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Supplementary Materials for

Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia

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Supplementary Materials and Methods.

Reagents, mice, and cell lines

The following endotoxin-free recombinant human (rh) cytokines were used: rhIL-2 (Prometheus Laboratories Inc.), rhIL-12 (Biolegend), rhIL-18 (InVivoGen), and rhIL-15 (Miltenyi). NSG mice were provided trimethoprim and sulfamethoxazole via drinking water throughout the experiments (0.25 mg/mL in water, changed weekly; Hi Tech Pharmacal). Cells were washed with PBS (Hyclone) before mouse injections.

NK cell purification and cell culture

To generate memory-like and control cells, PBMC or purified NK cells were plated at 3-5x10^6 cells/mL and pre-activated for 16±2 hours using rhIL-12 (10 ng/mL) plus rhIL-18 (50 ng/mL) and rhIL-15 (50 ng/mL) or control conditions (rhIL-15 1 ng/mL), washed 3 times to remove cytokines, and cultured in complete RPMI-1640 medium containing L-glutamine, HEPES, NEAA, penicillin/streptomycin, and 10% human AB serum (Sigma) supplemented with rhIL-15 (1 ng/mL) to support survival. Half of the medium was replaced every 2 days supplemented with rhIL-15 *(18)*.

Functional assays to assess cytokine production

In vitro differentiated or control treated cells were supplemented with 1 ng/mL rhIL-15 during stimulation assays (unstimulated, K562, primary AML). Viably cryopreserved AML samples were thawed and immediately used in the re-stimulation assays. Brefeldin A and monensin (GolgiStop/GolgiPlug, BD) were added after 1 hour, and cells were stained for surface NK markers and intracellular IFN-γ (Cytofix/Cytoperm, BD).

Flow-based killing assay (FloKA)

To determine percentage of specific killing, spontaneous K562 death (no effector control wells) was subtracted from total killing (in the presence of effector cells). In all cases, spontaneous death was less than 5%.

In vivo bioluminescence imaging

NSG mice were irradiated to 250 cGy 1 day before tumor cell injection. 1x10^6 K562-luciferase hematopoietic tumor cells in PBS were injected by tail vein (250 µl/mouse) on day 0. After 3 days, mice were weighed and imaged by BLI. On day 3, human NK cells were isolated and stimulated to generate control or memory-like NK

cells. On day 4, $4x10^6$ NK cells, control or pre-activated with IL-12/15/18, were administered by retroorbital injection. NK cell survival was maintained with 50,000 IU rhIL-2 i.p. on alternating days. For imaging, NSG mice were anesthetized with 2.5% isoflurane and imaged with a charge-coupled device (CCD) camera-based bioluminescence imaging system (IVIS 100; Caliper); exposure time 1-60 seconds, binning 16, field of view 12, f/stop 1, open filter. Signal was displayed as photons/sec/cm^2/sr *(35)*.

Mass cytometry

For all samples, EQ Four Element calibration beads were used during collection according to the manufacturer's instructions (Fluidigm). The non-barcoded (phenotypic panel) data were normalized using Fluidigm CyTOF2 Bead Normalization Tool; barcoded samples (phenotypic panel, functional panel) were normalized using bead-normalization (GitHub, Nolan lab) and de-barcoded using the Single Cell De-barcoder (GitHub, Nolan lab) *(38)*. Metal-tagged antibodies were purchased from Fluidigm or custom-conjugated using the Maxpar X8 Antibody Labeling Kit according to manufacturer's instructions (Fluidigm). All custom-conjugated antibodies were titrated. Staining was comparable to flow cytometry (Beckman Coulter Gallios, Kaluza 2.0). For staining, $0.25x10^6$ purified NK cells or $0.5\text{-}1.0x10^6$ PBMCs were stained with surface antibodies for 45 min at 4°C in CyFACS buffer (0.1% BSA, 0.02% NaN_2, 2mM EDTA in CyPBS, Rockland). Surface antibodies were washed away using CyPBS. Cells were then stained for viability with 2.5 µM cisplatin (Enzo life sciences) according to a standard protocol *(21, 22)*. For the functional assays and a subset of the phenotypic samples, barcoding was performed with Fluidigm Cell-ID 20-Plex Pd Barcoding Kit according to the manufacturer's protocol after the surface stain was performed. The cells were then permeabilized using BD Cytofix/Cytoperm according to the manufacturer's instructions (BD Biosciences). Intracellular staining was performed in BD Perm/Wash buffer for 45 minutes at 4°C. Cells were washed 3 times and stained with Cell-ID intercalator according to the manufacturer's instructions (Fluidigm).

Phenotypic mass cytometry data analysis. Donors were assessed by mass cytometry using the phenotypic panel (table S1). Briefly, individual control and memory-like NK cells (fig. S2) were analyzed using viSNE (Cytobank), with equal sampling, 10% down-sampling, and clustered on 21 parameters (table S1). Using the density plots (Fig. 2A), control and memory-like populations were gated in the tSNE1/2 fields. The resulting gated populations included 71% ± 1.8% (SEM) of the cells falling within their respective gates (>70% of the control-treated cells fell within the control gate). The viSNE-gated control and memory-like populations were

then assessed for the median expression of indicated markers, as well as percent positive for the indicated markers.

Functional mass cytometry data analysis. Control and memory-like purified NK cells (donor #1) or PBMCs (donor #2-8) were stimulated and stained as described above. SPADE analysis was performed on $CD56^+$ cells (fig. S2); clustering was performed on 21 parameters with a target node of 25-100 (~1% of analyzed events, for example 2,500 CD56+ events, 25 target nodes) (table S2; cytobank.org). HLA and KIR information for normal donors used in the functional assays are included in table S3. The primary AML HLA phenotypes are provided in table S4. SPADE nodes were designated as KIR/ KIR-ligand matched or mismatched based on the expression of KIR2DL2/2DL3 for AMLs 614, 895, and 734, because their ligand is HLA-C1, or on the expression of KIR2DL1 and KIR2DL2/2DL3 (for AML 925) because its ligand is C1/C2 (matched nodes included $KIR2DL1^+KIR2DL2/2DL3^-$, $KIR2DL1^+KIR2DL2/2DL3^+$, and $KIR2DL1^-KIR2DL2/2DL3^+$ nodes) *(28)*. Nodes >70% positive for the matched KIR were indicated as matched, and nodes <30% positive for matched KIR were indicated as mismatched. Nodes containing < 0.05% of cells within a sample or 30-70% positive for the matched KIR were excluded from the analysis (equaling < 5% of total cells). This strategy yielded 93.9% ± 1.2% (SEM) matched-KIR-positive (matched nodes) and 93.3% ± 1.1% (SEM) matched-KIR-negative (mismatched nodes). For bulk matched versus mismatched population analyses (fig. S3), node .FCS files were concatenated by match status using the Cytobank concatenation tool (cytobank.org), and bulk matched/mismatched populations were assessed for percentage positive for the indicated markers.

Clinical vignettes for all evaluable patients on the phase 1 clinical trial of MHC-haploidentical donor memory-like NK cell adoptive immunotherapy for rel/ref AML.

001: 73-year old male was diagnosed with AML (M2, normal karyotype, NPM1 mutation present) and was initially treated with decitabine (10 day schedule). After two cycles of decitabine, he achieved a complete remission (CR). He then received four additional cycles of decitabine, but had progressive AML 6 months after starting therapy. He next received CLAG-IDA (cladribine, cytarabine, G-CSF, and idarubicin) salvage chemotherapy, but had persistent disease after this therapy. Refractory to his last therapy, he was then enrolled on the memory-like NK cell clinical trial and received dose level 1 of memory-like NK cells approximately 7 months after his original diagnosis. His AML progressed after memory-like NK cell therapy.

006: 70-year old male was diagnosed with AML (M0, BCR/ABL1^{+} with complex karyotype). He did not achieve a remission after 7+3 (cytarabine and idarubicin) combined with dasatinib for induction, followed by 4 cycles of decitabine (10 day schedule) and then azacitidine with 6-thioguanine chemotherapy regimens. Refractory to all previous therapy, he was next enrolled on the memory-like NK cell clinical trial and received dose level 1 of memory-like NK cells approximately 1 year after his initial diagnosis, without ever achieving remission. His AML progressed after memory-like NK cell therapy.

007: 77-year old male was diagnosed with AML (normal karyotype) and was treated on a clinical trial combining azacitidine and lenalidomide. He initially achieved a CR after two cycles (3 months after his initial diagnosis) but then relapsed after the 8th cycle of this therapy. He was next enrolled on the memory-like NK cell clinical trial, received memory-like NK cells around 1 year after his initial AML diagnosis, and achieved a CR after receiving dose level 1 of memory-like NK cells. His AML relapsed after being in a CR for 6 months.

008: 76-year old male was diagnosed with AML (M4, Trisomy 8) and initially treated with 7+3 (cytarabine and idarubicin) chemotherapy plus oral Hedgehog inhibitor (PF-04449913) as part of a clinical trial, after which he achieved a CR. He then received two cycles of high dose cytarabine (HiDAC) consolidation followed by maintenance with the continuing oral Hedgehog inhibitor. He relapsed around 8 months after his initial diagnosis of AML. Afterwards he was treated with 4 cycles of decitabine (10 day schedule) without being able to achieve a remission. Refectory to his last therapy, he was enrolled on the memory-like NK cell clinical trial

and received dose level 2 of memory-like NK cells approximately 13 months after his initial diagnosis of AML. His AML progressed after memory-like NK cell therapy.

009: 73-year old female was diagnosed with AML (M1, normal karyotype) and initially treated with 7+3 chemotherapy, after which she achieved a CR. She then received two cycles of HiDAC consolidation. However, she relapsed approximately 6 months after her initial diagnosis of AML. She then failed to achieve a remission with one cycle of decitabine (10 day schedule). Refractory to her last therapy, she was then enrolled on the memory-like NK cell clinical trial and received dose level 2 of memory-like NK cells approximately 7 months after her initial diagnosis. She cleared her BM and blood blasts on day 14 after memory-like NK cell infusion, meeting criteria for morphologic leukemia free state (mLFS), but eventually her AML relapsed.

012 and 019: 71-year old female was diagnosed with AML (M5, normal karyotype) and initially treated with CLAM (cladribine, cytarabine, and mitoxantrone) chemotherapy plus imatinib mesylate, after which she achieved a CR. She then received 5 cycles of oral clofarabine maintenance as part of a clinical trial. However, her AML then relapsed, approximately 3 years after her initial diagnosis of AML. She was then treated with the MEC (mitoxantrone, etoposide, and cladribine) salvage chemotherapy regimen, but did not achieve a remission. Refractory to her last therapy, she was then enrolled on the memory-like NK cell clinical trial and received dose level 2 of memory-like NK cells approximately one month after her AML relapsed, about 3 years from her initial diagnosis. She achieved a CR after memory-like NK cell therapy. However, her AML relapsed 3 months later, and she was then re-enrolled on the memory-like NK cell clinical trial after IRB approval at a higher dose level. She received a second treatment with memory-like NK cell adoptive therapy at dose level 3 from the same MHC-haploidentical donor, after which she again achieved CR with MRD testing negative. At last follow-up, she was approximately 90 days out from the second memory-like NK cell therapy, continues to be in CR, and is both PRBC and platelet transfusion independent.

017: 64-year old male diagnosed with AML (therapy related, complex karyotype) was initially treated with 7+3 (cytarabine and idarubicin) chemotherapy. However, the patient had persistent disease after this regimen. He was then treated with CLAG (cladribine, cytarabine, and G-CSF) plus KPT-330 (oral selective inhibitor of nuclear export [SINE] inhibitor) salvage regimen on a clinical trial, after which he achieved a CR. However, he relapsed approximately 4 months after his initial diagnosis of AML. He was then treated with MEC salvage

chemotherapy, which did not result in a remission. Refractory to his last therapy, he was then was enrolled on the memory-like NK cell clinical trial and received dose level 3 of memory-like NK cells around 6 months after his initial diagnosis. His AML progressed after the infusion of the memory-like NK cells.

020: 60-year old male was diagnosed with AML (MDS-AML, normal karyotype) and initially treated with two cycles of decitabine (10-day course), but did not obtain a remission. Refractory to his last therapy he was enrolled on the memory-like NK cell clinical trial and received dose level 3 of memory-like NK cells approximately 5 months after his initial AML diagnosis. The patient achieved a CRi and remained in remission at last follow-up, approximately 60 days after his memory-like NK cell infusion.

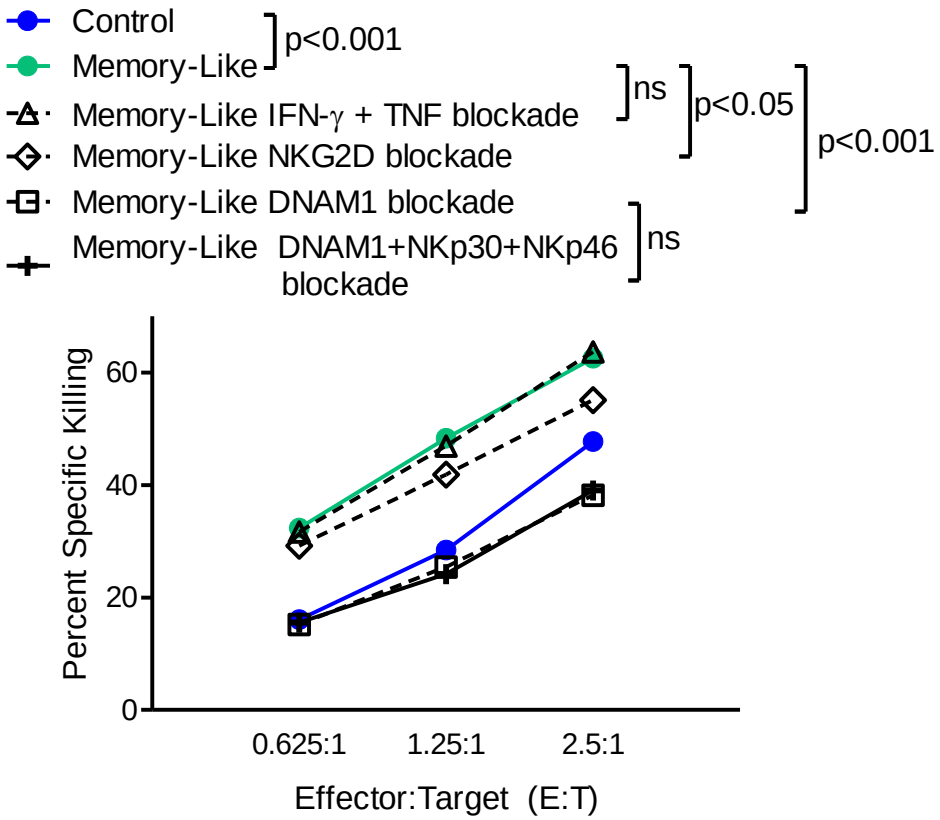

Figure S1. Reduction of memory-like NK cell cytotoxicity against K562 leukemia cells by blockade of NKG2D or DNAM-1. Memory-like or control NK cells were used in flow-based killing assays against K562 tumor targets at the indicated E:T ratios with or without pre-treatment with blocking antibodies against NK cell receptors (NKG2D, DNAM-1, NKp30, NKp46) or cytokines (IFN-γ, TNF). Results shown are from N=3-4 normal donors, and N=2 independent experiments. Statistical comparisons were performed by repeated measures ANOVA.

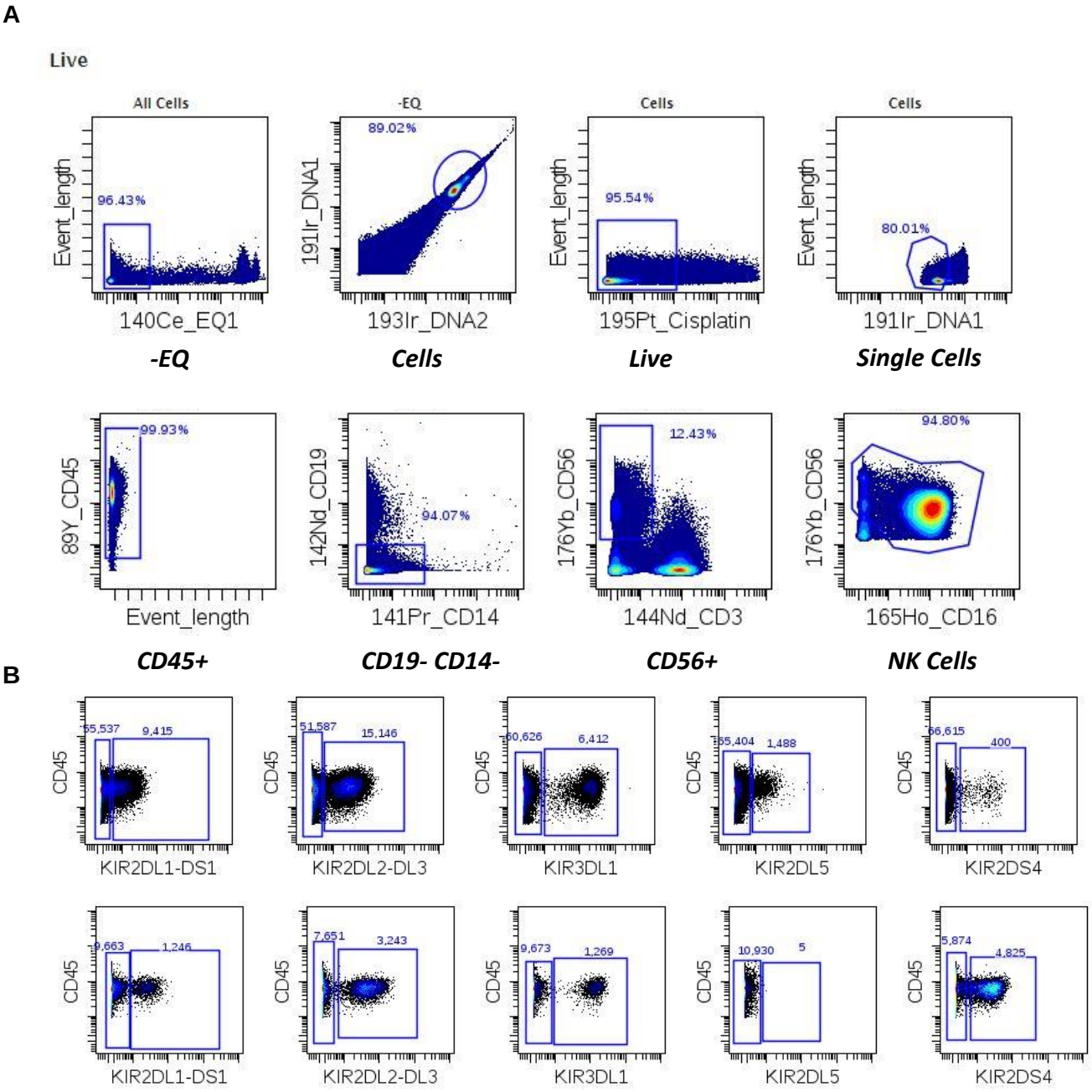

Figure S2. Mass cytometry gating strategies. (A) Representative mass cytometry gating strategy. NK cells were used for all SPADE and viSNE analyses. (B) Boolean gating strategy for Inverse-Simpson Diversity Index scoring. NK cells were gated on KIR2DL1/2DS1, KIR2DL2/2DL3, KIR3DL1, KIR2DL5, and KIR2DS4. Gating is shown from 2 representative individuals.

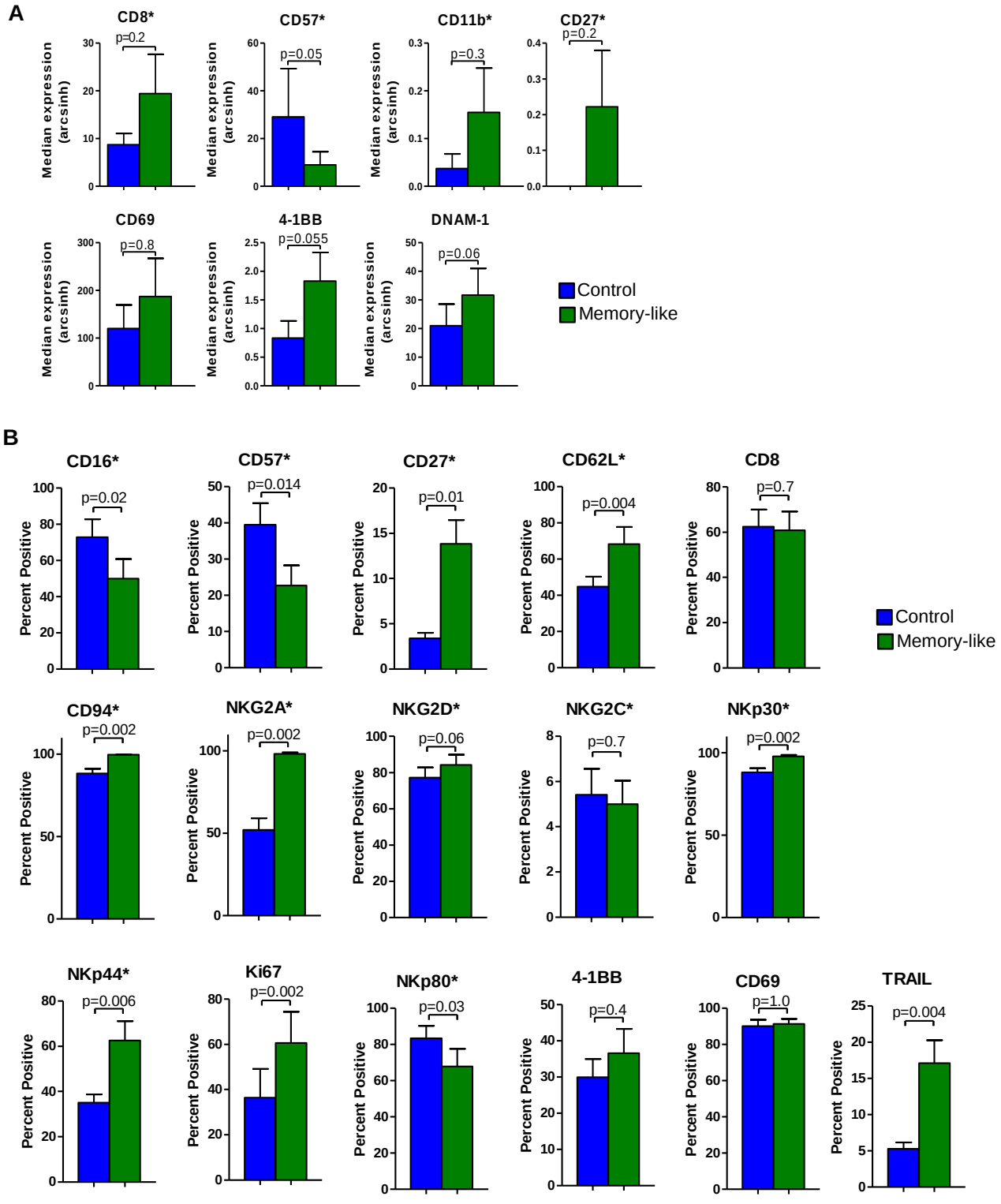

Figure S3. Phenotypic marker expression on viSNE gated control and memory-like NK cells. Control and memory like NK cells were generated as in Fig. 1A. Normal donor control and memory-like samples (N=9) were clustered using viSNE and gated (as in Fig. 2A). Memory-like and control populations were assessed for the (A) median or (B) percentage positive for the indicated markers. Control and memory-like NK cells were compared using Wilcoxon signed rank test. All graphs display mean +/- SEM. * Indicates viSNE clustering parameters.

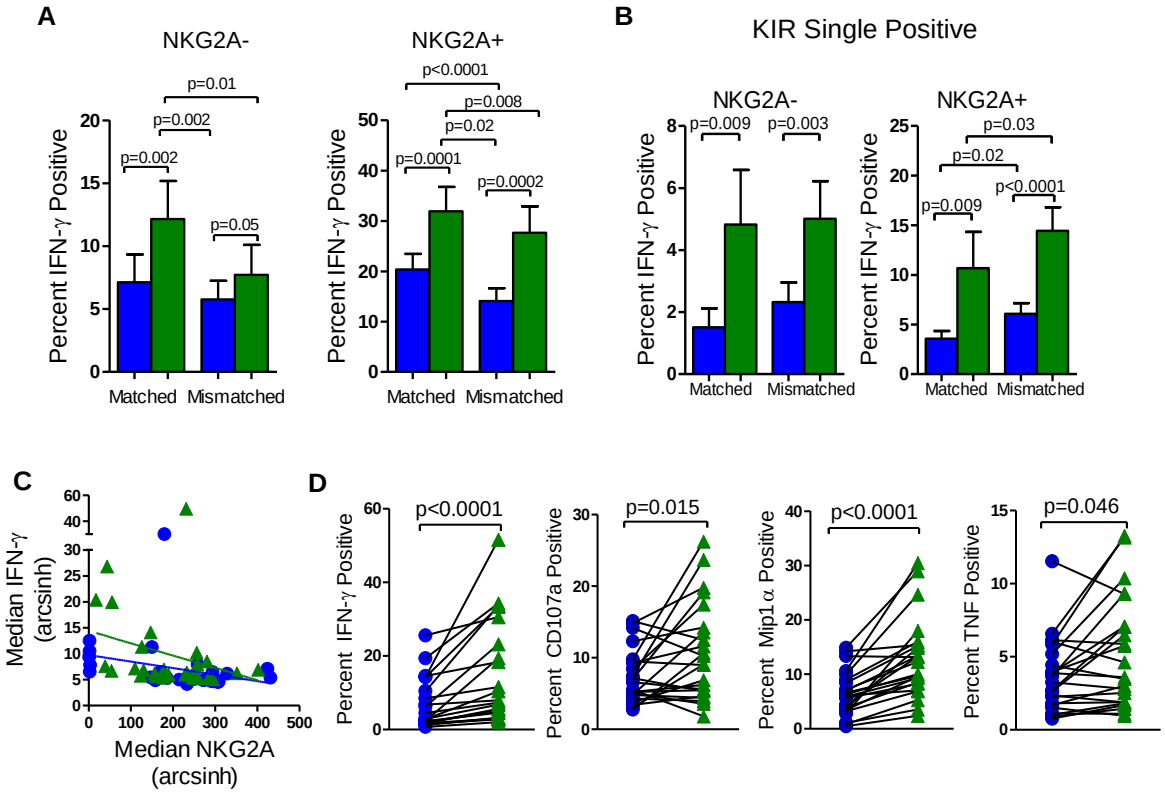

Figure S4. Enhanced effector responses of memory-like NK cells compared to control-treated NK cells. Control (blue) and memory-like NK cells (green) were stimulated for 6 hours with primary AML in the presence of 1 ng/mL IL-15, GolgiPlug, and GolgiStop. The cells were assessed for 32 markers using mass cytometry and analyzed with SPADE on 21 clustering parameters (as in Fig. 3). Nodes were assigned as KIR/KIR-ligand matched or mismatched. The cells within matched or mismatched nodes were then analyzed in bulk for percentage IFN-γ+ within the (A) NKG2A- or NKG2A+ populations and (B) KIR single positive NKGA-/NKG2A+. (C) IFN-γ median expression was correlated with NKG2A median expression from IFN-γ positive NK cells stimulated with primary AML. No significant association between NKG2A and IFN-γ expression was observed (control: R^2=0.09, p=0.10; memory-like: R^2=0.06, p=0.19). (D) The frequency of the indicated effector molecules was assessed in the bulk NK cell populations, regardless of match/mismatch status. Blue circles show control-treated, while green triangles show memory-like NK cells. Comparisons were made using Wilcoxon signed rank test. Data summarize N=4 primary AML and N=4-7 normal donors. Bar graphs display mean +/- SEM.

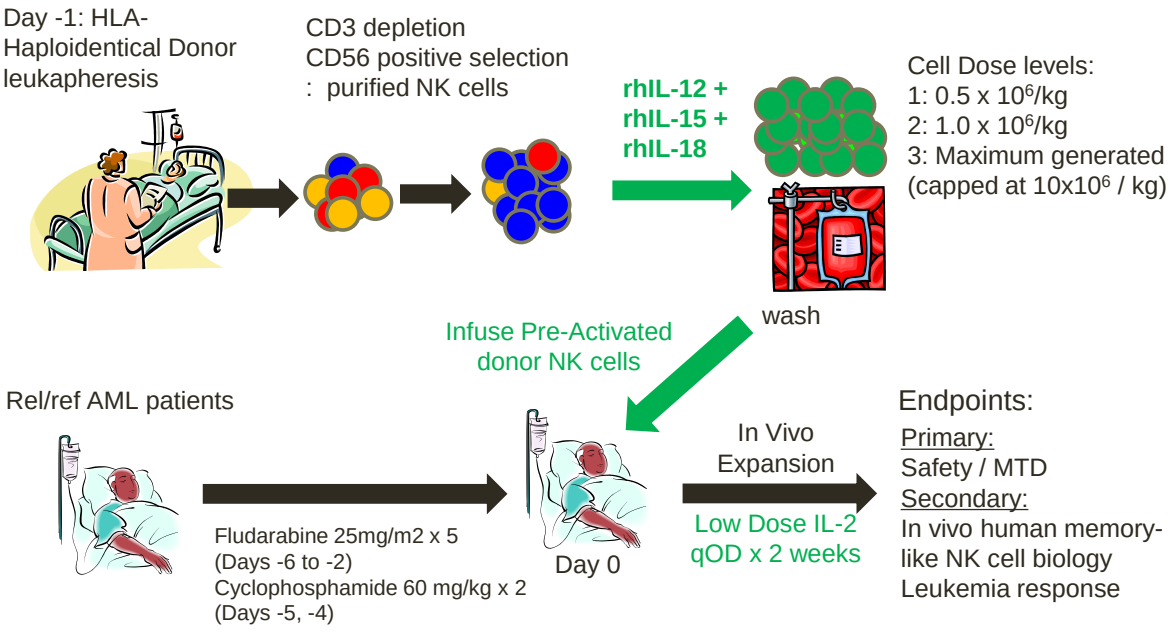

Figure S5. Schema of allogeneic memory-like NK cell phase 1 clinical trial (NCT01898793).

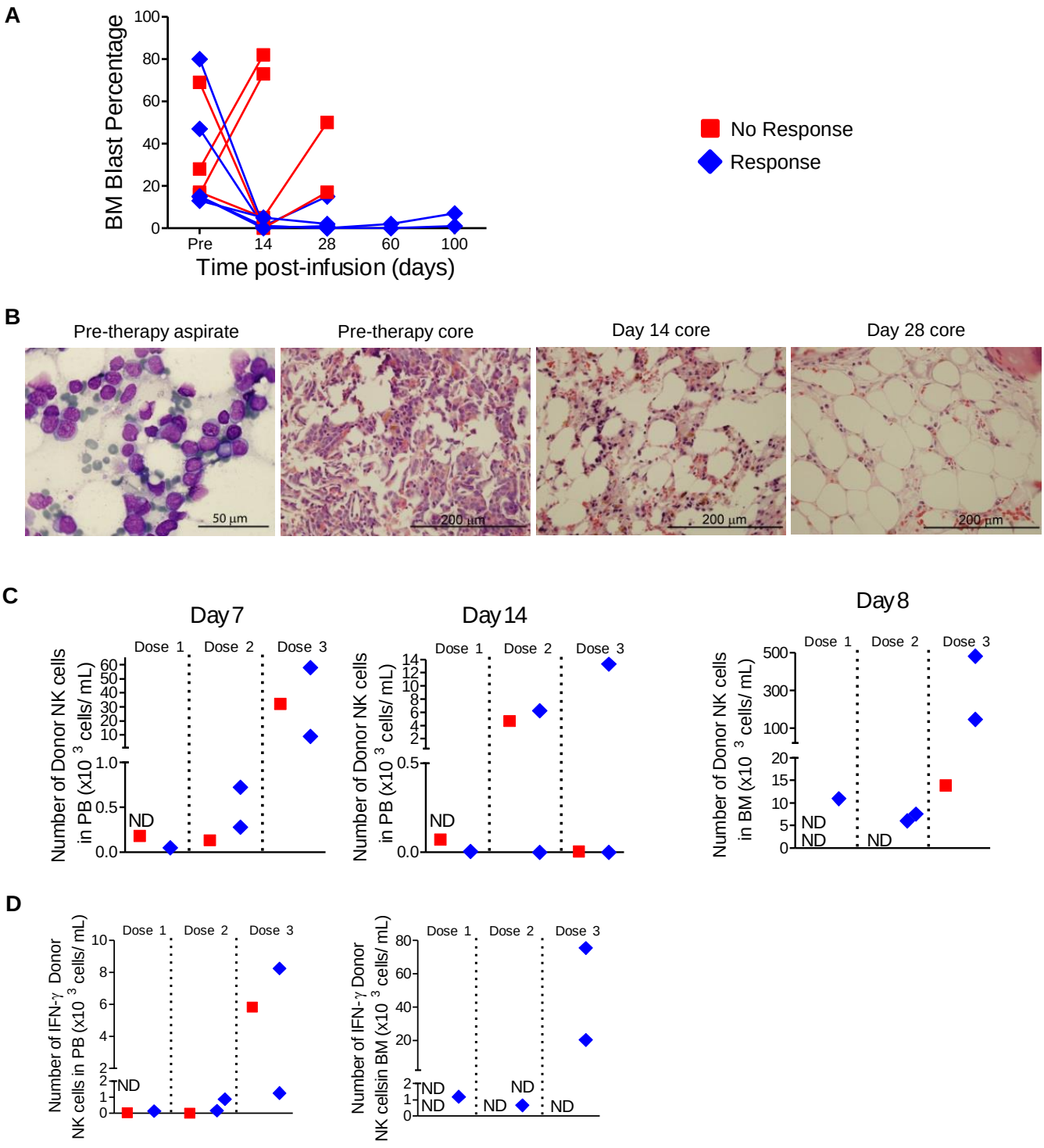

Figure S6. Distribution of BM blast percentages and donor NK cell numbers sorted by clinical outcomes. (**A**) Patient BM blast percentages determined by morphology immediately before and on the indicated days after memory-like NK cell infusion. (**B**) BM aspirate (far left) and morphology (H&E staining) in a representative patient (007) demonstrating blast clearance after memory-like NK cell infusion. (**C**) The number of donor NK cells in the PB (day 7 and 14) or BM aspirates (day 8) by dose level and response category. (**D**) The number of IFN-γ+ donor NK cells in the PB or BM at day 8. In some cases functional assays were not performed because of limited cell numbers. ND: not determined. Patient 001 (dose level 1) did not have a day 8 BM aspirate. Two patients (dose levels 1 and 2) did not have aspirate volume measurements available to calculate absolute numbers. Of the three patients shown, two patients treated at the highest dose have not reached the day 60 time point.

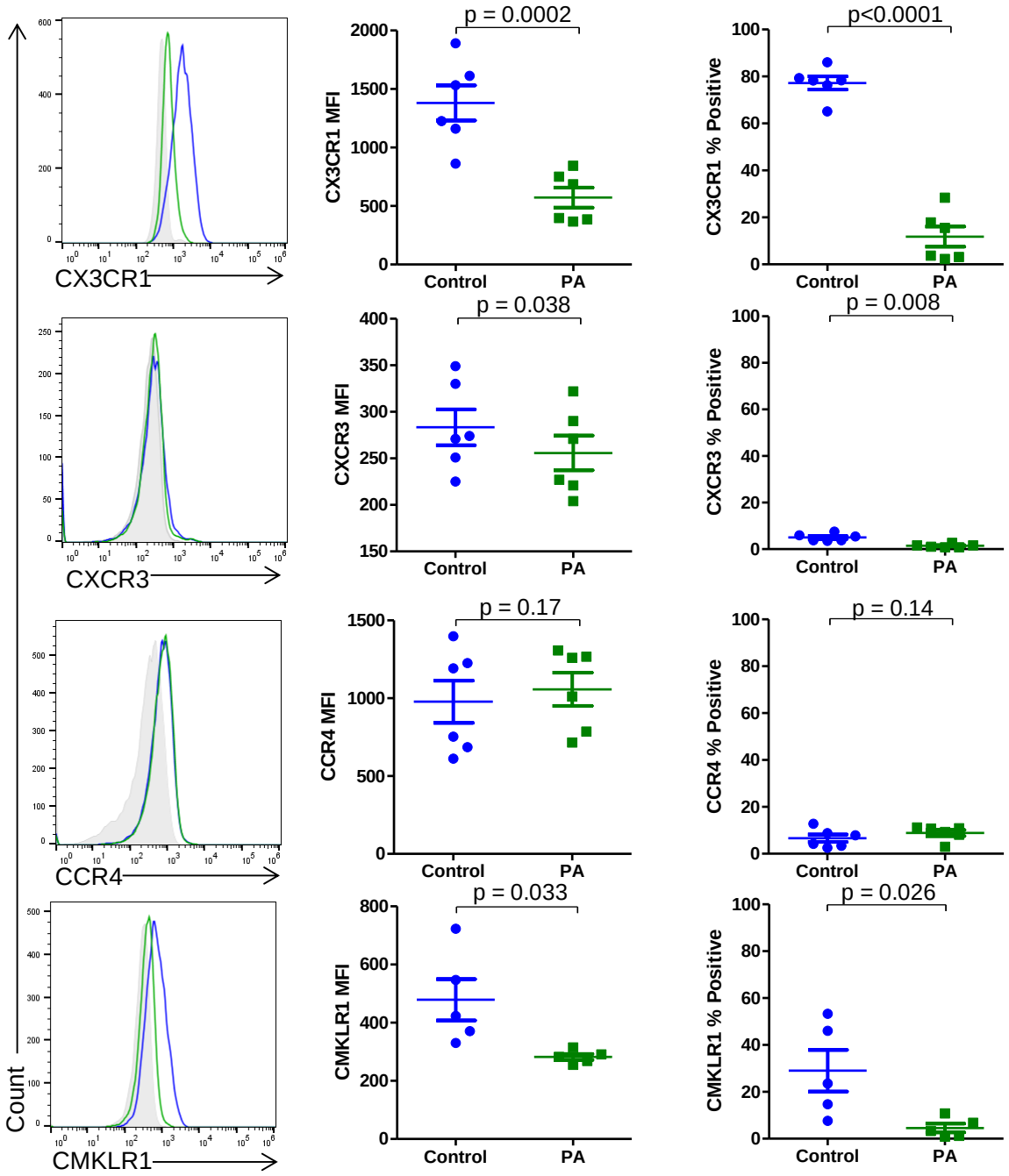

Figure S7. Chemokine receptor expression on control versus IL-12, IL-15, and IL-18–preactivated human NK cells. Freshly-isolated PBMCs were incubated for 16 hours with low-dose IL-15 (control) or IL-12 (10 ng/mL) + IL-15 (50 ng/mL) + IL-18 (50 ng/mL) (pre-activated; PA) then evaluated for expression of the following chemokine receptors: CX3CR1, CXCR3, CCR4, and CMKLR1. Flow cytometry histograms from representative donors are shown as well as summary data from n = 5-6 normal donors in 2 separate experiments. Gray = negative control (unstained or isotype), green = pre-activated NK cells, blue = control NK cells. Summary data display mean +/- SEM.

Phenotypic panel				
Tag	Antibody	Clone	Source	Clustering
89 Y	CD45	HI30	Fluidigm	
141 Pr	CD14	M5E2	BD Pharmingen*	
142 Nd	CD19	HIB19	Fluidigm	
143 Nd	KIR3DS1/L1 (CD158e1, e2)	Z27	R&D*	
144 Nd	CD3	UCHT1	Fluidigm	
145 Nd	KIR2DS4 (CD158i)	FES172	Beckman Coulter*	Figure 2a, 2b
146 Nd	KIR2DL1/DS1 (CD158a,h)	EB6B	Beckman Coulter*	Figure 2a, 2b
147 Sm	NKG2D	1D11	R&D*	Figure 2a, 2b
148 Nd	KIR2DL2/2DL3 (CD158b)	CH-L	BD Pharmingen*	Figure 2a, 2b
149 Sm	CD127	A019D5	Fluidigm	
151 Eu	TRAIL	RIK-2	Biolegend*	
152 Dy	CD305 (LAIR-1)	342219	R&D*	
152 Dy	CD8	OKT8	eBiosciences*	
153 Eu	CD62L	DREG-56	Fluidigm	Figure 2a, 2b
154 Sm	KIR2DL5 (CD158f)	UP-R1	Beckman Coulter*	Figure 2a, 2b
155 Gd	CD27	L128	Fluidigm	Figure 2a, 2b
156 Gd	KIR3DL1 (CD158e)	DX9	R&D*	Figure 2a, 2b
158 Gd	CD137 (41BB)	4B4-1	Fluidigm	
159 Tb	NKG2C	134591	R&D*	Figure 2a, 2b
160 Gd	CD69	FN50	Biolegend*	
161 Dy	NKp30	P30-15	Biolegend*	Figure 2a, 2b
162 Dy	Ki-67	B56	Fluidigm	
163 Er	CD94	DX22	Biolegend*	Figure 2a, 2b
165 Ho	CD16	3G8	Fluidigm	Figure 2a, 2b
166 Er	NKG2A	Z199	Beckman Coulter*	Figure 2a, 2b
167 Dy	NKp44	P44-8	Biolegend*	Figure 2a, 2b
168 Er	CD226 (DNAM-1)	DX11	Miltenyi*	
169 Tm	CD25	2A3	Fluidigm	Figure 2a, 2b
170 Er	NKp80	239127	R&D*	Figure 2a, 2b
171 Yb	Granzyme B	GB11	Fluidigm	
172 Yb	CD57	HCD57	Fluidigm	Figure 2a, 2b
174 Yb	NKp46	195314	R&D*	Figure 2a, 2b
175 Lu	Perforin	B-D48	Fluidigm	Figure 2a, 2b
176 Yb	CD56	HCD56	Fluidigm	Figure 2a, 2b
209 Bi	CD11b	ICRF44	Fluidigm	Figure 2a, 2b
	Cisplatinum		Enzo Life Sciences	
	Cell-ID intercalator Ir		Fluidigm	

Table S1. NK cell phenotypic mass cytometry panel design, reagents, and clustering usage. The metal isotope tag, maker name, antibody clone, source, and clustering usage is shown for a panel designed to deeply phenotype human NK cells. Clustering demarcates which channels were used for analysis in the indicated figure. Asterisks (*) included after the source indicate antibodies that were custom-conjugated using Fluidigm antibody labeling kits, per manufacturer's instructions.

Functional panel				
Tag	Antibody	Clone	Source	Clustering
89 Y	CD45	HI30	DVS Sciences	
141 Pr	CD14	M5E2	BD Pharmingen	
142 Nd	CD19	HIB19	DVS Sciences	
143 Nd	KIR3DS1/L1 (CD158e1, e2)	Z27	R&D	
144 Nd	CD3	UCHT1	DVS Sciences	
145 Nd	KIR2DS4 (CD158i)	FES172	Beckman Coulter	Figure 3
146 Nd	KIR2DL1/DS1 (CD158a,h)	EB6B	Beckman Coulter	Figure 3
147 Sm	NKG2D	1D11	R&D	Figure 3
148 Nd	KIR2DL2/2DL3 (CD158b)	CH-L	BD Pharmingen	Figure 3
150 Nd	MIP-1a	93342	R&D	
151 Eu	CD107a	H4A3	DVS Sciences	
152 Dy	TNF	Mab11	DVS Sciences	
153 Eu	CD62L	DREG-56	DVS Sciences	Figure 3
154 Sm	KIR2DL5 (CD158f)	UP-R1	Beckman Coulter	Figure 3
155 Gd	CD27	L128	DVS Sciences	Figure 3
156 Gd	KIR3DL1 (CD158e)	DX9	R&D	Figure 3
159 Tb	NKG2C	134591	R&D	Figure 3
160 Gd	CD69	FN50	Biolegend	
161 Dy	NKp30	P30-15	Biolegend	Figure 3
163 Er	CD94	DX22	Biolegend	Figure 3
165 Ho	CD16	3G8	DVS Sciences	Figure 3
166 Er	NKG2A	Z199	Beckman Coulter	Figure 3
167 Dy	NKp44	P44-8	Biolegend	Figure 3
169 Tm	CD25	2A3	DVS Sciences	Figure 3
170 Er	NKp80	239127	R&D	Figure 3
172 Yb	CD57	HCD57	DVS Sciences	Figure 3
174 Yb	NKp46	195314	R&D	Figure 3
175 Lu	IFN-g	B27	DVS Sciences	
176 Yb	CD56	HCD56	DVS Sciences	Figure 3
209 Bi	CD11b	ICRF44	DVS Sciences	Figure 3
	Cisplatinum		Enzo Life Sciences	
	Cell-ID intercalator-Ir		Fluidigm	

Table S2. NK cell functional mass cytometry panel design, reagents, and clustering usage. The metal isotope tag, maker name, antibody clone, source, and clustering usage is shown for a panel designed to deeply phenotype human NK cells. Clustering demarcates which channels were used for analysis in the indicated figure. Asterisks (*) included after the source indicate antibodies that were custom-conjugated using Fluidigm antibody labeling kits, per manufacturer's instructions.

Sample ID	KIR Genotype			HLA	
	2DL1	2DL2/2DL3	3DL1	HLA-B	HLA-C
1	pos	pos	pos	Bw4/Bw6	C1/C1
2	pos	pos	pos	Bw4/Bw6	C1/C2
3	pos	pos	pos	Bw4/Bw6	C1/C2
4	pos	pos	pos	Bw4/Bw6	C1/C2
5	pos	pos	pos	Bw4/Bw6	C1/C2
6	pos	pos	pos	Bw4/Bw4	C1/C2
7	pos	pos	pos	Bw4/Bw4	C1/C2
8	pos	pos	pos	Bw4/Bw6	C1/C2

Table S3. Characteristics of normal donors used in mass cytometry functional experiments. HLA phenotype, KIR genotype, and licensing status of donors used in Fig. 3. Pos: positive. Underline denotes an inhibitor KIR predicted to be unlicensed based on the lack of HLA ligand expression in the donor. The majority of KIR receptors were considered licensed in these individuals, if the receptor and HLA ligand were present.

UPN	Percent Blasts	WHO	Phenotype	HLA C1/C2	HLA Bw4/Bw6	% HLA A/B/C	% HLA-E	HLA-E MFI
176	90	AML M1	CD117+CD56+CD33-CD34-	C1/C2	Bw4/Bw6	N.D.	N.D.	N.D.
614*	97	AML M1	CD33+CD34-CD117+	C1/C1	Bw6/Bw6	97.4	15.3	0.89
895*	82	AML M5	CD33+CD56+CD64+CD117+DR+	C1/C1	Bw6/Bw6	99.5	75.2	2.55
734*	79	AML M4	CD33+CD34+CD117+	C1/C1	Bw6/Bw6	95.8	53.6	2.62
925*	79	AML M4	CD34-CD13+CD117+CD14+CD64+	C1/C2	Bw6/Bw6	99.7	97.6	3.22
101	94	AML M1	CD33+CD117+CD34+	C1/C2	Bw6/Bw6	N.D.	N.D.	N.D.

Table S4. **Characteristics of AML samples used for in vitro NK cell functional assays**. All samples were from the peripheral blood of patients with untreated AML. The percentage of blasts present in each AML sample assessed by clinical flow cytometry is indicated. Phenotypic information for selected AML markers is shown. The HLA C1, C2, and Bw4 that are ligands for KIR2DL2/3, KIR2DL1, and KIR3DL1 are shown. The percentage of AML blasts positive for MHC class I (all HLA-A/B/C) and HLA-E is shown. The median fluorescence intensity (MFI) is shown for the HLA-E positive subsets. *Indicates samples used for mass cytometry experiments. N.D.: not determined.

	Patient				Donor				KIR:KIRL Mismatch
UPN	HLA-A	Bw4	C	C Class	KIR2DL1	KIR2DL2/3	KIR3DL1	KIR2DS1	
001	14:02/07:02	Absent	08:02/07:02	C1/C1	Absent	Present	Present	Absent	Yes, 1
006	02/02	Absent	12:03/07:01	C1/C1	Present	Present	Present	Present	Yes, 2
007	01:01/25:01	Present	05:01/06:02	C2/C2	Present	Present	Present	Present	Yes, 1
008	32:01/02:01	Present	05:01/02:02	C2/C2	Present	Present	Present	Absent	Yes, 1
009	29:02/02:01	Present	16:01/05:01	C1/C2	Present	Present	Present	Present	No
012	24:02/11:01	Absent	04/05:01	C2/C2	Present	Present	Present	Absent	Yes, 2
017	03:01/24:02	Present	07:01/08:02	C1/C1	Present	Present	Present	Absent	Yes, 1
019	24:02/11:01	Absent	04/05:01	C2/C2	Present	Present	Present	Absent	Yes, 2
020	02:01/29:02	Absent	01:02/06:02	C1/C2	Present	Present	Present	Absent	Yes, 1

Table S5. Patient HLA and donor KIR characteristics for evaluable donor-patient pairs treated in the phase 1 clinical trial. KIR2DL1 was considered matched if HLA-C2 was present in patient. KIR2DL2/3 were considered matched if HLA-C1 was present in the patient. KIR3DL1 was considered matched if Bw4 was present in the patient. KIR to KIR ligand mismatch is noted in the donor versus patient vector, with numbers indicating the numbers of mismatched inhibitory KIR, which are underlined.

Antibody	Clone	Company
CD3	S4.1	Caltag
CD3	UCHT1	Beckman Coulter
CD8	SK1	BD
CD14	M5E2	BD
CD16	3G8	Caltag, BD
CD25	M-A251	BD
hCD45	J.33	Beckman Coulter
mCD45	30-F11	BD
CD56	N901	Beckman Coulter
CD69	FN50	BD
CD107a	H4A3	BD
IFN-γ	B27	BD
Ki67	B56	BD
Nkp46	9E2/NKp46	BD
TNF	MAb11	eBiosciences
CD4	RPA-T4	BD
CD34	581	BD
MIP1-α	39624	R&D
Granzyme B	GB12	Invitrogen
Perforin	Dg9	BioLegend
HLA-A/B/C	G46-2.6	BD
HLA-E	3D12	BioLegend
HLA-A2/A28	REA142	Miltenyi
HLA-A2	REA517	Miltenyi
HLA-A2	BB7.2	Biolegend
HLA-A3	GAP.A3	eBiosciences
HLA-A9	REA127	Miltenyi
HLA-A9/A4/A8	BVK5C4	Leids
HLA-Bw6	REA143	Miltenyi
Viability	7-AAD	Sigma

Table S6. Flow cytometry mAbs. Monoclonal antibodies, clones, and sources used for flow cytometry experiments.