Supplemental Tables and Figures

Supplemental Table 1: Demographics of the MDS Patient Cohort

Variables	No. of Patients (%)
Sex	
Male	40 (60%)
Female	27 (40%)
[a]Age (years)	47 (19-74)
Ethnicity	
Caucasian	62 (93%)
African-American	2 (3%)
Hispanic	2 (3%)
Other	1 (1%)
FAB Subtype	
RAEB-T	10 (15%)
RAEB	24 (36%)
RARS	8 (12%)
RA	9 (13%)
[b]Other	16 (24%)
[c]***Karyotype (IPSS)***	
Favorable	24 (36%)
Intermediate	9 (14%)
Poor	15 (22%)
Unknown	19 (28%)
[d]***Blast Percentage (IPSS)***	
>5%	35 (52%)
5 - 10%	14 (21%)
11 - 20%	3 (4%)
≥21%	5 (8%)
Unknown	10 (15%)
[e]***MDS Status***	
Lower Risk	24 (36%)
Higher Risk	41 (61%)
Unknown	2 (3%)

Abbreviations: FAB: French-American-British classification; RAEB-T: Refractory anemia with excess blasts in transformation; RAEB: Refractory anemia with excess blasts; RARS: Refractory anemia with ringed sideroblasts; RA: Refractory anemia; IPSS: International prognosis scoring system.

[a]Median (range) age at date of transplant

[b]Other: Paroxysmal nocturnal hemoglobinuria; Polycythemia vera; Essential thrombocythemia; Myelofibrosis with myeloid metaplasia; Myelofibrosis or myelosclerosis; Myelodysplasia or myloproliferative disorder.

[c]Favorable: normal karyotype, isolated –Y, del(5q) or del(20q); Intermediate: other abnormalities; Poor: complex (≥3 abnormalities) or chromosome 7 anomalies.

[d]Blast percentage at date of transplant

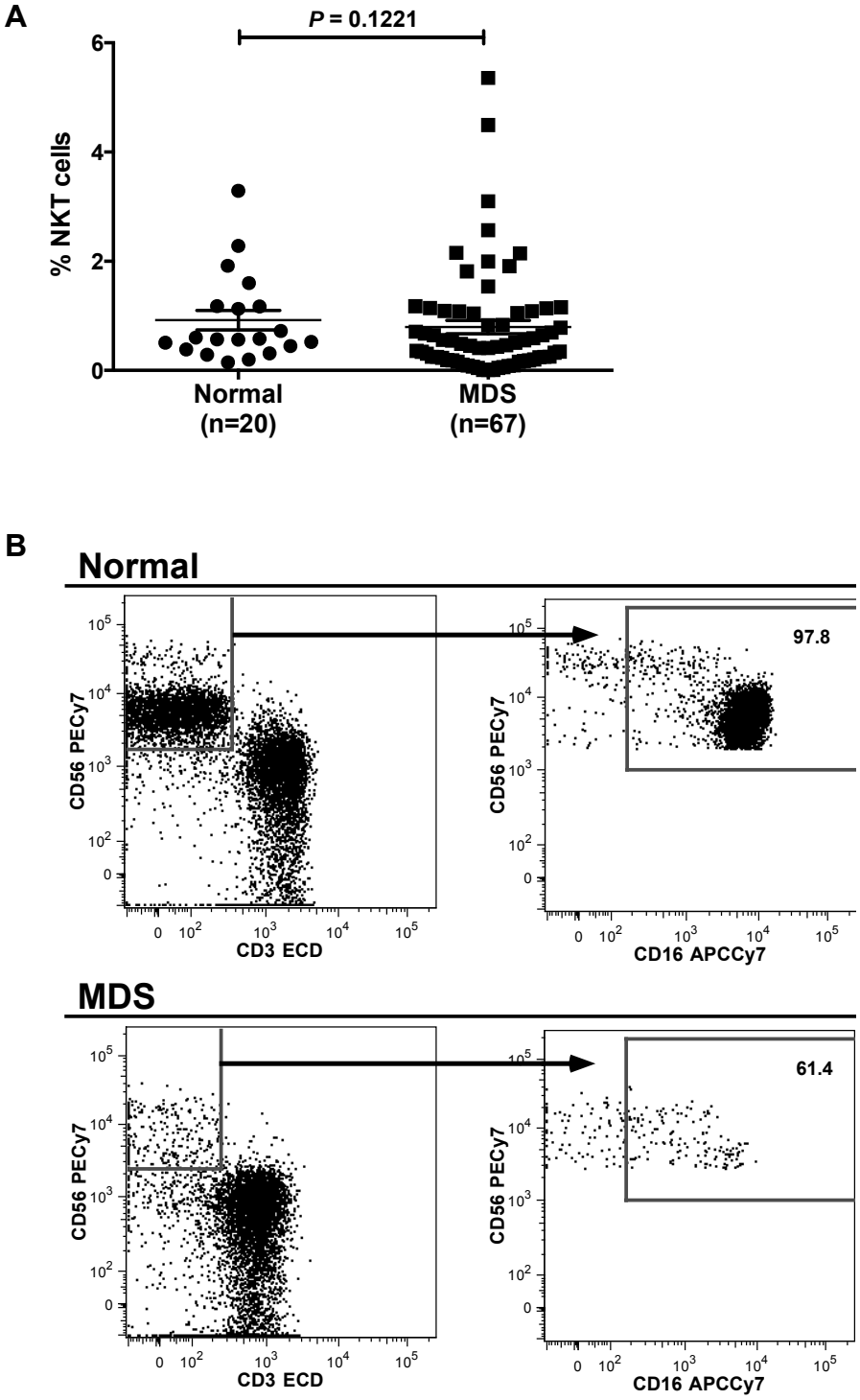
[e]Lower Risk: RA or RARS; Higher Risk: RAEB, RAEB-T or CMML. Data regarding specific cell counts were not available, thus we were unable to determine the overall IPSS scores

Supplemental Table 2: MDS cell population frequencies and CD16xCD33 BiKE-induced MDS NK cell function stratified by blast percentage (IPSS)

	[a]*Blast Percentage (IPSS)*				
	<5% (n=35)	5-10% (n=14)	11-20% (n=3)	≥21% (n=5)	Unknown (n=10)
% NK cells	2 ± 0.3%	3 ± 1%	0.2 ± 0.1%	2 ± 1%	1 ± 0.3%
% $CD33^+$	28 ± 3%	29 ± 6%	56 ± 12%	23 ± 7%	28 ± 7%
% MDSC	21 ± 3%	23 ± 4%	38 ± 9%	20 ± 6%	14 ± 3%
[b]**PBMC Alone**					
% CD107a+	38 ± 3%	33 ± 5%	33 ± 18%	30 ± 6%	42 ± 4%
% TNF-α^+	4 ± 1%	5 ± 2%	5 ± 4%	6 ± 4%	4 ± 1%
% IFN-γ^+	5 ± 1%	3 ± 0.4%	7 ± 3%	4 ± 1%	2 ± 1%
[c]**PBMC + HL-60**					
% CD107a+	42 ± 2%	42 ± 4%	34 ± 17%	46 ± 3%	45 ± 4%
% TNF-α^+	11 ± 2%	12 ± 2%	13 ± 8%	17 ± 4%	16 ± 2%
% IFN-γ^+	14 ± 2%	13 ± 2%	9 ± 5%	23 ± 6%	18 ± 3%

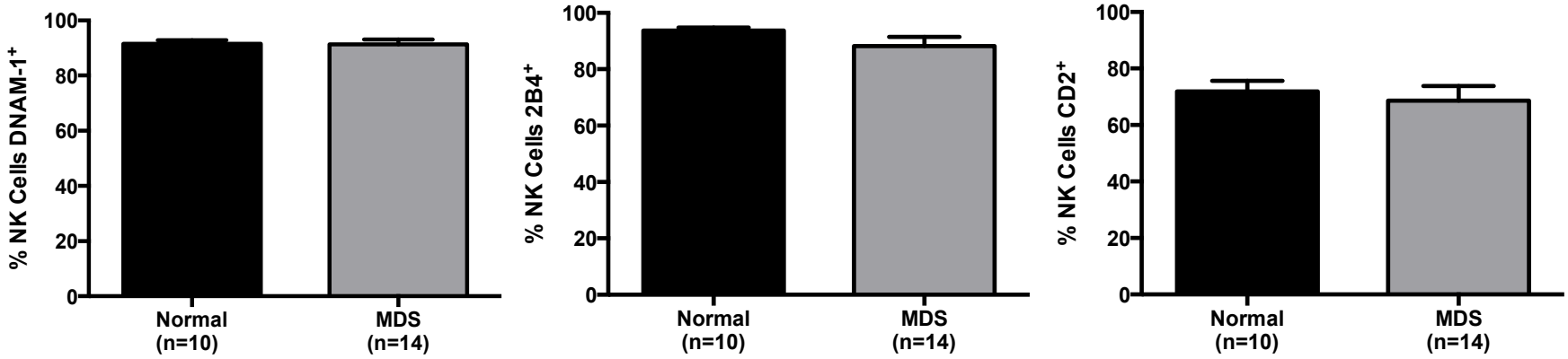
[a]Blast percentage at date of transplant
[b]CD16xCD33 BiKE induced MDS-NK cell function in the absence of HL-60 targets
[c]CD16xCD33 BiKE induced MDS-NK cell function in the presence of HL-60 targets

Supplemental Figure 1



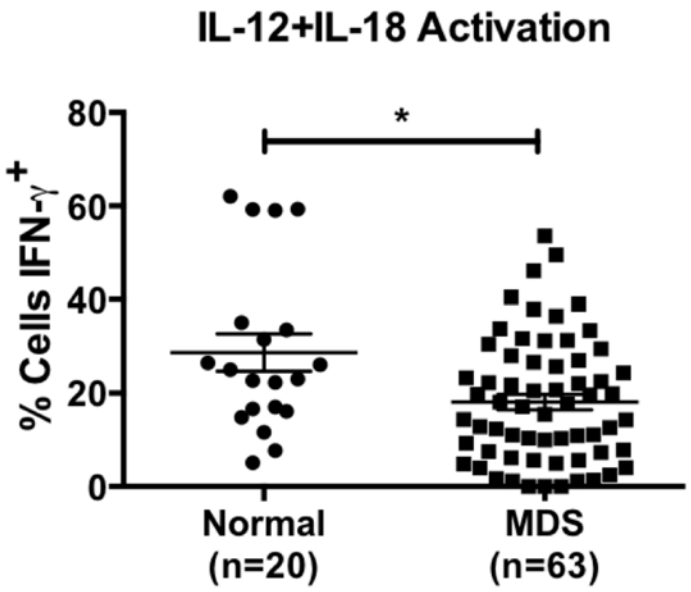
Supplemental Figure 1: NKT cell frequency and NK cell CD16 expression among MDS patients. (A) Percent of $CD56^+/CD3^+$ NKT cells (normalized to all cell fraction based on the FSC/SSC gate excluding debris). (B) Normal donor and MDS-NK cells were stained with anti-CD45, anti-CD7, ant-CD56, anti-CD3 and anti-CD16 mAb and analyzed by flow cytometry. Representative flow cytometry plots from one normal donor (#14) and one higher risk MDS patient (#31) of percent of $CD16^+$ NK cells are shown.

Supplemental Figure 2



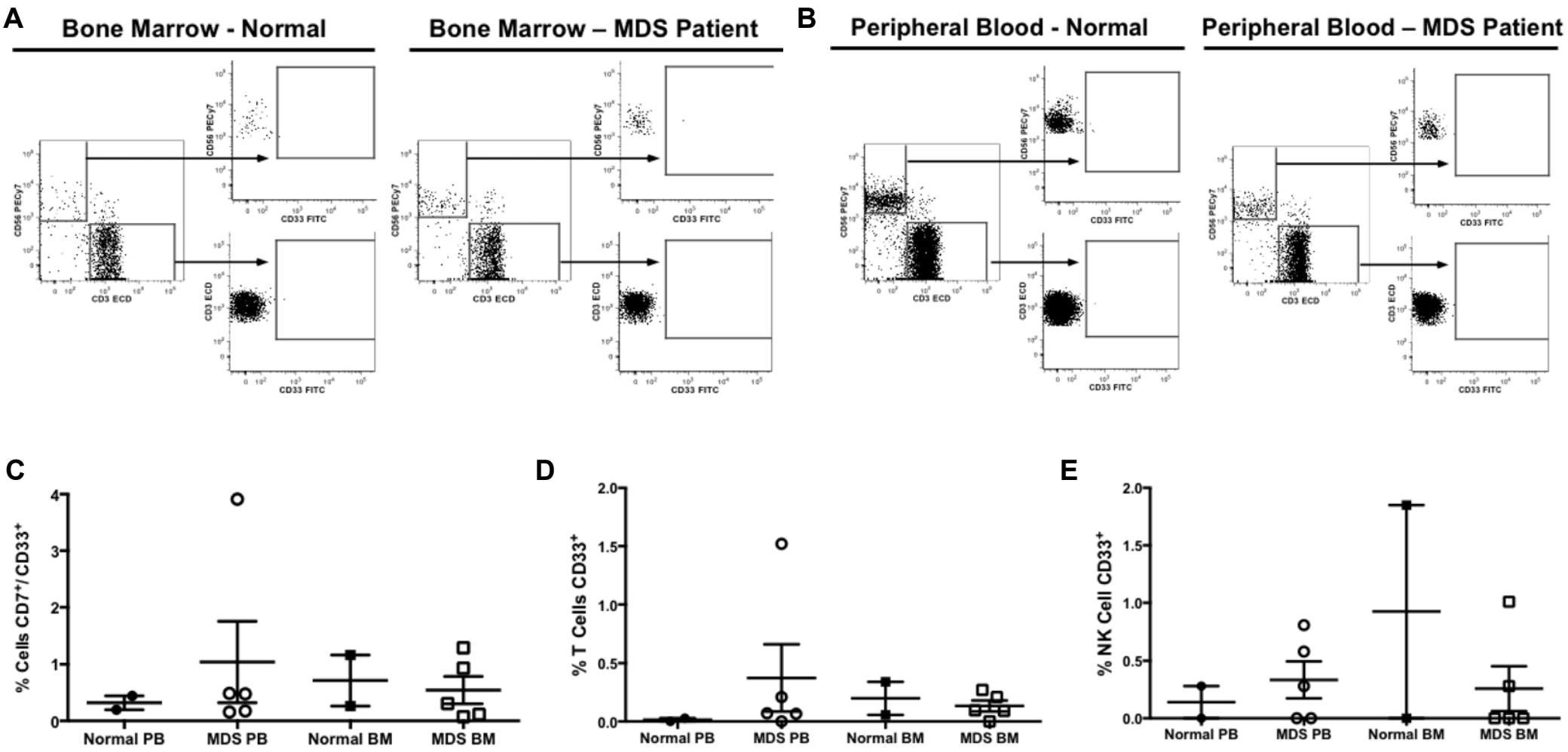
Supplemental Figure 2: DNAM-1, 2B4 and CD2 MDS-NK cell receptor expression. Normal donor and MDS-NK cells were stained with anti-CD45, anti-CD7, anti-CD56, anti-CD3, anti-DNAM-1, anti-2B4 and anti-CD2 mAb and surface receptor expression of DNAM-1, 2B4 and CD2 on NK cells were evaluated by flow cytometry.

Supplemental Figure 3



Supplemental Figure 3: IFN-γ production of MDS-NK cells is lower compared to normal donors after IL-12 and IL-18 activation. PBMC from normal donors and MDS patients were stimulated overnight with IL-12 (10ng/mL) and IL-18 (100ng/mL) and NK cell intracellular IFN-γ production was evaluated via FACS analysis (*P<0.05).

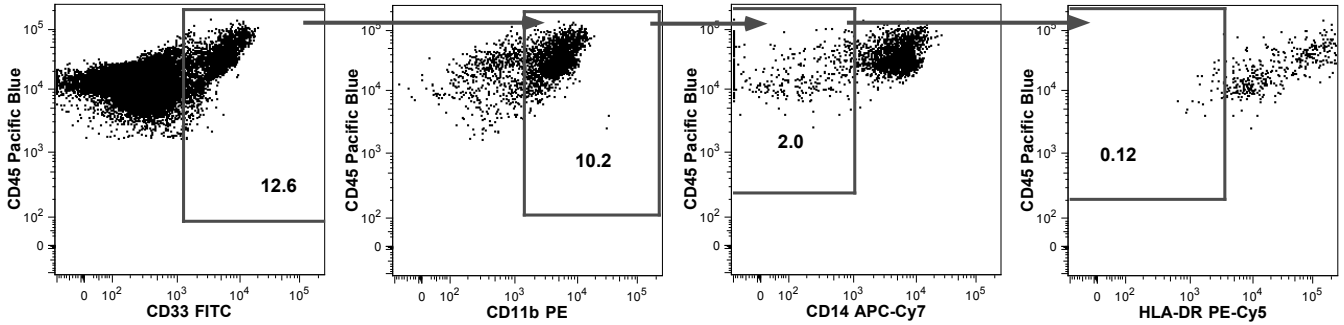
Supplemental Figure 4



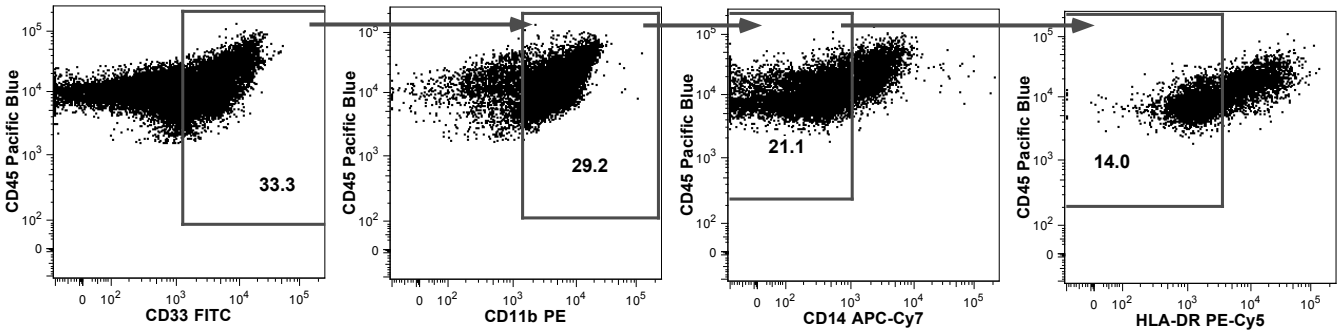
Supplemental Figure 4: CD33 Expression on lymphocyte populations. Bone marrow (BM) and peripheral blood (PB) from normal donors and MDS patients were stained with anti-CD45, anti-CD7, anti-CD56, anti-CD3 and anti-CD33 and CD33 expression was evaluated by flow cytometry on $CD7^+$, T and NK cell populations. Flow cytometry plots are representative of one normal donor and one MDS patient and show CD33 expression on (A) $CD56^-/CD3^+$ T cells and (B) $CD56^+/CD3^-$ NK cells. Aggregate data for CD33 expression on (C) $CD7^+$ lymphocytes, (D) T cells and (E) NK cells from normal donors (n=2) and MDS patients (n=5) from BM and PB are shown.

Supplemental Figure 5

A Normal Donor

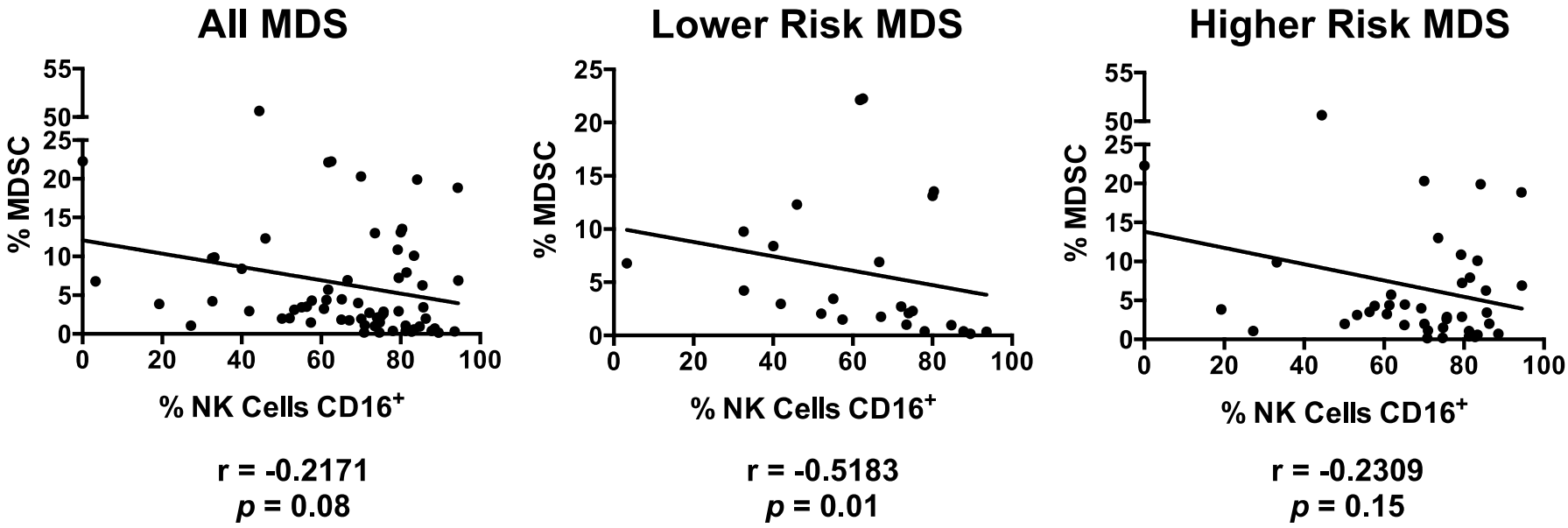


B MDS Patient



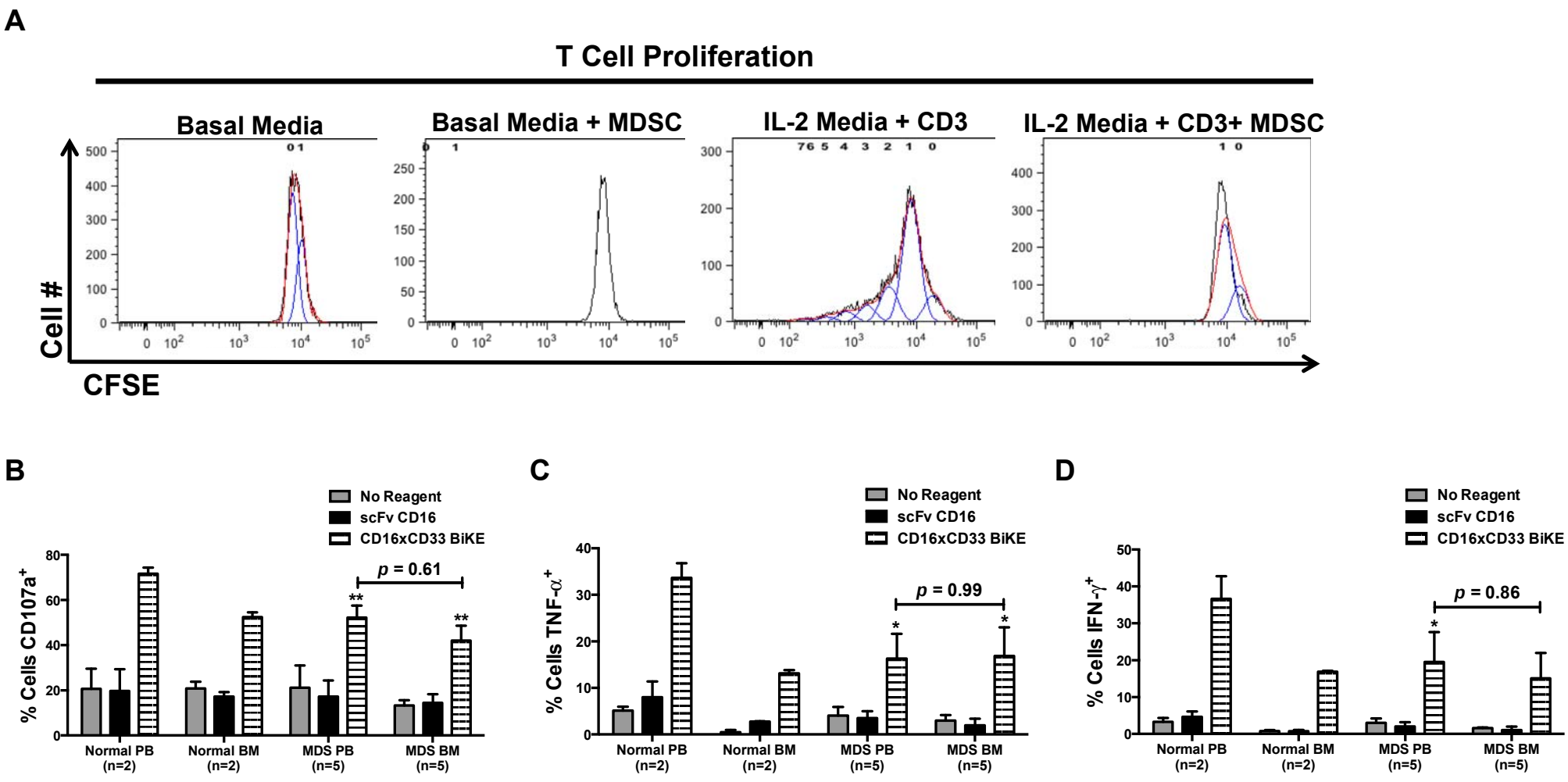
Supplemental Figure 5: MDSC gating strategy. PBMC from normal donors and MDS patients were stained with anti-CD45, anti-CD33, anti-CD11B, anti-CD14 and anti-HLA-DR mAbs. (A-B) Gating strategy for the evaluation of MDSC population in normal donors (A) and MDS patients (B). Plots are representative from one normal donor (#3 of 20) and one higher risk MDS patient (#25 of 67) and gate frequency indicates population percent normalized to the all cell fraction based on the FSC/SSC gate excluding debris.

Supplemental Figure 6



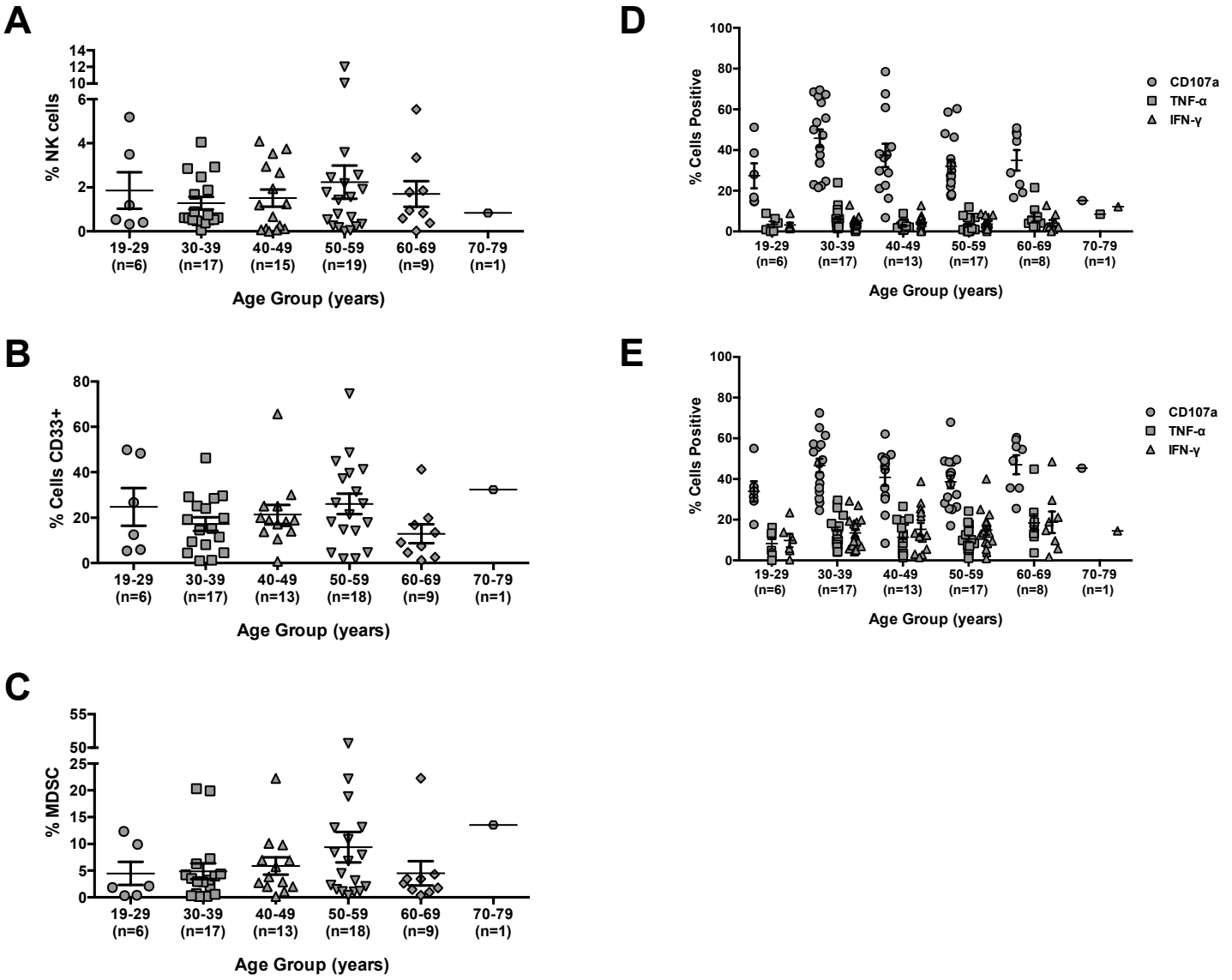
Supplemental Figure 6: MDS-NK cell CD16 expression negatively correlates with MDS-MDSC frequency. PBMC from MDS patients were stained with anti-CD45, anti-CD7, anti-CD56, anti-CD3, anti-CD16, anti-CD33, anti-CD11b, anti-CD14 and anti-HLA-DR mAbs. The correlations between % MDSC (normalized to all cell fraction excluding debris) and % NK cells $CD16^+$ among all MDS patients, lower risk MDS patients and higher risk MDS patients are presented. Correlation coefficients (r) and statistical significance are indicated in the figure.

Supplemental Figure 7



Supplemental Figure 7: CD16xCD33 BiKE enhances peripheral blood and bone marrow NK cell function against HL-60 targets. (A) CFSE-labeled T cells were cultured for 5 days in basal medium, medium supplemented with 100U/mL IL-2 and CD3 bead activation in the presence or absence (E:T ratio = 1:2) of cytokine-derived MDSC from normal PBMC and proliferation was evaluated via FACS analysis. Histogram plots represent one of six normal donors. (B-D) Mononuclear cells from peripheral blood (PB) and bone marrow (BM) were isolated from normal donors (n=2) and MDS patients (n=5), coated with 10μg/mL of CD16xCD33 BiKE or scFv CD16 control, co-cultured with HL-60 targets and NK cell (B) CD107a expression and intracellular (C) TNF-α and (D) IFN-γ production were evaluated via FACS analysis (*P<0.05, **P<0.01).

Supplemental Figure 8



Supplemental Figure 8: CD16xCD33 BiKE consistently enhances MDS-NK cell function among all age strata. The percent of $CD56^{+}/CD3^{-}$ MDS-NK cells (A), total $CD33^{+}$ cells (B) and MDS-MDSC (C) of the all cell fraction was stratified according to age groups (years). (D-E) CD16xCD33 BiKE-induced MDS-NK cell functions (degranulation (CD107a), intracellular TNF-α and IFN-γ production) in the absence of HL-60 targets (D) and in the presence of HL-60 targets (E) were stratified according to age groups. Y-axis of graphs (D and E) represent the percent of NK cells positive for each function listed in graph legend.