SNAI1* illustrated as histograms. Differential analysis of illustrated genes was found to be significant, with p-values <0.05.