Supplementary Data

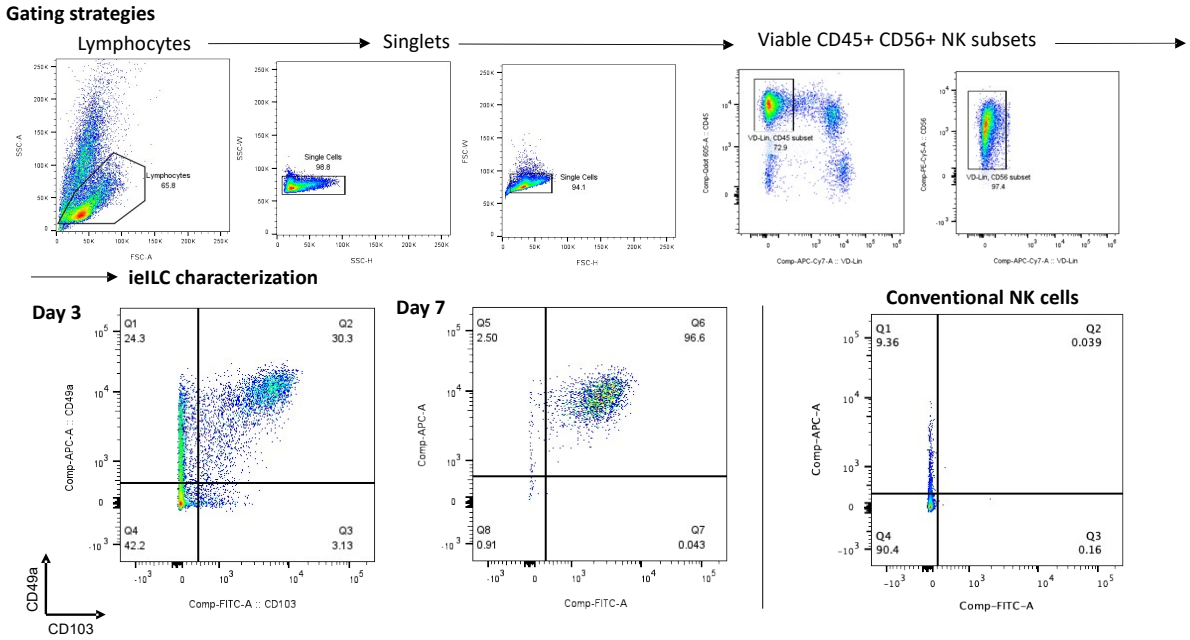

Figure S1: Gating Strategy for Distinguishing Conventional NK Cells from Tissue-Resident NK Cells in Flow Cytometry. CD49a+CD103+ intraepithelial lymphocyte (ieILC) characterization was performed on Days 3 and 7.

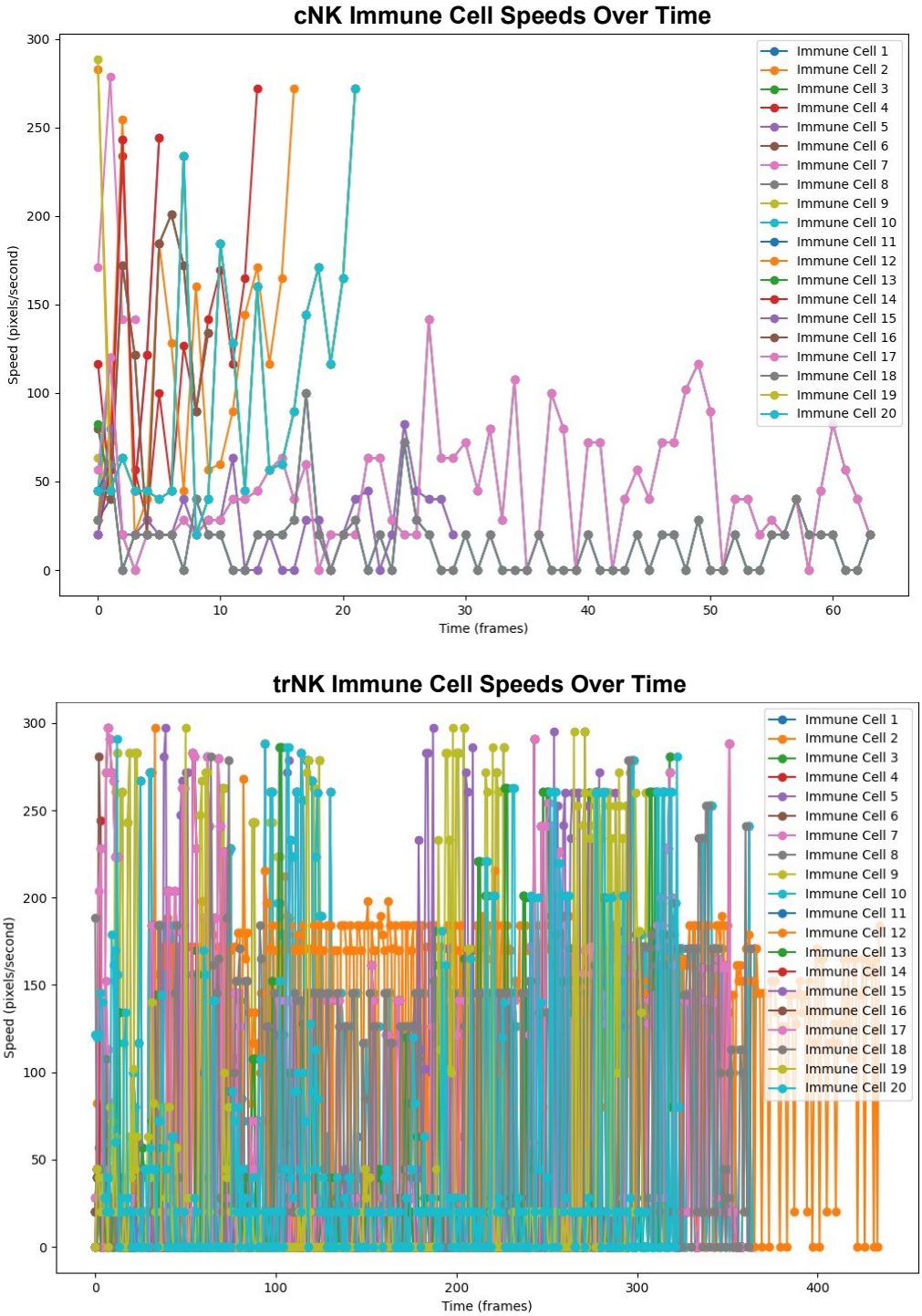

Figure S2. Speed analysis over time for cNK cells (top) and trNK cells (bottom), showing higher speeds and sustained motility for trNK cells compared to cNK cells.

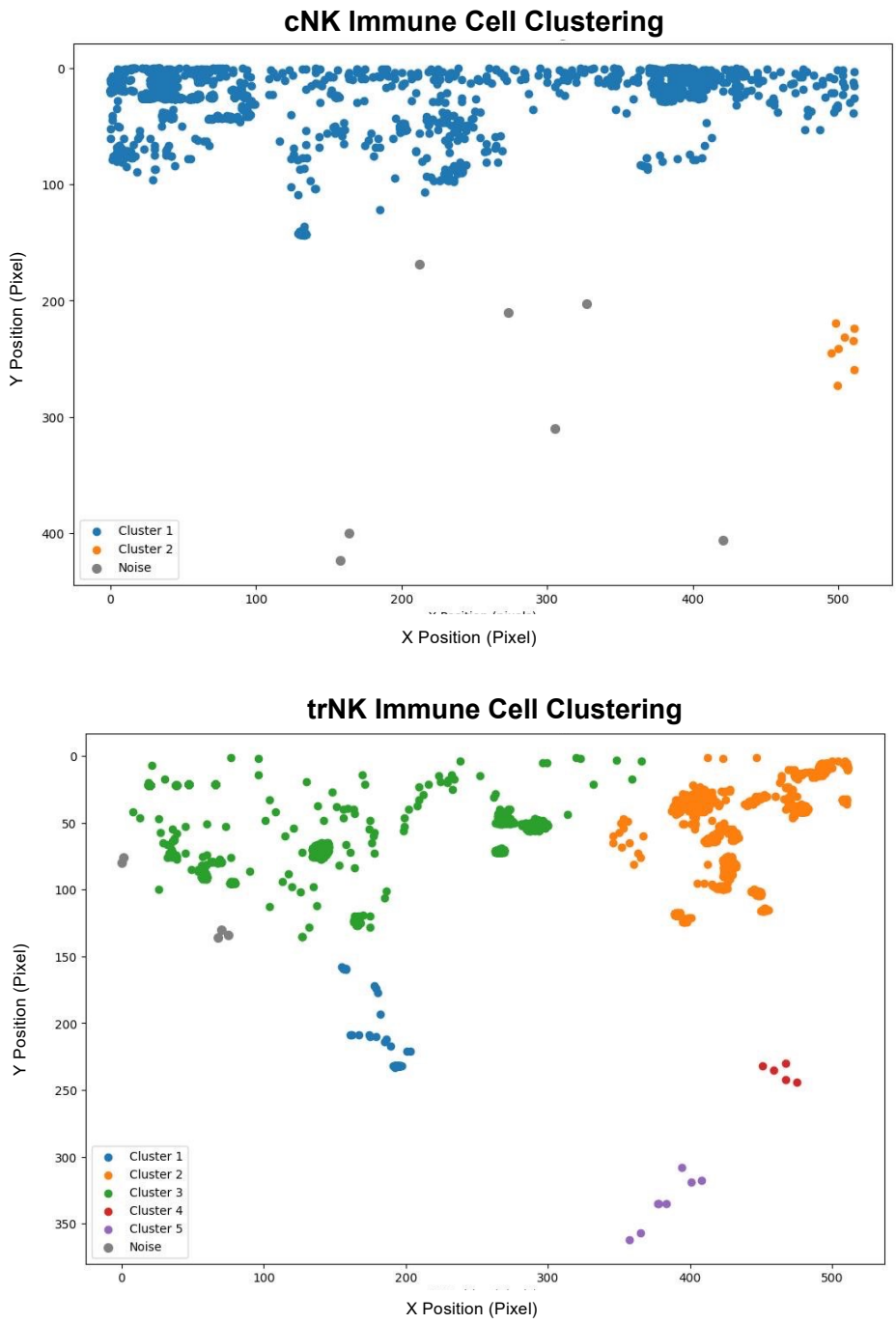

Figure S3: Clustering analysis of cNK cells (top) and trNK cells (bottom), highlighting distinct spatial distribution and clustering behaviors across multiple clusters.

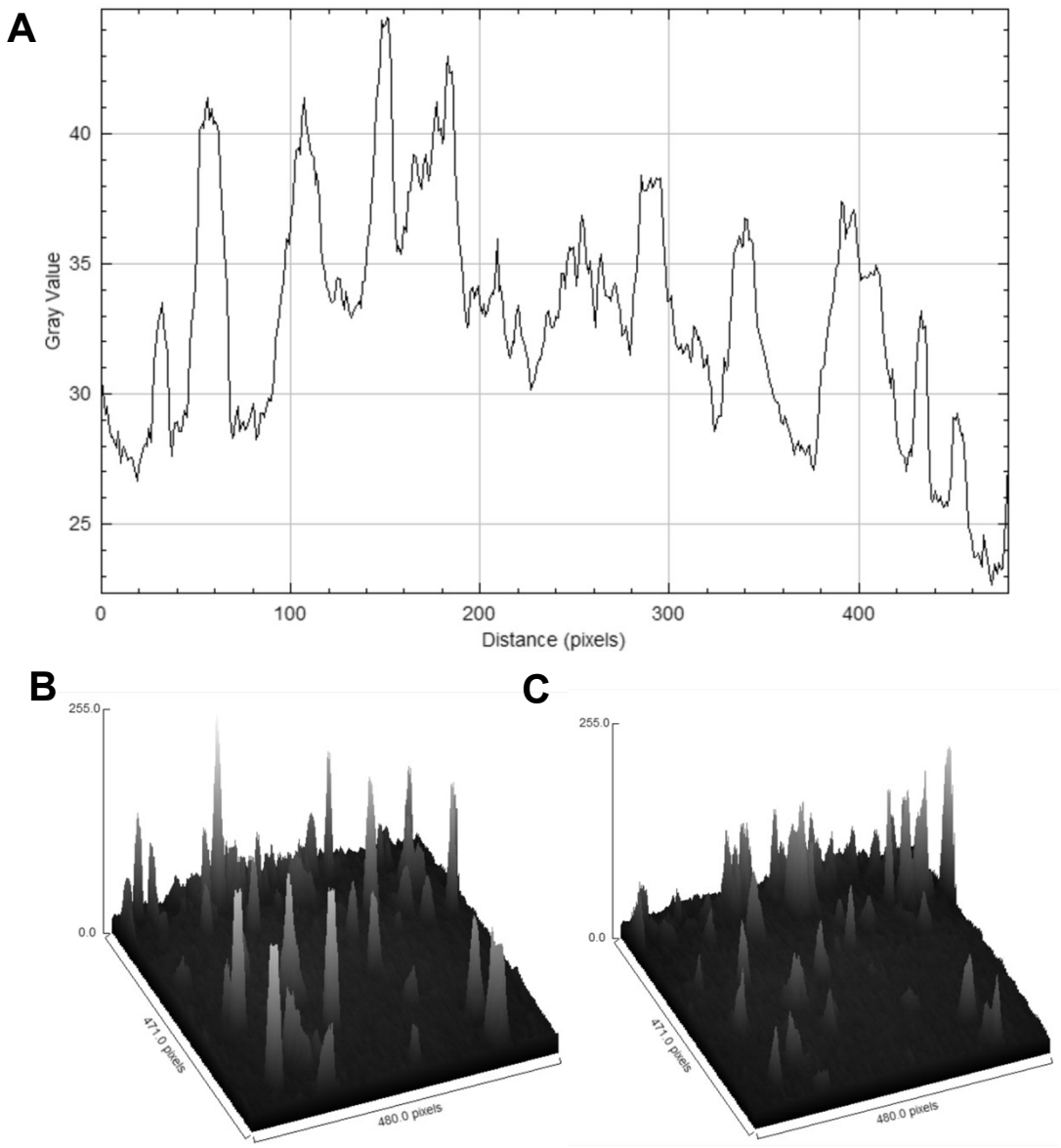

Figure S4. A. Gray value profile along a selected distance in live cell imaging. **(B, C)** 3D surface plots of the live cell imaging data, demonstrating intensity distribution and variation in cellular activity. Peaks in the surface plots correspond to regions of higher intensity, suggesting active or dense cell populations. These graphs were used to analyze intensity variations in the detected cells, highlighting differences in brightness and spatial distribution within the sample.

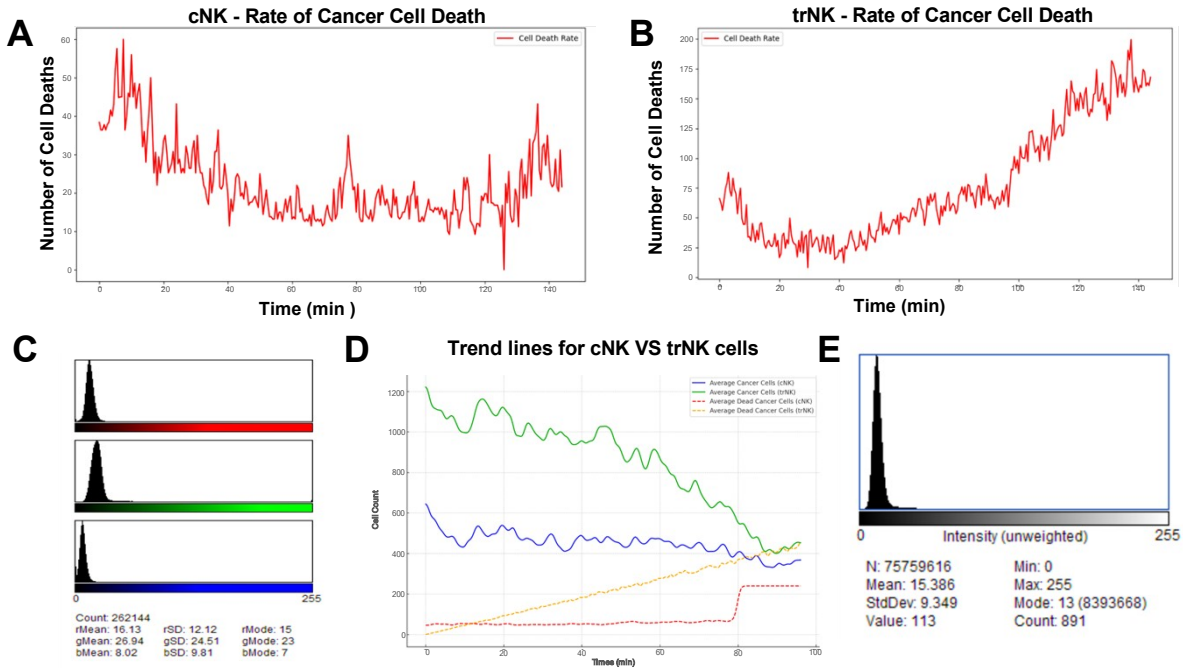

Figure S5: Comparative analysis of cNK and trNK cells based on cancer cell death rate and trend lines of cancer and dead cancer cell counts. **A.** cNK cancer cell death rate over time, showing fluctuations in cell death occurrences with an initial peak, followed by a gradual decline and subsequent stabilization. **B.** trNK cancer cell death rate over time, depicting a steady increase in cell deaths, with a continuous upward trend reaching a peak towards the end of the time frame. **C.** Histogram analysis of color intensities for cancer cells under different channels (Red, Green, Blue). The distribution of intensity values indicates the predominant channels involved in detecting specific cell types or conditions. **D.** Trend lines for cNK vs. trNK cell activity. trNK cells demonstrate a sharper decline in cancer cell counts and a faster increase in dead cell counts, while cNK cells show a more gradual trend, suggesting differential killing efficiencies. **E**. Overall intensity histogram for cell samples, detailing the distribution of pixel intensity across cell populations, providing insights into cell viability and detection sensitivity.

Supplementary Video S1: Brightfield imaging highlighting cytotoxic activity of trNK cells against cancer cells

Supplementary Video S2: Brightfield imaging highlighting cytotoxic activity of cNK cells against cancer cells

Supplementary Video S3: Video of trNK cell migration through the endothelial cell monolayer. This video shows trNK cells (white) migrating toward the tumor region, where they actively interact with and kill cancer cells (green). Dead cells are highlighted in red.

Supplementary Video S4: Video of cNK cell migration through the endothelial cell monolayer. The video shows conventional NK (cNK) cells (white) migrating through the endothelial cell monolayer, with limited migration to the tumor region. Fewer interactions and cancer cell deaths are observed compared to trNK cells.