Supporting Information

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SI Methods

MTOC Polarization Experiments. T2 cells were incubated overnight at 26 °C in the absence or presence of 10 μM peptide [VAP-FA, VAP-DA, or control peptide (SYDDAVYKL)]. DiO (1 μL) was added to 1×10^6 NKL-2DL3 cells in 1 mL RPMI and incubated for 5 min at 37 °C, then washed and resuspended in 1 mL R10 medium and incubated overnight. NKL-KIR2DL3 and T2 cells were coincubated at an E:T ratio of 1:1 for 15 min at 37 °C. Cells were pelleted, resuspended in 100 μL of PBS solution, and fixed with chilled methanol for 5 min at 20 °C, washed twice in PBS solution. To stain the cells, they were blocked with 5% BSA for 15 min at room temperature, then stained with mouse anti–γ-tubulin (Sigma-Aldrich) at 4 °C overnight, followed by goat anti-mouse Alexa 633 (Invitrogen). Cells were imaged using a Leica SP5 resonance scanning microscope (Leica Microsystems).

Antibodies. The following mAbs were used in flow cytometry: anti–CD56-PECy7, anti–CD3-APCCy7, anti–CD158b-FITC (CH-L), anti–CD107a-PE, anti–HLA-A2 (BB7.2), anti–mouse IgG1-PE (all from BD Pharmingen), and anti–CD158a-APC (EB6; Beckman Coulter). For microscopy studies, anti-CD158b mAb (GL183; Abcam) and Alexa Fluor–488 goat anti-mouse IgG were used (Invitrogen). For Western blotting, the following antibodies were used: phospho-Vav1 (Y174; Abcam), Vav1 (New England Biolabs), and HRP-conjugated goat anti-mouse IgG (Millipore).

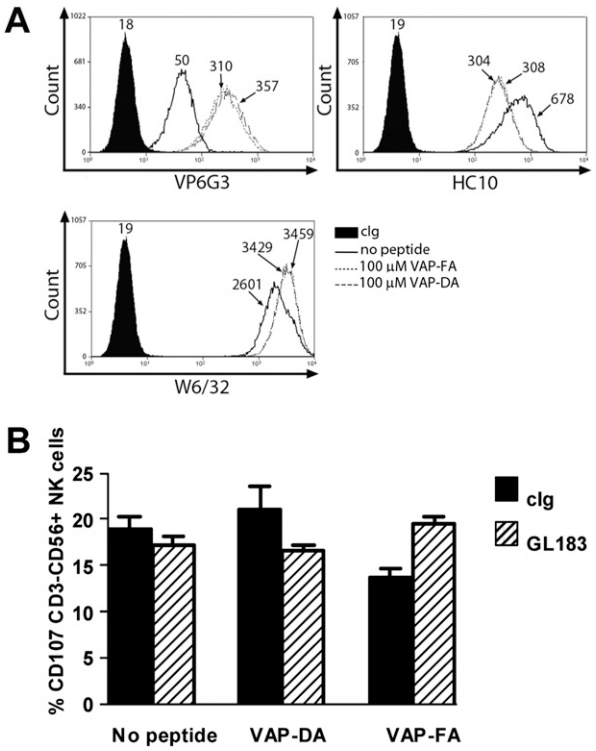

Fig. S1. HLA expression by T2 cells and inhibition of NK cells. (*A*) Flow cytometry histogram plots showing MHC class I expression as determined by VP6G3, HC10, and W6/32 following overnight incubation at 26 °C either in the absence of peptide, or with the peptide variants VAPWNSFAL (VAP-FA) or VAPWNSFAL (VAP-DA) at 100 μM. Mean fluorescence intensity (MFI) values are shown. (*B*) CD107a degranulation of $CD3^-CD56^+$ NK cells incubated with T2 cells in the presence or absence of the indicated peptides and the anti-KIR2DL2/3 antibody GL183 (black bars) or a control Ab (hatched bars). IL-15–treated PBMCs were incubated with 25 μg/mL GL183 (Abcam) or 25 μg/mL IgG1 control antibody (AbD Serotec) for 30 min at room temperature. Degranulation of $CD3^-CD56^+$ NK cells was assessed using the previously described protocol with the exception that the following antibodies were used: anti–CD107a-Alexa Fluor 647, anti–CD3 eFluor 450 (both from eBioscience), and anti–CD56-PECy7 (BD Pharmingen).

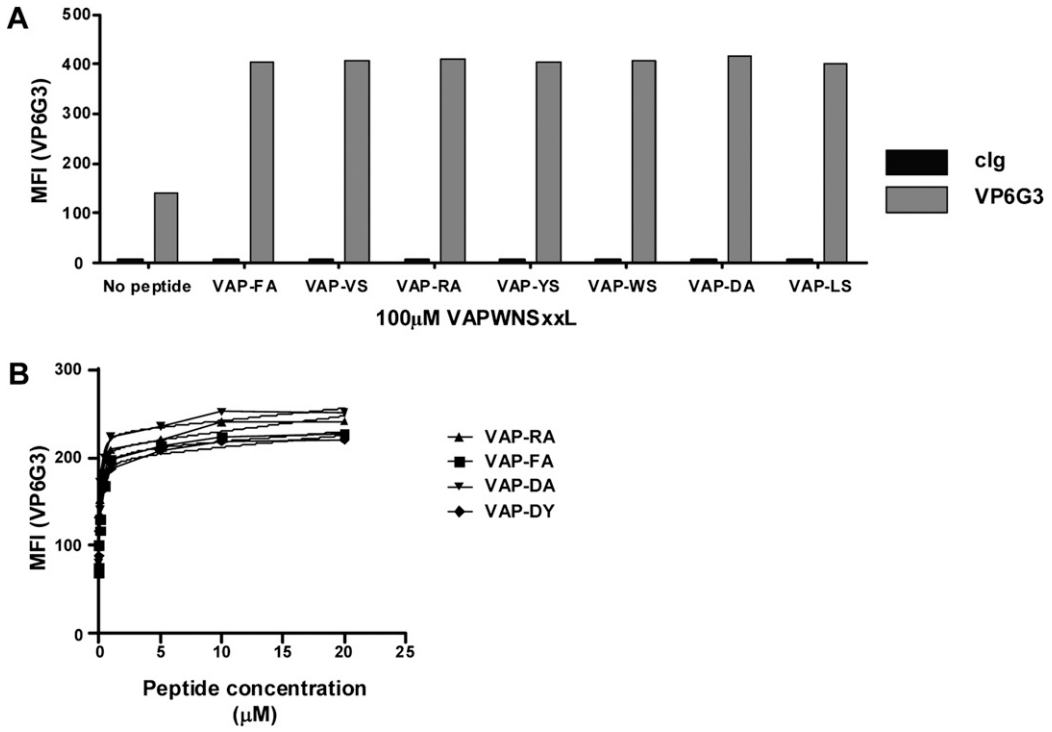

Fig. S2. Stabilization of HLA-Cw*0102 by the peptide variants of VAPWNSLSL. (*A*) T2 cells were incubated with the indicated peptides at a concentration of 100 μM and then stained with the VP6G3 antibody (hatched bars) or control Ig (black bars). The MFI is shown for the different peptides. (*B*) HLA-Cw*0102 expression on T2 cells by the indicated peptides at a concentration up to 20 μM as determined by VP6G3.

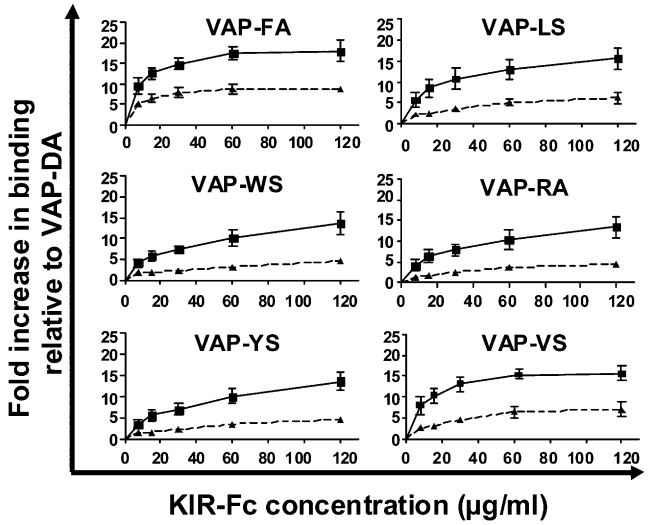

Fig. S3. Binding of KIR-Fc fusion constructs to peptide-loaded T2 cells. T2 cells loaded with 100 μM peptide were stained with increasing concentrations of KIR2DL2-Fc (solid line) or KIR2DL3-Fc (dashed line) and analyzed by flow cytometry. The fold increase in binding relative to VAP-DA and the means ± SEM of three independent experiments are shown.

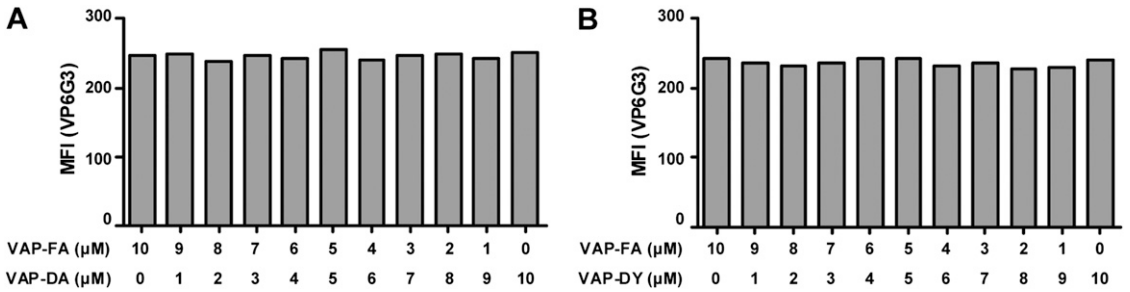

Fig. S4. Stabilization of HLA-Cw*0102 by peptide mixes. HLA-Cw*0102 stabilization of T2 cells incubated with VAP-FA/VAP-DA (*A*) and VAP-FA/VAP-DY (*B*) peptide mixes to a final concentration of 10 μM detected by VP6G3 antibody. The concentration of each peptide in the mix is shown.

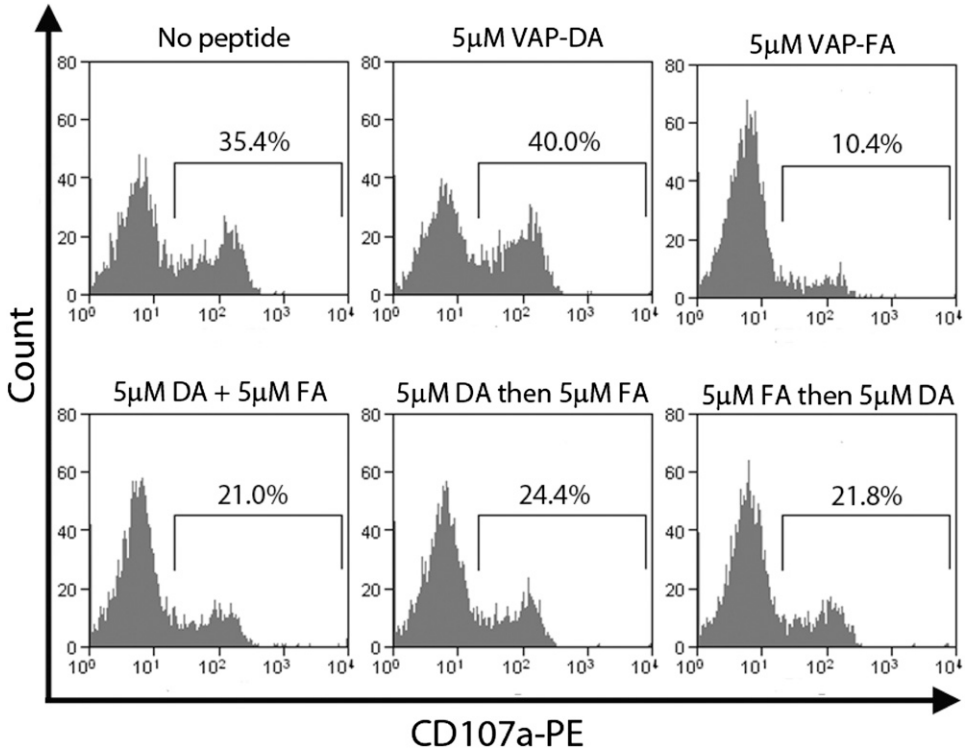

Fig. S5. The effect of serial addition of inhibition of CD158b$^+$ NK cells. T2 cells were incubated without peptide, with 5 μM of VAP-DA, with 5 μM of VAP-FA, or with both peptides at 26 °C overnight. For peptide mix, T2 cells were either loaded with VAP-DA and VAP-FA (DA+FA) simultaneously, loaded with VAP-DA for 1 h then with VAP-FA (DA then FA), or loaded with VAP-FA for 1 h then with VAP-DA (FA then DA). The following day, a degranulation assay was performed as previously using anti–CD107a-PE, anti–CD56-PECy7, anti–CD158b-FITC (all from BD Pharmingen) and anti–CD3-Pacific blue (eBioscience). Samples were analyzed by flow cytometry and one representative experiment of two is shown.

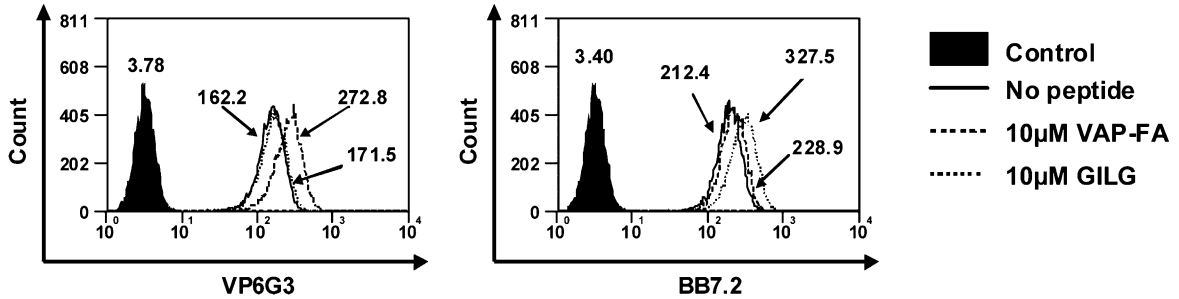

Fig. S6. The HLA-A2–binding peptide GILGFVFTL does not up-regulate HLA-Cw*0102. T2 cells were incubated with an HLA-Cw*0102–binding peptide VAPWNSFAL (VAP-FA) or an HLA-A*0201–binding peptide GILG. Stabilization of HLA-Cw*0102 as detected by VP6G3 (*Left*) and stabilization of HLA-A*0201 as detected by BB7.2 (*Right*) are shown. The MFIs of staining are indicated.

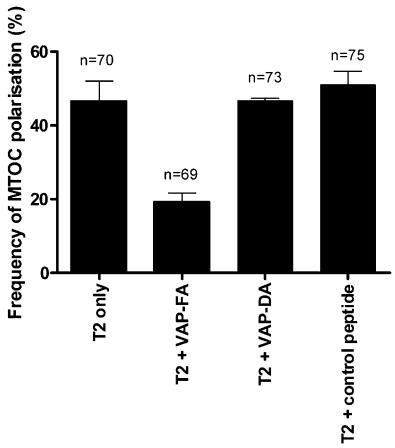

Fig. S7. Polarization of microtubule organizing center (MTOC) in response to T2 cells and peptides. T2 cells were incubated overnight in the presence or absence of the indicated peptide or a control peptide (SYDDAVYKL). DiO stained NKL-2DL3 cells were added and conjugates were allowed to form. The polarization of the MTOC in NKL-2DL3 cells was noted and the frequency with which it was polarized toward the synapse with a T2 cell was counted, and expressed as a percentage of conjugates formed. "*n*" indicates the total number of conjugates counted for each condition and is derived from three independent experiments.

Table S1. KIR-Fc binding to peptide loaded T2 cells

P7/P8	2DL2	2DL3	2DS2	VP6G3
FA	11.9	8.4	1.0	2.3
FS	11.4	5.0	1.1	2.1
LP	8.7	3.1	1.0	2.2
IS	8.4	4.5	1.0	2.8
LS	8.3	3.7	1.0	2.6
LA	8.3	2.1	1.0	2.2
RS	6.6	3.2	1.0	2.5
IA	6.1	3.0	0.9	2.0
LS	5.6	2.1	1.0	2.3
YS	4.8	1.6	1.1	2.1
VS	4.8	1.3	1.0	1.8
RA	4.6	1.0	1.0	2.0
WS	4.6	1.6	1.0	2.1
WA	3.3	1.6	1.1	1.7
KS	3.0	1.9	1.1	3.0
HS	2.7	1.4	1.0	2.4
VA	2.5	1.3	1.1	1.0
MS	2.3	1.1	1.0	1.9
MA	2.3	1.5	1.0	2.2
KA	2.2	1.5	1.1	2.5
YA	2.2	1.4	1.1	2.0
SS	2.1	1.3	1.1	1.8
HA	2.0	1.2	1.0	2.5
GS	1.8	1.4	1.0	3.0
TS	1.6	1.1	1.1	2.4
GA	1.5	0.9	1.0	2.7
SA	1.5	1.2	1.1	2.5
LT	1.5	1.1	1.0	2.1
LV	1.4	1.1	1.0	2.1
LG	1.4	0.9	0.9	1.9
QS	1.3	1.1	1.0	2.5
QA	1.3	1.0	1.0	2.8
AS	1.3	1.0	1.1	2.4
TA	1.3	1.4	1.0	1.9
LH	1.2	1.0	1.1	2.0
AA	1.2	1.2	1.0	2.7
PS	1.2	1.1	1.0	2.2
LK	1.2	1.0	0.9	1.9
LE	1.2	1.0	1.0	2.0
PA	1.2	1.1	1.1	2.5
ES	1.2	1.1	1.0	2.5
LL	1.2	1.0	1.1	2.0
NS	1.2	1.3	1.1	2.4
LR	1.2	1.1	0.9	1.6
LN	1.2	1.0	1.0	1.9
LI	1.1	1.0	1.0	2.2
LD	1.1	1.0	1.0	2.1
DS	1.1	1.2	1.1	2.9
CS	1.1	1.0	1.0	2.6
LM	1.1	1.2	1.0	2.3
DA	1.1	0.9	1.0	2.8
NA	1.1	1.0	1.0	2.3
LQ	1.1	1.0	1.0	2.3
LF	1.1	1.0	1.0	2.1
EA	1.1	0.9	1.0	2.7
LC	1.1	1.0	1.1	2.1
CA	1.1	1.0	1.0	2.5
LY	1.0	1.0	0.9	2.0
LW	1.0	1.0	1.0	2.0

Screening assay of KIR2DL2-Fc, KIR2DL3-Fc, and KIR2DS2-Fc binding to HLA-Cw*0102 stabilized with derivatives of VAPWNSLSL and differing at positions 7 and 8. Shown are the values for the fold increase in binding of the KIR-Fc proteins and VP6G3 in comparison with no exogenous peptide.

Table S2. KIR2DL2/KIR2DL3/KIR2DS2 and HLA-C genotypes of 11 donors and the degranulation levels observed in the CD107a assays

KIR type	HLA-C type	No peptide, % CD107a on CD158b+ NK cells	VAP-DA, % CD107a on CD158b+ NK cells	VAP-FA, % CD107a on CD158b+ NK cells	CD107a on CD158b+ NK cells at 50% inhibition, %	FA concentration at 50% inhibition
KIR2DL2/KIR2DS2	C2C2	22.4 ± 1.6	22.7 ± 0.8	6.0 ± 0.5	11.3 ± 0.4	2.7×10^{-7}
KIR2DL2/KIR2DL3/ KIR2DS2	C1C2	36.3 ± 2.2	35.3 ± 3.1	9.5 ± 1.9	17.7 ± 1.5	1.75×10^{-7}
KIR2DL2/KIR2DL3/ KIR2DS2	C1C2	39.7 ± 2.6	39.3 ± 2.0	10.8 ± 2.7	19.7 ± 1.0	2.89×10^{-7}
KIR2DL2/KIR2DL3/ KIR2DS2	C1C2	34.0 ± 2.9	34.0 ± 3.5	9.1 ± 1.5	17.0 ± 1.7	3.35×10^{-7}
KIR2DL2/KIR2DL3/ KIR2DS2	C1C1	40.1 ± 2.9	39.8 ± 0.9	9.4 ± 1.0	19.9 ± 0.4	3.26×10^{-7}
KIR2DL3	C1C1	38.9 ± 3.0	38.7 ± 3.4	11.0 ± 2.0	19.4 ± 1.7	1×10^{-7}
KIR2DL3	C1C1	40.6 ± 2.9	40.0 ± 3.4	12.3 ± 1.2	20.0 ± 1.7	3.1×10^{-7}
KIR2DL3	C1C2	27.3 ± 1.9	27.3 ± 1.3	8.7 ± 1.7	13.6 ± 0.6	3.62×10^{-7}
KIR2DL3	C1C2	31.0 ± 2.7	31.7 ± 1.3	8.4 ± 0.6	15.5 ± 0.7	2.79×10^{-7}
KIR2DL3	C2C2	39.1 ± 1.1	39.1 ± 1.8	10.8 ± 1.4	19.60.9	2×10^{-7}

The KIR and HLA genotypes of the 11 donors in Fig. 1*F* in the main text and the associated levels of CD107a expression following incubation with T2 cells either unpulsed or pulsed with VAP-DA (10 μM) or VAP-FA (10 μM). Also shown are the CD107a expression levels when 50% of responsive $CD158b^+$ NK cells were inhibited and the calculated VAP-FA concentration at that level of inhibition.