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	Figure 3A,B
144 Nd	CD3	UCHT1	DVS Sciences	
145 Nd	KIR2DS4 (CD158i)	FES172	Beckman Coulter	Figure 3A, B
146 Nd	KIR2DL1/DS1 (CD158a,h)	EB6B	Beckman Coulter	Figure 3A, B
147 Sm	NKG2D	1D11	R&D	
148 Nd	KIR2DL2/2DL3 (CD158b)	CH-L	BD Pharmingen	Figure 3A, B
149 Sm	MIP-1a	93342	R&D	
151 Eu	CD107a	H4A3	DVS Sciences	
152 Dy	TNF	Mab11	DVS Sciences	
153 Eu	CD62L	DREG-56	DVS Sciences	
154 Sm	KIR2DL5 (CD158f)	UP-R1	Beckman Coulter	Figure 3A, B
155 Gd	CD27	L128	DVS Sciences	
156 Gd	KIR3DL1 (CD158e)	DX9	R&D	Figure 3A, B*
158 Gd	CD137	4B4-1	DVS Sciences	
159 Tb	NKG2C	134591	R&D	
160 Gd	CD69	FN50	Biolegend	
161 Dy	NKp30	P30-15	Biolegend	
163 Er	CD94	DX22	Biolegend	
165 Ho	CD16	3G8	DVS Sciences	
166 Er	NKG2A	Z199	Beckman Coulter	Figure 3A, B
167 Dy	NKp44	P44-8	Biolegend	
168 Er	IFN-g	B27	DVS Sciences	
169 Tm	CD25	2A3	DVS Sciences	
170 Er	NKp80	239127	R&D	
171 Yb	Granzyme B	GB11	DVS Sciences	
172 Yb	CD57	HCD57	DVS Sciences	Figure 3A, B
174 Yb	NKp46	557911	R&D	
175 Lu	Perforin	B-D48	DVS Sciences	
176 Yb	CD56	NCAM16.2	DVS Sciences	Figure 3A, B
209 Bi	CD11b	ICRF44	DVS Sciences	
	Cisplatinum		Enzo Life Sciences	
	Cell-ID intercalator-Ir		Fluidigm	

Supplemental Table 1. NK cell 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 except KIR3DL1 (*) which was not used for donors 6-8.

Age (y)	WBC (k/μL)	Blast %	Lymph %	NK %	WHO	Cyto / FISH	Immunophenotype	FLT3 / NPM1	Figure	HLA-E % +	HLA-A/B/C % +	Figure Symbol
40	2.2	<1%	78	19	APL	t(15:17) / PML-RARA	CD34-33+117+13+HLA-DR-19-56-	No/No	S Fig 7A	NA	NA	◯
60	1.1	<1%	37	11	APL	t(15:17) / PML-RARA	CD34-33+117+13+HLA-DR-19p+56-	No/No	S Fig 7A	NA	NA	☒
76	48.7	98	NA	NA	AML M1	Normal / Neg	CD34-33-117+13-19-56+	ND/ND	Fig 6A	4	37	NA
47	65.8	70	15	NA	AML t(9;11)	t(9;11) / 11q23 abnl, +8	CD34-33+117+13-HLA-DR+19-56p+	D835/No	Fig 6A	94	100	NA
71	60	NA	NA	NA	AML M2	ND / Neg	CD34+33+117+13+HLA-DR+19-56-	No/Yes	Fig 6A	78	99	NA
47	49.8	45	13	NA	AML M2	Normal / Neg	CD34-33+117+13+HLA-DR+19-56-	D835/Yes	Fig 6A	39	92	NA
77	3.2	13	40	13	AML M6	del20(q11.2) / ND	CD34+33+117+13+HLA-DR+19+56+	ND/ND	Fig 6B	49	91	◯
69	6.1	22	73	2	AML M0	Trisomy 9 / Neg	CD34+33-117+13+HLA-DR+19-56-	ND/ND	S Fig 7A, Fig 6B	42	84	□
65	19.6	3	22	3	AML M4	Normal / Neg	CD34-33+117+13+HLA-	ITD/Yes	S Fig 7A, Fig 6B	67	99	△
62	3.3	4	30	9	AML M2	del9(q13a22) / ND	CD34+33+117+13+HLA-DR+19-56-	No/No	S Fig 7A, Fig 6B	50	88	◇
60	6.7	30	49	4	AML 5A	Complex / 11q23 abnl	CD34-33+117+13-HLA-DR-19-56-	No/ND	S Fig 7A, Fig 6B	41	100	▽
49	12.6	6	38	10	AML MDS	Trisomy 11 / ND	CD34+33+117+13+HLA-DR+19-56-	No/ND	S Fig 7A, Fig 6B	88	96	⬡
69	8.6	<1%	15	10	AML M5b	Comlex / HD	CD34-33-117-13+HLA-DR+19-56+	No/No	Fig 6B	93	99	⊗

Supplemental Table 2. AML patient characteristics for figures where primary AML blasts were utilized. AML blasts from newly-diagnosed patients were used as targets (Figure 6A, B) and/or AML patient PBMC were used in functional assays as effector cells (Figure 6B, Supplemental Figure 7A). APL = Acute Promyelocytic Leukemia, RARA = Retinoic Acid Receptor Alpha, FLT3 = FMS-Like Tyrosine Kinase 3, ITD = Internal Tandem Duplication, NPM1 = nucleophosmin 1. ND = Not Done. NA = Not Applicable. For FLT3, the mutation is listed if positive. For immunophenotype, if a subset of blasts expressed the marker, partial expression is indicated (p). If FISH was performed, the relevant positive findings are listed, or Negative if none of (5q, 7q, +8, PML-RARA, CBFB, ETO/AML) were identified. The fraction of blasts expressing HLA-E or HLA-A/B/C is indicated.

UPN	Age (years)	Gender	Status at Collection	Number of Prior Therapies	Most Recent Prior Therapy	CD138+ HLA-E Percent Positive	Figure Symbol
33718	67	Female	New Diagnosis	None	None	78%	○
67015	86	Male	New Diagnosis	None	None	90%	□
76440	74	Male	New Diagnosis	None	None	94%	△
14955	84	Female	New Diagnosis	None	None	88%	◇
87716 (CD138+)	66	Female	New Diagnosis	None	None	90%	NA
87716 (PBMC)	67	Female	100 Days Post-ASCT	1	4 cycles RVD – Melphalan ASCT	ND	▽

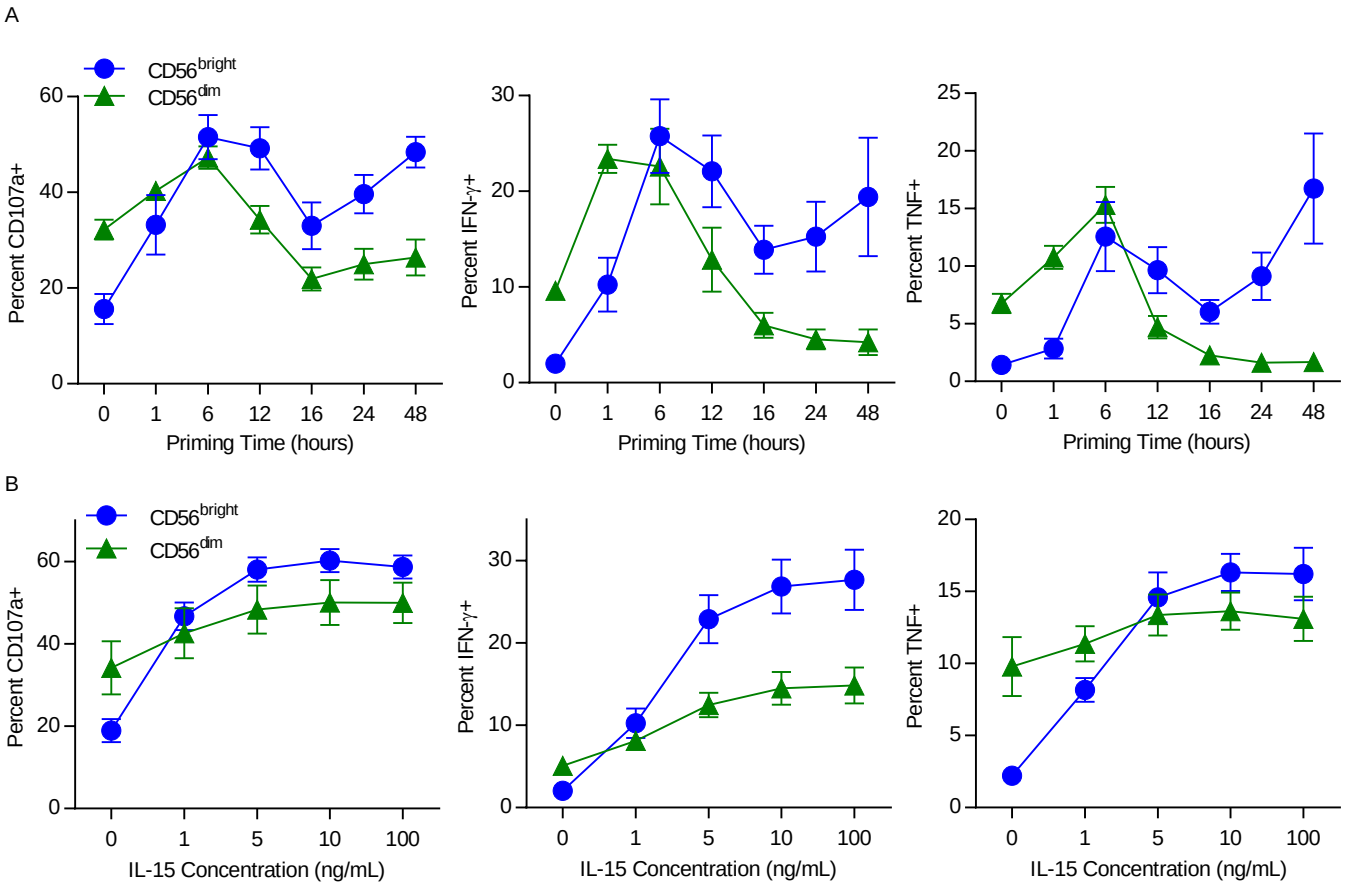
Supplemental Table 3. In vitro multiple myeloma patient characteristics. ASCT = autologous stem cell transplant, RVD = Revlimid, Velcade, Dexamethasone. ND, note done.

Subject ID	Age (years)	Gender	Number of Prior Therapies	Response to Last Prior Therapy	ALT-803 Dose	Patient Group	Figure Symbol
027006	53	Male	7	Refractory	10mcg/kg SQ	A	△
027007	80	Male	2	Refractory	10mcg/kg SQ	A	○
027002	55	Male	4	Refractory	3 mcg/kg IV	B	◇
027003	57	Male	4	Relapsed	3 mcg/kg IV	B	□
027004	70	Female	3	Refractory	6 mcg/kg IV	B	▽

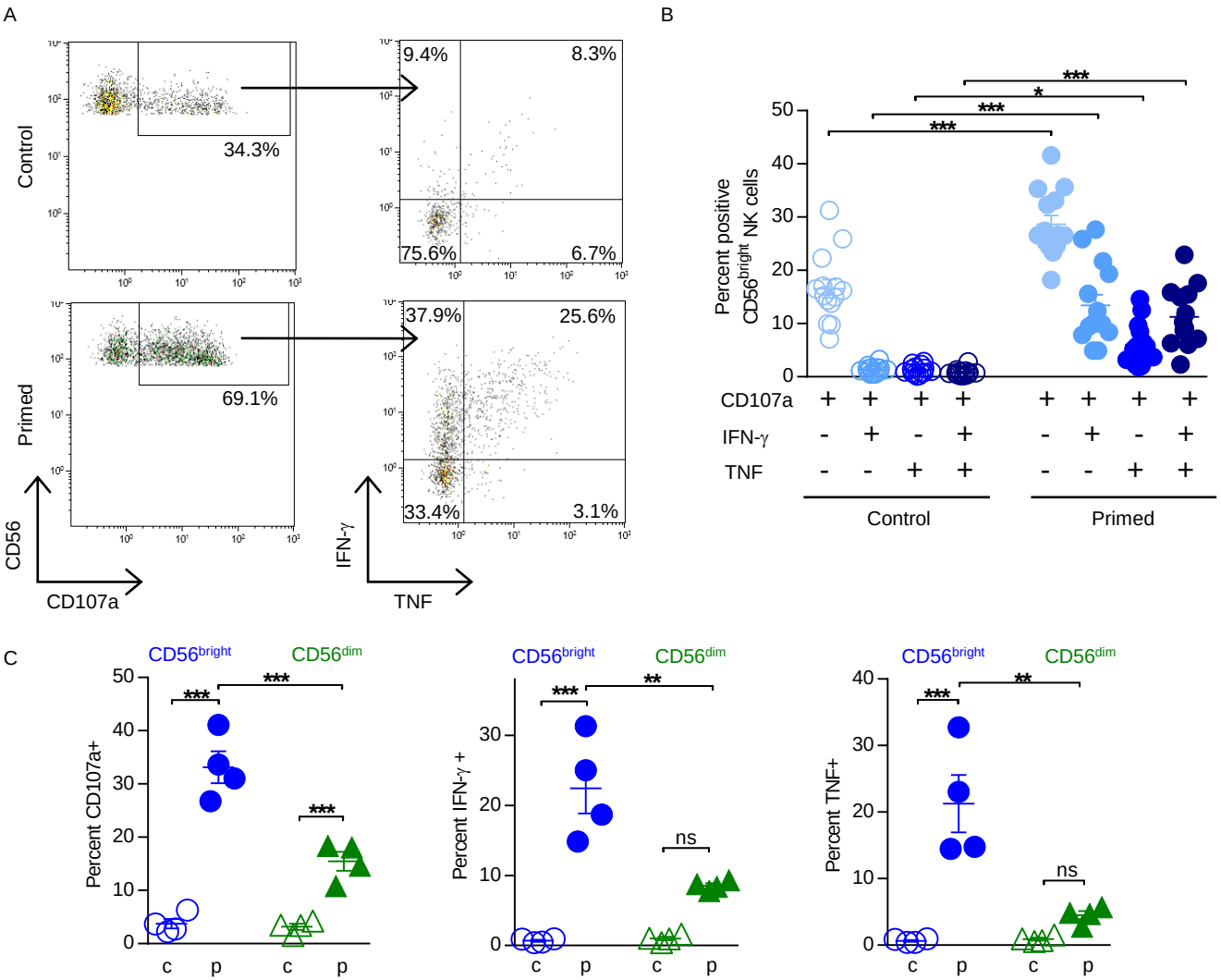
Supplemental Table 4. In vivo multiple myeloma patient characteristics.

Source	Antibody
Beckman Coulter	CD56 (N901), CD3 (UCHT1), CD45 (J.33), CD158b (CH-L), NKG2A (Z199.1), NKp46 (BAB281), CD158i (FES172)
BD Biosciences	CD2 (RPA-2), CD11a (HI111), CD16 (3G8), IFN-γ (B27), CD107a (H4A3), CD57(NK-1), CD69 (FN50), CD158a (HP3E4), CD158e (DX9), CD94 (HP-3D9), CD226 (DX11), CD137 (4-1BB), Perforin (δG9), Ki67, ERK1/2 (pT202,pY204), STAT5 (pY694), Akt (pS473) Purified: CD2 (RPA-2.10)
Biolegend	LFA-1 (m24) Purified: NKG2D (1D11), CD11a/LFA-1 (HI111), NKp30 (P30-15), NKp44 (P44-8), TRAIL (RIK-2), IgG1 control (MG1-45)
Caltag	Granzyme B (GB12)
eBioscience	CD244 (eBioDM244), TNF (MAb11)
Invitrogen	Granzyme B (GB12)
R & D Systems	NKG2C (134591), NKp80 (239127), CD158d (181703), purified TRAIL/TNSF10, goat IgG Control

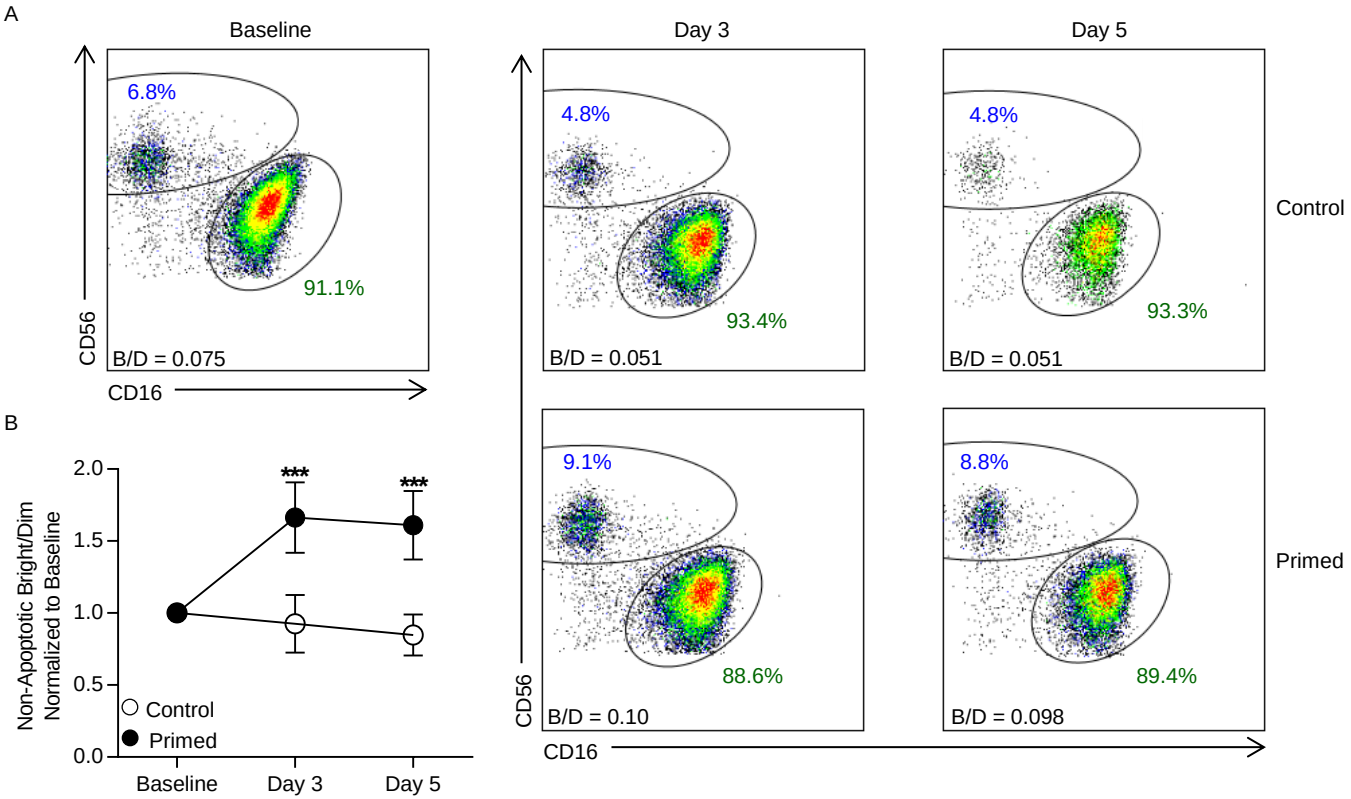
Supplemental Table 5. List of anti-human mAbs. The source of anti-human mAbs used is listed, with the clone indicated in parentheses.



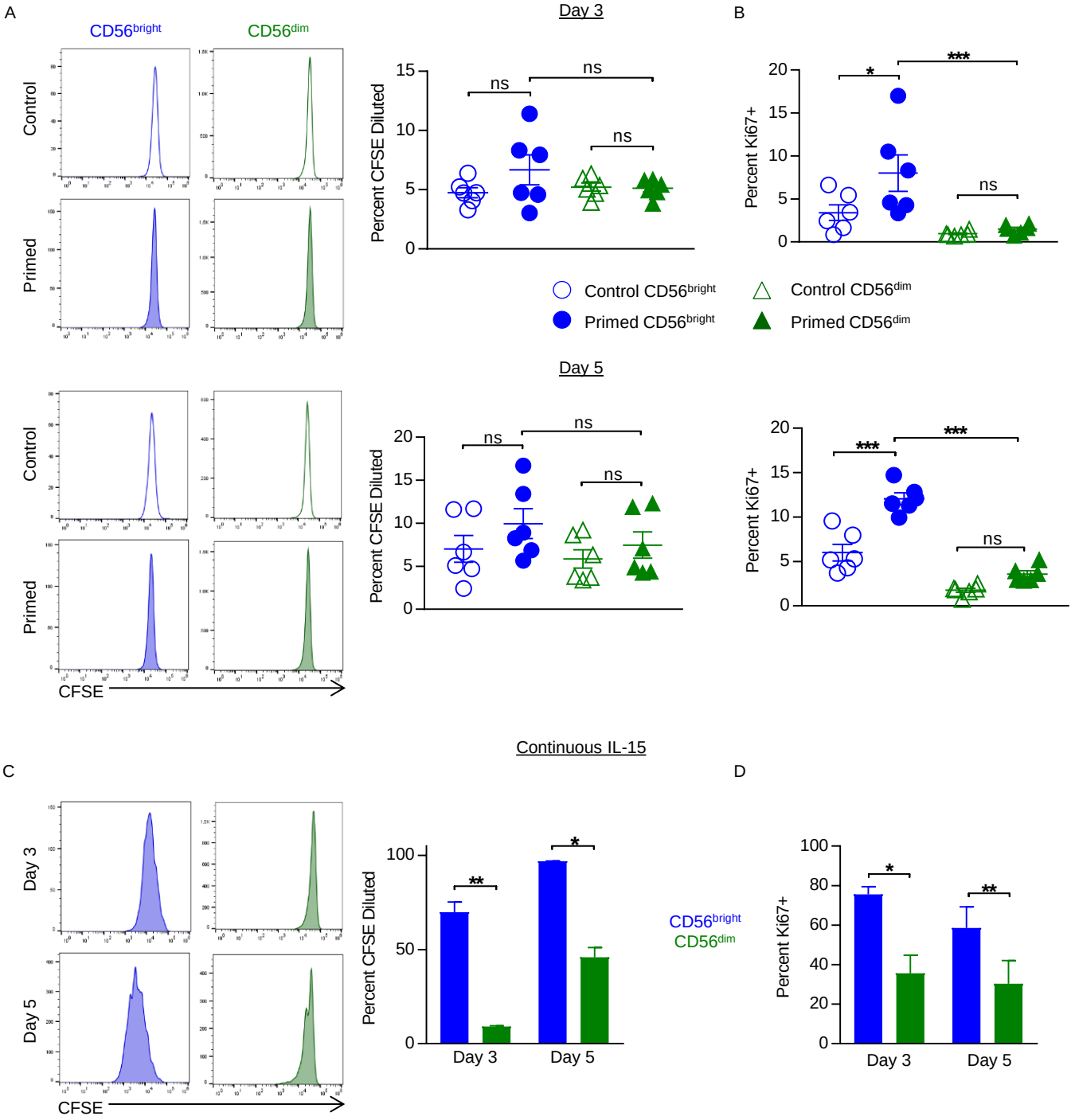
Supplemental Figure 1. IL-15 priming occurs rapidly and at low concentrations of IL-15. Time course and dose response of IL-15 priming of CD56^{bright} NK cells. (A) Purified NK cells were primed with 5ng/mL IL-15 for 0, 1, 6, 12, 16, 24, or 48 hours then washed and incubated with K562 tumor targets for 6 hours at a 5:1 E:T ratio. (B) Purified NK cells were primed with 0, 1, 5, 10, or 100ng/mL IL-15 for 6 hours then washed and incubated with K562 tumor targets for 6 hours at a 5:1 E:T ratio. Line graphs show mean ± SEM percentage of CD107a, IFN-γ, or TNF positive CD56^{bright} or CD56^{dim} NK cells for each priming time or concentration. N=5-6 normal donors, 2-3 independent experiments.



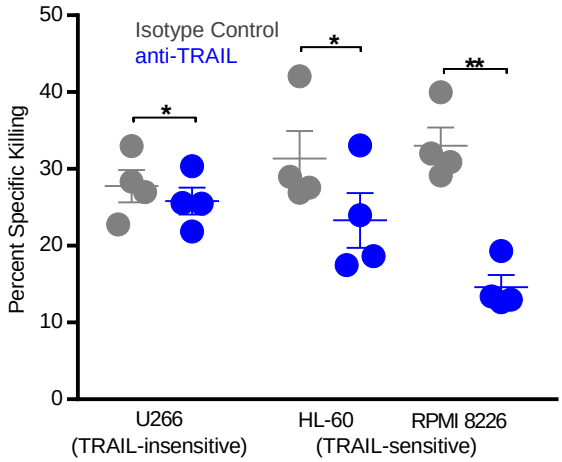
Supplemental Figure 2. IL-15 primes human CD56^{bright} NK cells for broad and polyfunctional responses to myeloid leukemia target cells. Control (c) or IL-15-primed (p) purified NK cells were triggered with K562 tumor targets for 6 hours at a 5:1 E:T ratio and assessed for response polyfunctionality. (A) Representative flow cytometry plots show IFN-γ and TNF expression in CD107a-positive control or primed CD56^{bright} NK cells following triggering with K562 target cells. (B) Summary data show mean ± SEM CD107a and/or IFN-γ and/or TNF percent positive control or primed CD56^{bright} NK cells. N=14 normal donors, 7 independent experiments. (C) Summary data show mean ± SEM CD107a, IFN-γ, or TNF positive control or primed CD56^{bright} and CD56^{dim} NK cells triggered with HL-60 AML targets. Data were compared using a one-way repeated measures ANOVA with Bonferroni's multiple comparisons testing of indicated groups.



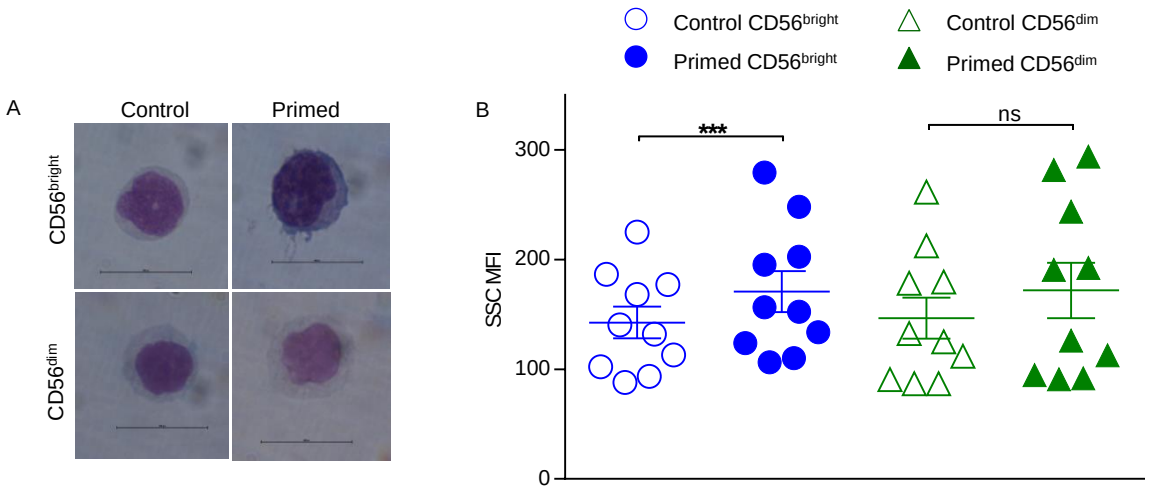
Supplemental Figure 3. IL-15 priming selectively enhances CD56^{bright} NK cell survival under cytokine-starved conditions. Purified NK cells were incubated with media alone (control) or media with 5ng/mL IL-15 (primed) for 12-16h hours. Next, cytokines were washed away and cells were cultured in media alone for 3 or 5 additional days. After 3 or 5 days, cells were harvested and stained for NK cell surface markers as well as Annexin V and activated caspase 3/7 expression (indicating apoptosis). Non-apoptotic cells were Annexin V negative/activated caspase negative. The CD56^{bright}/CD56^{dim} NK cell ratio (B/D) of non-apoptotic NK cells was calculated at the time of purification, as well as at Day 3 or 5 of culture without cytokines. At the time of purification, cells were determined to be >97% viable via AO/PI staining. (A) Flow cytometric plots from a representative donor show CD56^{bright} and CD56^{dim} non-apoptotic control or primed NK cell percentages and ratio (B/D) at baseline or after 3 or 5 days of culture without cytokines. (B) Summary data show CD56^{bright}/CD56^{dim} ratio of control or IL-15-primed NK cells after 3 or 5 days of culture without cytokines, normalized to the ratio at baseline. N = 6 normal donors, 2 independent experiments. Data were compared using a 2-way repeated measures ANOVA, with Bonferroni's multiple comparisons testing.



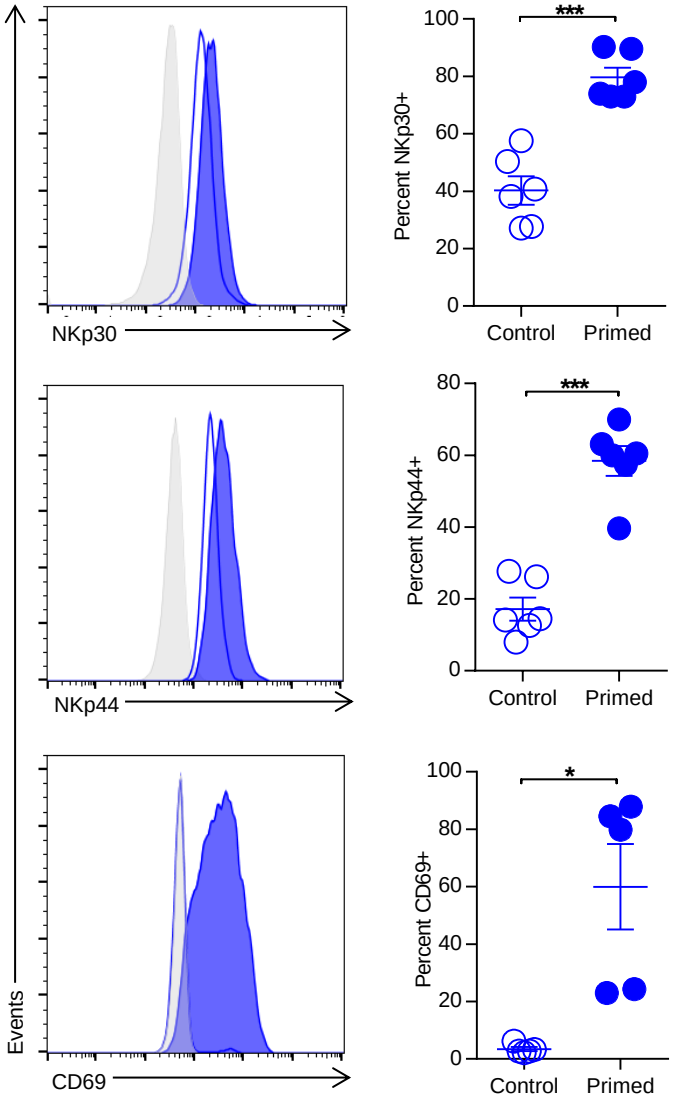
Supplemental Figure 4. IL-15 priming does not result in robust CD56^{bright} or CD56^{dim} NK cell proliferation. Purified, CFSE-labeled (A) or unlabeled NK cells (B) were incubated with 5ng/mL IL-15 (primed) or media alone (control) for 12-16h. Next, cytokines were washed away and cells were incubated in media alone for several days. Proliferation was assessed 3 and 5 days following cytokine wash-out via flow cytometric analysis of CFSE dilution (A) or intracellular Ki67 staining (B). (A) Representative flow histograms of CFSE dilution at Day 3 or 5 in control or primed NK cells. Summary data show percent of control or primed CD56^{bright} and CD56^{dim} NK cells that diluted CFSE (A) or express Ki67 (C) at the different time points. N=6 normal donors, 2 independent experiments. (C, D) A subset of donors were used to confirm that continuous culture in 5ng/mL IL-15 for 3 or 5 days induces proliferation (CFSE dilution) and Ki67 expression in CD56^{bright} and CD56^{dim} NK cells. Data were compared using a one-way repeated measures ANOVA with Bonferroni's multiple comparisons testing of indicated groups (A, B) or a paired student's t-test (C, D).



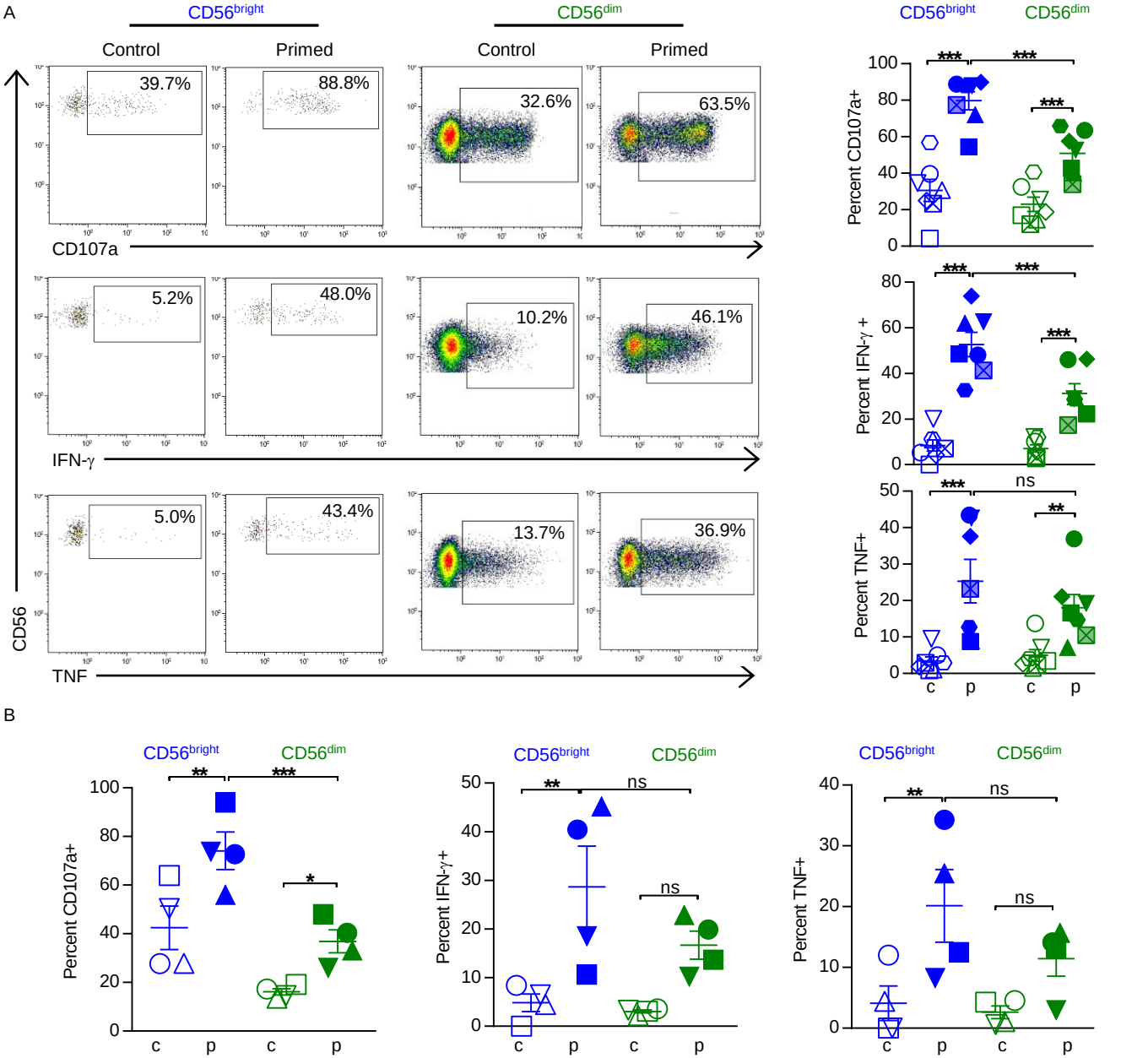
Supplemental Figure 5. TRAIL contributes to IL-15-primed CD56^{bright} NK cell killing of TRAIL-sensitive targets. Flow-sorted, IL-15-primed CD56^{bright} NK cells were triggered at a 5:1 E:T ratio with tumor target cell lines of differing TRAIL sensitivity in a 18 hour flow-based killing assay. Summary data shows mean ± SEM percent specific killing by CD56^{bright} NK cells in the presence of a TRAIL-neutralizing antibody or a isotype control antibody. N=4 normal donors, 2 independent experiments. Data were compared using a paired student's t-test.



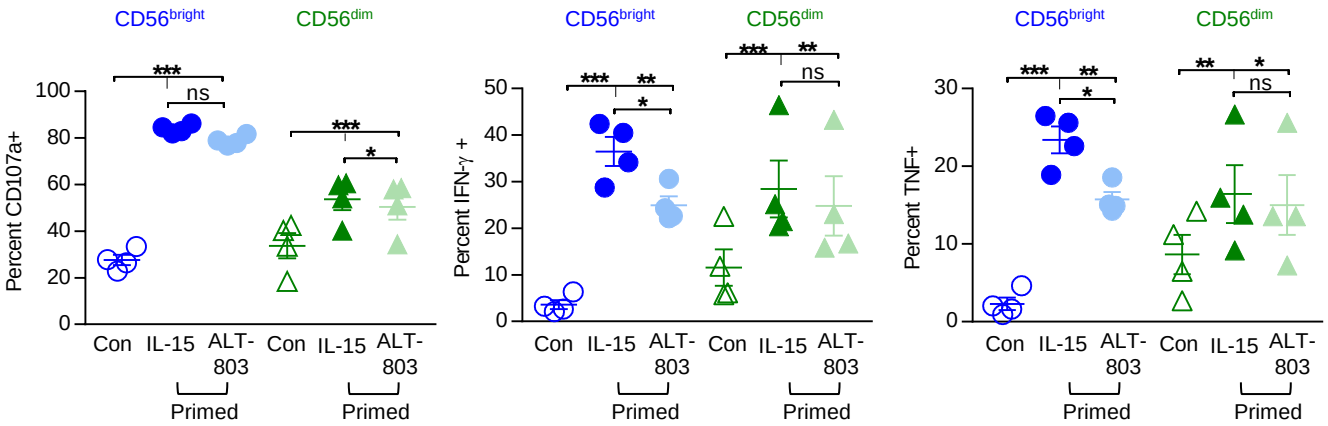
Supplemental Figure 6. IL-15 priming enhances CD56^{bright} NK cell granularity. (A) Flow-sorted, control or primed CD56^{bright} and CD56^{dim} NK cells were Wright/Giemsa stained then evaluated with a light microscope (1000x). Representative cells are shown, demonstrating increased granularity of IL-15-primed CD56^{bright} NK cells. (B) Summary data show mean ± SEM side scatter (indicating cellular complexity/granularity) of control or primed CD56^{bright} and CD56^{dim} NK cells. N = 10 normal donors, 5 independent experiments. Data were compared using a paired student's t-test.



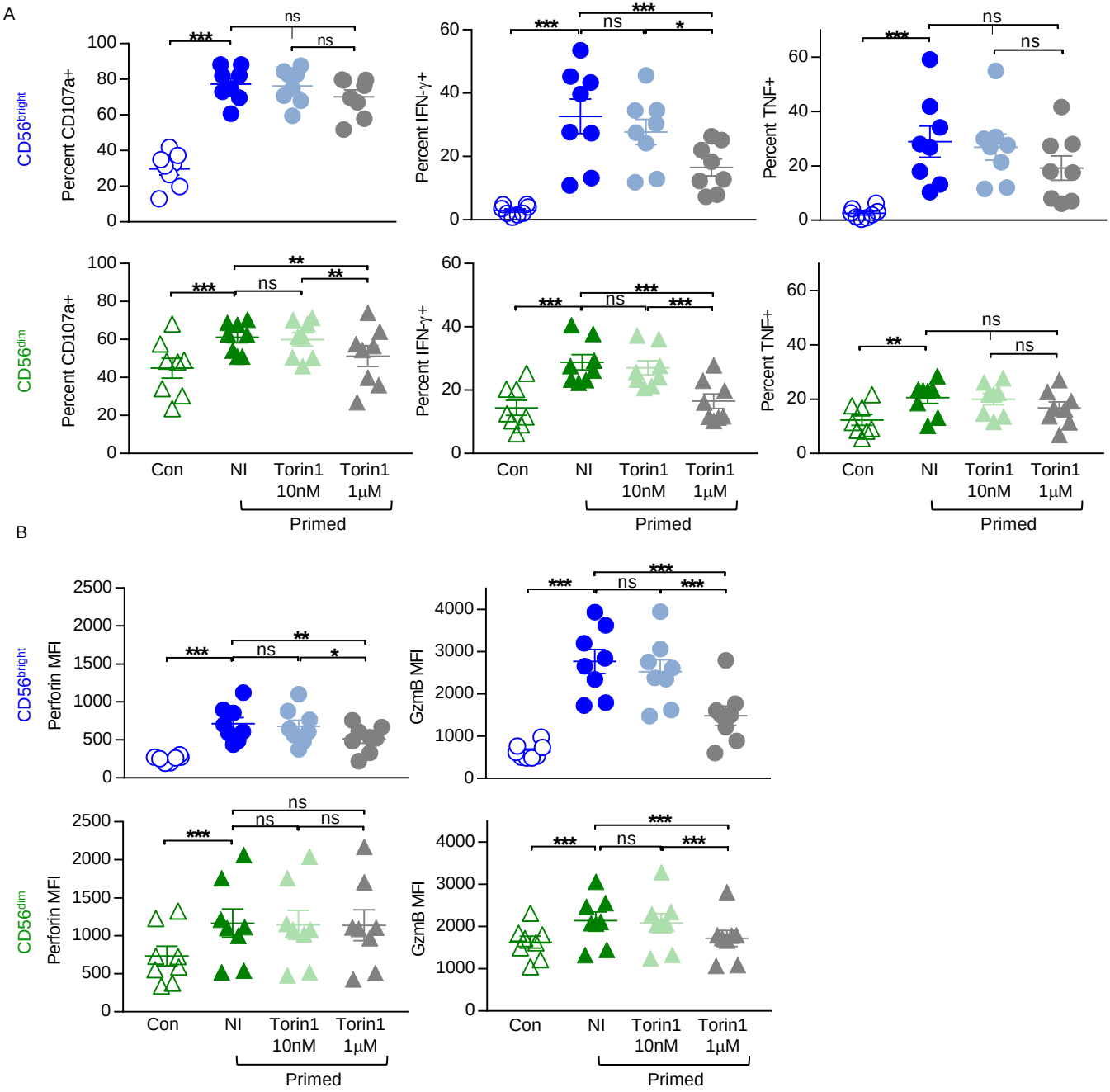
Supplemental Figure 7. IL-15-primed CD56^{bright} NK cells have increased NKp30, NKp44 and CD69 expression. Control or primed purified NK cells were assessed for NKp30, NKp44 and CD69 expression using flow cytometry. Representative histogram plots gated on CD56^{bright} NK cells show per cell expression of these markers, with grey histograms depicting unstained cells. Summary data show mean ± SEM percentage positive CD56^{bright} NK cells. N=5-6 normal donors, 2-3 independent experiments. Data were compared using a paired student's t-test.



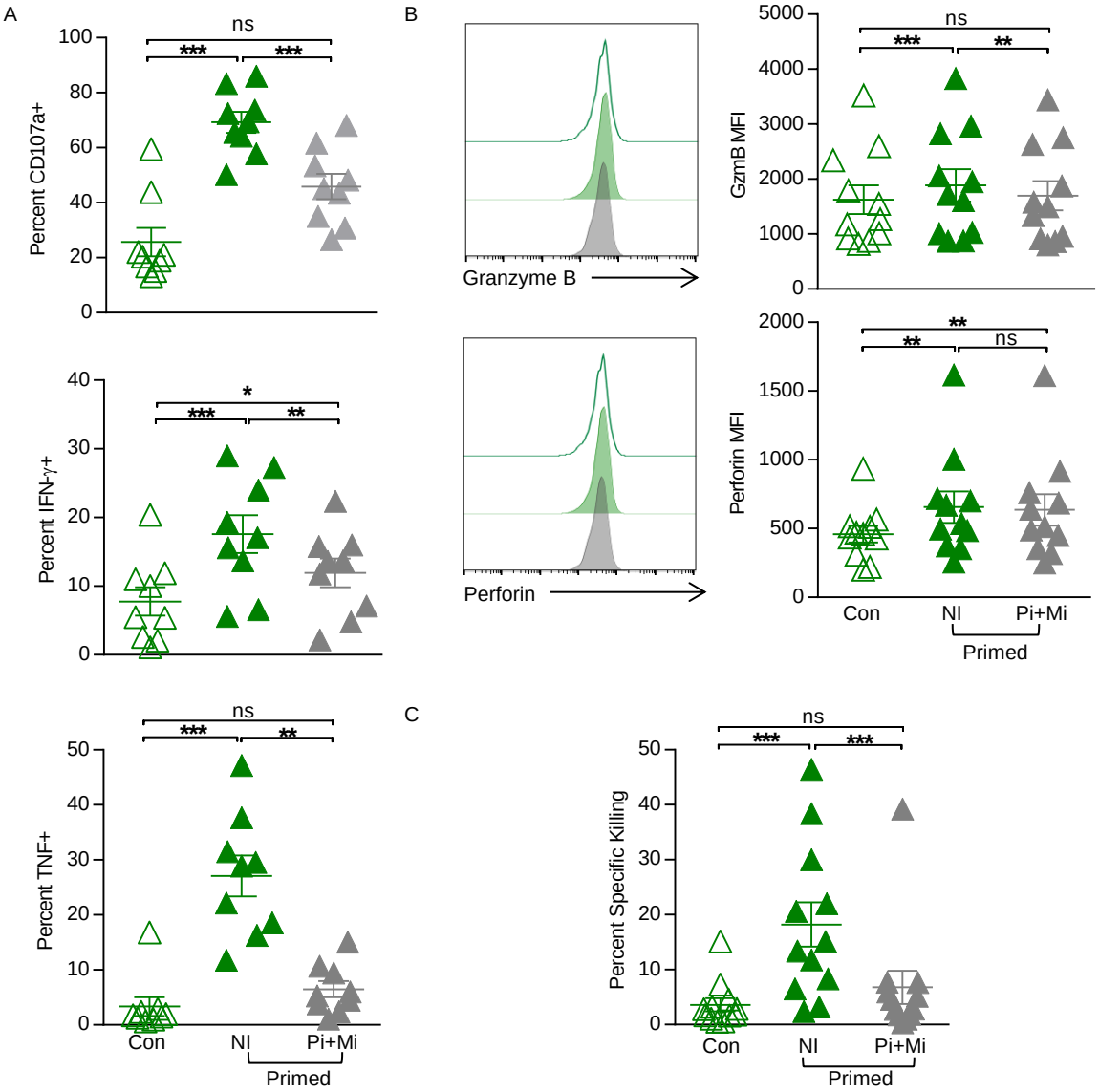
Supplemental Figure 8. IL-15 primes CD56^{bright} NK cells of patients with hematologic malignancies for enhanced functional responses to tumor cell lines. (A) Control (c) or IL-15-primed (p) PBMC from newly diagnosed, untreated AML patients were triggered with K562 leukemia targets for 6 hours at a 5:1 E:T ratio. Bivariate flow plots show the percentage of control or primed CD56^{bright} or CD56^{dim} NK cells from a representative AML patient positive for CD107a, IFN-γ, or TNF. Summary data show mean ± SEM percentage CD107a, IFN-γ, or TNF positive NK cells. N=7 patients, 5 independent experiments. (B) Control or IL-15-primed PBMC from newly-diagnosed, untreated multiple myeloma patients were triggered with U266 myeloma targets for 6 hours at a 5:1 E:T ratio. Summary data show mean ± SEM percentage CD107a, IFN-γ, or TNF positive NK cells. N=4 multiple myeloma patients, 4 independent experiments. Data were compared using a one-way repeated measures ANOVA with Bonferroni's multiple comparisons testing of indicated groups.



Supplemental Figure 9. ALT-803 primes CD56^{bright} NK cells for enhanced anti-tumor responses in vitro. Purified normal donor NK cells were cultured in media alone (control; con), media with 5ng/mL rhIL-15, or media with 17.5ng/mL (equimolar to 5ng/mL rhIL-15) ALT-803 for 12-16 hours. Cells were then washed and co-incubated with K562 tumor targets at a 5:1 E:T ratio and functional responses assessed. Summary data show mean ± SEM percentage CD107a, IFN-γ, or TNF positive CD56^{bright} or CD56^{dim} NK cells. N=4 normal donors, 2 independent experiments. Data were compared using a one-way repeated measures ANOVA with Tukey's multiple comparisons testing



Supplemental Figure 10. Selective mTOR blockade does not impede IL-15 priming of CD56^{bright} or CD56^{dim} NK cell anti-tumor responses. Purified NK cells were incubated with no inhibitor (NI) or Torin1 at 10nM or 1μM for 1 hour prior to 12-16 hours of priming with IL-15. Control (con) cells were incubated in media alone. Next, cells were washed and (A) co-incubated with K562 tumor target cells to assess functional responses or (B) assessed via intracellular flow cytometry for cytotoxic protein expression levels. (A) Summary plots show mean ± SEM percent of CD56^{bright} or CD56^{dim} NK cells positive for CD107a, IFN-γ, or TNF following co-incubation with K562 targets. (B) Summary plots show mean ± SEM perforin or granzyme B MFI in CD56^{bright} and CD56^{dim} NK cells. N=8 normal donors, 4 independent experiments. Data were compared using a one-way repeated measures ANOVA with Tukey's multiple comparisons testing.



Supplemental Figure 11. Combined PI3K/Akt/mTOR and Ras/Raf/MEK/ERK pathway blockade impairs flow-sorted, IL-15-primed CD56^{dim} NK cell anti-tumor responses and cytotoxicity. Flow-sorted CD56^{dim} NK cells were cultured with or without (no inhibitor; NI) small molecule inhibitors of PI3K (Ly294002, Pi) and MEK (PD98059, Mi) for 1 hour prior to 12-16 hours of IL-15 priming. (A) CD56^{dim} NK cells were then washed and incubated with K562 tumor targets for 6 hours at a 5:1 E:T ratio. Summary data show mean ± SEM CD107a, IFN-γ, or TNF percent positive cells. N=9 normal donors, 6 independent experiments. (B) CD56^{dim} NK cells were assessed for granzyme B and perforin expression via intracellular flow cytometry. Representative histogram plots show per cell cytotoxic protein expression. Summary data show mean ± SEM granzyme B or perforin MFI. N=11 normal donors, 6 independent experiments. (C) CD56^{dim} NK cells were incubated with K562 tumor targets (E:T = 2.5:1) in a 4 hour flow-based killing assay. Summary data show mean ± SEM percent specific killing. N=12 normal donors, 8 independent experiments. Data were compared using a one-way repeated measures ANOVA with Tukey's multiple comparisons testing.