Supplementary Information

The molecular basis of how buried human leukocyte antigen polymorphism modulates Natural Killer Cell function

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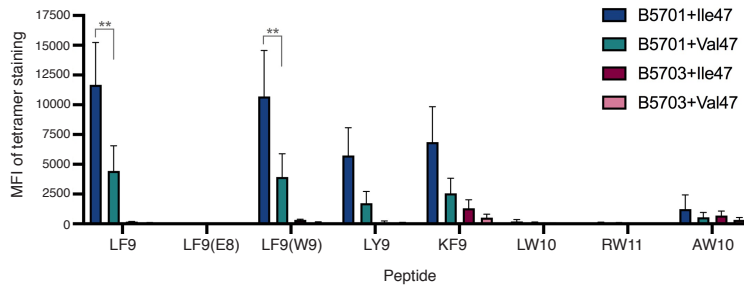2 supplementary figures

2 supplementary tables

Supplementary Figure 1
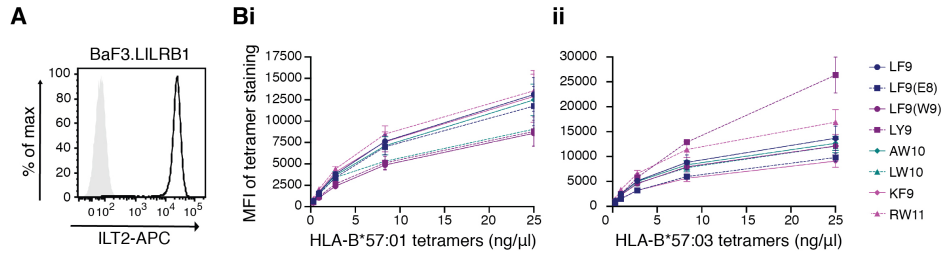
Supplementary Figure 1. *Validation of HLA-B57 tetramers staining on LILRB1-expressing cells.*
A. LILRB1 was detected on BaF3.LILRB1 cells, staining with ILT2-APC. *B*. HLA-B*57:01 *(i)* and
HLA-B*57:03 *(ii)* tetramers refolded with each peptide (LF9, LF9(E8), LF9(W9), LY9), AW10,
LW10, KF9 or RW11) were diluted three-fold and used to stain LILRB1-expressing Ba/F3 cells. The
MFI of tetramer staining was plotted, with the average of two experiments depicted with the SEM.

Supplementary Figure 2: *HLA-B*57:01 tetramers bind KIR3DL1^{Ile47+} NK cells more robustly than KIR3DL1^{Val47+} NK cells*. NK cells sorted and expanded from four KIR3DL1$^+$ donors were stained with PE-conjugated HLA-B*57:01 and HLA-B*57:03 tetramers loaded with eight different peptides (at 25ng/µl). The MFI of tetramer binding to the NK cells was stratified by HLA-B57 tetramer and KIR3DL1 subtype, where donors DD and DK possess KIR3DL1 allotypes with an isoleucine at residue 47, and donors DE and DJ a valine. Binding of HLA-B57 tetramers to KIR3DL1$^+$ NK cells (two independent experiments with two donors each; SEM depicted) was compared using a two way ANOVA with Tukey's multiple comparison test *(HLA-B*57:01/LF9 Ile47 vs. Val47: **p=0.0026; HLA-B*57:01/LF9(W9) Ile47 vs Val47 **p=0.0050).*

Supplementary Table 1: *Peptides refolded with HLA-B*57:01 and HLA-B*57:03*

Name	Sequence	Source	Notes
LF9	LSSPVTKSF	Immunoglobulin kappa constant (sp|P01834|IGKC_HUMAN)	Facilitates KIR3DL1 binding (12)
LF9 (E8)	LSSPVTK<u>E</u>F		E8 abrogates KIR3DL1 binding (12)
LF9 (W9)	LSSPVTKS<u>W</u>		"Flipped" C-2 (2)
LY9	LTVQVARVY	UPF0769 protein C21orf59 (sp|P57076|CU059_HUMAN)	"Flipped" C-2 (2)
AW10	ASLNLPAVSW	Catenin alpha-1 (sp|P35221|CTNA1_HUMAN)	
KF9	KSFDFHFGF	Protein unc-119 homolog A (sp|Q13432|U119A_HUMAN)	
LW10	LALSPVPSHW	B-cell antigen receptor complex-associated protein beta chain (sp|P40259|CD79B_HUMAN)	
RW11	RVLPPSHRVTW	Proto-oncogene vav (sp|P15498|VAV_HUMAN)	

Supplementary Table 2: *Data collection and refinement statistics*

Data collection statistics	KIR3DL1*001-HLA-B*57:03/LSSPVTKSF	HLA-B*57:03/ LSSPVTKSF	HLA-B*57:01/ ASLNLPAVSW	HLA-B*57:03/ ASLNLPAVSW
Space group	$P1$	$P\,2_1\,2_1\,2_1$	$P\,2_1\,2_1\,2_1$	$P\,2_1\,2_1\,2_1$
Cell Dimensions	a = 51.8, b =60.8, c =65.4, α= 96.6 , β= 97.4, γ= 109.2	a = 50.9, b=82.2, c=110.8, α=90.0, β= 90.0, γ= 90.0	a = 50.8, b=82.0, c=110.5, α=90.0, β= 90.0, γ= 90.0	a = 51.0, b=82.3, c=110.9, α=90.0, β= 90.0, γ= 90.0
Resolution Å	50 – 1.98 (2.08 – 1.98)	46.27– 1.60 (1.63 – 1.60)	46.13 – 1.27 (1.29 – 1.27)	46.32 - 1.30 (1.32 – 1.30)
Total no. observations	221369 (31894)	458505 (23157)	821731 (35648)	647166 (29099)
No. unique observations	50170 (7050)	62191 (3036)	121661 (5564)	115263 (5638)
Multiplicity	4.4 (4.5)	7.4 (7.6)	6.8 (6.4)	5.6 (5.2)
Data completeness (%)	97.3 (93.7)	100.0 (100.0)	99.3 (93.0)	100.0 (100.0)
$1/\sigma_I$	10.3 (2.5)	11.5 (2.6)	15.1 (2.2)	10.4 (2.1)
R_{merge}[1]	0.076 (0.483)	0.127 (1.038)	0.049 (0.693)	0.079 (1.049)
Refinement statistics				
Non-hydrogen atoms				
Protein	5406	3958	3220	3228
Sugar	42	-	-	-
Solvent	401	660	582	515
R_{factor} (%)[2]	19.0	16.3	13.8	14.1
R_{free} (%)[2]	23.6	19.5	16.0	16.8
r.m.s.d. from ideality				
Bond lengths (Å)	0.005	0.011	0.007	0.008
Bond angles (°)	1.040	1.150	0.970	0.970
Dihedrals (°)	13.0	8.846	8.094	9.146
Ramachandran plot				
Favoured regions (%)	96.23	98.68	98.68	98.42
Allowed regions (%)	3.77	1.32	1.32	1.58
B-factors (Å^2)				
Average main chain	37.3	18.9	21.3	20.9
Average side chain	43.3	26.6	27.5	26.4
Average sugar	66.3	-	-	-
Average solvent	42.9	34.7	33.9	31.8

[1] $R_{merge} = \sum_{hkl} \sum_{j} |I_{hkl,j} - <I_{hkl}>| / \sum_{hkl} \sum_{j} I_{hkl,j}$.

[2] $R_{factor} = \sum_{hkl} ||F_o| - |F_c|| / \sum_{hkl} |F_o|$ for all data excluding the 5% that comprised the R_{free} used for cross-validation.