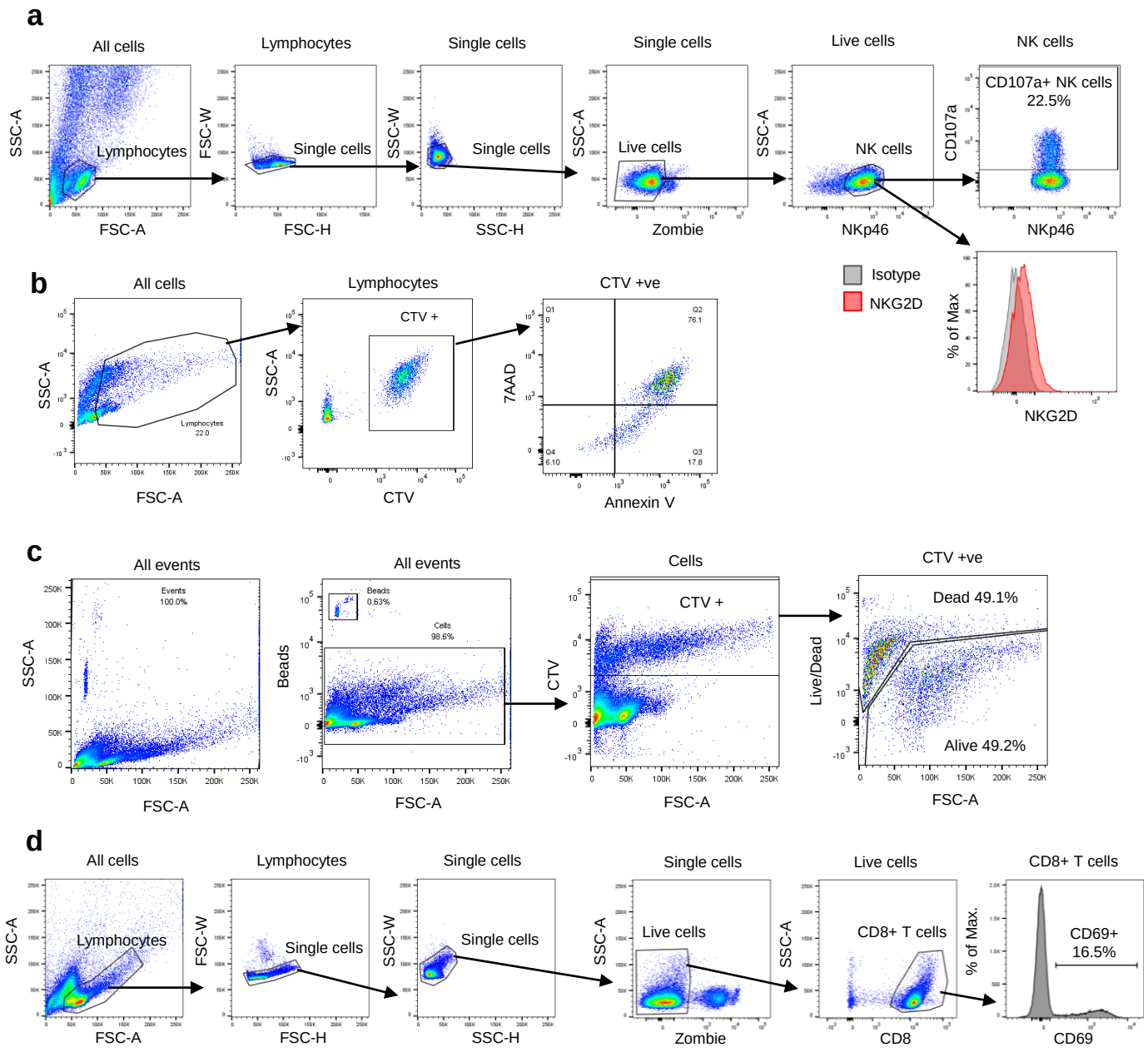
Supplementary Fig. 1.



Supplementary Fig. 1. Flow cytometry gating strategies for NK cell degranulation, T cell activation and cytotoxicity assays. a, Gating strategy for the identification of degranulating (CD107a+) and NKG2D+ NK cells upon co-culture of naïve NK cells with NB targets. Plots represent NK cells co-cultured with SHEP cells at an E:T ratio of 1:2. CD107a+ gate was drawn based on NK cells alone. **b,** Gating strategy for determining 7AAD+ and Annexin V+ NB cells following co-culture with NK cells. Plots represent SH-EP cells co-cultured with NK cells at an E:T ratio of 3:1. For all targets, target cell alone was used as a negative control. **c,** Gating strategy for identifying viable and dead NB cells following co-culture with PRAME-CTLs. Plots represent CHP-212 cells co-cultured with PRAME-CTLs at an E:T ratio of 10:1. For all targets, target cell alone was used as a negative control. **d**, Gating strategy for quantifying CD8+ CD69+ OT-1 cells upon co-culture of naïve OT-1 cells with H-2Kb^{lo} or H-2Kb^{hi} -NB-9464 cells. Plots represent OT-1 cells co-cultured with H-2Kb^{hi} cells pulsed with OVA peptide at an E:T ratio of 1:1.

Supplementary Protocols

Double Immunofluorescence staining of tumor sections

Staining was performed by Applied Pathology Systems (Shrewsbury, MA). Formalin-fixed-paraffin-embedded (FFPE) murine tumor sections (5 µm) were baked at 60^{o} C for 1h, deparaffinized, rehydrated, washed and placed in a loosely covered container with 1x AR6 buffer (Akoya Biosciences) and microwaved on high for 90 seconds and on low power for 15min. After cooling to RT, slides were rinsed with distilled water and TBST, and sequentially incubated with Opal antibody diluent/blocking reagent (Akoya Biosciences) for 10min at RT and anti-Prrx1 antibody overnight at 4^{o}C. Following 3 x 2-min. washes in TBST, slides were sequentially incubated with Opal polymer anti-rabbit HRP secondary antibody and Opal fluorophore 690 reagent (Akoya Biosciences) for 10min at RT. Slides were washed in TBST and microwaved in 1x AR9 buffer (Akoya Biosciences) as described above and sequentially incubated with Opal antibody diluent/blocking reagent for 10min at RT and anti-MYCN antibody overnight at 4^{o} C, followed by TBST washes and sequential incubation with anti-rabbit HRP secondary antibody and fluorophore 480 reagent as described above. Next, slides were microwaved in 1x AR9 buffer as above, and following sequential washes in water and TBST, counterstained with Hoechst (1:500 dilution, Thermo Fisher Scientific) for 5min at RT. Following sequential rinses in TBST and water, slides were mounted in Prolong Diamond Antifade Mountant (Invitrogen). Images were acquired with an EVOS M5000 imaging system (Invitrogen) using the following channels: DAPI (Hoechst stain), Cy5 (690 reagent/Prrx1), and GFP (480 reagent/MYCN). Antibodies are detailed in **Supplementary Table 7**.

Immunohistochemistry (IHC) analysis of human tumors

IHC analysis of human tumor samples was performed by the Veterinary Pathology Core at St. Jude Children's Research Hospital. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. FFPE blocks were sectioned at 4 µm thickness and mounted on Superfrost

Plus glass slides (Thermo Fisher Scientific). IHC staining was performed on an auto-stainer. Following heat-induced epitope retrieval with cell conditioning solution 1 (Ventana Medical Systems), slides were incubated with antibodies against PHOX2B for 60min, and vimentin and CD8 for 20min **(Supplementary Table 7)**. PHOX2B and CD8 staining were visualized using DISCOVERY OmniMap anti-rabbit HRP and DISCOVERY ChromoMap DAB kit (Ventana Medical Systems). Vimentin staining was visualized using rabbit anti-mouse IgG (Abcam, 133469) and anti-rabbit HRP as above. Scanned images were visualized using the QuPath software platform for whole-slide image analysis (https://qupath.github.io/).