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LETTER

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HIV-1 adaptation to NK-cell-mediated immune pressure

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Natural killer (NK) cells have an important role in the control of viral infections, recognizing virally infected cells through a variety of activating and inhibitory receptors[1–3]. Epidemiological and functional studies have recently suggested that NK cells can also contribute to the control of HIV-1 infection through recognition of virally infected cells by both activating and inhibitory killer immunoglobulin-like receptors (KIRs)[4–7]. However, it remains unknown whether NK cells can directly mediate antiviral immune pressure *in vivo* in humans. Here we describe KIR-associated amino-acid polymorphisms in the HIV-1 sequence of chronically infected individuals, on a population level. We show that these KIR-associated HIV-1 sequence polymorphisms can enhance the binding of inhibitory KIRs to HIV-1-infected $CD4^+$ T cells, and reduce the antiviral activity of KIR-positive NK cells. These data demonstrate that KIR-positive NK cells can place immunological pressure on HIV-1, and that the virus can evade such NK-cell-mediated immune pressure by selecting for sequence polymorphisms, as was previously described for virus-specific T cells and neutralizing antibodies[8]. NK cells might therefore have a previously underappreciated role in contributing to viral evolution.

We hypothesized that HIV-1 can evade recognition by NK cells through the selection of sequence polymorphisms in regions targeted by KIRs, and that KIR-associated polymorphisms in the HIV-1 sequence can be identified on a population level. To test this hypothesis, we examined the relationship between KIR genotypes and HIV-1 polymorphisms in a cohort of 91 untreated, chronically HIV-1-infected individuals (Supplementary Table 1), in whom full-length HIV-1 sequences were determined and HLA-class-I-associated polymorphisms were previously described[9]. We used a decision-tree approach that corrects for phylogenetic structure among the sequences and allows for a multivariate analysis to identify KIR-associated sequence polymorphisms[10]. This analysis led to the identification of 22 positions in the HIV-1 genome at which amino-acid polymorphisms were significantly associated with the presence of a specific KIR gene (Table 1 and Supplementary Fig. 1). Taken together, these data show that HIV-1 can adapt to host KIR genotypes on a population level.

To assess the consequences of these KIR-associated amino-acid polymorphisms for NK-cell-mediated recognition of cells infected with HIV-1, we initially evaluated polymorphisms in a region of HIV-1 that encodes an overlapping segment that spans the carboxy-terminal end of Vpu and the amino-terminal end of Env (polymorphisms 15–18 in Table 1 and Supplementary Fig. 1). We selected this region because these polymorphisms were present in both reading frames at significantly higher frequencies in individuals that possessed at least one copy of the *KIR2DL2* gene than in individuals that did not (Table 2 and Supplementary Tables 2 and 3). In amino-acid positions 71 and 74 of Vpu, HIV-1 sequences derived from KIR2DL2-positive individuals encoded a methionine in position Vpu(71) and a histidine in position Vpu(74) in more than 70% of cases. Because of the overlapping Vpu/Env coding region, these positions of Vpu corresponded to a tryptophan in position Env(17) and a methionine in position Env(20), respectively. The Vpu(71M/74H) (Env(17W/20M)) sequence was significantly less frequent in HIV-1-infected individuals that did not encode KIR2DL2 ($P < 0.0001$). Furthermore, the presence of the Vpu(71M) polymorphism was in strong linkage disequilibrium with the Vpu(74H) polymorphism ($P = 3.17^{-12}$). Taken together, these data demonstrate a significant enrichment of HIV-1 viruses containing the Vpu(71M/74H) (Env(17W/20M)) polymorphism in individuals encoding KIR2DL2.

To determine the functional consequences of these KIR2DL2-associated polymorphisms in Vpu and Env, we constructed HIV-1 viral variants using the backbone of the HIV-1 NL4-3 strain[11,12]. These variants encoded either the Vpu(71M/74H) (Env(17W/20M)) sequence that was seen in $KIR2DL2^+$ individuals (referred to as $Vpu–Env^{V/V}$, with 'V' standing for variant), or the Vpu(71R/74L) (Env(17G/20L)) sequence that was most commonly seen in $KIR2DL2^-$ subjects (referred to as $Vpu–Env^{WT/WT}$). No significant differences were observed in the ability of the viral variants to replicate in primary $CD4^+$ T cells *in vitro* (Supplementary Fig. 2a). We subsequently assessed whether the different viral variants had any impact on NK-cell recognition and/or antiviral activity. Primary $CD4^+$ T cells were infected with viruses containing either the $Vpu–Env^{V/V}$ or the $Vpu–Env^{WT/WT}$ sequence, and then placed in co-culture with autologous NK

Table 1 | KIR footprints in HIV-1 sequence

	Protein	Amino-acid position	KIR association	Consensus amino acid*	*Q* value
1	Gag	93	KIR2DS3	E	0.1389
2	Gag	138	KIR2DL2	L	0.1852
3	Gag	138	KIR2DS2	L	0.1852
4	Gag	371	KIR2DS5	T	0
5	Gag	389	KIR3DS1	T	0
6	Gag	479	KIR2DS1	I	0
7	Vpr	37	KIR2DS3	I	0.0909
8	Tat	3	KIR2DL2	S	0.0246
9	Tat	3	KIR2DS2	S	0
10	Tat	3	KIR3DS1	S	0.1311
11	Tat	9	KIR2DS3	P	0.1311
12	Tat	28	KIR2DS1	V	0.1311
13	Tat	28	KIR2DS5	V	0.1339
14	Vpu	3	KIR2DL3	S	0.0833
15	Vpu	71	KIR2DL2	M	0.125
16	Vpu	74	KIR2DL2	H	0.1354
17	Env	17	KIR2DL2	W	0
18	Env	20	KIR2DL2	M	0.1667
19	Env	46	KIR3DS1	K	0.1667
20	Env	347	KIR2DS1	L	0.2
21	Env	595	KIR2DS1	I	0.2
22	Nef	9	KIR2DL2	S	0.0833

* HIV-1 consensus sequence in the 91 study subjects.

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Table 2 | Frequency of amino-acid polymorphisms among KIR2DL2$^+$ and KIR2DL2$^-$ subjects

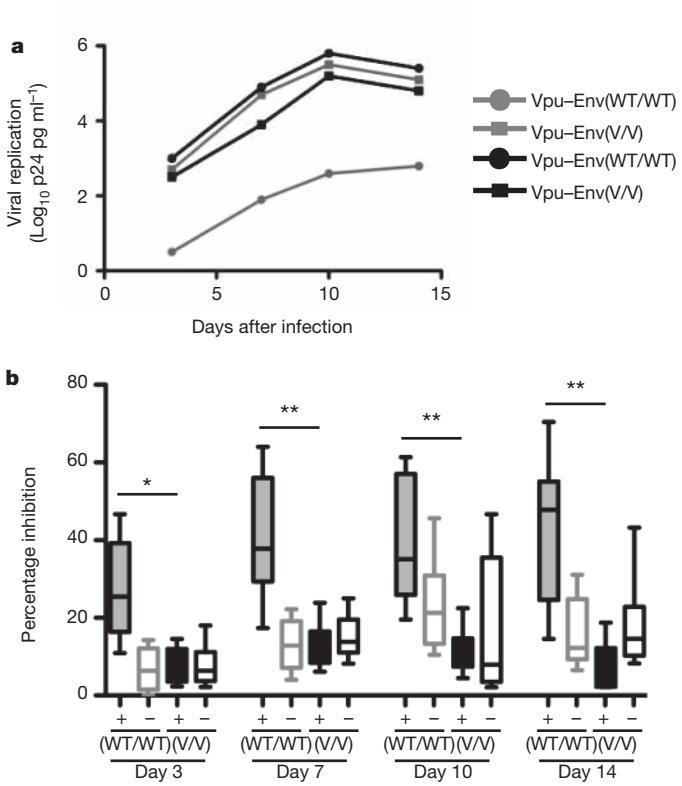
KIR2DL2 status	Vpu (amino-acid position)										Percentage of individuals	Number of individuals
	E (70)	**M (71)**	G (72)	H (73)	**H (74)**	A (75)	P (76)	W (77)	D (78)	V (79)		
KIR2DL2$^+$	·/P/D	·	·	·	·	·	·	·	N/·	I/·	72	(34/47)
	·	**L/Q/-/·**	·	·	**D/L/·**	·	·	·	·	I/·	24	(11/47)
	·	**R**	·	·	**L**	·	·	·	·	I/·	4	(2/47)
KIR2DL2$^-$	·/D	·	·/E	·/Q	·	·/D	·	·	·	·	32	(14/44)
	·/H/M	**N/V/Q/G**	·/A	·	**D/R**	·	·/L	·/G	·/V	·/I	34	(15/44)
	·/H	**R**	·	·/R	**L**	·	·/L	·/G/R/L	·/V	·/I	34	(15/44)

KIR2DL2 status	Env (amino-acid position)										Percentage of individuals	Number of individuals
	R (16)	**W (17)**	G (18)	T (19)	**M (20)**	L (21)	L (22)	G (23)	M (24)	L (25)		
KIR2DL2$^+$	·/K	·	·	·/I	·	·	·	·	·/L/I	·	85	(40/47)
	·/K	**R/·**	·	I	**T/·**	·	·	·	·	·	11	(5/47)
	·/K	**G**	·	I/A	**L**	·/F	·	·	·/I	·	4	(2/47)
KIR2DL2$^-$	·/K	·	·	·/I	·	·	·	·	·/I	·	43	(19/44)
	·	**R/M**	·	·/I	**·/V**	·	·	·	·/I	·	23	(10/44)
	·/K	**G**	·	·/I	**L**	·	·	·	·/I	·	34	(15/44)

KIR2DL2 status	Gag (amino-acid position)										Percentage of individuals	Number of individuals
	I (134)	V (135)	Q (136)	N (137)	**L (138)**	Q (139)	G (140)	Q (141)	M (142)	V (143)		
KIR2DL2$^+$	·	·	·	·	·	·	·	·	·	·/I	68	(32/47)
	·/V	·	·	·	**M/V/A**	·	·	·	·	·/I	32	(15/47)
	·	·	·	·	**I**	·	·	·	·	·	0	(0/47)
KIR2DL2$^-$	·	·	·/R	·	·	·	·	·	·	·	57	(25/44)
	·	·	·/R	·	**M/V/A**	·	·	·	·	·	25	(11/44)
	·	·	·	·	**I**	·	·	·	·	·	18	(8/44)

KIR2DL2 status	Nef (amino-acid position)										Percentage of individuals	Number of individuals
	W (5)	S (6)	K (7)	R (8)	**S (9)**	V (10)	V (11)	G (12)	W (13)	P (14)		
KIR2DL2$^+$	·/X	·	·	·/X	·	·/X	·/X	·/X	·	·/X	75	(35/47)
	·/X	·	·	·/X	**E/R/N/C/L/-**	·/X	·/X	·/E	·	·/X	23	(11/47)
	·	·	·	·	**K**	E	N	·	·	S	2	(1/47)
KIR2DL2$^-$	·/C	·	·/R	·/X	·	·/X	·/X	·/X	·	·/X	52	(23/44)
	·/C	·	·/R	·/X	**-/P/R/I/G/W**	·/X	·/X	·/X	·	·/X	27	(12/44)
	·	·	·	·/X	**K**	·/X	·/X	·/D	·	·/S	21	(9/44)

The KIR2DL2-associated sequence polymorphisms in Vpu, Env, Gag and Nef are highlighted in bold. The consensus sequence in the 91 studied individuals is listed in the respective headings. The most common amino acid substitutions are shown for the respective positions. X, positions in which several different amino acids were observed; -, amino-acid deletions; ·, identical amino acids as in consensus sequence.



cells derived from KIR2DL2$^+$ or KIR2DL2$^-$ HIV-1-negative individuals (Fig. 1). Both viruses replicated well in the presence of KIR2DL2$^-$ NK cells, with less than half a log inhibition of viral replication in the presence of NK cells compared to replication in CD4$^+$ T cells alone (Fig. 1a, b). In contrast, the Vpu–Env$^{WT/WT}$ virus was markedly inhibited by NK cells derived from KIR2DL2$^+$, but not KIR2DL2$^-$, subjects (Fig. 1b). Thus, the Vpu–Env$^{V/V}$ virus, which contains polymorphisms that were strongly associated with the presence of KIR2DL2 on the population level, was not inhibited by KIR2DL2$^+$ NK cells *in vitro*, whereas the 'wild-type' variant that was rarely observed in KIR2DL2$^+$ individuals was strongly inhibited by KIR2DL2$^+$ NK cells, consistent with the selection of Vpu–Env$^{V/V}$ viruses in KIR2DL2$^+$ individuals.

To elucidate further the mechanism by which KIR2DL2$^+$ NK cells inhibit replication of Vpu–Env$^{WT/WT}$ viruses, we monitored the induction of CD107a expression on NK cells *in vitro* after stimulation with autologous CD4$^+$ T cells infected with either the Vpu–Env$^{WT/WT}$

Figure 1 | KIR2DL2-associated sequence polymorphisms result in a loss of inhibition of HIV replication by NK cells *in vitro*. **a**, The Vpu–Env$^{WT/WT}$ virus was inhibited more robustly than the Vpu–Env$^{V/V}$ virus by NK cells derived from a KIR2DL2$^+$ individual (grey lines). NK cells derived from a KIR2DL2$^-$ individual (black lines) did not inhibit either virus. **b**, The Vpu–Env$^{WT/WT}$ virus (dark grey bars) was inhibited significantly more strongly than the Vpu–Env$^{V/V}$ virus (black bars) by NK cells derived from individuals that expressed KIR2DL2 ($n = 6$). NK cells derived from individuals that did not express KIR2DL2 (white bars, $n = 6$) did not significantly inhibit either virus. *, $P < 0.05$; **, $P < 0.005$; +, KIR2DL2-positive; −, KIR2DL2-negative. All results are given as mean and s.e.m.

or the Vpu–Env$^{V/V}$ virus (Fig. 2a–c). Consistent with the viral inhibition data, CD158b$^+$ NK cells from KIR2DL2$^+$ individuals were strongly activated by CD4$^+$ T cells infected with the Vpu–Env$^{WT/WT}$ virus, but not by CD4$^+$ T cells infected with the Vpu–Env$^{V/V}$ virus (Fig. 2a–c). These data are consistent with a model in which the inhibitory NK-cell receptor KIR2DL2 does not bind to cells infected with HIV-1 strains containing the Vpu–Env$^{WT/WT}$ sequence, but can bind to cells infected with HIV-1 Vpu–Env$^{V/V}$, providing a strong inhibitory signal to KIR2DL2$^+$ NK cells and thereby protecting cells infected with Vpu–Env$^{V/V}$ viruses from lysis by NK cells.

KIR2DL2 segregates with *KIR2DL3*, as an allele of the same locus[13], and is in strong linkage disequilibrium with *KIR2DS2* (W*n*, weighted normalized statistic for linkage disequilibrium = 0.976, $P < 0.001$), therefore most individuals in our cohort expressed both these KIRs. However, the three individuals in our cohort who expressed KIR2DL2 in the absence of KIR2DS2 also encoded the Vpu(71M) polymorphism, indicating that KIR2DL2, not KIR2DS2, is responsible for the association with this polymorphism. To test whether KIR2DL2 was directly involved in the recognition of the viral variants, a KIR2DL2–IgG fusion construct was used to assess whether the Vpu–Env$^{V/V}$ polymorphism modulated the interaction of KIR2DL2 with HIV-1-infected CD4$^+$ T cells. The KIR2DL2 fusion construct bound robustly to all uninfected CD4$^+$ T cells, and also bound to CD4$^+$ T cells infected with the HIV-1 Vpu–Env$^{V/V}$ variant significantly better than to CD4$^+$ T cells from the same donor infected with the Vpu–Env$^{WT/WT}$ variant (Fig. 2d–f). In contrast, the binding of a KIR2DL3 fusion construct to HIV-1-infected CD4$^+$ T cells was not significantly affected by the KIR2DL2-associated polymorphism (Fig. 2e, f). HLA-C group 1 and group 2 molecules serve as the ligands for the inhibitory receptor KIR2DL2, which has been shown to bind with greater affinity to HLA-C group 1 than group 2 (ref. 13). Staining with the KIR2DL2 fusion construct was consistent with these results, because it bound most strongly to cells from individuals homozygous for HLA-C group 1 (Supplementary Fig. 3). Consistent with these binding data, the Vpu–Env$^{V/V}$ polymorphisms were significantly enriched in KIR2DL2$^+$ individuals homozygous for HLA-C group 1 ($P = 0.008$ for Vpu(71M) and $P = 0.01$ for Vpu(74H)). Taken together, these data indicate that the Vpu–Env$^{V/V}$ polymorphism enhances the ability of the inhibitory receptor KIR2DL2 to bind to HIV-1-infected cells, in particular those expressing the ligands with highest affinity for KIR2DL2.

In addition to the Vpu(71M/74H) polymorphism, two additional amino-acid polymorphisms (Gag(138L/I) and Nef(9S/K)) were associated with the presence of the *KIR2DL2* gene in the study population (Table 1, polymorphisms 2 and 22). We next determined whether these KIR2DL2-associated polymorphisms were also associated with differential recognition of HIV-1-infected cells by KIR2DL2$^+$ NK cells. Viruses containing the polymorphisms replicated similarly in CD4$^+$ T cells (Supplementary Fig. 2b). As observed for the Vpu and Env variants, viruses containing the amino acids that were rarely observed in KIR2DL2$^+$ individuals (Gag^{WT} and Nef^{WT}, Table 2 and Supplementary Tables 4 and 5) were inhibited more strongly by NK cells derived from KIR2DL2$^+$ individuals than were viruses containing the variants selected in KIR2DL2$^+$ subjects (Fig. 3a, c). Furthermore, KIR2DL2$^+$ NK cells degranulated more robustly in response to CD4$^+$ T cells infected with the Gag^{WT} and Nef^{WT} viruses (Fig. 3b, d), and KIR2DL2–IgG fusion constructs bound significantly less to CD4$^+$ T cells infected with the Gag^{WT} and Nef^{WT} viruses than to cells infected with Gag^{V} and Nef^{V} viruses (Fig. 4a–c). Taken together, these data demonstrate that HIV-1 may evolve in KIR2DL2$^+$ individuals to enrich particular amino-acid polymorphisms, in an effort to escape recognition by KIR2DL2$^+$ NK cells.

Increasing amounts of evidence indicate that NK cells have an important role in the control of HIV-1 infection[4–6]. Here we report several amino-acid polymorphisms within the HIV-1 clade B sequence that are significantly associated with the expression of specific KIR genes on the population level. We demonstrate in functional studies that these 'KIR footprints' can modulate the interaction of KIR$^+$ NK cells with HIV-1-infected CD4$^+$ T cells. The selection of particular

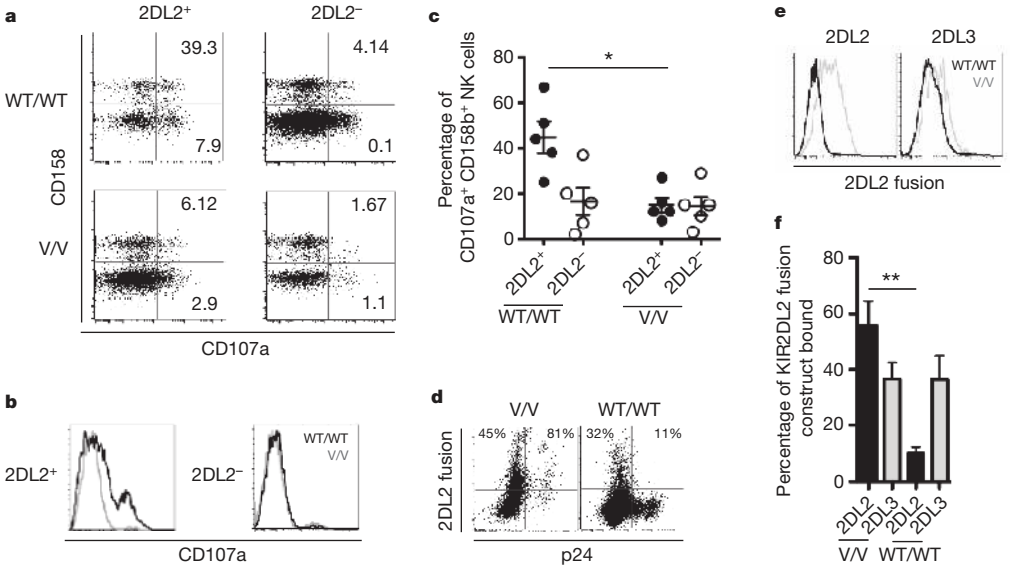


Figure 2 | Amino-acid polymorphisms at positions 71 and 74 in Vpu inhibit KIR2DL2, but not KIR2DL3, recognition and binding. **a**, Flow cytometric plots depicting the percentages of total CD158b(KIR2DL2/2DL3/2DS2)$^+$ NK cells that degranulated after co-culture with autologous CD4$^+$ T cells infected with the Vpu–Env$^{WT/WT}$ virus or the Vpu–Env$^{V/V}$ virus for two representative subjects (left panels, KIR2DL2$^+$ subjects; right panels, KIR2DL2$^-$ subjects). **b**, The percentage of degranulating CD158$^+$ NK cells in the total CD158$^+$ NK-cell population is also represented in histograms for both the KIR2DL2$^+$ donor (left panel) and KIR2DL2$^-$ donor (right panel), for both the WT/WT virus (black line) and the V/V virus (grey line). **c**, Combined data for NK-cell degranulation in KIR2DL2$^+$ ($n = 5$) and KIR2DL2$^-$ ($n = 5$) individuals. *, $P < 0.05$. **d**, Staining pattern of a KIR2DL2–IgG fusion construct on HIV-infected CD4$^+$ T cells from an HLA-C1/C2 heterozygous donor. Percentages indicate the percentage of p24$^-$ or p24$^+$ cells that were stained with the KIR2DL2–IgG fusion construct. **e**, Staining of KIR2DL2–IgG and KIR2DL3–IgG fusion constructs on the same donors infected with the WT/WT and V/V viral variants. **f**, Summary of binding data for the KIR2DL2–IgG (black) and KIR2DL3–IgG (grey) fusion constructs for five different HLA-C1 heterozygous CD4$^+$ T-cell donors after infection with the two viral variants. **, $P < 0.005$. All results are given as mean and s.e.m.

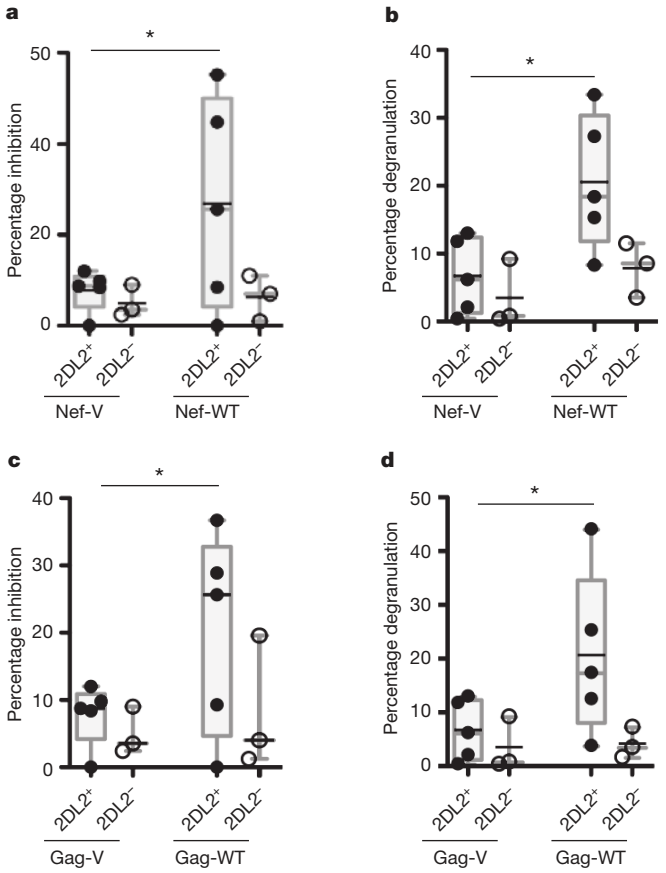


Figure 3 | Two additional KIR2DL2-associated amino-acid polymorphisms reduce KIR2DL2-mediated NK-cell recognition of virally infected cells. **a**, **c**, NK cells from KIR2DL2$^+$ individuals ($n = 5$) inhibited the replication of the Nef-WT virus and the Gag-WT virus significantly better than they inhibited replication of the Nef-V and Gag-V viruses. **b**, **d**, NK cells derived from KIR2DL2$^+$ individuals ($n = 5$) were activated significantly more by cells infected with the Nef-WT and Gag-WT viruses than by cells infected with Nef-V and Gag-V viruses. However, NK cells derived from KIR2DL2$^-$ individuals ($n = 3$) did not inhibit viral replication or degranulate in response to cells infected with either of the wild-type or variant viruses. *, $P < 0.05$. All results are given as mean and s.e.m.

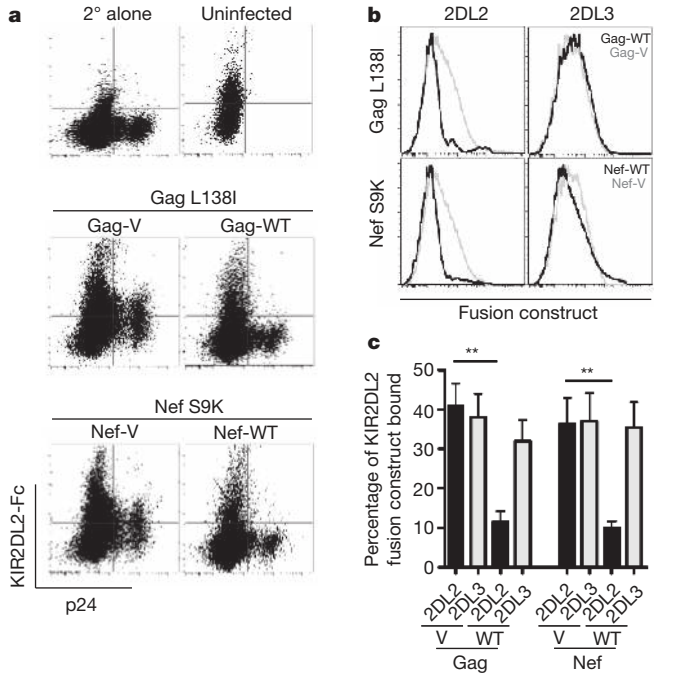


Figure 4 | KIR2DL2-associated amino-acid polymorphisms affect binding of KIR2DL2, but not KIR2DL3, to infected CD4$^+$ T cells. CD4$^+$ T cells infected with variant viruses were stained with KIR2DL2–IgG and KIR2DL3–IgG fusion constructs. **a**, **b**, The dot plots (**a**) and histograms (**b**) show the staining pattern of KIR2DL2–IgG and KIR2DL3–IgG fusion constructs on HIV-1-infected CD4$^+$ T cells from a HLA-C1/C2 heterozygous donor. **c**, The bar graph summarizes binding data for KIR2DL2 (black) or KIR2DL3 (grey) IgG fusion constructs for five different HLA-C1/C2 heterozygous CD4$^+$ T-cell donors infected with viral variants. **, $P < 0.005$. All results are given as mean and s.e.m.

amino-acid residues that result in enhanced binding of inhibitory KIRs to infected cells represents a novel approach by which HIV-1 can evade NK-cell-mediated immunity. The molecular mechanisms and precise receptor–ligand interactions involved in this evasion of NK-cell recognition require further investigation. Previous *in vitro* studies have demonstrated that sequence variations in HLA-class-I-presented epitopes[14–23] and small changes in the peptide repertoire presented on HLA class I molecules[24] can both modulate the binding of KIR, providing a potential mechanism for virus-sequence-dependent recognition of infected cells by NK cells. The KIR2DL2-associated sequence polymorphisms studied here had no impact on KIR2DL2 binding to transporter associated with antigen processing (TAP)-deficient T2 cells expressing HLA-Cw*0102 (Supplementary Fig. 4), despite some degree of HLA-Cw*0102 stabilization. However, several different HLA-C group 1 molecules might present epitopes in these regions of HIV-1, resulting in differential recognition by KIR2DL2. Additionally, other mechanisms besides the modulation of KIR binding to HLA class I might account for the observed reduction in recognition of variant-virus-infected cells by KIR2DL2$^+$ NK cells. KIR-associated sequence polymorphisms in HIV-1 proteins might directly modulate the ability of these proteins to be processed and presented[25], might subtly alter hydrostatic interactions with KIR, or might change the profile of NK-cell receptor ligands expressed on infected cells, as has been described for HIV-1 Nef, Vpu and Vpr[26,27].

Protective effects of specific KIRs or combinations of KIR and HLA class I have been described for many infectious diseases, including HIV, hepatitis C virus and human papilloma virus. Although *KIR2DL2* has not been identified as a protective *KIR* gene in any of these viral infections, homozygosity of its allotypic counterpart, *KIR2DL3*, has been associated with the resolution of hepatitis C infection when *KIR2DL3* is co-expressed with its ligand, HLA-C group 1 (ref. 28). This protective KIR2DL3/HLA-C group 1 combination provides a weaker inhibitory signal, resulting in weaker inhibition of NK cells[28]. Here we describe a mechanism by which HIV-1 selects for sequence polymorphisms in KIR2DL2$^+$ individuals that lead to an enhanced binding of this inhibitory KIR to infected cells, resulting in the inhibition of NK-cell function and thereby enabling HIV-1 to escape the potential protective role of this KIR. Overall, the data from different viral infections are consistent with a model in which enhanced NK-cell activity can contribute to the control of viral replication, and indicate that viruses can evade this NK-cell-mediated immune pressure by selecting for variants that modulate the recognition of infected cells by KIR.

METHODS SUMMARY

Viral sequencing. Genomic DNA was extracted from samples of peripheral blood mononuclear cells and nested PCR protocols were used to amplify HIV-1 genomes[9].

Phylogenetic analysis of KIR-associated sequence polymorphisms. A decision-tree approach[10], followed by adjustment for multiple comparisons, was used to identify KIR-associated sequence polymorphisms.

Construction of viruses containing sequence polymorphisms. Mutations of interest were inserted into the HIV-1 NL4-3 backbone using the GeneTailor site-directed mutagenesis system[11,12].

Viral inhibition assay. Viral inhibition assays were performed after infecting CD4$^+$ T cells with viral constructs, as indicated. The level of viral inhibition was

then calculated as the difference between viral production (p24 Gag) in wells containing autologous NK cells and production in wells containing $CD4^+$ T cells alone[6].

NK-cell degranulation assay. Degranulation of $KIR2DL2^+$ NK cells was examined by flow cytometry after co-culture of NK cells with autologous $CD4^+$ T cells infected with the viral construct indicated, in the presence of Golgi-stop and anti-CD107a-PECy5 for 6 h (ref. 29). NK cells were stained with anti-CD3, anti-CD56, anti-CD16 and anti-CD158b (KIR2DL2/2DL3/2DS2) antibodies, and the level of degranulation was assessed as the proportion of $CD107a^+$ NK cells among the $CD158b^+$ NK cells.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions G.A. conducted the immunology experiments and L.F. performed the KIR-staining experiments on T2 cell lines. A.S. and C.O.-N. constructed the viral variants. D.H., C.M.K. and J.M.C. performed the data analysis identifying KIR-associated polymorphisms. B.L. and T.M.A. performed the viral sequencing, M.C. and M.M. performed the HLA and KIR typing, and L.F. and S.I.K. provided the KIR fusion construct. G.A. and M.A. planned the studies, prepared the manuscript and supervised the project.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to M.A. (maltfeld@partners.org).

METHODS

Study subjects. Ninety-one untreated subjects chronically infected with HIV-1 subtype B, for which HLA class I and KIR genotypes were available (Supplementary Table 1), were included in this study[9]. In addition, 100 HIV-1 negative controls were genotyped for KIR and HLA genotypes. Of this large cohort of uninfected controls, 15 subjects that were KIR2DL2$^+$ (KIR2DL2$^+$/KIR2DL3$^+$) and 15 subjects that were KIR2DL2$^-$ (KIR2DL3$^+$/KIR2DL3$^+$) were enrolled to provide samples for the generation of NK cells and autologous CD4$^+$ target cells (Supplementary Table 6). For these *in vitro* studies, only individuals that did not encode KIR3DS1 and HLA-B Bw4-80I were selected, because we had previously observed very strong inhibition of HIV-1 replication *in vitro* in individuals with this combined KIR/HLA genotype[6]. All study subjects were enrolled in Boston through the Massachusetts General Hospital, the Lemuel-Shattuck Hospital and the Fenway Community Health Center. The study was approved by the Massachusetts General Hospital Review Board and all subjects gave written informed consent.

Viral sequencing. Genomic DNA was extracted from peripheral blood mononuclear cell samples and nested PCR protocols were used to amplify HIV-1 genomes as described previously[9]. Five independent PCR products of each sample were pooled and directly population-sequenced at the Massachusetts General Hospital DNA sequencing core facility using clade B consensus sequencing primers, as previously described[9].

HLA class I and KIR typing. High-resolution HLA class I typing and KIR genotyping were performed as described previously[6].

Phylogenetic analysis of KIR-associated sequence polymorphisms. We used the decision-tree approach[10], which corrects for phylogenetic structure among the sequences and allows for a multivariate analysis, to identify KIR-associated sequence polymorphisms. All results were adjusted for multiple comparisons (both P and Q values are assigned to each result). For each protein analysed, a maximum likelihood phylogenetic tree was constructed from the corresponding sequences. For every KIR and HLA gene, amino-acid position and amino acid at that position, we created two generative or directed graphical models of the observed presence or absence of the amino acid in each sequence: one representing the null hypothesis that the observations are generated by the phylogenetic tree alone and the other representing the alternative hypothesis that additional escape or reversion takes place owing to KIR/HLA pressure in the subjects for which the sequences are observed. The likelihood of the observations was then maximized over the parameters of both models using an expectation-maximization algorithm, and a P value was computed using a likelihood ratio test based on those likelihoods[10]. To increase power, the tests were binarized, such that the presence or absence of a given KIR or HLA gene was correlated with the presence or absence of a given amino acid. In addition, KIR–polymorphism pairs were analysed only when the actual or expected count in every cell of the corresponding two-by-two contingency table was ≥ 3. For every amino acid at each position, the KIR or HLA gene with the strongest association (and its corresponding P value) was added to the list of identified associations. The analysis was then repeated after removing individuals having or possibly having this KIR or HLA gene. This procedure was iterated until no KIR or HLA gene yielded an association with a P value less than 0.05. A Q-value statistic, estimating the proportion of false positives among the associations identified, was computed for each association by repeating this analysis on null data (generated by permuting the KIR/HLA data). Correction for multiple comparisons was undertaken using both $Q < 0.05$ (estimating 5% false positives) and $Q < 0.2$ (estimating 20% false positives).

Construction of viruses containing sequence polymorphisms. The HIV-1 strain NL4-3 was modified to express one or two mutations in *vpu/env*, *nef* or *gag* using the GeneTailor site-directed mutagenesis system (Invitrogen) or the QuikChange Lightning site-directed mutagenesis system (Stratagene)[11,12]. In brief, mutagenesis was performed using 5′ oligonucleotide primers Vpu_M71R-f (CTTGTGGAGATGGGGGTGGAAAGGGGGCACCAT (nucleotide (nt) 6279)), Vpu_M71R-r (TTTCCACCCCCATCTCCACAAGTGCTGATACTTCT (nt 6234)), Vpu_H74L-f (ATGGGGGTGGAAATGGGGCACCTTGCTCCTTGG), Vpu_H74L-r (GGTGCCCCATTTCCACCCCCATCTCCACAAG (nt 6247)), Vpu_M71R/H74L-f (ATGGGGGTGGAAAGGGGGCACCTTGCTCCTTGG (nt 6247 and nt 6288)) and Vpu_M71R/H74L-r (GGTGCCCCCTTTCCACCCCCATCTCCACAAG (nt 6247)); Nef/S9K-f (GTGGTCAAAAAGTAAAGTGATTGGATGGCC (nt 8827)) and Nef/S9K-r (GGCCATCCAATCACTTTACTTTTTGACCAC (nt 8798)); Gag/L138I-f (CCTATAGTGCAGAACATCCAGGGGCAAATGG (nt 1216)) and Gag/L138I-r (CCATTTGCCCCTGGATGTTCTGCACTATAGG (nt 1186)). Mutated nucleotides are underlined and primer positions are numbered according to the numbering of NL4-3 (GenBank accession number AF324493). The complete HIV-1 coding region of the variant proviruses was sequenced on an ABI3730 XL DNA analyser. Propagation of provirus and generation of viral stocks was performed as previously described[11,12]. Although the full-length sequence of the NL4-3 viruses differed from the autologous sequence of the respective study subjects, the areas flanking the *vpu*, *gag* and *nef* sequences studied were identical between the NL4-3 virus and the respective areas of interest, and only differed in the amino acids indicated in Supplementary Tables 2–5.

Viral inhibition assay. Viral inhibition assays using NK cells were performed as previously described[6]. CD4$^+$ T cells that were generated after 4 days in culture with a bi-specific antibody to CD3 and CD8 were infected with laboratory strains containing amino-acid polymorphisms at a multiplicity of infection of 0.01 for 4 h at 37 °C. Cells were washed twice. Equal numbers of CD4$^+$ T cells were plated at NK-cell/CD4$^+$ T cell ratios of 10:1, or alone, for 14 days in the presence of 50 U ml^{-1} interleukin-2. Supernatant was collected every 3–4 days for quantification of p24 Gag production by enzyme-linked immunosorbent assay (p24 ELISA; Perkin Elmer).

NK-cell degranulation assay. To examine whether KIR2DL2$^+$ NK cells specifically degranulated in response to autologous CD4$^+$ T cells infected with either of the viral variants, we monitored the level of CD107a upregulation on KIR2DL2$^+$ or KIR2DL2$^-$ NK cells[29]. We selected HIV-1-negative donors that expressed KIR2DL2 in the absence of KIR2DS2 (referred to as KIR2DL2$^+$), or KIR2DL3 in the absence of KIR2DL2 and KIR2DS2 (referred to as KIR2DL2$^-$). An anti-CD158b antibody was used to detect NK cells expressing KIR2DL2, KIR2DL3 and KIR2DS2. The level of degranulation was assessed as the proportion of CD107a$^+$ NK cells among the CD158b$^+$ NK cells. NK cells thus purified were co-cultured in the presence of autologous CD4$^+$ T cells infected *in vitro* with the respective HIV-1 strains for 7 days. Monensin was added to co-cultures on day 7 at 0.3 μg ml^{-1}, in the presence of 20 μl CD107a-PE-Cy5, for 6 h. Cells were washed and stained with CD3-Pacific Blue, CD56-PE-Cy7, CD16-APC-Cy7 (BD Biosciences) and CD158b-PE (Beckman Coulter) for 30 min, then washed and fixed in 1% paraformaldehyde until flow cytometric analysis was performed (FACSCalibur; BD Biosciences).

KIR–IgG fusion construct binding assay. Differences in the ability of KIR2DL2 or KIR2DL3 to interact with CD4$^+$ T cells infected with either of the viral variants were ascertained using KIR2DL2–IgG and KIR2DL3–IgG fusion constructs (provided by S. Khakoo and O. Mandelboim). Peripheral blood mononuclear cells were obtained from HLA-C1/C1 homozygotes, HLA-C1/C2 heterozygotes or HLA-C2/C2 homozygotes, and were treated with 0.3 μg of a bispecific CD3/CD8 antibody in the presence of 50 U interleukin-2 per ml of complete medium. After 3 days, the cells were infected with one of the three KIR2DL2-associated variants (Vpu-71/74, Gag-138 or Nef-9), or with wild-type virus, for 2 days. The cells were then collected and stained with 2 μl of the KIR2DL2-IgG or KIR2DL3-IgG for 1 h on ice. The cells were then washed and stained with a secondary allophycocyanin (APC)-conjugated goat anti-human IgG antibody for an additional 20 min on ice. In parallel, infected cells were stained with the anti-human antibody alone to define the background level of staining. All cells were then fixed with 100 μl of Fix A solution (Invitrogen) for 10 min, washed and permeabilized using 100 μl of Perm B (Invitrogen). The cells were then stained for intracellular p24 using the KC-57-RD1 antibody for 20 min on ice, and then washed. Cells were fixed in 1% paraformaldehyde until flow cytometric analysis was performed (FACSCalibur; BD Biosciences).

HLA class I stabilization assays. HLA-C stabilization was assessed in 2×10^5 T2 cells that were incubated with 0.04 mg ml^{-1} peptide, as indicated, overnight at 26 °C. The following day, peptide-pulsed T2 cells were stained with W632-PE (eBioscience) antibody (HLA-A/B/C) or DT9 antibody (HLA-C/E) for 30 min at 4 °C. Cells were then washed in PBS before staining with anti-mouse IgG-PE (Sigma) for 30 min at 4 °C. Cells were washed twice with PBS and fixed in Perm A solution (BD Biosciences). KIR2DL2–IgG binding to peptide-pulsed T2 cells was assessed as previously described[24].

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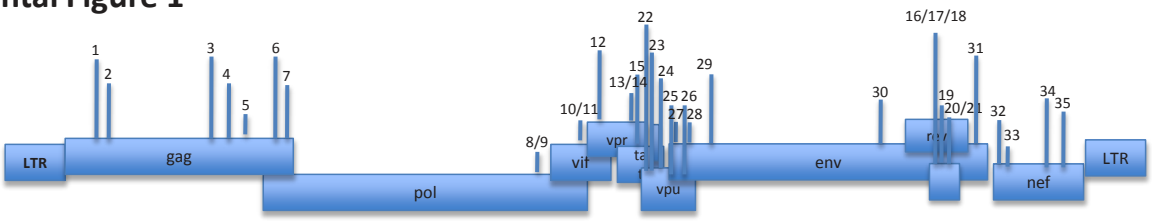
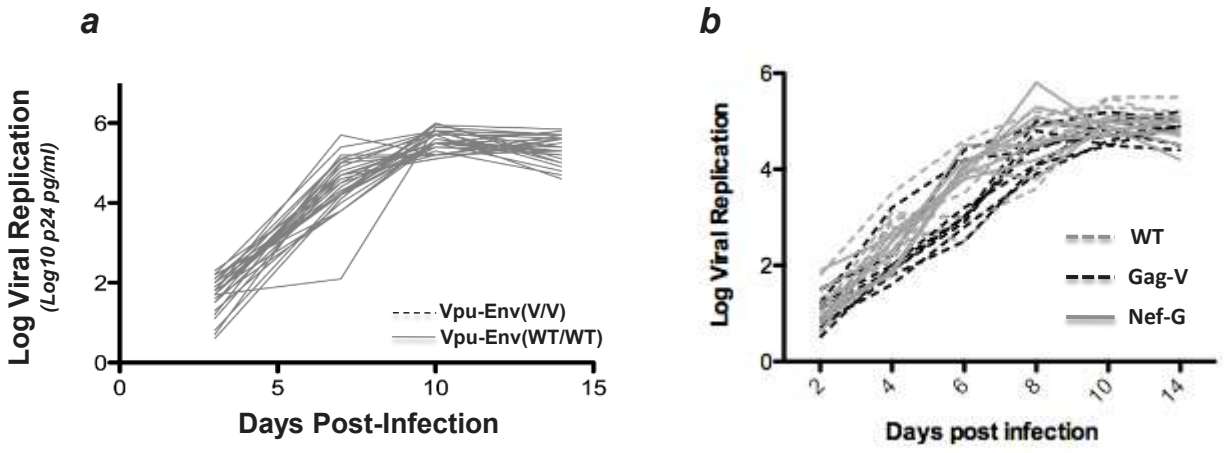
Supplemental Figure 1



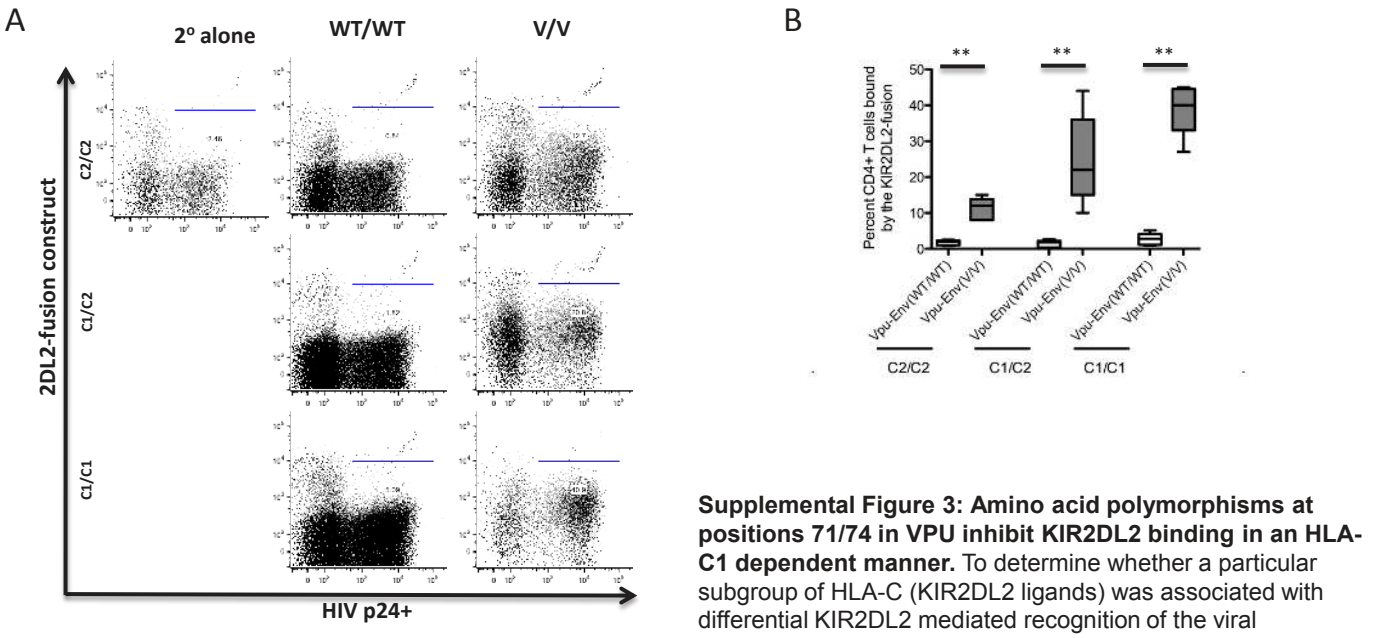
Supplemental Figure 1: Distribution of KIR associated polymorphisms across the viral genome. The figure depicts the distribution of KIR –associated polymorphisms across the HIV genome. The bulk of the mutations cluster within variable genes that are expressed early during the viral life-cycle. Numbers correspond to numbers in Table 1.

Supplemental Figure 2



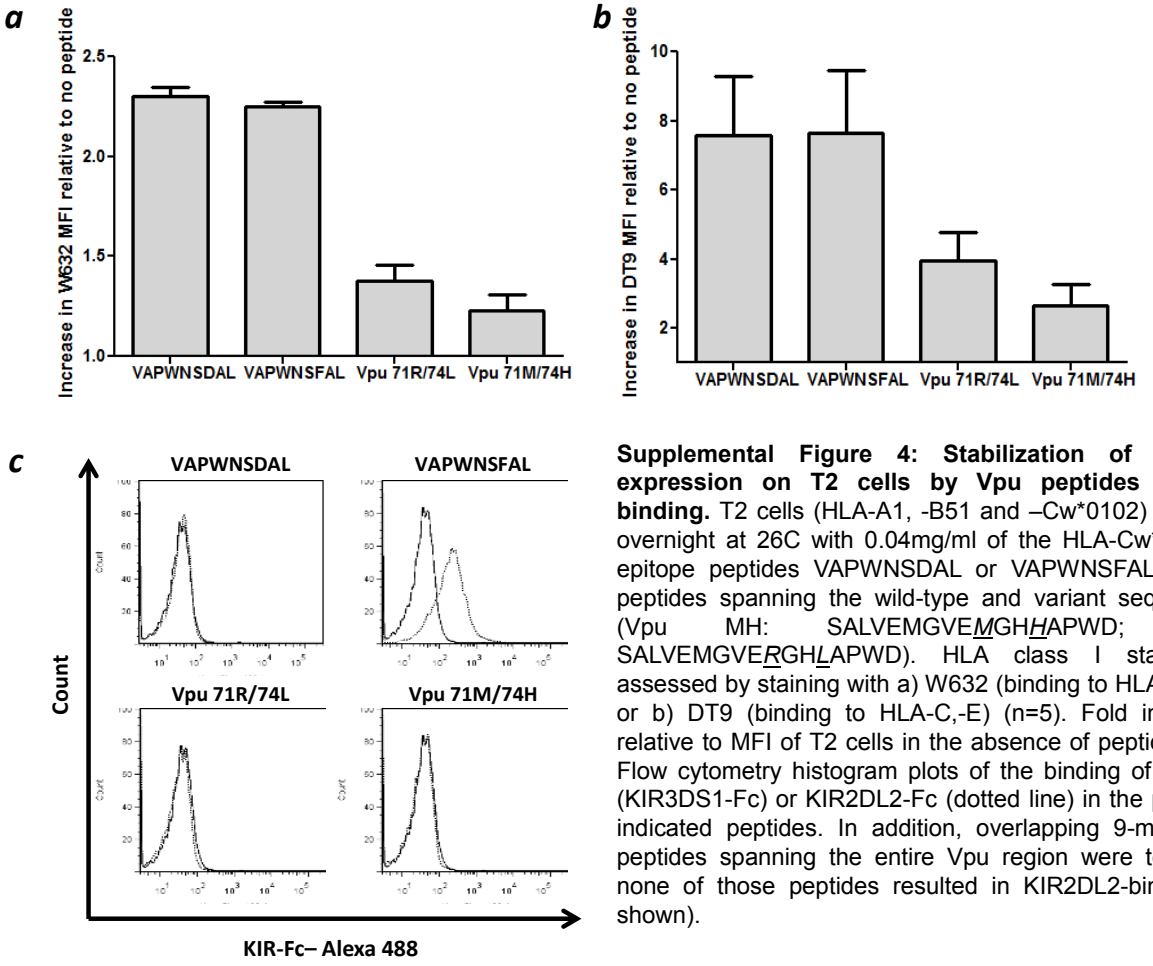
Supplemental Figure 2: Replication of the different viral constructs in CD4+ T cells in the absence of NK cells. No differences in the replication capacity were observed among the wildtype (WT) and variant (V) viruses.

Supplemental Figure 3



Supplemental Figure 3: Amino acid polymorphisms at positions 71/74 in VPU inhibit KIR2DL2 binding in an HLA-C1 dependent manner. To determine whether a particular subgroup of HLA-C (KIR2DL2 ligands) was associated with differential KIR2DL2 mediated recognition of the viral polymorphisms, differences in the capacity of KIR2DL2 to interact with CD4+ T cells infected with the mutant or wild-type virus was compared using a KIR2DL2-IgG fusion construct. HIV-1-infected CD4+ T cells isolated from donors encoding for HLA-C group 1/1, 2/2, or 1/2 were stained. Significantly stronger binding to C1/C1+ than to C2/C2+ CD4+ T cells infected with the variant virus was observed. ** $p < 0.005$.

Supplemental Figure 4



Supplemental Figure 4: Stabilization of HLA-Cw*0102-expression on T2 cells by Vpu peptides and KIR2DL2 binding. T2 cells (HLA-A1, -B51 and –Cw*0102) were incubated overnight at 26C with 0.04mg/ml of the HLA-Cw*0102-restricted epitope peptides VAPWNSDAL or VAPWNSFAL[24], or with the peptides spanning the wild-type and variant sequences in Vpu (Vpu MH: SALVEMGVE*M*GH*H*APWD; Vpu RL: SALVEMGVE*R*GH*L*APWD). HLA class I stabilization was assessed by staining with a) W632 (binding to HLA-A,-B,-C) (n=5) or b) DT9 (binding to HLA-C,-E) (n=5). Fold increase in MFI relative to MFI of T2 cells in the absence of peptide is shown. C) Flow cytometry histogram plots of the binding of isotype control (KIR3DS1-Fc) or KIR2DL2-Fc (dotted line) in the presence of the indicated peptides. In addition, overlapping 9-mer and 10-mer peptides spanning the entire Vpu region were tested, however none of those peptides resulted in KIR2DL2-binding (data not shown).

Supplemental Table 1. HLA class I and KIR typing of HIV-infected subjects

Subject	A1	A2	B1	B2	Cw1	Cw2	2DL1	2DL2	2DL3	2DL4	2DL5	2DS1	2DS2	2DS3	2DS5	3DL1	3DS1	3DL2	3DL3
1	'0101	'0201	'3701	'5101	'0602	'1602	'0	'1	'0	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
2	'0201	'2402	'0720	'4402	'0501	'0702	'1	'1	'0	'1	'1	'1	'1	'1	'0	'1	'1	'1	'1
3	'3002	'3303	'4403	'4403	'0304	'0401	'1	'1	'0	'1	'1	'0	'1	'1	'1	'1	'0	'1	'1
4	'0201	'2601	'3801	'4402	'0501	'1203	'1	'1	'0	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
5	'0101	'2902	'1510	'3910	'0401	'1203	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1	'0	'1	'1
6	'0101	'0301	'1402	'5701	'0602	'0802	'1	'1	'0	'1	'1	'1	'1	'1	'1	'1	'1	'1	'1
7	'0301	'2601	'0702	'4402	'0501	'0702	'0	'1	'0	'1	'1	'1	'1	'0	'1	'0	'1	'1	'1
8	0201	2402	1801	5501	0303	0701	'1	'1	'0	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
9	'0101	'1101	'3501	'2705	0202	'0401	'1	'1	'0	'1	'1	'1	'1	'1	'1	'1	'1	'1	'1
10	0101	0201	0801	4402	0501	0701	'1	'1	'0	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
11	'1101	'2402	'1402	'5501	'0304	'0802	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
12	'2301	'7400	'0801	'1402	'0701	'0802	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
13	0201	1101	1501	4402	0303	0501	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'1	'1	'1
14	'2402	'3201	'0705	'1302	'0602	'1505	'1	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'1	'1
15	'2601	'2902	'4403	'4901	'0701	'1601	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
16	'2902	'6801	'4002	'5201	'0202	'1505	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
17	2601	7401	3701	4901	0602	0701	'1	'1	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
18	'0201	'3101	'5101	'5101	'0102	'0401	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
19	2402	3012	3503	5703	0701	1203	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
20	'7401	'8001	'1801	'5703	'0202	'0701	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
21	'0101	'6801	'0801	'5702	'0701	'1800	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
22	0201	3201	4402	5101	0501	1502	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'1
23	'0201	'3002	'1503	'5101	'0210	'1602	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'1	'1	'1
24	'0101	'2601	'2705	'5601	'0102		'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
25	'3303	'7401	'1503	'5801	'0202	'0701	'1	'1	'1	'1	'1	'0	'0	'0	'1	'1	'0	'1	'1
26	2902	6802	4403	5802	1601	0602	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
27	'1101	'6801	'1501	'1803	'0303	'0701	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
28	2402	8001	2705	5301	0202	0401	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'1	'1	'1
29	'0201	'3303	'0705	'4403	'0303	'1505	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
30	0205	2902	1402	5801	0701	0802	'1	'1	'1	'1	'1	'1	'1	0	'1	'1	'1	'1	'1
31	0201	0301	1501	3501	0303	0401	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'1	'1	'1
32	'0101	'0101	'0801	'5701	'0602	'0701	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
33	'0202	'6801	'1401	'1401	'0210	'0804	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
34	'0301	'3002	'0702	'1801	'0501	'0702	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
35	0201	6801	0702	4402	0702	0704	'1	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'1	'1
36	0101	1101	3501	5501	1402	0303	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'1
37	'2301	'6801	'1402	'5802	'0602	'0802	'1	'1	'1	'1	'1	'0	'0	'0	'1	'1	'0	'1	'1
38	'2301	'3402	'4403	'8101	'0401	'1801	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
39	6801	6802	0702	0801	0702	0701	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
40	'1101	'6801	'4402	'5802	'0501	'0602	'1	'1	'1	'1	'1	'1	'1	'1	'1	'1	'1	'1	'1
41	0101	0201	2705	3701	0602	1203	'1	'1	'1	'1	'1	'0	'1	'0	'1	'1	'0	'1	'1
42	'0201	'3101	'0702	'1531	'0407	'0702	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
43	0201	3402	3501	4501	0401	1601	'1	'1	'1	'1	'1	'0	'1	'1	'0	'1	'0	'1	'1
44	'2301	'2601	'0702	'5301	'0602	'0702	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
45	'0201	'3002	'1801	'0801	'0501	'20	'1	'1	'1	'1	'0	'0	'1	'0	'0	'1	'0	'1	'1
46	'0202	'3012	'5301	'5801	'0802	'0401	'1	'1	'1	'1	0	0	'1	0	0	'1	0	'1	'1
47	0201	3601	5301	2705	0102	0401	'1	'1	'1	'1	'1	'1	'1	'0	'1	'1	'1	'1	'1
48	'0202	'3002	'4901	'7801	'0701	'1601	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
49	'0301	'2501	'1501	'4001	'0304	'0304	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
50	'0101	'0301	'0702	'0702	'0702	'0702	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
51	'2402	'6801	'0702	'1302	'0702	'0802	'1	'0	'1	'1	'1	'1	'0		'1	'1	'1	'1	'1
52	'0301	'3002	'1801	'0801	'0501	'0702	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
53	0202	2902	0702	4901	0701	0701	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
54	'0101	'3201	'0801	'1801	'0701	'0701	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
55	'0101	'0301	'0702	'3508	'0401	'0702	'1	'0	'1	'1	'1	'1	'0	'0	'1	'1	'0	'1	'1
56	1101	3201	0702	3503	0702	1202	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
57	'0201	'2601	'0801	'4402	'0501	'0702	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
58	0201	0301	4001	5101	0102	0304	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
59	'0301	'1101	'0702	'3901	'0702	'1203	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
60	'0301	'6802	'1510	'3501	0304	'0401	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
61	'2902	'3101	'3512	'4002	'0202	'0401	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
62	'2301	'2902	'4403	'4403	'1601	'1601	'1	'0	'1	'1	'1	'0	'0	'1	'0	'1	'0	'1	'1
63	'0101	'0201	'1801	'2705	'0202	'1203	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
64	'0101	'0201	'0702	'3512	'0401	'0702	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
65	'0101	'1101	'0801	'3504	'0401	'0701	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'1	'1	'1
66	'2402	'3201	'1502	'5101	'0801	'1602	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
67	'0101	'2402	'0801	'1801	'0701	'1203	'1	'0	'1	'1	'1	'1	'0	'0	'1	'1	'1	'1	'1
68	'0201	'2301	'4501	'4501	'0602	'0602	'1	'0	'1	'1	'1	'1	'0	'0	'1	'1	'1	'1	'1
69	0101	0101	1402	4403	0501	1601	'1	'0	'1	'1	'1	'1	'0	'1	'1	'1	'1	'1	'1
70	'1101	'2501	'3501	'3901	'0401	'1203	'1	'0	'1	'1	'1	'1	'0	'0	'1	'1	'1	'1	'1
71	'0101	'3001	'0702	'4201	'1505	'1700	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
72	'0201	'0301	'1801	'4403	'0701	'1602	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
73	'0101	'0201	'0801	'5101	'0701	'1502	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
74	33	68	1510	5301	0304	0401	'1	'0	'1	'1	'1	'1	'0	'0	'1	'1	'1	'1	'1
75	'0301	'2301	'0702	'4501	'0602	'0702	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
76	'2902	'6801	'4002	'5201	'0202	'1502	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
77	0217	2601	0801	4002	0305	0701	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
78	'0217	'2601	'0801	'4002	'0305	'0701	'1	'0	'1	'1	'1	'1	'0	'0	'1	'1	'1	'1	'1
79	'0201	'0204	'4402	'5101	'0501	'1502	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
80	'3601	'6802	'1510	'1516	'0304	'1402	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
81	0201	6801	1501	5501	0303	0501	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
82	'0301	'2423	'3501	'5101	'0401	'1402	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
83	'0204	'0205	'5101	'5301	'0401	'1502	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
84	'0201	'0202	'3501	'4501	'1402	'1601	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
85	'0101	'0201	'5101	'5703	'1601	'0701	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
86	'0301	'3601	'4402	'0401	'0501		'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
87	'0301	'6802	'4403	'5301	'0401	'0401	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
88	'0201	'0301	'3502	'4402	'0401	'0704	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1
89	'0201	'0301	'3901	'0702	'0701	'0702	'1	'0	'1	'1	'0	0	'0	'0	'0	'1	'0	'1	'1
90	'0201	'1101	'3503	'4001	'0304	'0401	'1	'0	'1	'1	'0	0	'0	'0	'0	'1	'0	'1	'1
91	0101	6802	1510	5802	0304	0602	'1	'0	'1	'1	'0	'0	'0	'0	'0	'1	'0	'1	'1

Suppl. Table 2. Vpu alignments

	E	M	G	H	H	A	P	W	D	V	D
1	.	.	.	.	.	.	.	.	.	I	.
2	.	L	.	.	D	.	.	.	.	I	N
3	.	.	.	.	L	.	.	.	.	.	N
4	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	.	.	.	.	I	.
6	.	.	.	.	.	.	.	.	.	I	.
7	.	R	.	.	L	.	.	.	.	.	.
8	.	.	.	R	L	V	.	R	N	.	.
9	.	.	.	.	.	.	.	.	.	.	A
10	P	.	.	N	.	.	.	.	.	.	.
11	.	.	.	.	D	.	.	.	V	I	.
12	.	.	.	.	.	.	.	.	.	I	N
13	D	.	.	.	.	.	.	.	.	.	N
14	.	.	.	.	.	.	.	.	.	.	N
15	D	.	.	.	.	.	.	.	.	.	N
16	.	.	.	.	.	.	.	.	.	.	.
17	.	.	.	.	D	.	.	.	.	.	N
18	.	.	.	.	.	.	.	.	.	.	.
19	.	.	.	.	D	.	.	.	V	.	N
20	.	.	.	.	.	.	.	G	.	I	N
21	.	.	.	.	.	.	.	.	.	I	.
22	A	.	.	.	.	.	.	.	.	I	.
23	.	.	.	.	D	.	.	.	.	.	N
24	.	.	.	.	.	.	.	.	P	I	.
25	.	.	.	.	.	.	.	.	.	I	N
26	.	.	.	.	.	.	.	.	.	I	.
27	.	.	.	.	.	.	.	.	.	I	.
28	.	.	.	.	.	.	.	.	.	.	N
29	.	.	.	.	.	.	.	.	.	.	N
30	.	R	.	.	L	T	.	.	.	I	.
31	.	.	.	.	.	.	.	.	.	.	.
32	.	.	.	.	L	I	.	G	A	I	N
33	-	-	-	.	.	.	.	-	.	.	.
34	.	.	.	.	.	.	.	.	.	.	.
35	.	.	.	.	.	.	.	.	.	.	A
36	H	Q	A	.	.	.	.	.	.	I	.
37	.	.	.	.	.	.	.	.	.	.	N
38	.	.	.	.	.	.	.	.	.	.	N
39	.	.	.	.	.	.	.	.	N	.	N
40	.	.	.	.	.	.	.	.	.	.	N
41	.	.	.	.	.	.	.	.	N	.	.
42	.	.	.	.	.	.	.	.	V	.	.
43	.	.	.	.	.	.	.	.	.	.	N
44	.	.	.	.	.	.	.	.	N	.	.
45	.	.	.	.	D	.	.	.	.	.	.
46	P	.	.	.	.	.	.	.	.	.	N
47	D	.	.	P	.	D	L	.	N	I	N
48	.	.	.	.	R	.	.	G	.	I	N
49	.	.	.	.	.	.	.	.	.	I	.
50	.	.	.	.	.	.	.	.	.	I	.
51	D	.	.	.	.	.	.	.	.	.	.
52	.	V	.	.	.	.	.	.	.	.	.
53	.	R	.	.	L	.	.	.	.	.	N
54	.	R	.	.	L	.	.	.	.	I	.
55	.	.	.	.	.	.	.	.	.	.	N
56	.	R	.	.	L	.	.	.	N	I	.
57	.	.	.	R	L	.	.	.	.	I	N
58	.	.	.	.	.	.	.	.	.	.	N
59	.	.	.	Y	.	.	.	.	.	I	.
60	.	.	.	.	.	.	.	.	.	.	N
61	.	R	.	.	L	.	.	.	.	.	.
62	.	R	.	.	L	.	.	.	.	.	N
63	.	R	.	.	L	.	.	.	.	.	N
64	.	.	.	.	.	.	.	.	.	.	N
65	.	R	.	.	L	.	.	.	.	I	.
66	.	R	.	.	L	.	.	.	.	.	N
67	.	.	.	.	D	.	.	.	.	.	.
68	.	.	.	.	D	.	.	.	.	.	.
69	.	R	.	.	L	.	.	.	N	I	.
70	.	.	.	.	.	.	.	.	.	.	N
71	H	Q	A	.	D	.	.	.	.	.	.
72	.	.	.	.	L	.	.	.	.	I	.
73	.	R	.	.	.	.	.	.	.	.	.
74	.	R	.	.	L	.	.	.	.	I	N
75	H	N	A	.	.	.	.	.	.	I	.
76	.	.	.	.	D	.	L	.	V	.	N
77	.	.	.	.	L	.	L	G	.	.	N
78	.	R	.	.	L	.	.	.	.	.	N
79	.	R	.	.	L	.	.	.	.	.	N
80	.	.	.	.	.	.	.	.	.	I	.
81	.	R	.	.	L	.	.	.	N	.	.
82	.	.	.	.	D	.	.	.	.	.	N
83	.	R	.	.	L	.	.	.	.	.	N
84	.	.	.	.	.	D	.	G	.	.	.
85	.	R	.	.	L	.	.	R	.	I	.
86	D	.	E	Q	.	.	L	L	L	I	.
87	.	.	.	.	.	.	.	.	.	.	N
88	.	.	.	.	L	.	.	.	.	.	N
89	.	.	.	.	L	.	.	.	.	.	.
90	M	G	.	.	D	.	.	.	.	.	N
91	.	.	.	.	.	.	.	.	.	.	N

Suppl. Table 3. Env Alignments

	W	R	W	G	T	M	L	L	G	M	L
1	.	K	.	.	I	.	.	.	.	T	.
2	.	S	.	.	.	.	.	.	.	I	.
3	.	.	.	.	I	L	.	.	.	.	.
4	.	.	.	.	I	T	.	.	.	.	.
5	.	.	.	.	I	.	.	.	.	I	.
6	.	.	.	.	.	.	.	.	.	T	.
7	.	K	G	.	I	L	.	.	.	.	.
8	.	.	.	.	A	L	F	.	.	.	.
9	.	.	.	.	I	.	.	.	.	.	.
10	L	.	.	.	.	.	.	.	.	.	.
11	.	.	.	.	M	.	.	.	.	L	.
12	.	.	.	.	.	.	.	.	.	I	.
13	G	I	.	.	.	.	.	.	.	.	.
14	.	K	.	.	I	.	.	.	.	.	.
15	G	I	.	.	.	.	.	.	.	.	.
16	.	.	.	.	.	.	.	.	.	.	.
17	.	.	.	.	M	.	.	.	.	.	.
18	.	K	.	.	.	.	.	.	.	.	.
19	.	.	.	.	.	.	.	.	.	L	.
20	.	.	.	.	I	.	.	.	E	I	.
21	.	K	.	.	.	.	.	.	.	I	.
22	.	.	.	.	.	.	.	.	.	I	.
23	.	K	.	.	M	.	.	.	.	.	.
24	.	.	.	.	.	.	.	.	.	L	.
25	.	.	.	.	.	.	.	.	.	I	.
26	.	K	.	.	I	.	.	.	.	T	.
27	.	.	.	.	.	.	.	.	.	I	.
28	.	K	.	.	.	.	.	.	.	.	.
29	.	K	.	.	I	.	.	.	.	.	.
30	.	K	G	.	I	L	.	.	.	I	.
31	.	.	.	.	I	.	.	.	.	.	.
32	G	.	.	.	I	L	F	.	.	L	.
33	.	.	.	.	.	.	.	.	.	.	.
34	.	K	.	.	.	.	.	.	.	.	.
35	.	.	.	.	I	.	.	.	.	.	.
36	G	T	R	L	.	.	.	.	.	I	.
37	.	.	.	.	.	.	.	.	.	.	.
38	.	.	.	.	I	.	.	.	.	.	.
39	.	.	.	.	I	.	.	.	.	.	.
40	.	.	.	.	I	.	.	.	.	.	.
41	.	.	.	.	.	.	.	.	.	.	.
42	.	.	.	.	I	.	.	.	.	L	.
43	.	K	.	.	.	.	.	.	.	.	.
44	.	.	.	.	I	.	.	.	.	.	.
45	.	.	.	.	M	.	.	.	.	.	.
46	L	Q	.	.	.	.	.	.	.	.	.
47	.	T	.	.	L	.	I	F	.	I	.
48	.	.	.	.	.	V	.	.	.	I	.
49	.	.	.	.	.	.	.	.	.	I	.
50	.	K	.	.	.	.	.	.	.	I	.
51	R	T	.	.	.	.	.	.	.	.	.
52	.	.	.	.	.	.	.	.	.	.	.
53	.	K	G	.	.	L	.	.	.	.	.
54	.	.	G	.	.	L	.	.	.	I	.
55	.	.	.	.	.	.	.	.	.	.	.
56	.	.	G	.	.	L	.	.	.	I	.
57	.	.	.	.	V	L	.	.	.	I	.
58	.	K	.	.	I	.	.	.	.	.	.
59	.	K	.	.	I	.	.	.	.	I	.
60	.	.	.	.	I	.	.	.	.	.	.
61	.	.	G	.	.	L	.	.	.	.	.
62	.	.	G	.	.	L	.	.	.	.	.
63	.	.	G	.	.	L	.	.	.	.	.
64	.	.	.	.	I	.	.	.	.	.	.
65	.	K	G	.	I	L	.	.	.	I	.
66	.	.	G	.	.	L	.	.	.	.	.
67	.	K	.	.	.	.	.	.	.	.	.
68	.	.	.	.	M	.	.	.	.	.	.
69	.	.	G	.	.	L	.	.	.	I	.
70	.	.	.	.	I	.	.	.	.	.	.
71	G	T	R	L	M	.	.	.	.	.	.
72	.	.	.	.	I	L	.	.	.	I	.
73	.	.	G	.	.	.	.	.	.	.	.
74	.	.	G	.	I	L	.	.	.	I	.
75	G	T	M	L	.	.	.	.	.	I	.
76	.	.	.	.	M	.	.	F	.	L	.
77	.	K	.	.	.	L	.	.	.	.	.
78	.	.	G	.	.	L	.	.	.	.	.
79	.	K	G	.	I	L	.	.	.	.	.
80	.	K	.	.	I	.	.	.	.	I	.
81	.	.	G	.	I	L	.	.	.	.	.
82	.	K	.	.	.	.	.	.	.	.	.
83	.	K	G	.	I	L	.	.	.	.	.
84	.	.	.	.	.	.	I	.	.	.	.
85	.	K	G	.	I	L	.	.	.	I	.
86	.	T	.	S	S	.	.	F	C	L	.
87	.	.	.	.	.	.	.	.	.	.	.
88	.	.	.	.	I	L	.	.	.	.	.
89	.	.	.	.	I	L	.	.	.	.	.
90	K	W	G	.	M	.	.	.	.	.	.
91	.	.	.	.	.	.	.	.	.	.	.

Suppl. Table 4. Nef Alignments

	K	W	S	K	R	S	V	V	G	W	P
1	.	.	.	.	.	.	I	.	.	.	.
2	.	.	.	.	N	.	I	.	.	.	.
3	.	.	.	.	S	.	.	T	.	.	.
4	.	.	.	.	S	.	I	.	.	.	.
5	.	.	.	.	.	.	E	P	.	.	F
6	.	.	.	.	P	.	F	P	.	.	A
7	.	.	.	.	S	.	.	.	.	.	.
8	N	.	.	.	.	.	M	P	.	.	S
9	.	.	.	.	-	E	-	-	.	.	.
10	G	.	A	A	.	E	R	M	Q	R	A
11	.	.	.	.	S	.	.	.	.	.	S
12	.	.	.	.	S	.	.	.	.	.	.
13	.	.	.	.	S	.	.	.	.	.	.
14	.	.	.	.	P	R	.	P	.	.	S
15	.	.	.	.	S	.	.	.	.	.	.
16	.	.	.	.	.	.	M	G	.	.	S
17	.	.	.	.	S	.	.	.	.	.	.
18	.	.	.	.	-	N	M	C	.	.	.
19	.	.	.	.	S	.	.	.	.	.	.
20	.	.	.	.	.	.	E	N	.	.	S
21	.	.	.	.	-	C	M	S	.	.	.
22	.	.	.	.	S	.	K	.	.	.	.
23	.	.	.	.	S	.	L	.	.	.	.
24	.	.	.	.	.	.	M	G	.	.	S
25	.	.	.	.	S	.	I	.	.	.	.
26	.	.	.	.	.	.	.	P	.	.	T
27	.	.	.	.	.	.	R	D	.	.	S
28	.	.	.	.	S	.	.	.	.	.	S
29	.	.	.	.	.	.	.	P	.	.	S
30	.	L	.	.	.	-	M	R	.	.	A
31	.	.	.	.	S	.	M	G	.	.	S
32	.	.	.	.	.	.	A	P	.	.	S
33	.	.	.	.	-	-	L	S	.	.	N
34	.	.	.	.	.	K	E	N	.	.	S
35	.	.	.	.	E	.	K	E	E	.	.
36	.	C	.	.	.	-	M	P	.	.	.
37	.	L	.	.	.	.	A	P	.	.	S
38	.	-	G	S	K	L	S	K	.	.	Q
39	.	-	G	S	K	L	S	K	.	.	Q
40	.	C	.	.	.	.	-	.	E	.	N
41	.	.	.	.	-	.	I	.	.	.	.
42	.	.	.	.	C	.	P	I	.	.	.
43											S
44	.	.	.	.	M	.	-	-	.	.	.
45	.	.	.	.	-	C	-	S	.	.	.
46	.	.	.	.	.	.	M	S	.	.	.
47	.	.	.	.	S	.	I	.	.	.	.
48	.	.	.	.	.	.	G	.	.	.	.
49	.	.	.	.	S	K	I	F	.	.	S
50	.	.	.	.	S	.	.	I	.	.	.
51	.	.	.	.	S	.	.	.	.	.	.
52	.	.	.	.	S	.	.	.	.	.	.
53	.	.	.	.	S	K	M	F	.	.	S
54	.	.	.	.	.	-	M	A	.	.	.
55	.	.	.	.	S	.	.	.	.	.	.
56	.	.	.	.	.	.	.	P	.	.	.
57	.	.	.	.	.	.	G	G	.	.	E
58	.	.	.	.	C	.	L	.	.	.	.
59	.	.	.	.	H	P	G	S	.	.	A
60	.	.	.	.	.	.	.	T	E	.	.
61	.	.	.	.	S	K	.	A	.	.	S
62	.	.	.	.	S	K	M	G	.	.	S
63	.	.	.	.	S	.	I	.	.	.	.
64	.	.	.	.	S	R	G	F	.	.	Q
65	.	.	.	.	.	-	L	G	.	.	.
66	.	.	.	.	S	K	M	G	.	.	S
67	.	.	.	.	S	.	M	A	K	.	.
68	.	.	.	.	.	.	L	G	.	.	.
69	.	.	.	R	S	I	G	A	.	.	S
70	.	.	.	.	.	.	R	D	.	.	Q
71	.	.	.	.	N	K	I	.	D	.	.
72	.	.	.	.	S	I	G	S	.	.	S
73	.	.	.	.	S	.	K	I	.	.	.
74	.	.	.	.	Y	.	-	-	.	.	A
75	.	C	.	.	.	-	M	P	.	.	.
76	.	.	.	.	G	G	L	G	K	.	.
77	.	.	.	.	S	W	P	A	V	R	E
78	.	.	.	.	-	-	F	S	.	.	A
79	.	.	.	.	.	K	A	E	.	.	S
80	.	.	.	.	S	.	.	.	.	.	A
81	.	.	.	.	.	.	-	-	Q	.	S
82	.	.	.	.	-	.	K	L	.	.	.
83	.	.	.	.	-	-	F	C	.	.	.
84	.	.	.	.	.	G	.	P	.	.	.
85	.	.	.	.	.	.	N	K	D	.	.
86	.	.	.	.	.	.	.	P	.	.	.
87	.	.	.	.	.	K	R	D	.	.	S
88	.	.	.	.	S	.	.	.	.	.	.
89	.	.	.	.	.	.	K	I	.	.	.
90	.	.	.	.	S	.	.	.	.	.	.
91	.	.	.	.	N	K	I	P	.	.	.

Suppl. Table 5. Gag Alignments

	P	I	V	Q	N	L	Q	G	Q	M	V
1	.	.	.	.	.	M	.	.	.	.	.
2	.	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	.	.	.	.	.	.	.
6	.	.	.	.	.	M	.	.	.	.	I
7	.	.	.	.	.	.	.	.	.	.	.
8	.	.	.	.	.	M	.	.	.	.	.
9	.	.	.	.	.	.	.	.	.	.	.
10	.	.	.	.	.	.	.	.	.	.	.
11	.	.	.	.	.	.	.	.	.	.	.
12	.	.	.	.	.	.	.	.	.	.	.
13	.	.	.	.	.	.	.	.	.	.	.
14	.	.	I	.	.	.	.	.	.	.	.
15	.	.	.	.	.	.	.	.	.	.	.
16	.	.	.	.	.	.	.	.	.	.	.
17	.	.	.	.	.	M	.	.	.	.	.
18	.	.	.	.	.	.	.	.	.	.	.
19	.	.	.	.	.	.	.	.	.	.	.
20	.	.	.	.	.	.	.	.	.	.	.
21	.	.	.	.	.	.	.	.	.	.	.
22	.	V	.	.	.	M	.	.	.	.	.
23	.	.	.	.	.	.	.	.	.	.	.
24	.	V	.	.	.	.	.	.	.	.	.
25	.	.	.	.	.	M	.	.	.	.	.
26	.	.	.	.	.	M	.	.	.	.	.
27	.	.	.	.	.	.	.	.	.	.	.
28	.	.	.	.	.	M	.	.	.	.	.
29	.	.	.	.	.	.	.	.	.	.	.
30	.	.	.	.	.	.	.	.	.	.	.
31	.	.	.	.	.	M	.	.	.	.	.
32	.	.	.	.	.	M	.	.	.	.	.
33	.	.	.	.	.	.	.	.	.	.	.
34	.	.	.	.	.	M	.	.	.	.	.
35	.	.	.	.	.	.	.	.	.	.	.
36	.	.	.	.	.	.	.	.	.	.	.
37	.	.	.	.	.	V	.	.	.	.	.
38	.	.	.	.	.	.	.	.	.	.	.
39	.	.	.	.	.	.	.	.	.	.	.
40	.	.	.	.	.	.	.	.	.	.	.
41	.	.	.	.	.	.	.	.	.	.	.
42	.	.	.	.	.	.	.	.	.	.	.
43	.	.	.	.	.	M	.	.	.	.	.
44	.	.	.	.	.	.	.	.	.	.	.
45	.	.	.	.	.	V	.	.	.	.	.
46	.	.	.	.	.	.	.	.	.	.	.
47	.	.	.	.	.	A	.	.	.	.	.
48	.	.	.	.	.	I	.	.	.	.	.
49	.	.	.	.	.	M	.	.	.	.	.
50	.	.	.	.	.	.	.	.	.	.	.
51	.	.	.	.	.	.	.	.	.	.	.
52	.	.	.	.	.	.	.	.	.	.	.
53	.	.	.	.	.	M	.	.	.	.	.
54	.	.	.	.	.	.	.	.	.	.	.
55	.	.	.	.	.	.	.	.	.	.	.
56	.	.	.	.	.	.	.	.	.	.	.
57	.	.	.	.	.	.	.	.	.	.	.
58	.	.	.	.	.	I	.	.	.	.	.
59	.	.	.	.	.	V	.	.	.	.	.
60	.	.	.	.	.	I	.	.	.	.	.
61	.	.	.	.	.	M	.	.	.	.	.
62	.	.	.	.	.	.	.	.	.	.	.
63	.	.	.	.	.	M	.	.	.	.	.
64	.	.	.	.	.	.	.	.	.	.	.
65	.	.	.	.	.	M	.	.	.	.	.
66	.	.	.	.	.	.	.	.	.	.	.
67	.	.	.	.	.	A	.	.	.	.	.
68	.	.	.	.	.	I	.	.	.	.	.
69	.	.	.	.	.	.	.	.	.	.	.
70	.	.	.	.	.	M	.	.	.	.	.
71	.	.	.	.	.	.	.	.	.	.	.
72	.	.	.	.	.	I	.	.	.	.	.
73	.	.	.	R	.	.	.	.	.	.	.
74	.	.	.	.	.	.	.	.	.	.	.
75	.	.	.	.	.	.	.	.	.	.	.
76	.	.	.	.	.	.	.	.	.	.	.
77	.	.	.	.	.	.	.	.	.	.	.
78	.	.	.	.	.	M	.	.	.	.	.
79	.	.	.	.	.	.	.	.	H	.	
80	.	.	.	.	.	.	.	.	.	.	.
81	.	.	.	.	.	.	.	.	.	.	.
82	.	.	.	.	.	I	.	.	.	.	.
83	.	.	.	.	.	.	.	.	.	.	.
84	.	.	.	.	.	.	.	.	.	.	.
85	.	.	.	.	.	.	.	.	.	.	.
86	.	.	.	.	.	.	.	.	.	.	.
87	.	.	.	.	.	I	.	.	.	.	.
88	.	.	.	.	.	M	.	.	.	.	.
89	.	.	.	.	.	I	.	.	.	.	.
90	.	.	.	.	.	A	.	.	.	.	.
91	.	.	.	.	.	.	.	.	.	.	.

Supplemental Table 6. KIR and HLA class I genotypes of Healthy Donors

	2DL1	2DL2	2DL3	2DL4	2DL5	2DS1	2DS2	2DS3	2DS5	3DL1	3DS1	3DL2	3DL3	HLA-A1	HLA-A2	HLA-B1	HLA-B2	HLA-C1	HLA-C2	C group	
A	1	1	1	1	0	0	1	0	0	1	0	1	1	0301	6802	0702	1402	0702	0802	C1	C1
B	1	1	1	1	0	0	1	0	0	1	0	1	1	0201	0201	1801	3901	0501	1203	C2	C1
C	1	1	1	1	1	0	1	1	0	1	0	1	1	0201	2402	3502	3931	0401	1203	C2	C1
D	1	1	1	1	0	0	1	0	0	1	0	1	1	0301	2402	0702	3906	0702	0702	C1	C1
E	1	1	1	1	1	0	1	1	0	1	0	1	1	0301	3201	0702	5001	0602	0702	C2	C1
F	1	1	1	1	0	0	1	0	0	1	0	1	1	2902	6802	1402	2705	0202	0804	C2	C1
G	1	1	1	1	0	0	1	0	0	1	0	1	1	0301	3004	1801	5108	1203	1602	C1	C2
H	1	1	1	1	1	0	1	1	0	1	0	1	1	0205	6801	3503	4901	0401	0701	C2	C1
I	1	1	1	1	0	0	1	0	0	1	0	1	1	0101	0201	0702	4102	0602	0701	C2	C1
J	1	1	1	1	1	1	1	0	1	1	0	1	1	0201	0302	3508	5201	0401	1202	C2	C1
K	1	1	1	1	1	0	1	1	0	1	0	1	1	0101	1101	3501	3701	0401	0701	C2	C1
L	1	1	1	1	0	0	1	0	0	1	0	1	1	2902	3101	4402	4501	0602	0704	C2	C1
M	1	1	0	1	1	1	1	1	0	1	0	1	1	0201	0201	0702	4102	1203	1602	C1	C2
N	1	1	1	1	0	0	1	0	0	1	0	1	1	2601	3101	2705	4901	0102	0701	C1	C1
O	1	1	1	1	1	1	1	0	1	1	0	1	1	0101	2402	3501	3501	0401	0701	C2	C1
P	1	0	1	1	0	0	0	0	0	1	0	1	1	0101	0201	0801	3501	0401	0701	C2	C1
Q	1	0	1	1	0	0	0	0	0	1	0	1	1	0201	2601	1501	2705	0102	0303	C1	C1
R	1	0	1	1	0	0	0	0	0	1	0	1	1	2501	3101	1401	1801	0802	1203	C1	C1
S	1	0	1	1	0	0	0	0	0	1	0	1	1	0205	2601	3801	5001	0602	1203	C2	C1
T	1	0	1	1	0	0	0	0	0	1	0	1	1	0301	3201	0801	3508	0401	0701	C2	C1
U	1	0	1	1	0	0	0	0	0	1	0	1	1	0201	1101	1501	3501	0303	0401	C1	C2
V	1	0	1	1	0	0	0	0	0	1	0	1	1	0201	0206	4011	4402	0304	0501	C1	C2
W	1	0	1	1	0	0	0	0	0	1	0	1	1	1101	2402	1301	1525	0304	0403	C1	C2
X	1	0	1	1	0	0	0	0	0	1	0	1	1	0201	0206	4011	4402	0304	0501	C1	C2
Y	1	0	1	1	0	0	0	0	0	1	0	1	1	0201	3303	1501	5801	0302	0801	C1	C1
Z	1	0	1	1	0	0	0	0	0	1	0	1	1	0201	0201	0702	5601	0102	0702	C1	C1
AA	1	0	1	1	0	0	0	0	0	1	0	1	1	0101	0301	0702	1501	0304	0702	C1	C1
AB	1	0	1	1	0	0	0	0	0	1	0	1	1	0201	3002	0702	2705	0102	0702	C1	C1
AC	1	0	1	1	0	0	0	0	0	1	0	1	1	0101	2601	0702	0801	0701	0702	C1	C1
AD	1	0	1	1	0	0	0	0	0	1	0	1	1	0101	0201	1801	5101	0602	1402	C2	C1