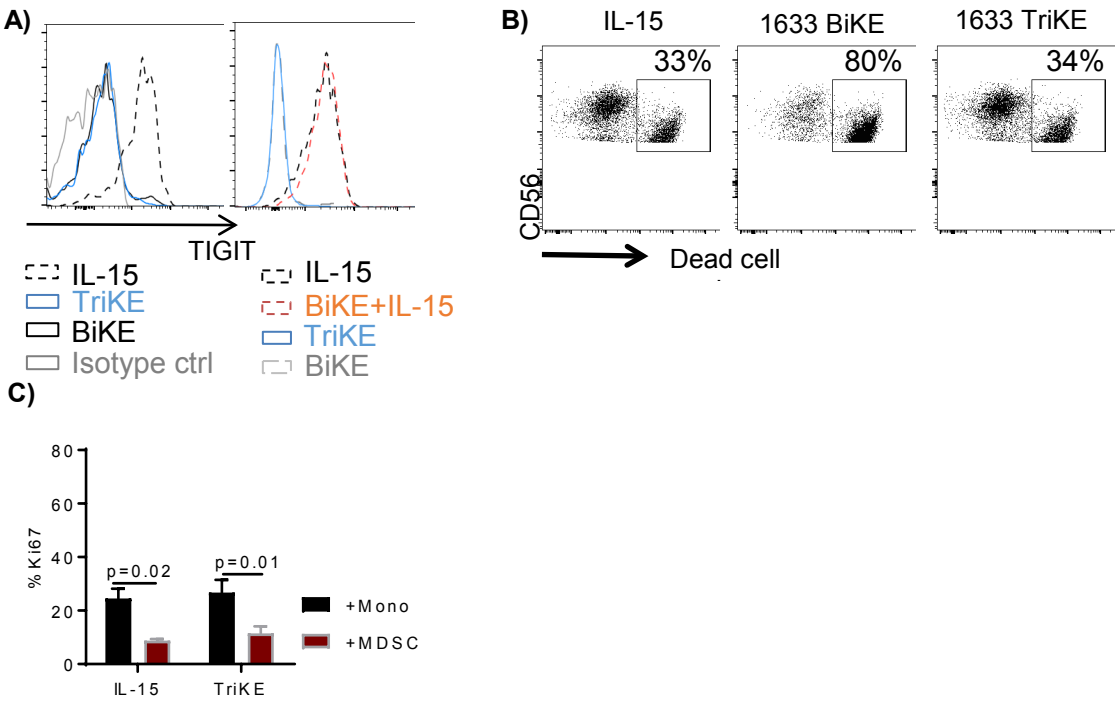
Supplemental Table 1. Antibodies used for study.

Marker	Clone	Fluorochrome	Manufactory
CD3	OKT3	BV785	Biolegend
CD56	NCAM	PE/CY7	Biolegend
CD16	3G8	AF700	Biolegend
TIGIT	741182	APC	R&D systems
CD45	HI30	BV605/BV711	BD Biosciences
CD7	M-T701	APC	BD Biosciences
pSTAT5	pY694	AF488/APC	BD Biosciences
CellTrace		Violet	Invitrogen
CellTrace		Far Red	Invitrogen
CellTrace Calcein		Green	Invitrogen
CD107a	H4A3	PerCP/Cy5.5	Biolegend
IFNγ	4S.B3	BV650	Biolegend
Ki67	B56	AF700	BD Biosciences
Fixable dead cell marker		Near-IR	Invitrogen

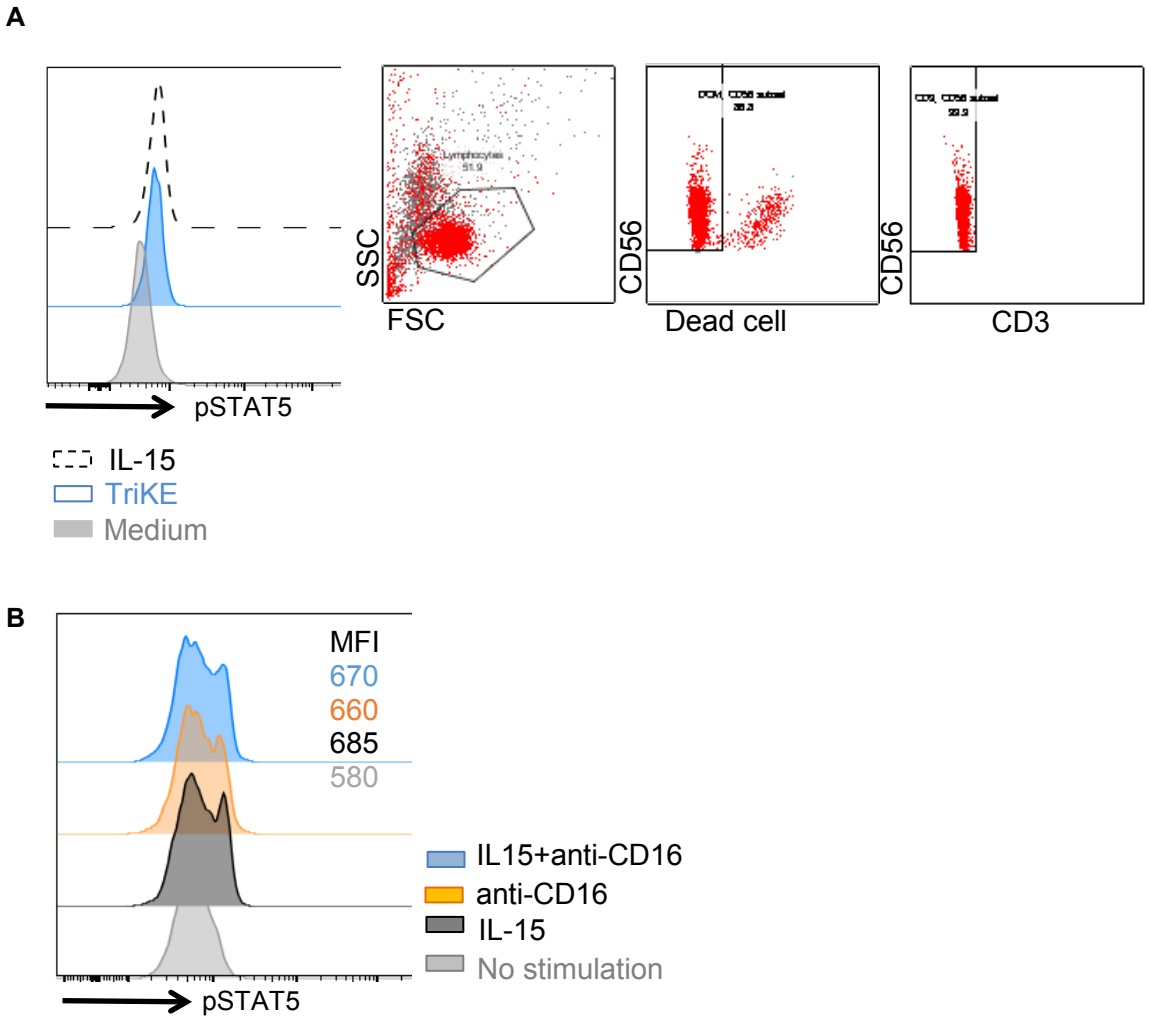
Supplemental Figure 1.



Supplemental Figure 1.

A) Purified HD-NK cells (n=5) HD were stimulated with IL-15 (10 nM), agonistic anti-CD16 (1 µg/ml), IL-15+anti-CD16, or left unstimulated for 25 min prior analysis of STAT5 phosphorylation. Representative gating strategy is shown. **B)** Purified HD-NK cells (n=6) were co-cultured with autologous monocytes for 5 days in the presence of IL-15 (equal-molar concentration to the IL-15 in the TriKE), Bike (50 nM), or TriKE (50 nM). NK cells were analyzed for viability. One representative donor is shown. **C)** Purified HD-NK cells (n=4) were pre-treated with IL-15 or TriKE (50 nM) overnight prior extensive wash. Subsequently, NK cells were co-cultured with autologous monocytes or MDSC for 5 days and analyzed for proliferation (Ki67). Data are shown as mean±SEM and statistical analyses were performed using paired t-test.

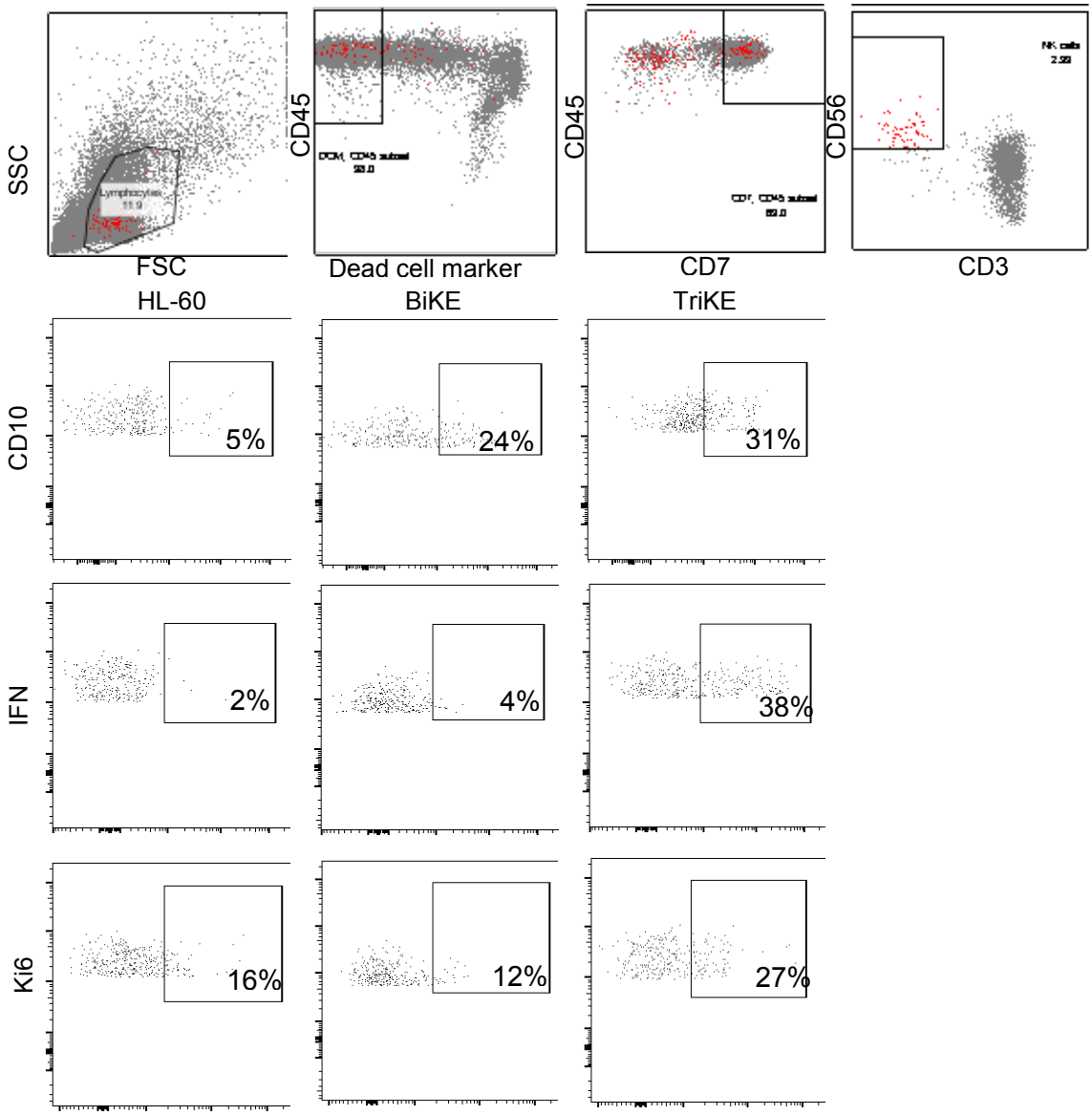
Supplemental Figure 2.



Supplemental Figure 2.

A) Isolated NK cells (n=9), by negative depletion, from healthy blood donors were stimulated with IL-15 (equal-molar concentration to the IL-15 in the TriKE (50 nM), Bike (50 nM), BiKE + IL-15 (both 50 nM), or TriKE (50 nM) with or without HL60 targets for 25 minutes before fixation and STAT5 phosphorylation analysis. Alternately, purified HD-NK cells (n=5) HD were stimulated with IL-15 (10 nM), agonistic anti-CD16 (1 µg/ml), IL-15+anti-CD16, or left unstimulated prior analysis of STAT5 phosphorylation. Representative data are shown.

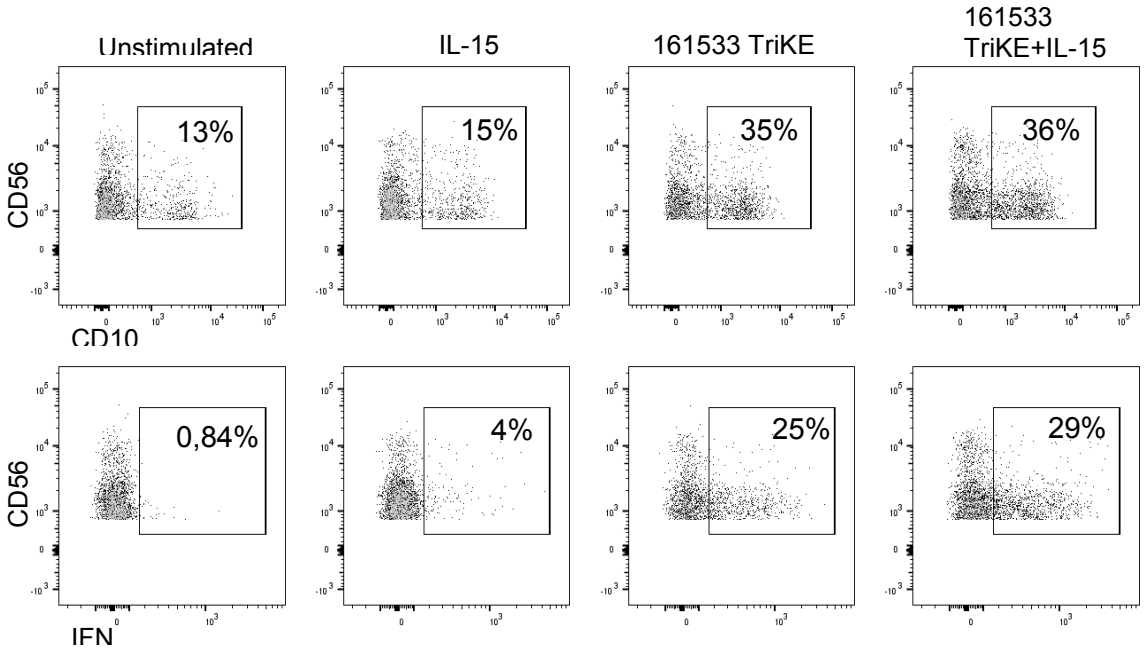
Supplemental Figure 3.



Supplemental Figure 3.

Cryopreserved PBMC from MDS patients were activated overnight with BiKE (50 nM), or TriKE (50 nM). Target cells were then added 6h prior to staining. NK cell degranulation (CD107a), IFNγ production, and proliferation (Ki67) were evaluated. A representative gating strategy is shown.

Supplemental Figure 4.



Supplemental Figure 4.

Cryopreserved PBMC from HD were activated overnight with IL-15, 161533 TriKE, 161533 TriKE+IL-15, or left untreated and analyzed for NK cell degranulation and IFNγ. One representative donor (n= 4) is shown.