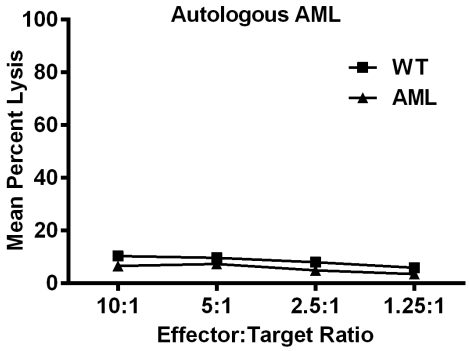
Supplemental Figure 1

A



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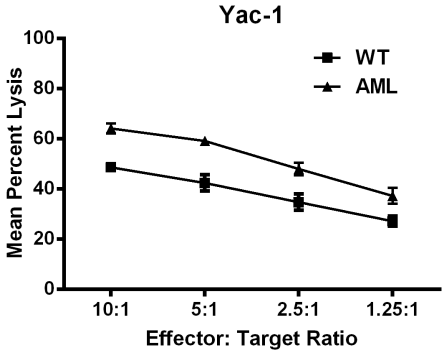

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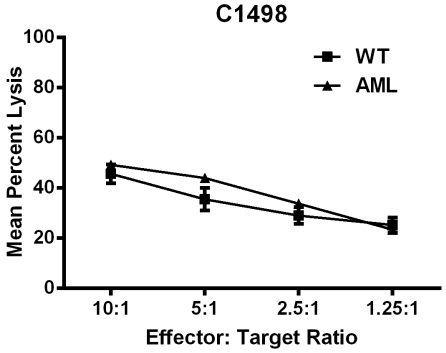
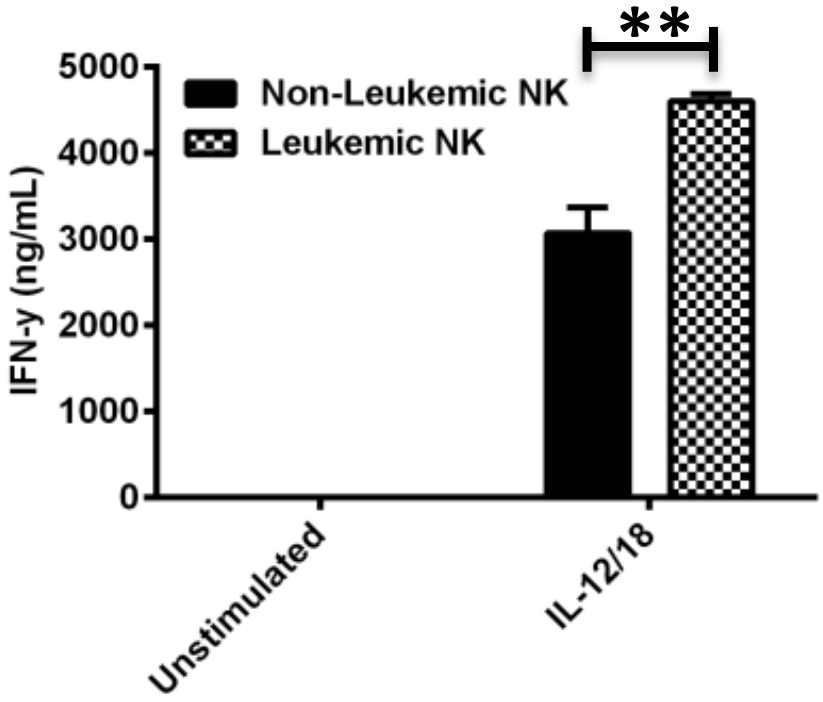


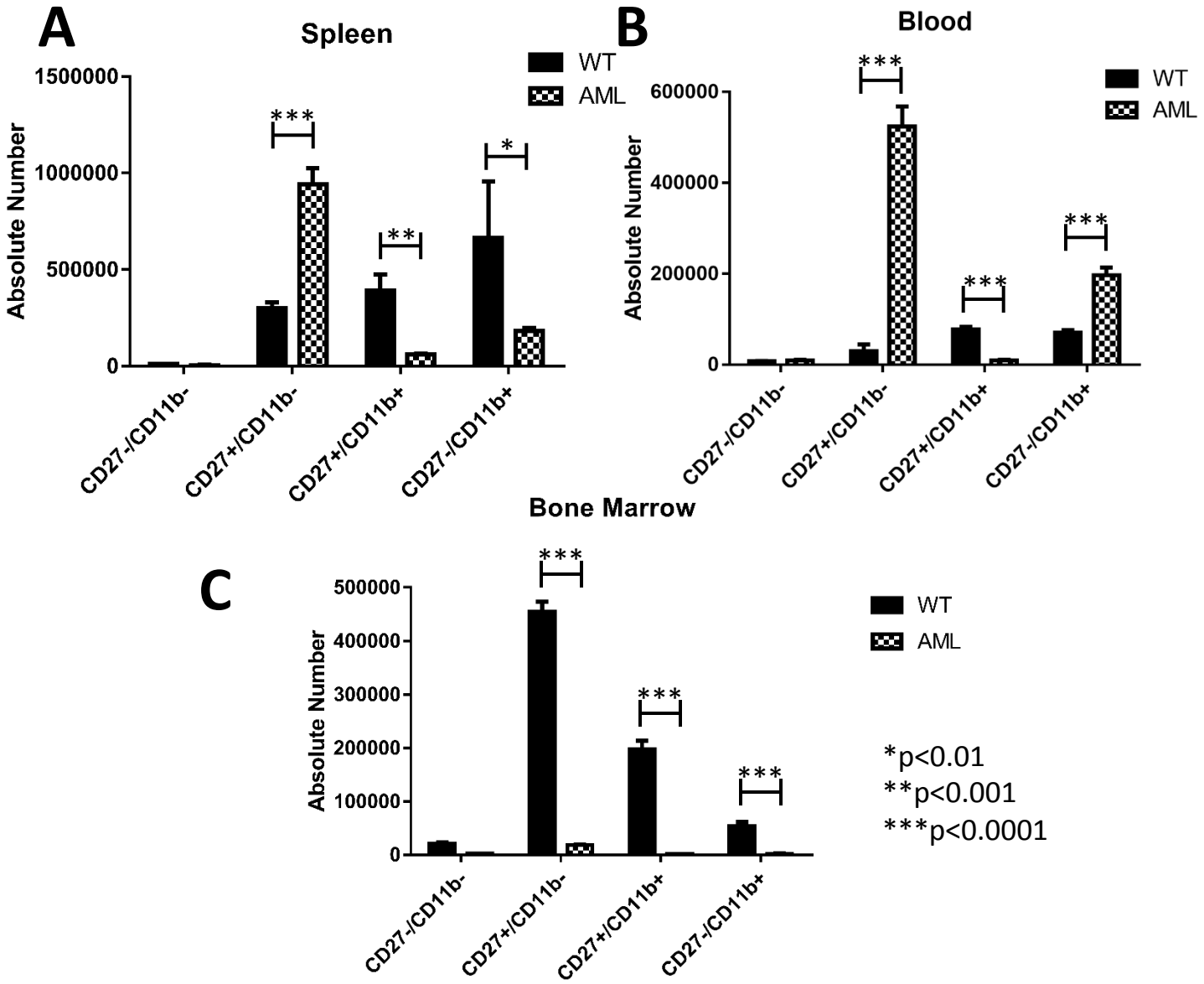
Supplemental Figure S1. NK1.1(+)CD3(-) NK cells were isolated from splenocytes of leukemic mice and sorted for purity. Autologous blasts were also isolated from the spleens of the same leukemic mice. A standard chromium release cytotoxicity assay was performed with **(A)** autologous blasts, **(B)** Yac-1 murine lymphoma, or **(C)** C1498 murine acute myeloid leukemia cells as targets at the indicated ratios (minimum of n=3 independent mice/graph).

Supplemental Figure 2



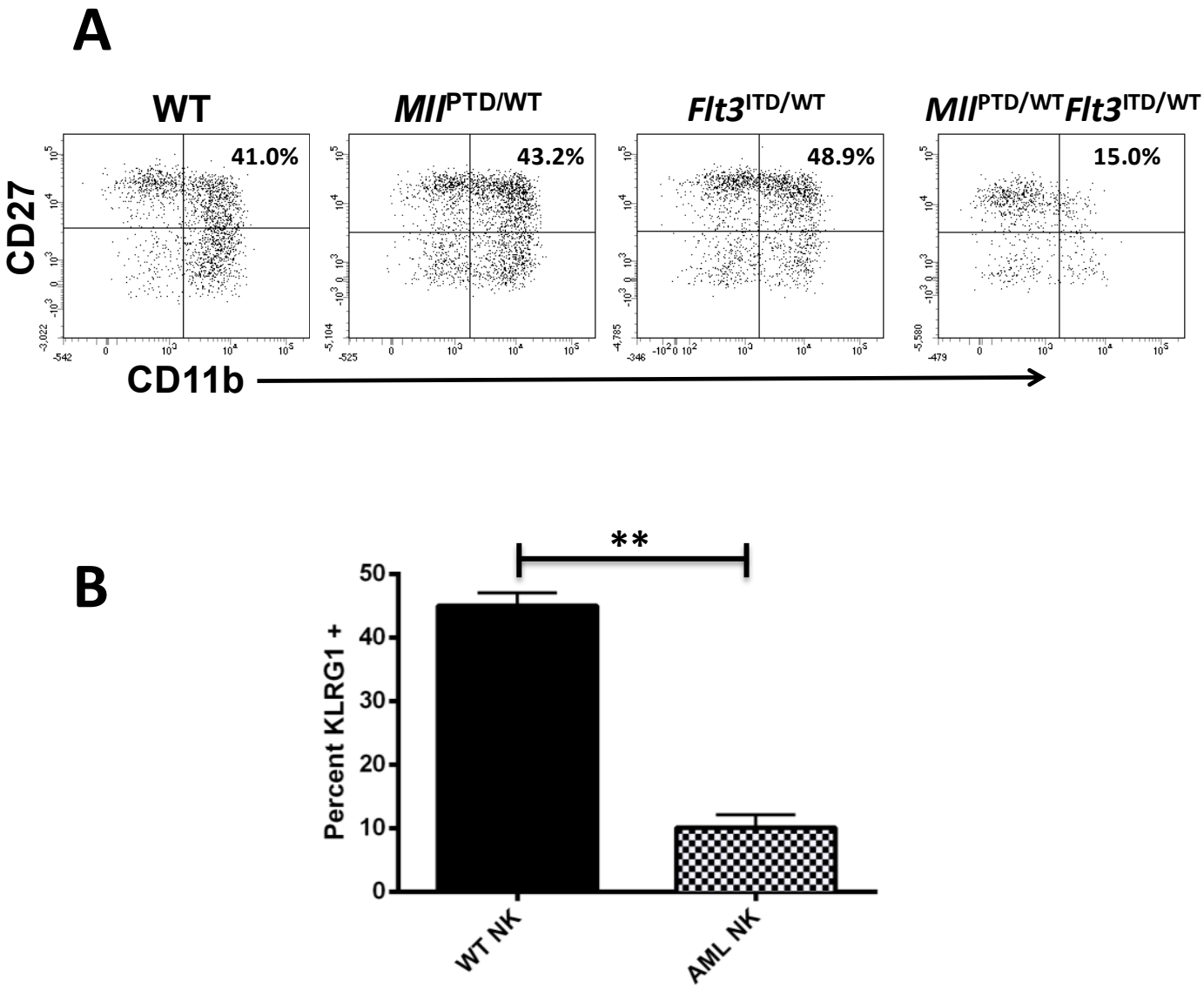
Supplemental Figure S2. (C)IFN-γ was measured in freshly isolated splenic NK cells by ELISA, with or without IL-12 and IL-18 (50ng/mL) stimulation after 24 hours *ex vivo* (p=0.0011; n=minimum 3 mice/group, repeated two independent studies with a representative study depicted). Two sample t-tests with Holm's adjustment was used for multiple comparisons within the same mice were used for statistical modeling.

Supplemental Figure 3



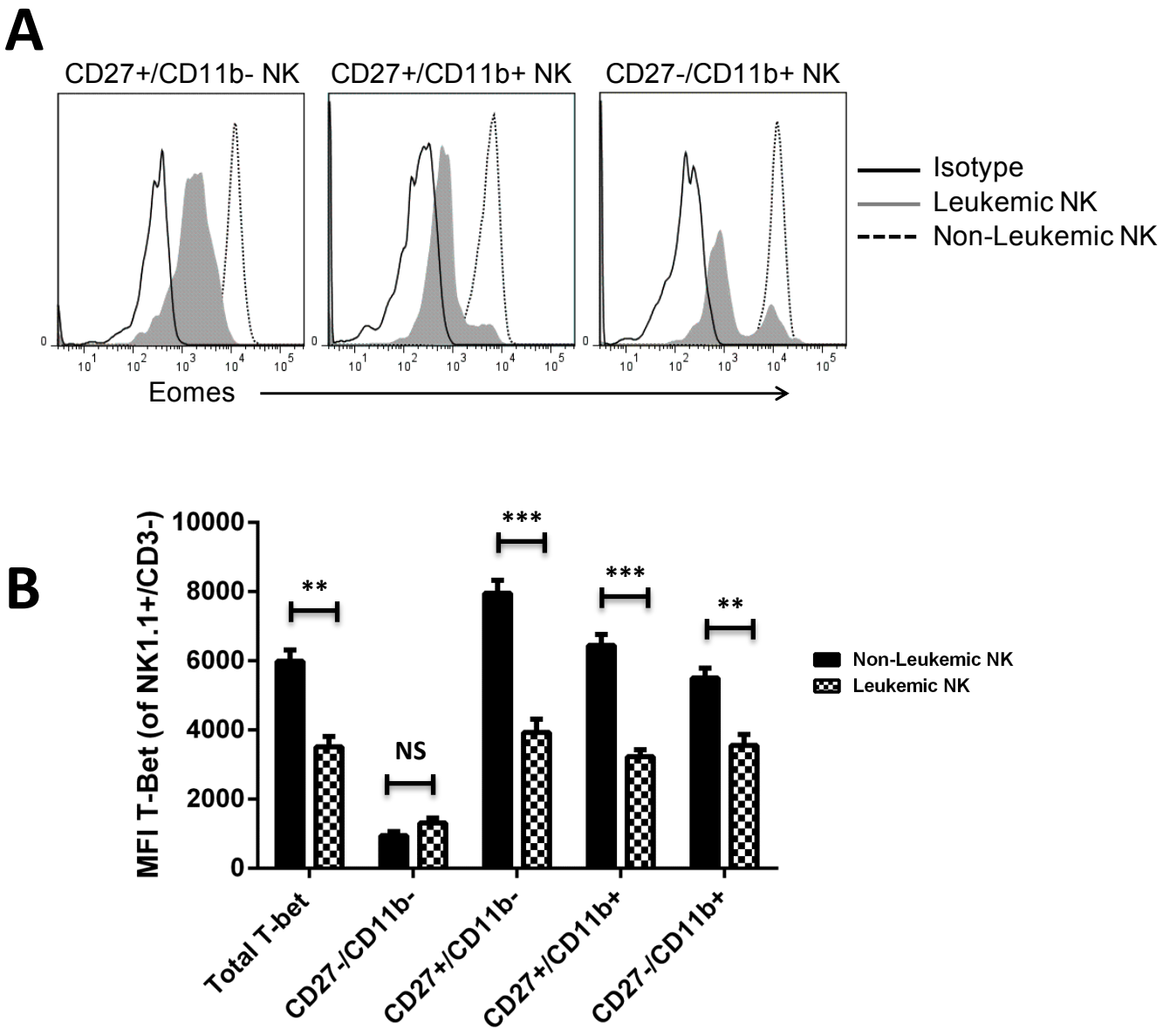
Supplemental Figure S3. Freshly isolated cells from the **(A)** spleen, **(B)** blood, or **(C)**bone marrow were labeled with anti-NK1.1, CD3, CD45.1, CD27 and CD11b and evaluated by flow cytometry. Absolute number of NK cell subsets were calculated based on the total NK cell count. (***p<0.0001, **p<0.001, *p<0.01; n=5-8 mice/group). Two sample t-tests with Holm's adjustment was used for multiple comparisons within the same mice were used for statistical modeling.

Supplemental Figure 4



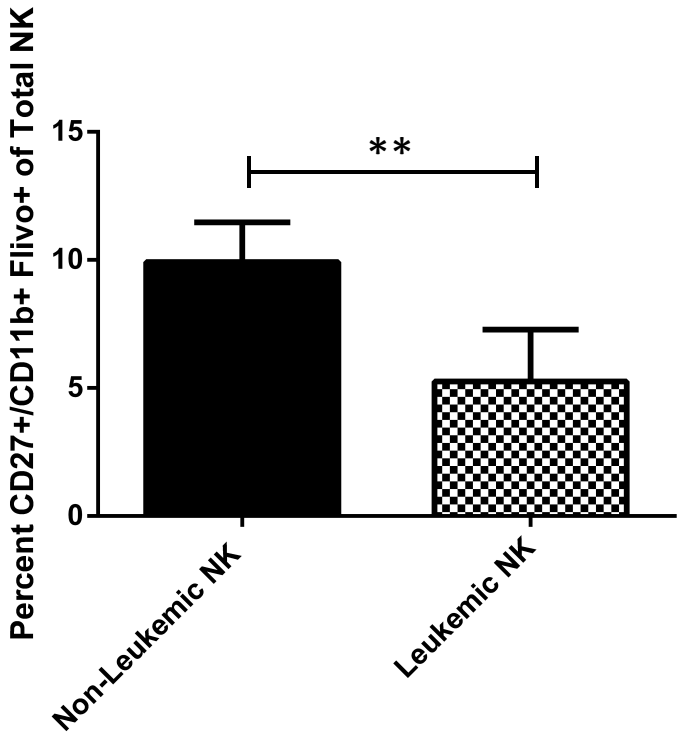
Supplemental Figure S4. (A) Splenocytes from WT, single mutant *Mll*$^{PTD/WT}$, single mutant *Flt3*$^{ITD/WT}$, or double mutant *Mll*$^{PTD/WT}$*Flt3*$^{ITD/WT}$ were labeled with anti-NK1.1, CD3, CD45.1, CD27 and CD11b and evaluated by flow cytometry. Representative flow dot plots demonstrate CD27 and CD11b expression within the NK1.1(+)CD3(-) NK cell population. **(B)** KLRG1 protein expression was evaluated in freshly isolated splenic NK cells by flow cytometry (n=4/group, repeated two independent studies with a representative study depicted; p=0.003). Student's t-test was used for statistical modeling.

Supplemental Figure 5



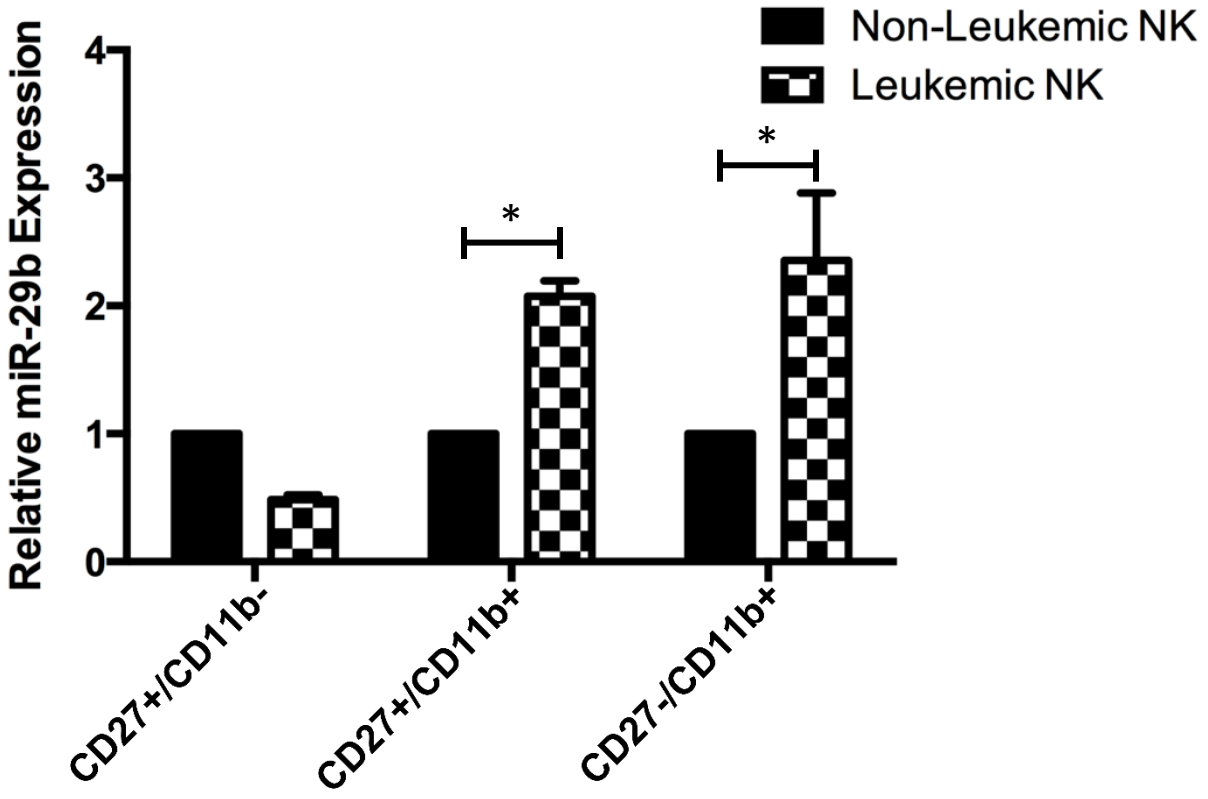
Supplemental Figure S5. Freshly isolated splenocytes were labeled with anti-NK1.1, CD3, CD45.1, CD27 and CD11b surface antibodies, then fixed, permeabilized, and labeled with Eomes or T-bet. **(A)** Representative flow histogram of NK cell subsets depicted. **(B)** T-bet expression was evaluated in bulk NK cells and NK cell subsets and MFI (Mean fluorescent intensity sample-corresponding isotype control) was calculated to determine level of protein expression (**p<0.01, ***p<0.001). Linear mixed effects models were used for multiple comparisons with Holm's adjustment.

Supplemental Figure 6



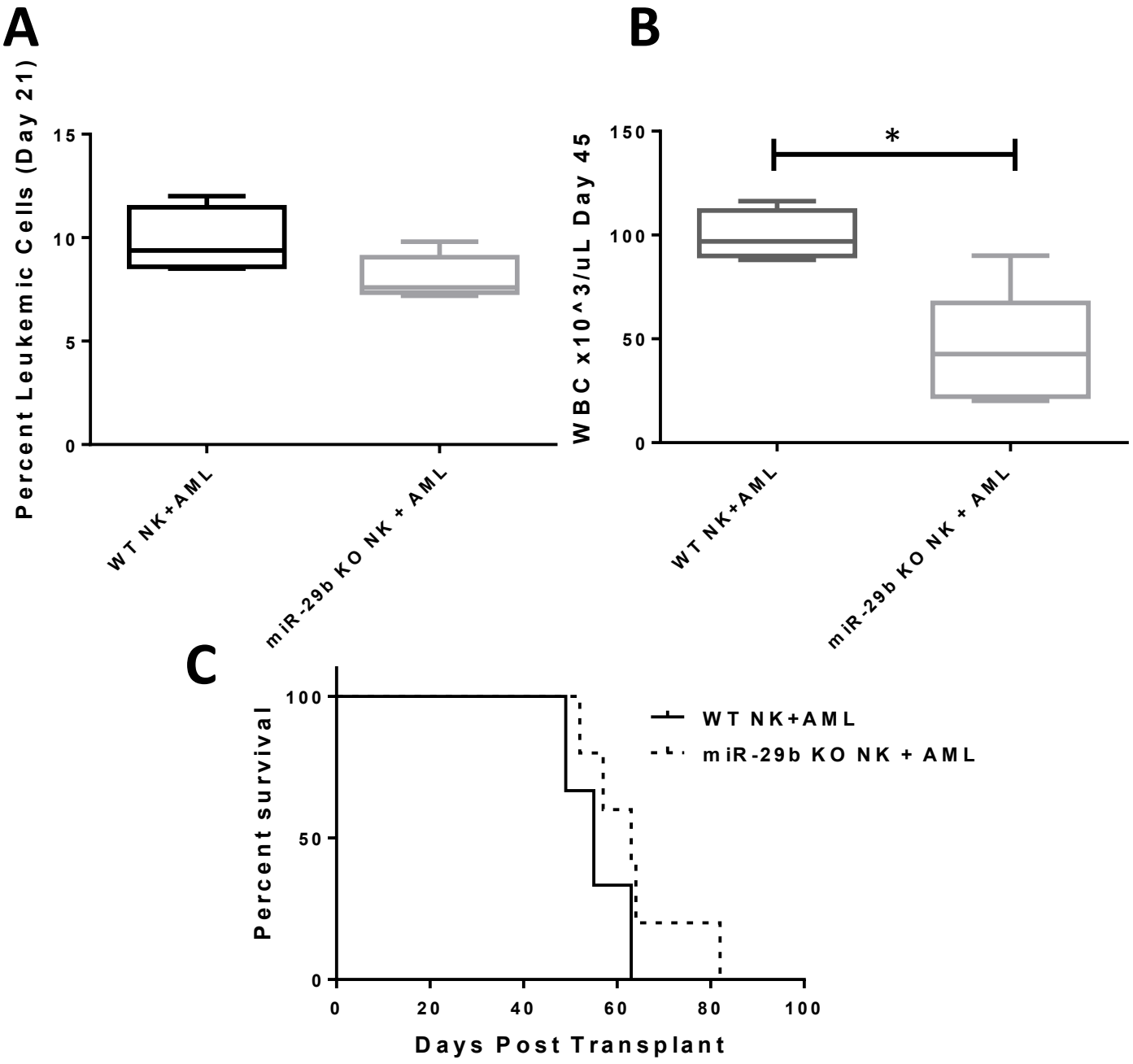
Supplemental Figure S6. NK1.1(+)CD3(-)CD27(+)CD11b(+) NK cells ($5x10^4$) were isolated from spleens of WT congenic CD45.1(+) mice and adoptively transferred into non-leukemic or leukemic mice. Spleens were harvested and cells were labeled with anti-NK1.1, CD3, CD45.1, CD27 and CD11b and evaluated by flow cytometry**.** CD27(+)CD11b(+) DP splenic NK cells were harvested three days after adoptive transfer and evaluated for apoptosis using Flivo *in vivo* imaging dye (**p=0.018; n=4/group). Student's t-test was used for statistical modeling.

Supplemental Figure 7



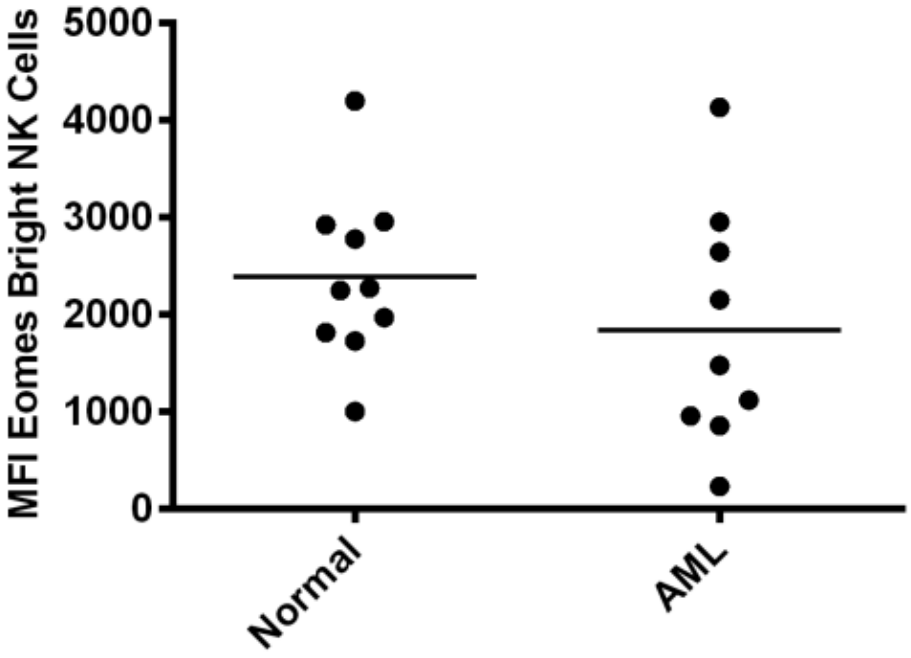
Supplemental Figure S7. NK1.1(+)CD3(-) NK cells were isolated from spleens and sorted for CD27 and CD11b expression by FACS from non-leukemic or leukemic mice. Levels of miR-29b were evaluated by quantitative Real-time PCR, and expression in leukemic mice was compared to non-leukemic mice (n=3 mice/group); *$p<0.05$. Two sample t-tests with Holm's adjustment was used for multiple comparisons within the same mice were used for statistical modeling.

Supplemental Figure 8



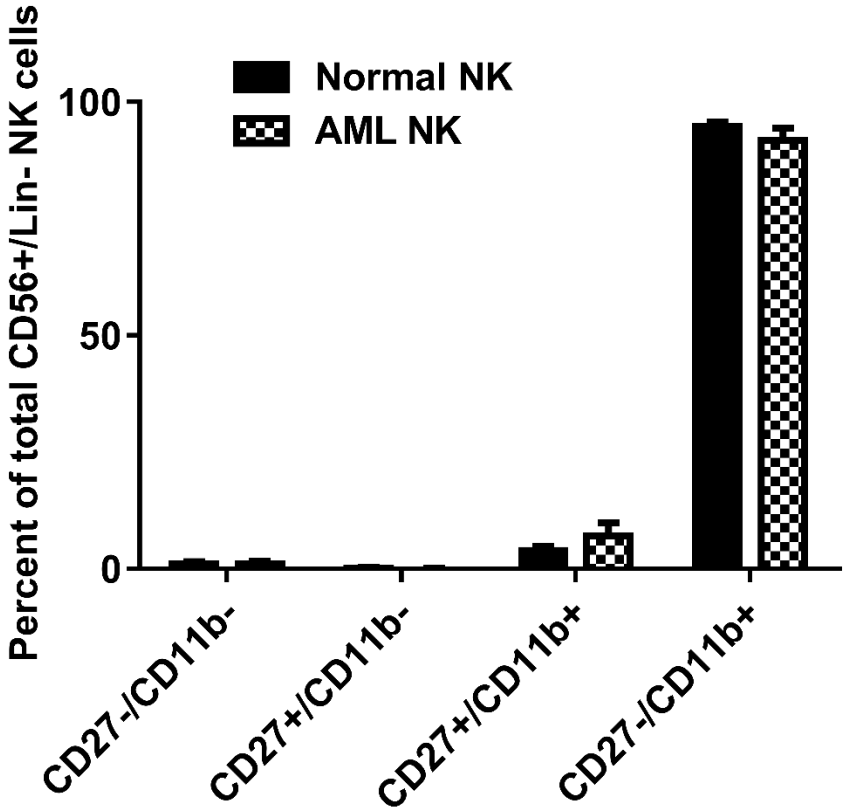
Supplemental Figure S8. CD45.2(+) AML blasts were transplanted into congenic CD45.1(+) recipients with miR-29b KO NK or WT NK cells. **(A)** The percent of circulating blasts (CD45.2+) was evaluated by flow cytometry at day 21 post-transplant (p=0.09; n=4-5/group; two sample t-test). **(B)** Total WBC count was assessed 45 days after transplant (p=0.008; n=4-5 mice/group). **(C)** Survival of mice transplanted with AML blasts + WT NK or AML blasts + miR-29b KO NK was evaluated (p=0.06; n=5). A log-rang test was used for statistical analysis.

Supplemental Figure 9



Supplemental Figure S9. CD56+Lin-CD94+CD16- Bright NK cells taken from AML patients and normal donors were evaluated for Eomes expression by intracellular flow cytometry (n=9 normal donors and n=5-9 untreated AML patients; p=0.024). The data was first log2 transformed and then ANOVA was used for analysis.

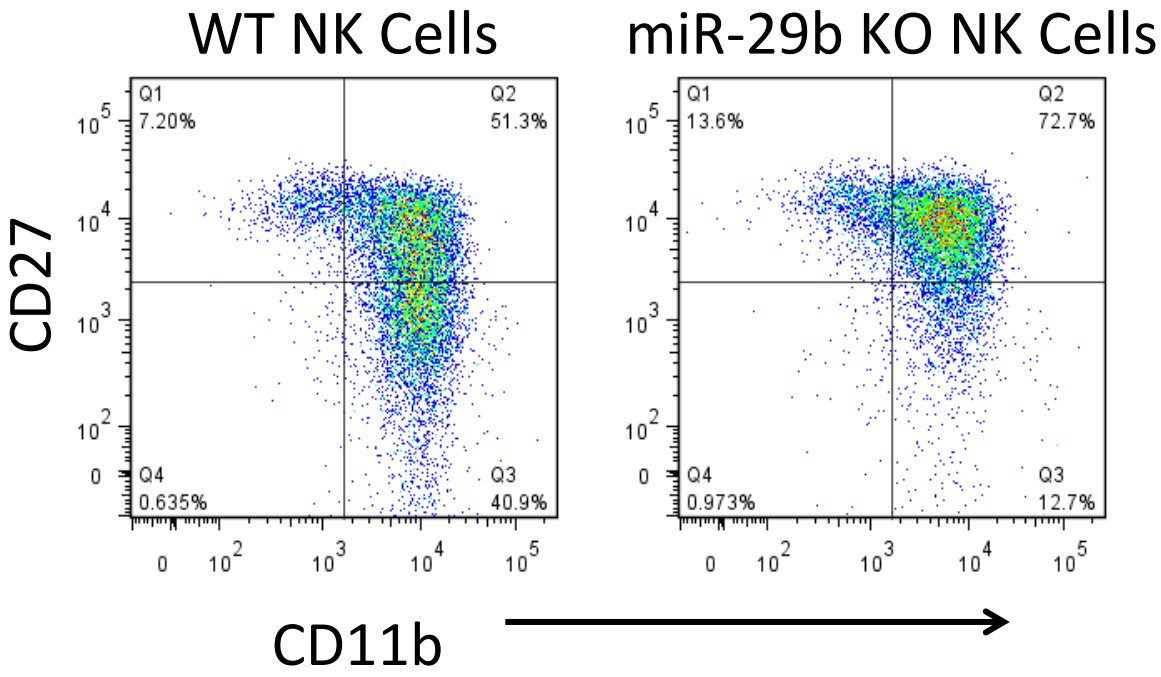
Supplemental Figure 10



Supplemental Figure S10. Peripheral blood was obtained from untreated newly diagnosed AML patients or normal donors. NK cells were enriched by negative selection, labeled with anti-CD94, CD56, CD16, CD3, CD15, CD64, CD13, CD3, CD14, CD20, CD27, and CD11b and then evaluated using highly selective gating by flow cytometry. CD27 and CD11b expression were then evaluated in the CD56(+)/Lineage(-) NK cell population. There were no significant differences found between the NK cell subsets as defined by CD27 and CD11b (n=6 AML and 6 normal donors). Linear mixed effects models were used for multiple comparisons with Holm's adjustment.

Supplemental Figure 11

A



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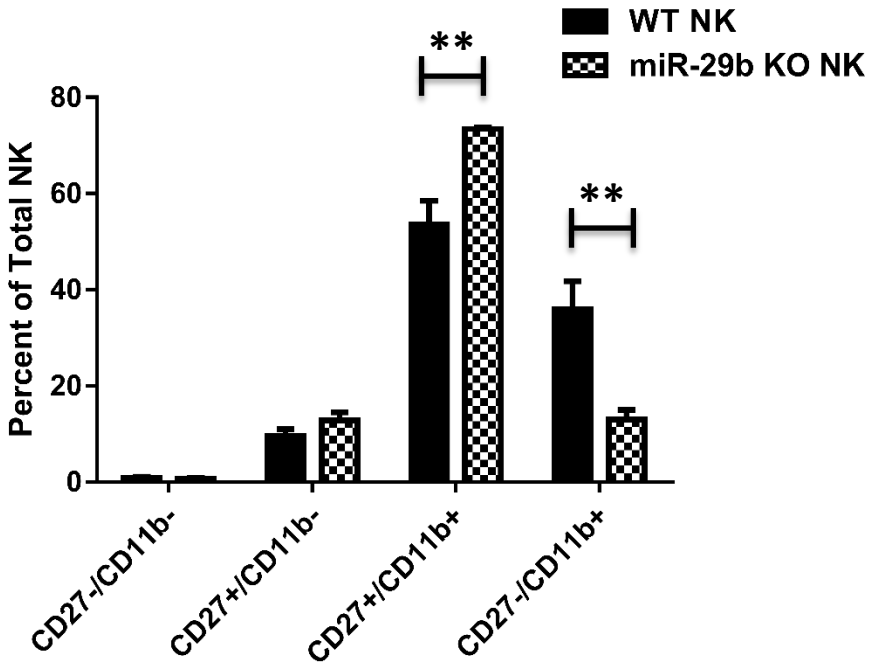
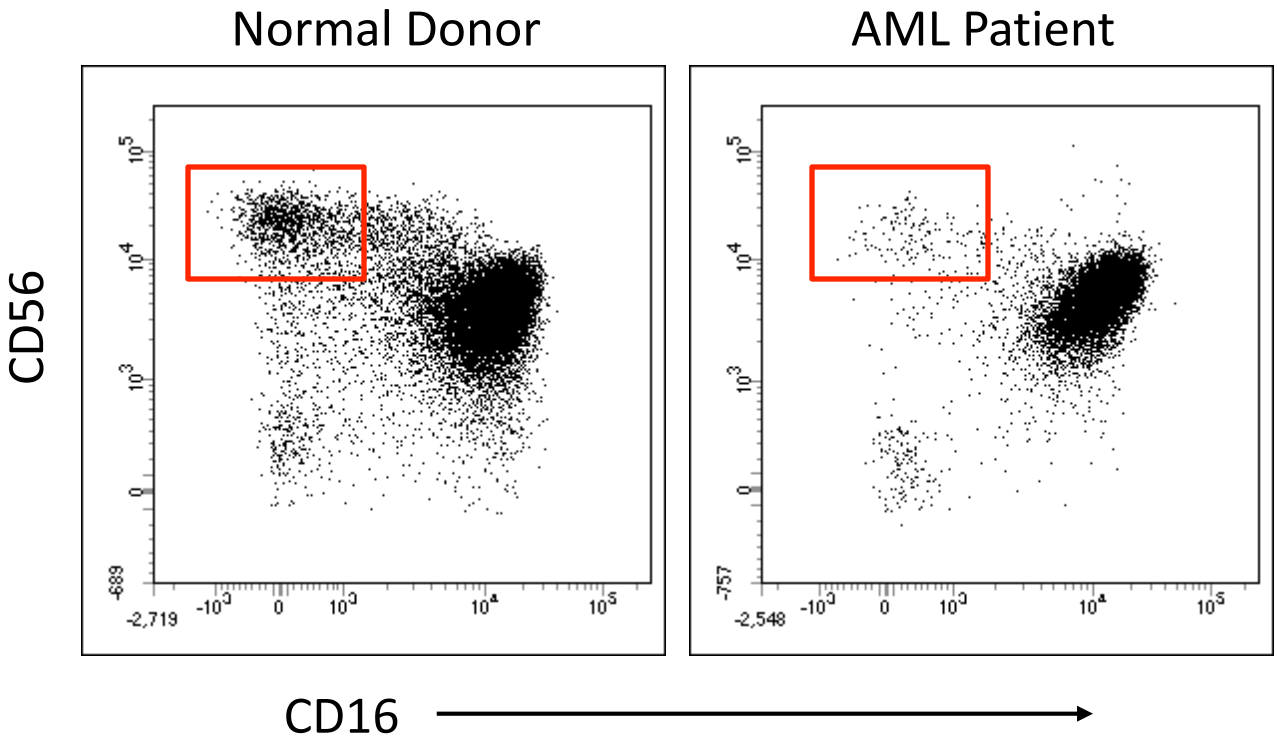


Supplemental Figure S11. (A) Representative flow dot plot of CD27 and CD11b expression within the NK population of WT and miR-29b KO splenocytes. **(B)** Percentages of all four NK cell subsets as defined by CD29 and CD11b expression within total NKp46(+)CD3(-) splenic NK cells in miR-29b KO NK or WT littermate control NK cells (** $p<0.01$; n=4 mice/group). Linear mixed effects models were used for multiple comparisons with Holm's adjustment.

Supplemental Figure 12



Supplemental Figure S12. Peripheral blood was obtained from untreated newly diagnosed AML patients or age and gender-matched normal donors. NK cells were enriched by negative selection, labeled with anti-CD94, CD56, CD16, CD3, CD15, CD64, CD13, CD3, CD14, and CD20 and then evaluated by flow cytometry. Representative flow dot plot of CD56 and CD16 expression within the total Lineage negative PBMC population of an AML patient and normal donor. Red rectangle represents CD16 negative population within CD56 Bright NK cells.

Supplemental Table 1			
Mouse Antibodies			
CD3	Percp-Cy5.5	BD	145-2C11
CD11b	FITC	BD	M1/70
CD27	PE-Cy7	Ebioscience	LG.7F9
CD45.1	V450/PerCP-Cy5.5/FITC	BD	A20
CD45.2	PE	BD	104
CD49b	PE-CF594	BD	DX5
Ki67	PE	BD	B56
NK1.1	PE/APC/V450/BV	BD	PK136
NKp46	APC/V450	BD	29A1.4
KLRG1	PE	Miltenyi	2F1
Integrin Alpha V	PE	Ebioscience	RMV-7
Perforin	PE	Ebioscience	eBioOMAK-D
T-bet	PE	BD	Apr-46
Eomes	PE	BD	DAN11mag

Human Antibodies			
CD3	VG	Miltenyi	BW264/56
CD13	PerCP-Cy5.5	BD	WB15
CD14	VG	Miltenyi	TÜK4
CD15	PerCP-Cy5.5	BD	HI98
CD16	APCH7	BD	3G8
CD20	VG	Miltenyi	LT20
CD34	PerCP-Cy5.5	Miltenyi	AC136
CD45	FITC	Miltenyi	5B1
CD56	BV	BD	NCAM16.2
CD64	PerCP-Cy5.5	BD	10.1
CD94	PE-VIO770	Miltenyi	REA113
TBET	Efluor 660	Ebioscience	4B10
EOMES	Efluor 660	Ebioscience	WE 1928
NKp80	APC	Miltenyi	4A4.D10