Supplementary Materials and Methods

Flow cytometry for intracellular cytokine production and degranulation

NK cells were cocultured for 6 hours with unlabeled and AFM13 (10 pM, 30 minutes)-labeled HuT-78 and Raji cells at 10:1 E:T ratio. The bispecific antibody AFM12 (CD19/CD16A) and Raji cells ($CD30^-$) were used as negative control for AFM13 (CD30/CD16A) binding specificity. After 6 hours of incubation, cells were surface stained, fix/permed, and stained, as described (1). Cells were acquired in a Navios flow Cytometer (Beckman Coulter) and data analyzed using FlowJo V10 (BD Biosciences). CB-NK cells were co-cultured with Karpas 299 cells for 5 hours at an E:T ratio of 1:1 in the presence of Brefeldin A (Sigma). Cytokine production and CD107a degranulation were measured by flow cytometry using a BD LSRFortessa™ X-20 cell analyzer (BD Biosciences).

Cytotoxicity assays

The cytotoxicity of CB-NK cells and conventional and ML NK cells was assessed by in a standard four-hour ^{51}Chromium (^{51}Cr)-release assay as described elsewhere (2,3). For the IncuCyte live imaging, Karpas 299 cells were labeled with Vybrant® Dye Cycle™ Ruby (Essen BioScience). NK cells and labeled Karpas cells were then incubated with or without Caspase-3/7 Green Detection Reagent (Thermo Fisher) at 37°C. The number of viable target cells was monitored by fluorescence imaging over 24 hours using an IncuCyte Live Cell Analysis System (Essen BioScience). The number of live cells was quantified using IncuCyte Zoom software.

Mass cytometry staining and analysis

CB-NK cells were harvested, washed twice with cell staining buffer (0.5% bovine serum albumin/PBS) and incubated with 5 µl of human Fc receptor blocking solution (Trustain FcX, Biolegend, San Diego, CA) for 10 minutes at room temperature. Cells were then stained with a freshly prepared CyTOF

antibody mix against cell surface markers as described previously.[39] Samples were acquired at 300 events/second on a Helios instrument (Fluidigm) using the Helios 6.5.358 acquisition software (Fluidigm).

For PBMC staining, 1x106 PBMCs were stained with surface antibodies for 45 minutes in CyFACS buffer (0.1% BSA, 0.02% NaN2, 2 mM EDTA in CyPBS, Rockland) before staining with 2.5 μM cisplatin (Enzo life sciences). The cells were then permeabilized using Cytofix/Cytoperm buffer (eBiosciences) according to the manufacturer's instructions. Intracellular staining was performed in 1X Permeablization wash buffer (eBiosciences) for 45 minutes at 4°C and cells washed to remove unbound antibodies. Cells were barcoded (20-plex Barcode, Fluidigm), pooled and stained with Cell-ID intercalator according the manufacturer's instructions (Fluidigm). Metal-tagged antibodies were purchased from Fluidigm or custom-conjugated using the Maxpar X8 Antibody Labeling Kit (Fluidigm). All custom conjugated antibodies were titrated and staining was comparable to flow cytometry (Beckman Coulter Gallios, Kaluza 2.0). Samples were normalized using bead-normalization (GitHub, Nolan lab), and then de-barcoded using the Single Cell De-barcoder (GitHub, Molan lab). Mass Cytometry data were normalized based on EQTM four element signal shift over time using the Fluidigm normalization software 2. Initial data quality control was performed using FlowJo version 10.2. Calibration beads were gated out and singlets were chosen based before exclusion of dead cells. For analysis of CB-NK cells, CD45+ cells were gated followed by selection of NK cells (CD3-CD56+). For analysis of PBMC, CD45+ cells were gated, then CD14-CD19- cells were selected and NK cells identified as CD56+CD3- cells. Data were analyzed using automated dimension reduction including (viSNE) and SPADE analysis. Clustering channels used to run SPADE analysis is identified in table S1. A total of 140,000 cells were evenly sampled from 28 samples which from 7 cords to perform automated clustering. The data were processed using the R package cytofkit (v1.11.3). The markers expressions were arcsine transformed with a cofactor of 5. Dimension reduction of the data was performed using the R package Rtsne (v0.15). The R package Rphenograph (v0.99.1) were used to

cluster all the cells into 17 clusters. Both the R package Rstne (v0.15) and the R package Rphenograph (v0.99.1) were implemented in the R package cytofkit (v1.11.3). The t-SNE plots were generated using the R package ggplot2 (v3.3.2). The normalized mean values of marker expressions in each cluster were plotted as heat map using the function "pheatmap" from R package pheatmap (v1.0.12). We used min-max normalization to scale each marker's mean expressions range to [0,1].

RNA sequencing

Total RNA was collected from 5 million expanded CB-NK cells (with or without pre-activation) and extracted and purified using RNeasy Plus Mini Kit (Qiagen). SmartSeq2 RNA-seq libraries were prepared as described (3). The differentially expressed genes were identified using DEseq2 (4), and the GSEA analysis Bioconductor library package with FDR (5) (false discovery rate) cutoff <0.01 and fold change > 1.5. The Toil RNA-seq workflow (6) was used to convert RNA sequencing data into gene- and transcript-level expression quantification. FastQC (7) was used to for quality control of the sequencing data. CutAdapt (8) was used to remove extraneous adapters. Sequencing reads were aligned to the human reference genome (hg38) using STAR (9). The gene expression levels were measured by counting the mapped reads using RSEM (10) based on an hg38 GENCODE v25 gene model. The differentially expressed genes were performed using DEseq2 (4), and differentially expressed genes were identified at the adjusted p-value < 0.05 and absolute log 2 fold change > 1.5. GSEA analysis was performed using the Bioconductor library gage (5).

Supplementary References

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