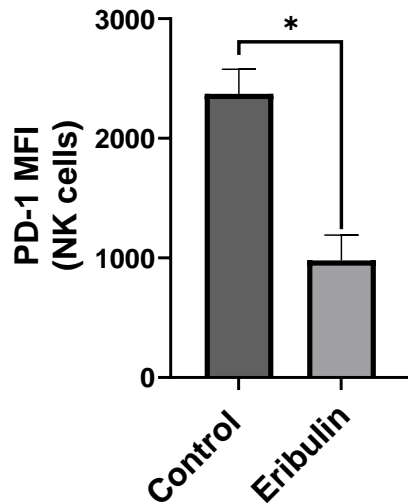


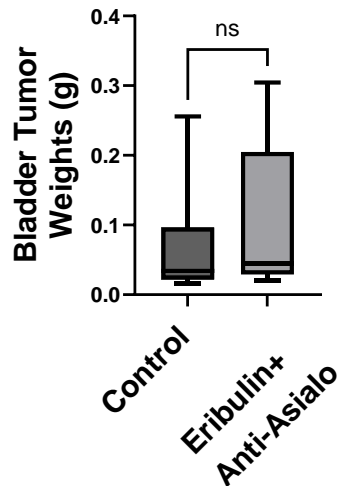
## Supplementary Figures

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



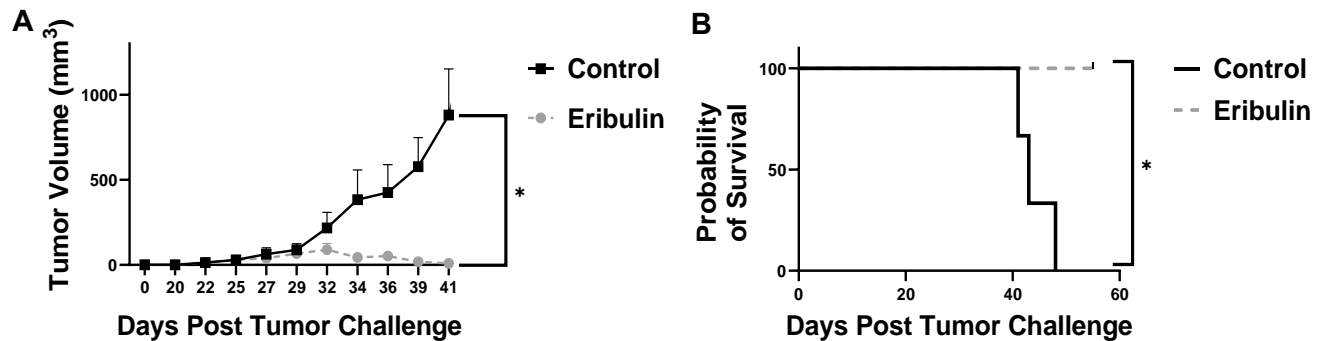
**Supplementary Figure S1. Eribulin treatment decreases the expression of exhaustion markers on NK cells *in vivo*** C57BL/6 mice were challenged by intravesical instillation of MB49 murine BCa cells and treated with 1.8 mg/kg of eribulin or 100  $\mu$ L of DPBS by intravesical instillation weekly for 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**

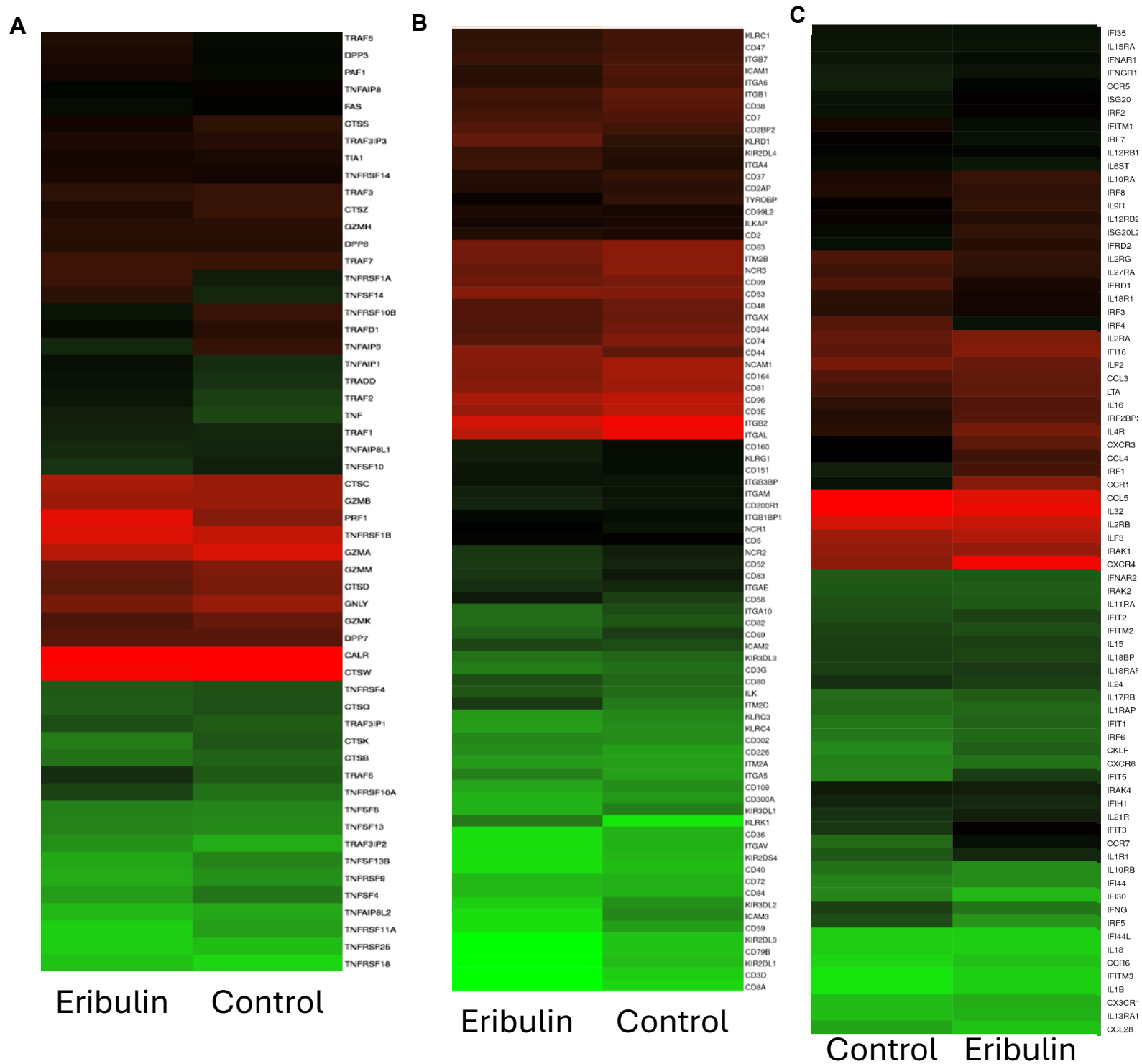


**Supplementary Figure S2. NK cell depletion abolishes the anti-tumor effects of eribulin treatment in bladder cancer** C57BL/6 mice were challenged by intravesical instillation of MB49 murine BCa cells and treated with 1.8 mg/kg of eribulin or 100  $\mu$ L of DPBS by intravesical instillation weekly 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.

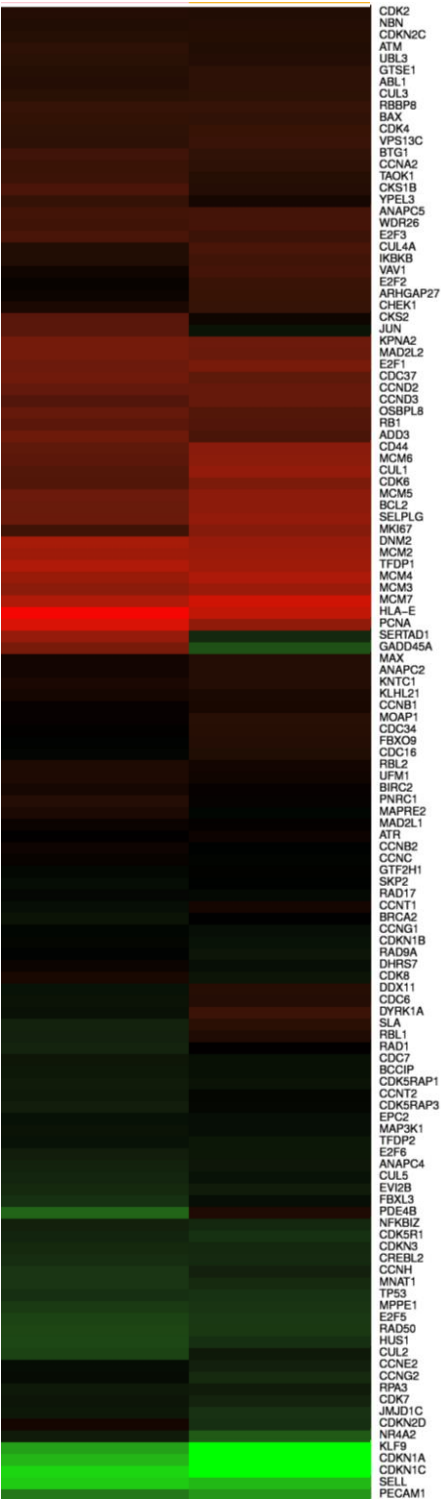
### Supplementary Figure S3. Eribulin treatment reduces tumor burden in humanized murine BCa model



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



D



Control Eribulin

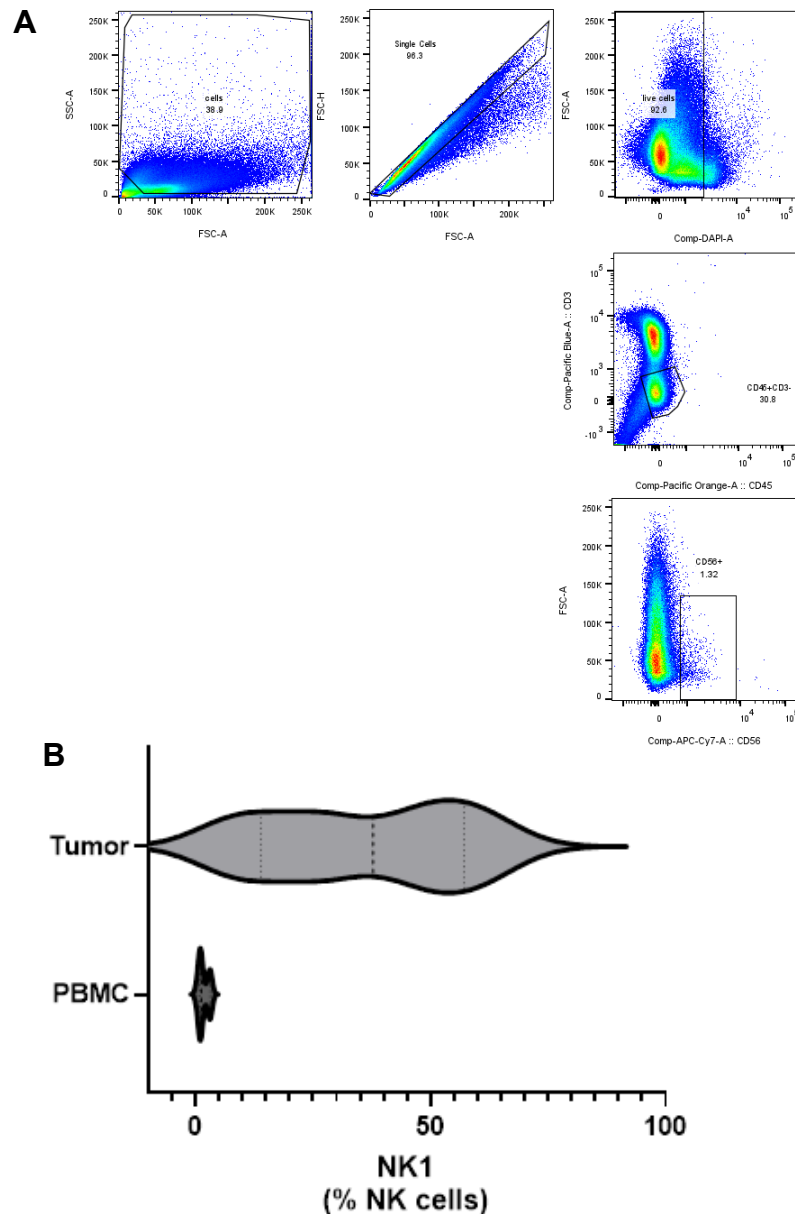
E



Eribulin Control

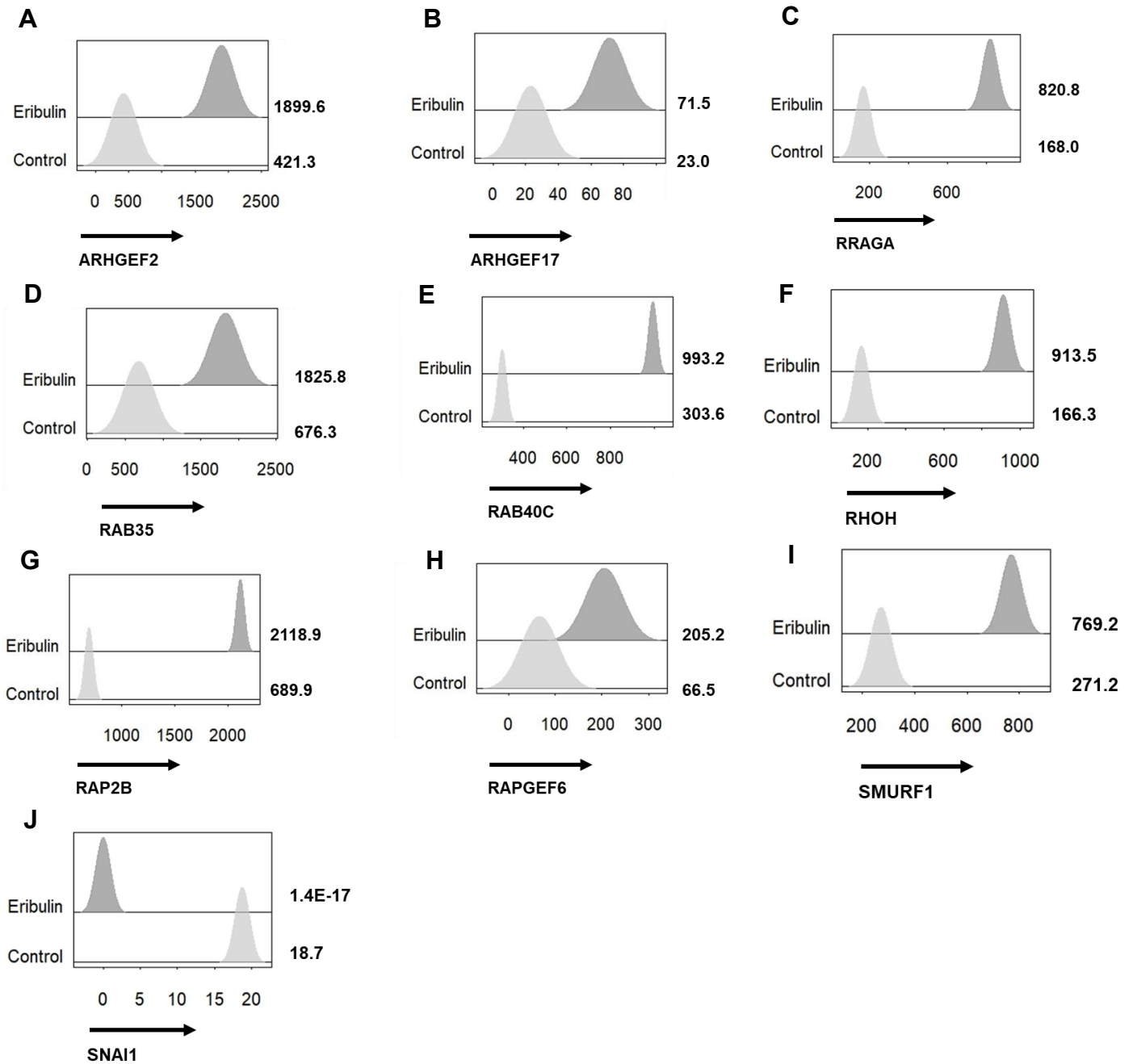
**Supplementary Figure S4. Eribulin treatment significantly alters the RNA expression of genes involved in NK cell functionality** Heatmap of bulk RNAseq data of NK92 cells treated with 100 nM of eribulin for 3 hours. Genes related to **(A)** cytotoxicity, **(B)** adhesion, **(C)** secreted cytokines, **(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<sup>-</sup>). NK cells, defined as CD56<sup>+</sup> cells, were gated from the total CD45<sup>+</sup>CD3<sup>-</sup> population. **(B)** Shown is the proportion of peripheral or tumor-infiltrating NK cells that are CD49a<sup>+</sup>CD103<sup>+</sup>. Values were based on pooled data from 3 patients (PBMC) and 4 patients (Tumor).

# **Supplementary Figure S6. Eribulin treatment influences TGF $\beta$ signaling related genes in NK cells**



## **Supplementary Figure S6 Eribulin treatment influences TGF $\beta$ 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.