Supplemental Table 1

Disease status at time of 3F8 administration

Disease Status	**Missing ≥ 1 ligand** ***n*=153**	**All ligands present** ***n*=89**
First CR/VGPR	69 (45.1%)	37 (41.6%)
Second CR	14 (9.2%)	5 (5.6%)
Refractory	54 (35.3%)	37 (41.5%)
Others*	16 (10.4%)	10 (11.2%)

*patients who received 3F8 as part of induction therapy in combination with chemotherapy

Supplemental Table 3
NK subpopulations with exclusive expression of a single inhibitory KIR

	Single Positive / Total NK cells (%)			KIR Ligands	
Donor no	2DL1	2DL2/L3/S2	3DL1	HLA-Bw	HLA-C
1	2.64	**5.32**	**25.9**	Bw4/Bw6	C1/C1
2	6.53	**8.22**	**0.13**	Bw4/Bw6	C1/C1
3	3.97	**7.01**	**6.24**	Bw4/Bw4	C1/C1
4	**14.8**	14.7	**9.97**	Bw4/Bw6	C2/C2
5	2.91	**29.7**	1.75	Bw6/Bw6	C1/C1
6	**5**	**10.8**	**3.33**	Bw4/Bw6	C1/C2
7	4.13	**17.6**	**3.22**	Bw4/Bw6	C1/C1
8	10.7	**12.2**	**3.16**	Bw4/Bw6	C1/C1
9	3.12	**12.3**	**2.22**	Bw4/Bw6	C1/C1
10	7.43	**8.09**	**7.26**	Bw4/Bw6	C1/C1
11	**9.9**	**5.55**	13	Bw6/Bw6	C1/C2
12	**9.23**	9.94	**6.55**	Bw4/Bw6	C2/C2
13	8.42	**12**	**5.96**	Bw4/Bw6	C1/C1
14	4.3	**13.5**	**5.07**	Bw4/Bw4	C1/C1
15	6.34	**16**	**5.18**	Bw4/Bw4	C1/C1
16	2.48	**30.3**	1.26	Bw6/Bw6	C1/C1
17	12.7	**15.3**	22	Bw6/Bw6	C1/C1
18	**7.57**	**12.1**	**11.2**	Bw4/Bw6	C1/C2
19	**0.52**	**22.9**	**11.8**	Bw4/Bw6	C1/C2
20	**8.79**	**9.07**	**14.1**	Bw4/Bw4	C1/C2

Boldface indicates licensed NK subgroups with single positive staining for a self-HLA-specific inhibitory KIR

Supplemental Table 4

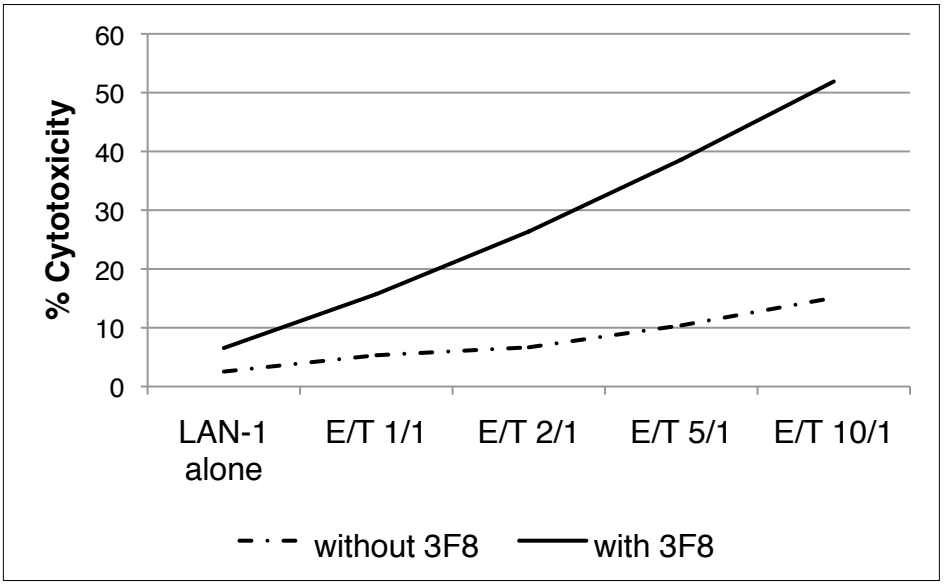
KIR ligands of neuroblastoma cell lines

	HLA KIR ligands	
NB cell line	HLA-Bw	HLA-C
LAN-1	Bw4/Bw6	C1/C1
IMR-32	Bw4*/Bw6	C1/C1
SKNLP	Bw4/Bw4	C1/C2
NB-1691	Bw6/Bw6	C1/C2
BE(1)N	Bw4/Bw4	C1/C2
BE(2)N	Bw4/Bw4	C1/C2

*Bw4 epitope contributed by an HLA-A allele

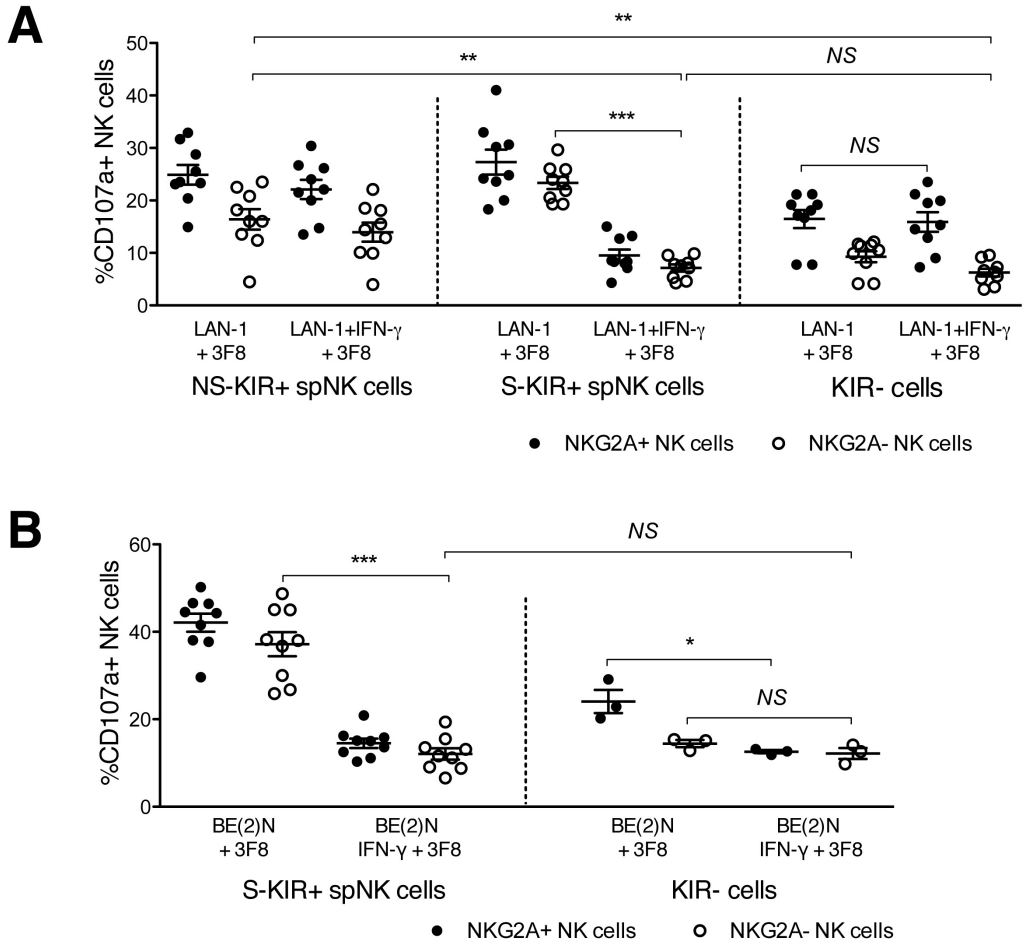
Supplemental Figure 2

3F8 increases NK-mediated cytotoxicity against the NB cell line LAN-1. NK cells were incubated with LAN-1 at different effector/target (E/T) ratios in the presence or absence of 3F8 and compared to spontaneous cytotoxicity among LAN-1 cells alone. Target cytotoxicity was assessed as the percentage of dead cells identified by TO-PRO-3 positive cells within the PKH-26 labeled LAN-1 cell population.



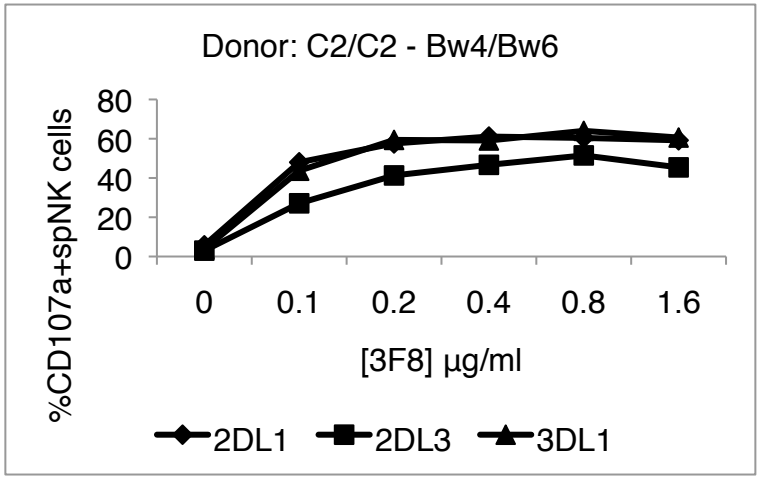
Supplemental Figure 3

NKG2A co-expression increases CD107 response among all NK subsets to NB targets in the presence of 3F8 but does not affect HLA class I-mediated inhibition of S-KIR-positive NK cells. Aggregate CD107 response among NKG2A-positive and NKG2A-negative NK subsets from (**A**) 9 HLA-C1/C1 individuals (donors 7, 8, 9, 10, 13, 14, 15, 16 and 17) to the HLA-C1/C1 target LAN-1 and (**B**) 3 HLA-C1/C2/Bw4 ("all ligands present") individuals (donors 18, 19 and 20) to the HLA-C1/C2/Bw4 target BE(2)N are shown. NKG2A co-expression enhances response of all NK subsets. Following incubation with IFN-γ, upregulation of self-HLA class I expression on the target cell inhibits S-KIR-positive NK cells. IFN-γ induced expression of the non-classical HLA class I HLA-E molecule on BE(2)N inhibits response of NKG2A-bearing NK cells. Standard errors of mean are shown. **P*<0.05, ***P*<0.01, ****P*<0.001, *NS*=non-significant.



Supplemental Figure 4

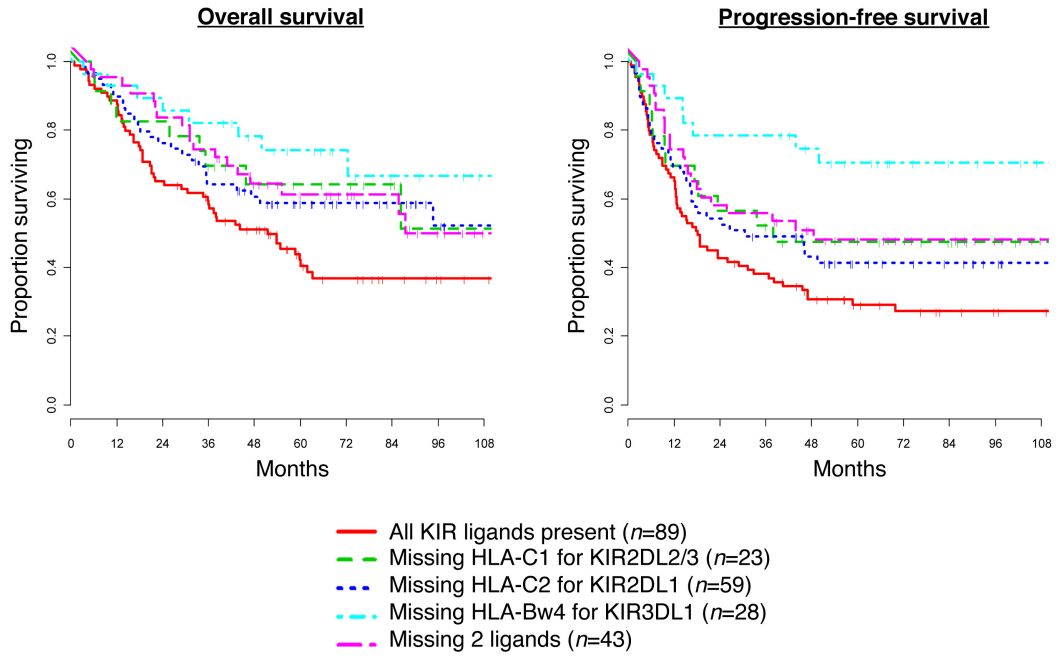
Titration assays for 3F8 demonstrate NK cell activation in a dose-dependent manner. CD107 degranulation in the presence of LAN-1 NB target with 3F8 at different concentrations among S-KIR and NS-KIR spNK cells were analyzed using cells from individual #4. All NK subsets showed an increase CD107a response with increasing doses of 3F8, reaching a plateau at a 3F8 concentration of 0.8 µg/ml.



Supplemental Table 2 KIR and KIR ligands in 20 donors.

	KIR Genotype										HLA KIR ligands	
	Inhibitory KIR#				Activating KIR							
Donor no	2DL1	2DL2	2DL3	3DL1	2DS1	2DS2	2DS3	2DS4	2DS5	3DS1	HLA-Bw	HLA-C
1	+	−	**+**	**+**	−	−	−	+*	−	−	Bw4/Bw6	C1/C1
2	+	**+**	**+**	**+**	+	+	−	+*	+	−	Bw4/Bw6	C1/C1
3	+	**+**	**+**	**+**	−	+	+	+*	−	−	Bw4/Bw4	C1/C1
4	**+**	−	+	**+**	−	−	−	+*	−	−	Bw4/Bw6	C2/C2
5	+	**+**	**+**	+	+	+	+	+	+	+	Bw6/Bw6	C1/C1
6	**+**	**+**	**+**	**+**	+	+	+	+	−	+	Bw4/Bw6	C1/C2
7	+	**+**	**+**	**+**	−	+	−	+	−	−	Bw4/Bw6	C1/C1
8	+	−	**+**	**+**	+	−	−	+	+	−	Bw4/Bw6	C1/C1
9	+	**+**	**+**	**+**	+	+	−	+	+	+	Bw4/Bw6	C1/C1
10	+	**+**	**+**	**+**	−	+	+	+	−	−	Bw4/Bw6	C1/C1
11	**+**	−	**+**	+	−	−	−	+	−	−	Bw6/Bw6	C1/C2
12	**+**	+	+	**+**	−	+	−	+	+	−	Bw4/Bw6	C2/C2
13	+	−	**+**	**+**	−	−	−	+	−	−	Bw4/Bw6	C1/C1
14	+	**+**	**+**	**+**	−	+	−	+	−	−	Bw4/Bw4	C1/C1
15	+	**+**	**+**	**+**	−	+	−	+	−	−	Bw4/Bw4	C1/C1
16	+	**+**	**+**	+	−	+	−	+	−	−	Bw6/Bw6	C1/C1
17	+	−	**+**	+	−	−	−	+*	−	−	Bw6/Bw6	C1/C1
18	**+**	−	**+**	**+**	−	−	−	+	−	−	Bw4/Bw6	C1/C2
19	**+**	**+**	−	**+**	−	+	+	+*	−	−	Bw4/Bw6	C1/C2
20	**+**	−	**+**	**+**	−	−	−	+	−	−	Bw4/Bw4	C1/C2

bold-face indicates “licensing” or self-HLA-specific KIR
*denotes the presence of allele 2DS4 *00

Supplemental Figure 1

Lack of any one KIR ligand for individual inhibitory KIR is associated with improved overall survival and progression-free survival in patients with high-risk NB treated with 3F8. Patients lacking HLA-Bw4 ligand for KIR3DL1 have the highest OS (HR 0.38 (95% CI, 0.18-0.79), *P*=0.01) and PFS (HR 0.28 (95% CI, 0.13-0.58), *P*=0.001) compared to patients with all ligands present. Patients lacking HLA-C1 ligand for KIR2DL2/3 have more favorable hazard ratios for OS (HR 0.58 (95% CI, 0.28-1.17), *P*=0.128) and PFS (HR 0.63 (95% CI 0.34-1.17), *P*=0.141); and patients lacking HLA-C2 ligand for KIR2DL1 have more favorable hazard ratios for OS (HR 0.63 (95% CI, 0.39-1.02), *P*=0.06) and PFS (HR 0.73 (95% CI 0.48-1.1), *P*=0.135), although not statistically significant.