Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary information for:

Single-cell analysis of the dynamics and functional outcomes of interactions between human natural killer cells and target cells

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Supplemental methods

Fabrication of arrays of nanowells

Arrays of nanowells containing either 30 μm (248,832 wells/array) or 50 μm (84,672 wells/array) cubic wells were prepared on 75 × 25 mm^2 glass slides (Corning) by injecting a silicone elastomer (poly(dimethylsiloxane) (PDMS; Dow Corning), 10:1 ratio of base:catalyst) into a mold containing a microfabricated silicon master. PDMS was cured at 80°C for 4 h and then released from the mold. Shortly before depositing cells into the array, the arrays were treated with an oxygen plasma (Harrick PDC-32G) for 30 s to sterilize the array and render the PDMS hydrophilic. Arrays were stored in phosphate-buffered saline (PBS) until use, and were washed with complete media prior to cell loading.

Microengraving to detect secreted proteins

Capture antibodies against MIP-1β (R&D Systems), IFN-γ (Mabtech), and human immunoglobulin G (hIgG) (ZyMax; Invitrogen) (10 μg/mL each in borate buffer; pH 9) were coated onto poly(L-lysine)-coated glass microscope slides for 1 h at room temperature. Slides were then blocked in 1.5% bovine serum albumin (BSA; EMD Chemicals) / PBS-TWEEN20 (0.05%; Sigma-Aldrich) (PBST) for 30 min, washed once in PBS, dipped in water, and spun dry. Prior to microengraving, the cell-loaded arrays of nanowells were rinsed with serum-free media containing hIgG (34.5 ng/mL; Athens Research & Technology) to provide a positive background signal in every well and facilitate the registration of the features during image analysis of the captured protein microarrays. Excess media was aspirated and the capture antibody-coated glass slides were placed face down on top of the cell-loaded arrays (Fig. 4A). Compression was applied using a microarray hybridization chamber (Agilent). The clamped arrays were incubated for 1 h (30 μm wells) or 2 h (50 μm wells) to allow the capture of secreted proteins onto the antibody-coated glass slide. The resulting microarrays of secreted proteins were then separated from the PDMS array, washed in PBS, blocked with 1.5% BSA-PBST, and hybridized (45 min, room temperature) with the following detection antibodies: biotinylated anti-MIP-1β, anti-IFN-γ-AlexaFluor555, and anti-hIgG-AlexaFluor700 (1 μg/mL each in 0.1% BSA-PBST, all from same manufacturers as corresponding capture antibodies). Arrays were washed in PBS and PBST and then hybridized an additional 30 min at room temperature with streptavidin-AlexaFluor647 (1

μg/mL; Invitrogen). After a final series of washes in PBS, PBST, and water, the protein microarrays were dried and imaged with 5-μm resolution using a commercial microarray scanner (GenePix 4200AL, Molecular Devices).

The microarray of secreted proteins was analyzed using commercial image processing software (GenePix Pro 6, Molecular Devices). The median fluorescence intensity (MFI) in each channel was calculated for each spot in the array to determine the relative intensity of secretion of the cells in the corresponding nanowell. Data were filtered to exclude spots with saturated pixels or high coefficients of variation (>70–90). Spots with a high signal-to-noise ratio (>3–5), low relative local background, and MFI > [global background + 2 standard deviations] were marked as positive spots.

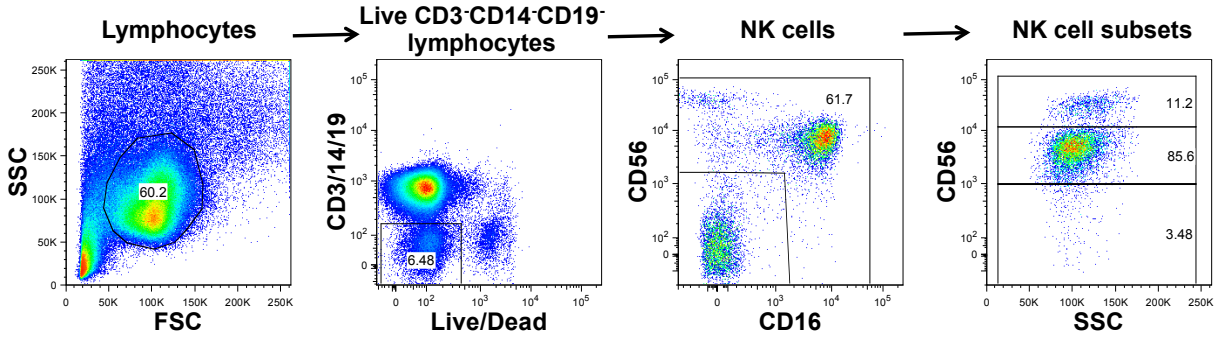

Fig. S1 Flow cytometry gating scheme for NK cells. Numbers indicate percentages within each gate. NK cells were defined as live $CD3^-CD14^-CD19^-$ lymphocytes expressing CD56 and/or CD16 (i.e., $CD56^+CD16^-$ ($CD56^{bright}$ NK cells), $CD56^+CD16^+$ ($CD56^{dim}$ NK cells) or $CD56^-CD16^+$ ($CD56^{neg}$ NK cells)).

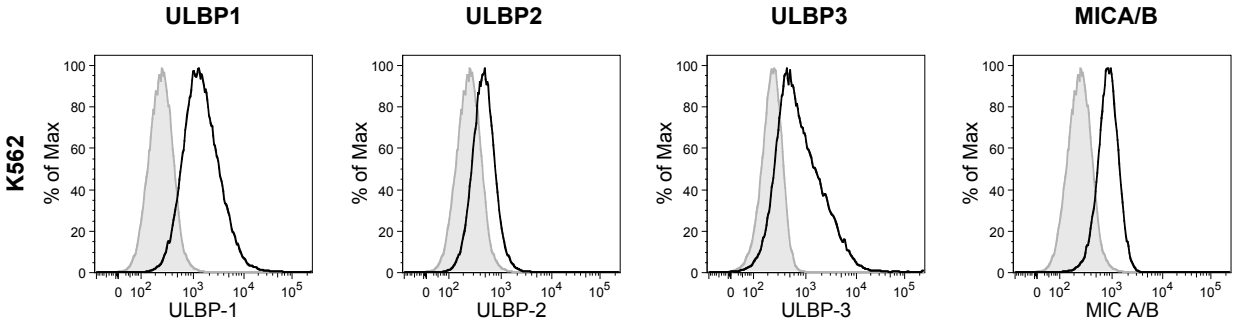

Fig. S2 Surface expression of NKG2D ligands on K562 target cells. Black lines represent the K562 cells stained with the indicated antibody; grey shaded areas show the respective isotype controls.

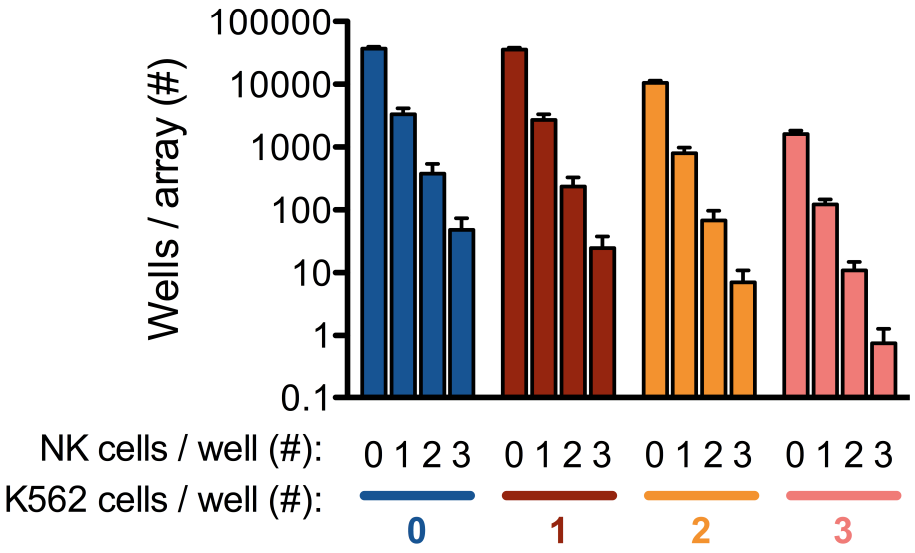

Fig. S3 Distribution of NK cells and K562 target cells in arrayed nanowells. Bar graph of the mean and standard deviation (SD) of the numbers of nanowells on each array that contained the indicated combinations of NK cells and target cells. Results were compiled from eight arrays containing 30 μm cubic nanowells. Each 75 × 25 mm^2 array contained 248,832 wells. In all experiments, to minimize any potential edge effects, only wells located in the center of the array were considered (147,456 wells). Further filtering was performed to exclude wells with dead cells at the t = 0 h time-point and wells in which the cell occupancy changed between the images acquired at t = 0 h and t = 4 h.

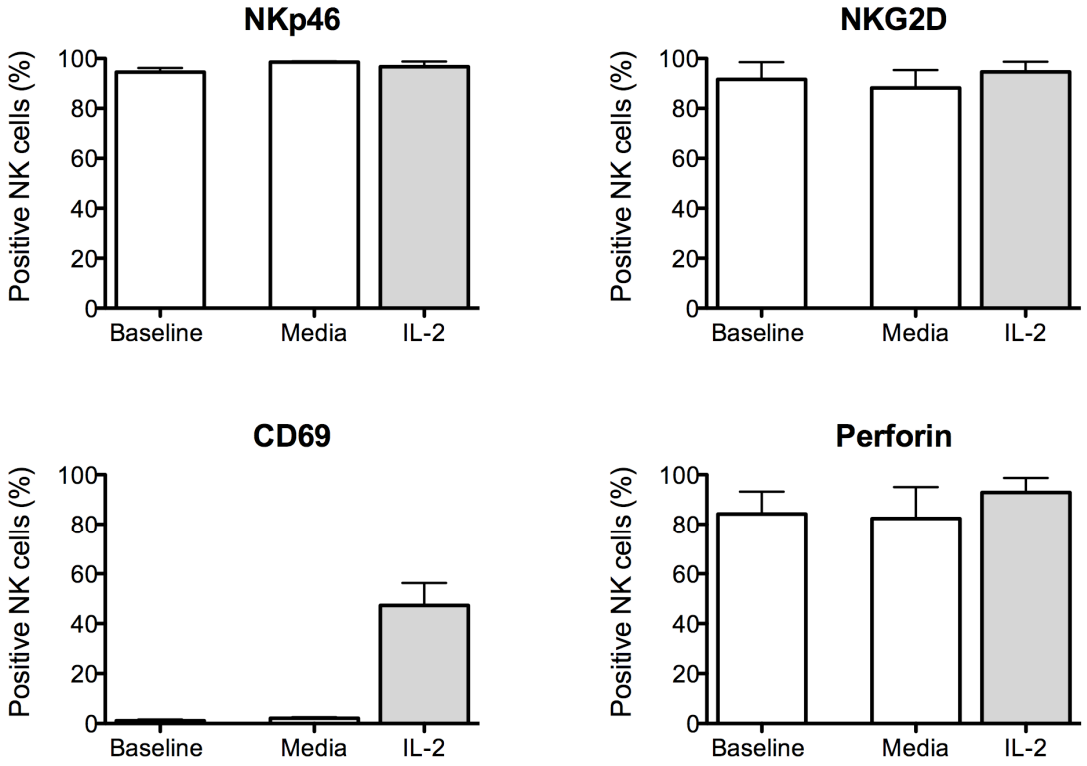

Fig. S4 Phenotypes of NK cells after 44 h stimulation with IL-2. NKp46, NKG2D, CD69, and intracellular perforin were measured by flow cytometry for freshly isolated NK cells (Baseline) or for NK cells stimulated for 44 h in media or in 50 U/ml IL-2. Bar graphs show mean and SD for three donors per condition.