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Inhibitory killer-cell immunoglobulin-like receptors strengthen CD8$^+$ T cell-mediated control of HIV-1, HCV and HTLV-1

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One sentence summary: Inhibitory killer-cell immunoglobulin-like receptors (iKIRs) help maintain the CD8$^+$ T cell response to chronic viral infections; in contrast to many reported iKIR-disease associations, these observations are remarkably wide-ranging, being seen for all iKIRs and all 3 viral infections studied.

Abstract

Killer-cell immunoglobulin-like receptors (KIRs) are expressed predominantly on natural killer cells, where they play a key role in the regulation of innate immune responses. Recent studies show that inhibitory KIRs can also impact adaptive T cell-mediated immunity. In mice and in human T cells *in vitro*, inhibitory KIR ligation enhanced $CD8^+$ T cell survival. To investigate the clinical relevance of these observations, we conducted an extensive immunogenetic analysis of multiple, independent cohorts of HIV-1, hepatitis C virus (HCV) and human T cell leukemia virus (HTLV-1)-infected individuals in conjunction with *in vitro* assays of T cell survival, analysis of *ex vivo* KIR expression and mathematical modeling of host-virus dynamics. Our data suggest that functional engagement of inhibitory KIRs enhances the $CD8^+$ T cell response against HIV-1, HCV and HTLV-1 and is a significant determinant of clinical outcome in all three viral infections.

Introduction

Killer-cell immunoglobulin-like receptors (KIRs) are a family of transmembrane glycoproteins with activating and inhibitory isoforms; their ligands include the HLA class I molecules (*1, 2*). KIRs are expressed predominantly on natural killer (NK) cells and are primarily associated with innate NK cell-mediated immunity. However, recent studies have shown that KIRs can also impact adaptive T cell-mediated immunity.

Two distinct routes whereby inhibitory KIRs (iKIRs) affect T cell responses have been described (*3, 4*). First, iKIR expression on $CD8^+$ T cells directly affects their longevity and function. KIRs are expressed on T cells with an effector memory TEM(RA) phenotype ($CD28^-$ $CD45RA^{+/-}CD45RO^{-/+}CCR7^-$); the frequency of KIR^+ T cells increases with age and some persistent viral infections (*5-9*). In mice, inhibitory Ly49 receptors (the functional homolog of iKIRs) enhance $CD8^+$ T cell survival (*10*). Similarly, in KIR-transgenic mice, ligation of iKIRs on T cells reduces activation-induced death (*11, 12*). Furthermore, iKIR expression on human T cells is associated with higher levels of the survival molecule Bcl-2, reduced cell death and impaired functionality (*13-15*). Second, KIRs indirectly modify the $CD8^+$ T cell response via their expression on NK cells. NK cells regulate T cell immunity by cytokine secretion and killing activated $CD4^+$ and $CD8^+$ T cells; this regulation may be modified by the expression of inhibitory receptors on NK cells (*4, 16, 17*). In mice, lymphocytic choriomeningitis virus-specific $CD8^+$ T cell responses are modulated via NK cell mediated-killing of activated $CD4^+$ T cells (*18*) and in murine cytomegalovirus infection, IL-10 production by NK cells impairs the $CD8^+$ T cell response (*19*). Additionally, human NK cells can lyse hepatitis B virus-specific $CD8^+$ T cells *in vitro* (*20*).

The clinical relevance of an interaction between iKIRs and $CD8^+$ T cells is difficult to infer from these earlier studies, because it is impossible to tell whether changes in $CD8^+$ T cell lifespan in mice or *in vitro* alter the course of human disease *in vivo*. To investigate whether iKIRs have a clinically significant impact on the $CD8^+$ T cell response, our starting point was the well-documented association between certain HLA class I alleles and disease outcome as this represents compelling evidence that $CD8^+$ T cells are clinically relevant. We reasoned that if KIRs significantly affect the $CD8^+$ T cell response then KIRs will affect HLA class I disease

associations. In a previous study, we validated this approach by showing that *KIR2DL2* exaggerates both protective and detrimental HLA class I associations with disease progression in individuals infected with hepatitis C virus (HCV) or human T cell leukemia virus type 1 (HTLV-1) (*21*). However, it remains unclear whether this constitutes a generalizable phenomenon that extends to other viruses and iKIRs. Furthermore, the mechanism underlying the effect was not investigated.

In this study we used epidemiological data from multiple, independent cohorts of HIV-1, HCV and HTLV-1-infected individuals; *in vitro* T cell survival assays; *ex vivo* analyses of KIR expression and mathematical modelling of host-virus dynamics. Our data indicate that both two-domain and three-domain iKIRs, together with their HLA class I ligands, enhance the $CD8^+$ T cell response to HIV-1, HTLV-1 and HCV by prolonging $CD8^+$ T cell survival and are a significant determinant of clinical outcome in all three viral infections.

Results

We studied a well-characterized cohort of HIV-1 seroconverters from sub-Saharan Africa who were identified when seronegative and followed under Protocol C of the International AIDS Vaccine Initiative (IAVI) (*22*). First we sought to identify KIR-HLA associations that could potentially confound our study. We define a "functional KIR gene" as a KIR gene together with a gene encoding its HLA class I ligand. Both functional *KIR3DS1* (*KIR3DS1* with the gene encoding its putative ligand HLA-Bw4-80I) and functional *KIR3DL1* (*KIR3DL1* with the gene for its ligand HLA-Bw4) have previously been associated with good NK cell-mediated control of HIV-I infection (*23*). In the IAVI cohort, the protective effect of functional *KIR3DS1,* but not functional *KIR3DL1* was replicated, **Table S1**. To prevent confounding effects arising from strong linkage disequilibrium between the KIR genes we therefore excluded all individuals with functional *KIR3DS1*. As expected, *HLA-B*57* was significantly associated with low early set-point viral load (Coefficient = –0.42, P = 0.004) and slow progression to low CD4 count (Hazard Ratio = 0.44, P = 0.02). Where the Coefficient (Coeff) is the change in $\log_{10}$[early viral load set point] associated with possession of *HLA-B*57* and the Hazard Ratio (HR) is the relative risk of progression to low CD4 count associated with possession of *HLA-B*57*.

iKIR genes are associated with an enhancement of the protective effect of *HLA-B*57*

To determine whether *HLA-B*57* was more protective in the presence of functional *KIR2DL2,* we stratified the cohort into individuals with functional *KIR2DL2* and without functional *KIR2DL2* and analyzed the protective effect of *HLA-B*57* in each stratum. Two definitions of functional *KIR2DL2* were considered: (i) strong functional *KIR2DL2* (*KIR2DL2* with genes encoding its strong HLA-C1 ligand); and (ii) weak functional *KIR2DL2* (*KIR2DL2* with genes encoding its weaker HLA-C2 ligand). For both early set-point viral load and time to low CD4 count, *HLA-B*57* was significantly protective in the stratum with functional *KIR2DL2* but weaker and non-significant in its absence; additionally, the magnitude of the protection was greater with the stronger ligand (**Fig. 1A, Table 1**). These differences were not attributable to cohort size because there were fewer *HLA-B*57*$^{+}$ individuals in the stratum with functional *KIR2DL2* than in the stratum without functional *KIR2DL2*. We then extended the analysis to other iKIRs with known ligands (*KIR2DL1* with *HLA-C2* and *KIR2DL3* with *HLA-C1*). Functional *KIR3DL1* could not be analysed because there were no individuals with *HLA-B*57* who lacked

functional *KIR3DL1*. iKIRs with disputed ligands were excluded from the analysis. Both functional *KIR2DL1* and functional *KIR2DL3* enhanced the protective effect of *HLA-B*57* on both early set-point viral load and time to low CD4 count (**Fig. 1A, Table 1**). The odds of seeing this enhancement by chance are $P = 2x10^{-6}$ (Permutation test).

Having investigated the effect of the presence or absence of individual functional iKIR we next investigated the effect of the number of functional iKIRs. For each subject in the cohort we assigned two numbers: (i) a 'count of functional iKIR' (a count of the number of iKIR+ligand pairs); and (ii) an 'inhibitory score' (similar to the count but weighted to reflect the strength of iKIR signalling; **Methods**). We first stratified the cohort based on the inhibitory score (all cut-offs that gave balanced strata were considered) and analyzed the strength of *HLA-B*57*-mediated protection in each stratum. Again, we found that the protective effect of *HLA-B*57* on both early set-point viral load and progression to low CD4 count was enhanced in the high inhibitory score stratum and that the size of the protective effect increased with the magnitude of the score (**Fig. 1A & B, Table 1**). Repeating the analysis using the count of functional iKIRs gave similar results indicating that our findings were not dependent on the choice of scoring system. Within *HLA-B*57*$^{+}$ individuals (but not *HLA-B*57*$^{-}$ individuals), both the inhibitory score and the count of functional iKIRs were significantly associated with a decrease in early set-point viral load (**Fig. 1C**). This result indicates that iKIRs enhance the *HLA-B*57* protective effect in a gene dose-dependent manner and shows that functional iKIRs alone (i.e. not in the context of *HLA-B*57*) are not significantly protective; this latter conclusion was confirmed by multivariate analysis (**Table S2**).

The iKIR-associated enhancement of *HLA-B*57*-mediated protection was wholly dependent on the presence of the KIR ligands. If we counted iKIRs in the absence of ligand, we found no enhancement of *HLA-B*57*-mediated protection (**Fig. S1**).

Repeating the analysis of the impact of iKIRs on *HLA-B*57* using a different approach (introducing a covariate with different levels for *HLA-B*57*$^{-}$, *HLA-B*57*$^{+}$*KIR*$^{-}$ and *HLA-B*57*$^{+}$*KIR*$^{+}$ and performing multivariate regression and Cox survival analysis on the whole, unstratified cohort) yielded identical conclusions: the iKIRs *KIR2DL1*, *KIR2DL2* and *KIR2DL3*

with genes encoding their HLA ligands, a high inhibitory score and a high count of functional iKIRs all enhanced the protective effect of *HLA-B*57* on both outcome measures: early viral load and time to low CD4 count (**Table S3**).

To check that the iKIR-associated enhancement we observed was not driven by leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2) binding (*24*) (which was significantly protective in our cohort, **Table S4**) or by functional *KIR3DL1* (which was not protective in our cohort, **Table S1**, but has been reported elsewhere (*23*)), we included these terms as covariates in our model. In both cases, the conclusions were unchanged (**Table S5**), indicating that functional iKIR enhancement was not attributable to these potentially confounding factors. The conclusions were also unchanged when we included *KIR3DL2* with genes for its classical HLA ligands (HLA-A3, -A11 and -B27) or genes for its classical and non-classical HLA ligands (HLA-A3, -A11, -B27 and -F (*25*)) in the count of functional iKIRs and the inhibitory score.

Which iKIR is responsible for the enhancement of *HLA-B*57*-mediated protection?

The KIR genes are in strong linkage disequilibrium so the enhancement of *HLA-B*57*-mediated protection by functional *KIR2DL1*, functional *KIR2DL2* and functional *KIR2DL3* are not independent. Establishing which iKIR gene(s) was/were associated with the enhancement was difficult because the cohort size limited the power of further sub-stratification. Across the cohort, functional *KIR2DL1* and functional *KIR2DL2* were both negatively associated with functional *KIR2DL3* while functional *KIR2DL1* and functional *KIR2DL2* were unassociated. Given that all three functional iKIR enhanced *HLA-B*57* and none of them were positively associated this suggests that more than one functional iKIR was enhancing *HLA-B*57*-mediated protection (the requirement for ligand makes enhancement via 'absence of a KIR' unlikely). Furthermore, among *HLA-B*57*$^{+}$ subjects, both the count of functional iKIRs and the inhibitory score were better predictors of early viral load than any of the individual functional iKIRs, again suggesting that more than one iKIR was enhancing the protective effect of *HLA-B*57*. Henceforth, because of the difficulty in determining which iKIR(s) were responsible for the enhancement of *HLA-B*57* together with the evidence that more than one iKIR was implicated we focus on the count of iKIRs and the inhibitory score rather than individual iKIR.

Maintenance of the protective effect of *HLA-B*57* depends on iKIRs

Next, we investigated longitudinal viral load in the IAVI cohort (a total of 5197 person-visits between 0–2000 days post-infection). The cohort was stratified in three ways: (i) functional iKIR-negative and –positive; (ii) low and high functional iKIR count; or (ii) low and high inhibitory score. In each case, the local non-parametric regression lines were calculated (**Fig. 2A, Fig. S2**). A consistent picture emerged for each of the three functional iKIRs and for both the iKIR count and inhibitory score. In the presence of a strong inhibitory signal (functional iKIR-positive or a high inhibitory score/count), the protective effect of *HLA-B*57* on viral load was sustained over time. Conversely, in the absence of a strong inhibitory signal (functional iKIR-negative or a low inhibitory score/count), the protective effect of *HLA-B*57* on viral load was weak. Interestingly, in the absence of a strong inhibitory signal (**Fig. 2A**, left panel), *HLA-B*57* appeared to protect at very early time points post-infection, but this protective effect was eroded with time. Bootstrap estimation of the correlation between *HLA-B*57*-mediated protection and time confirmed this observation: the protective effect of *HLA-B*57* decreased significantly more rapidly in people with a low inhibitory score, **Fig. 2B**. These findings are consistent with our hypothesis that a strong inhibitory signal enhances T cell survival.

What is the impact of iKIRs on the detrimental effect of *HLA-B*35Px?*

In the context of HTLV-1 infection, *KIR2DL2* has also been shown to enhance the detrimental effects of the susceptibility allele *HLA-B*54* (*21*). We therefore investigated the impact of iKIRs on the well-established detrimental association between *HLA-B*35Px* and the outcome of HIV-1 infection (*26*). In the IAVI cohort, *HLA-B*35Px* was associated with an increase in set-point viral load (Coeff = +0.35, P = 0.0007); but this effect was not consistently enhanced by iKIRs (**Table S6**). However, when we studied longitudinal viral dynamics, it could again be seen that the detrimental impact of *HLA-B*35Px* on viral load was progressively eroded in individuals with a low inhibitory score and maintained in individuals with a high inhibitory score (**Fig. S3**). This observation explains why iKIR enhancement of the *HLA-B*35Px* effect was not visible in the original outcome measure, early set-point viral load, which focuses on the first 3–9 months post-infection. Furthermore, bootstrap estimates of the correlation between time and the detrimental effect of *HLA-B*35Px* showed that the detrimental effect weakened in individuals with a low inhibitory score but was maintained in individuals with a high

inhibitory score (P=6x10^{-7}, P=5x10^{-9}, P=9x10^{-18} for inhibitory score cut-offs of 2.0, 2.5, 2.75 respectively, **Fig. S3**). *HLA-B*35Px* did not influence the time to low CD4 count in the IAVI cohort (HR = 1.04, P = 0.8), so we did not investigate the interplay between iKIRs and *HLA-B*35Px* for this outcome measure.

Results are replicated in independent cohorts

We considered two independent replication cohorts: IAVI-partners and US.

IAVI-partners. IAVI-partners is a cohort of 315 HIV-1$^+$ individuals from sub-Saharan Africa (the partners of the incident cases in the main IAVI cohort). In this cohort, *HLA-B*57* showed a trend to reduce median viral load (Coeff = −0.33, P = 0.06) and *HLA-B*35Px* was non-significant. Pooling IAVI and IAVI-partners provided a much larger cohort (N = 776). In this pooled cohort, *HLA-B*57* was strongly protective (Coeff = −0.43, P = 0.00003) and *HLA-B*35Px* showed a trend to be detrimental (Coeff = +0.16, P = 0.06). In both the unpooled and the pooled cohorts, the effects of *HLA-B*57* and *HLA-B*35Px* were enhanced in people with a high inhibitory score (**Table 2**). Strikingly, *HLA-B*35Px,* which only showed a trend for susceptibility in the pooled cohort and nothing in the unpooled cohort, was highly significantly detrimental in people with a high inhibitory score.

US. The US is a cohort of 548 HIV-1$^+$ individuals with known seroconversion date. 57.8% of subjects were white, 38.7% African American and 3.5% Hispanic/"Other"; we therefore stratified by ethnicity. Among whites, but not African Americans, both functional *KIR3DS1* (*KIR3DS1*$^+$*Bw4-80I*$^+$) and functional *KIR3DL1* (*KIR3DL1*$^+$*Bw4*$^+$) were protective. We therefore excluded functional *KIR3DS1*$^+$ individuals and included functional *KIR3DL1* as a covariate in the white stratum (exclusion of functional *KIR3DS1*$^+$ individuals was not possible due to their high frequency). Although neither of these steps were necessary in the African American stratum, we repeated the analysis with both steps to check that the weak, insignificant effects of functional *KIR3DS1* or functional *KIR3DL1* were not responsible for our results.

Among whites, 15 *HLA-B*57*$^+$ and 16 *HLA-B*35Px*$^+$ individuals remained after exclusion of functional *KIR3DS1*$^+$ individuals. Stratification of these groups left at most 8 people in each arm, so analysis was not possible. In contrast, among African Americans, there were 31 *HLA-*

*B*57*$^{+}$ and 45 *HLA-B*35Px*$^{+}$ individuals. Analysis showed that both the protective effect of *HLA-B*57* and the detrimental effect of *HLA-B*35Px* on the time to CD4 count < 200 cells/mm^3 were consistently enhanced by functional iKIRs (**Table 2**). On pooling white, Hispanic/"Other" and African Americans (with ethnicity as a covariate), the protective effect of *HLA-B*57* and the detrimental effect of *HLA-B*35Px* were still enhanced, although the enhancement of *HLA-B*57* appeared weaker (**Table 2**) suggesting that the iKIR effect on *HLA-B*57* was largely driven by the African American population. These findings were replicated when high-expressing *KIR3DL1* alleles were included as a covariate (**Table S7**).

We conclude that in HIV-1 infection, in three independent cohorts, the effects of both protective and detrimental HLA alleles on viral load and disease progression are stronger in the presence of genes coding for iKIRs with their ligands.

iKIRs enhance HLA associations with asymptomatic status in HTLV-I infection

We previously reported that *KIR2DL2* enhances HLA class I associations in HTLV-1 infection, but found little evidence for enhancement by other iKIRs (*21*). In contrast, the findings reported here, show that all iKIRs amenable to analysis enhanced *HLA-B*57* and *HLA-B*35Px* disease associations in HIV-1 infection. We therefore re-examined the HTLV-1 data.

We confirmed that, in the HTLV-1-infected Kagoshima cohort, functional *KIR2DL2* strongly enhanced the protective effect of *HLA-C*08* and the detrimental effect of *HLA-B*54* on the odds of the inflammatory disease HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Previously, we reported that, unexpectedly, functional *KIR2DL2* did not enhance the protective effect of *HLA-A*02* (*21*). However, we recently found that only *HLA-A*02:07*, and not the more frequent *HLA-A*02:01* and *HLA-A*02:06*, associates with protection in the Kagoshima cohort (**Table S8**). We therefore repeated the analysis at the level of 4-digit HLA resolution. Although subject numbers were low, we found evidence that *KIR2DL2* did indeed enhance the protective effect of *HLA-A*02:07* (halving the risk of developing HAM/TSP). Investigating the other iKIRs, we found that the low frequency of *HLA-C2* precluded a meaningful analysis of functional *KIR2DL1* (too few functional *KIR2DL1*$^{+}$ individuals) and functional *KIR2DL3* (too few functional *KIR2DL3*$^{-}$ individuals), explaining why we had not

previously seen an effect for these other iKIRs. However, for functional *KIR3DL1* both the positive and negative stratum were of sufficient size to permit analysis and there we found good evidence that functional *KIR3DL1* enhanced both the detrimental effect of *HLA–B*54* and the protective effects of *HLA–A*02:07* and *HLA–C*08*. Furthermore, the effects of *HLA–B*54, HLA–A*02:07* and *HLA–C*08* were, in each case, strongest in individuals with a high inhibitory score and were weak and non-significant in individuals with a low inhibitory score (**Table 3**).

The high inhibitory score stratum was heavily enriched for people with strong functional *KIR2DL2* ($P < 10^{-14}$ cut-off = 1.5, $P < 10^{-15}$ cut-off = 1.75; Chi squared) so the *KIR2DL2* enhancement and high iKIR score enhancement of *HLA–B*54, –A*02:07* and *–C*08* are not independent. However, functional *KIR3DL1* is not a surrogate for functional *KIR2DL2 (P = 0.86* Chi-squared), so the enhancement due to *KIR2DL2* is independent of the enhancement due to *KIR3DL1*. We conclude that, although most iKIRs cannot be analysed (due to the low frequency of *HLA-C2* alleles in Japan) there is evidence that, independent of *KIR2DL2,* another iKIR, *KIR3DL1* and a high inhibitory score enhance HLA class I disease associations in HTLV-1 infection.

iKIRs enhance HLA associations with spontaneous clearance of HCV

We previously reported that functional *KIR2DL2* enhances the protective effect of *HLA-B*57* on spontaneous clearance of hepatitis C virus (HCV). Again, the earlier analysis found no evidence that other iKIRs showed similar behaviour (*21*). Reanalysis of this cohort confirmed the *KIR2DL2* enhancement. It was not possible to analyze any other individual iKIR (insufficient numbers of HLA-*B57*$^{+}$ functional *KIR2DL1*$^{-}$ and HLA-*B57*$^{+}$ functional *KIR3DL1*$^{+}$ individuals; and functional *KIR2DL3* alone is protective (*27*), **Table S2**). We also found a strong enhancement of the *HLA-B*57* effect in people with a high inhibitory score (**Table S9**). However, the high inhibitory score stratum was heavily enriched for people with strong functional *KIR2DL2* for all three cut-offs considered ($P < 2.10^{-16}$ in each case; Chi squared) and so the functional *KIR2DL2* enhancement and strong iKIR enhancement of *HLA-B*57* are not independent. We conclude that functional *KIR2DL2* and a high inhibitory score enhance the

protective effect of *HLA-B*57* in HCV infection but it is not possible to test whether or not other iKIRs show the same behaviour.

Primary HLA class I associations are unlikely to be attributable to NK cells

In theory, an HLA class I disease association could be attributable to the role of these molecules as recognition targets for either CD8$^+$ T cells (via somatically rearranged T cell antigen receptors) or NK cells (via germline-encoded KIRs). If a given HLA association is attributable to NK cells rather than CD8$^+$ T cells, then other HLA class I molecules with similar KIR-binding properties should be similarly protective/detrimental (in the context of the relevant KIRs). This pretext forms the basis of classical KIR-HLA analyses (*28-31*). We investigated this possibility for each of the HLA class I alleles included in this study (*HLA-B*57* and *HLA-B*35Px* in the HIV-1-infected IAVI cohort, *HLA-A*02:07*, *HLA-C*08* and *HLA-B*54* in the HTLV-1-infected Kagoshima cohort, and *HLA-B*57* in the HCV-infected cohort). The results are detailed in the **Supplementary Material** & **Table S10**. We found no evidence to suggest that any of the HLA class I associations could be explained by a ligand preference for certain KIRs. This is consistent with existing evidence that these HLA class I associations are attributable to CD8$^+$ T cells (*32-37*). Furthermore, simply considering iKIR:ligand pairs (without also considering the protective or detrimental HLA class I alleles) finds no significant protective or detrimental effect of any individual iKIR:ligand or of the count of functional iKIR or of the Inhibitory score in HIV-1, HTLV-1 or HCV (**Table S2**).

iKIR ligation enhances CD8$^+$ T cell survival *in vitro*

Studies in mice and *in vitro* have shown that inhibitory NK receptors on T cells reduce activation-induced cell death (*10-12*). An iKIR-mediated enhancement of CD8$^+$ T cell survival may therefore explain our immunogenetic findings. However, whilst our immunogenetics findings show presence of the HLA ligand is crucial (**Fig. S1**), the role of ligand in the *in vitro* survival studies is less clear. Two studies reported that iKIRs are associated with CD8$^+$ T cell survival independent of ligand (*13, 38*), while a third indicated that KIR-ligation is required for the increased survival (*15*). To clarify and extend this work we investigated the impact of blocking iKIR ligation on CD8$^+$ T cell survival *in vitro*. Three iKIR-expressing CD8$^+$ T cell lines were established: two expressing KIR2DL3 and one expressing KIR3DL1, (**Fig. S4**). The lines were co-cultured with the HLA class-I-deficient B cell line 721:221 (henceforth 221) that was

either untransfected, transfected to express a non-cognate HLA molecule (HLA-B57:01 or HLA-C04:01 in the case of KIR2DL3 and HLA-C03:04 or HLA-C04:01 in the case of KIR3DL1) or transfected to express a cognate HLA molecule (HLA-C03:04 in the case of KIR2DL3 and HLA-B57:01 in the case of KIR3DL1). Activation was induced with staphylococcal enterotoxin E (SEE). T cell survival was quantified in the presence of antibodies that block KIR (GL183, Dx9 (*39*)), or block HLA class I (Dx17 (*40*)); or an isotype control. The gating strategy is illustrated in **Fig. S5.** We found that blocking the iKIR-HLA interaction either by blocking KIR or by blocking HLA class I significantly decreased the count of live T cells compared to isotype control (median decrease = 40% and 48% respectively; **Fig. 3A & 3B**). Survival was similarly impaired in the absence of ligand (221 transfected with non-cognate HLA or untransfected; median decrease =57% and 53% respectively; **Fig. 3A & 3B**).

Next, we extended this work to primary $CD8^+$ T cells. Peripheral blood mononuclear cells (PBMCs) from three *HLA-B*27*$^+$ individuals with ankylosing spondylitis, who have a high proportion of PBMCs expressing KIR3DL2 (*6*), were stimulated with staphylococcal enterotoxin B. KIR3DL2 binds HLA-B27 homodimers (*6*). Survival of $KIR3DL2^+$ $CD8^+$ T cells was quantified in the presence of antibodies that block KIR3DL2 (DX31), that block HLA-B27 homodimers (HD6 (*41*)) or an isotype control, **Fig. S6**. We found that blocking the iKIR-HLA interaction reduced the count of live $KIR3DL2^+$ $CD8^+$ T cells compared to isotype control (block KIR: median difference=63%; block HLA: median difference=50%; **Fig. 3C**).

Finally, iKIR ligation has been shown to inhibit $CD8^+$ T cell killing under some conditions (*42*). To assess this possibility, which could mitigate the biological impact of enhanced survival, we quantified the impact of iKIR ligation on the mobilization of CD107a, a surrogate marker of degranulation. Greater numbers of live $CD107a^+$ cells were observed in the presence of a functional KIR-HLA interaction after stimulation of the KIR2DL3-expressing $CD8^+$ T cell line VTE1 with SEE (**Fig. 3D**). In summary, KIR ligation enhances the number of viable cells and the number of functional cells both for $CD8^+$ T cell lines and for primary T cells *in vitro*.

Virus-specific CD8$^+$ T cells express iKIR *ex vivo*

To probe the relevance of these findings, we quantified the fraction of virus-specific CD8$^+$ T cells which expressed iKIRs (KIR2DL1, KIR2DL2/L3, KIR3DL1 and KIR3DL2) in PBMCs from HIV-1-positive and HTLV-1-positive donors. We found considerable proportions of iKIR-expressing pentamer$^+$ CD8$^+$ T cells in both groups (HIV-1: mean = 27%; HTLV-1: mean = 15%), **Fig. 3E, Fig. S7, S8**. On analysing the differentiation stage of virus-specific KIR-expressing CD8$^+$ T cells we found, consistent with previous work on bulk CD8$^+$ T cells (*9*), that approximately 40% of iKIR$^+$ pentamer$^+$ cells lay within the CD45RA$^+$CD28$^-$ T effector memory revertant gate; the remainder were distributed approximately equally between CD45RA$^-$CD28$^+$ central memory T cells, CD45RA$^-$CD28$^-$ effector memory T cells and CD45RA$^+$CD28$^+$ stem cell memory/naïve T cells, **Fig. S9**.

We did not investigate iKIR expression on pentamer$^+$ CD8$^+$ T cells from HCV-seropositive individuals because the frequency of HCV-specific CD8$^+$ T cells in the blood is known to be extremely low (*43, 44*), due in part to T cell homing to the liver (*45*). Instead we investigated the relationship between functional iKIR expression and cell death *ex vivo* using bulk CD8$^+$ and CD4$^+$ T cells from HCV-seropositive individuals and healthy donors. We found that iKIR$^+$ CD8$^+$ and iKIR$^+$ CD4$^+$ T cells were both less likely to stain with annexin V in individuals where the cognate HLA ligand was present (**Supplementary Results & Fig. S10**).

Mathematical modelling can explain the enhancement of both protective and detrimental HLA associations

The hypothesis that iKIRs enhance HLA class I disease associations by enhancing T cell survival sounds reasonable, but closer inspection reveals a number of difficulties. First, iKIRs would be expected to increase the survival of all CD8$^+$ T cells not just those restricted by protective HLA alleles. In an immunogenetic study, a protective HLA effect reflects the difference in viral control between average and protective HLA alleles, so it is not obvious how a non-specific increase in T cell survival would enhance protective HLA associations. Second, is it difficult to envisage how the same mechanism could also have the opposite effect and enhance detrimental HLA associations. Finally, there is evidence that iKIRs can suppress the lytic activity of CD8$^+$ T cells under some conditions (*14*) (although this was not replicated in our

work with CD107a). This raises another question: is it reasonable to suggest that in people with a high iKIR score, and potentially a higher level of functional inhibition, $CD8^+$ T cell protection is actually enhanced? To address these questions we constructed a mechanistic mathematical model of host-virus dynamics for HIV-1. In this model, $CD8^+$ T cells restricted by protective HLA class I molecules were assumed to lyse targets more efficiently than average, whereas $CD8^+$ T cells restricted by detrimental HLA class I molecules were assumed to lyse targets less efficiently than average. In the first instance, we assumed that the only effect of iKIRs was to enhance T cell survival; in later analyses, we assumed that iKIRs also impaired $CD8^+$ T cell lysis. We generated 100,000 random parameter combinations by sampling from the physiological range for each parameter (**Table S11**) and solved the system numerically (**Fig. 4A)**. For each parameter combination we recorded whether the protective effect (decrease in set-point viral load associated with possession of a protective HLA class I molecule compared with an average HLA class I molecule) was enhanced in people with a high number of functional iKIRs. We found that the majority of runs (97%) showed iKIR-mediated enhancement of protective HLA class I associations (**Fig. 4B**). Next, we repeated the analysis for detrimental HLA class I molecules. This showed that detrimental HLA class I-associated effects were also enhanced across a wide range of parameter space (**Fig. 4C**). Comparison of the region of parameter space where protective HLA class I associations were enhanced and where detrimental HLA class I associations were enhanced showed that there was broad overlap; i.e. there exist parameter regions where both protective and detrimental HLA class I associations were simultaneously enhanced. Additionally, when iKIRs were assumed to inhibit T cell lysis, enhancement of protective and detrimental HLA class I effects was decreased, but the majority of runs still showed enhancement (**Fig. 4D & E**). Further analysis showed these results were robust to both structural and parametric changes in the model (**Supplementary Methods**).

To understand how an increase in $CD8^+$ T cell survival can enhance both protective and detrimental HLA class I associations, we simulated viral load as a function of $CD8^+$ T cell lifespan for three representative individuals: one with average HLA class I molecules, one with a protective HLA class I molecule and one with a detrimental HLA class I molecule (**Fig. 4F**). On seeing this figure, the explanation immediately becomes obvious. In the limit, when $CD8^+$ T cell lifespan is very short, it makes little difference whether an individual possesses average,

protective or detrimental alleles; everyone has a similar viral load independent of their HLA genotype. In an immunogenetics study this would manifest as an absence of association between either protective or detrimental alleles and outcome. As $CD8^+$ T cell lifespan is increased (for example by iKIRs) then the "quality" of the $CD8^+$ T cell response becomes important, and a large difference in viral load emerges between people with average, protective and detrimental HLA class I alleles. In an immunogenetics study this would manifest as large protective and detrimental effects i.e. as $CD8^+$ T cell lifespan increases both protective and detrimental effects are enhanced. Although this model does not prove the hypothesis it does show that the hypothesis predicts our findings and provides a plausible and intuitive explanation for a seemingly contradictory observation.

Discussion

In this study, we conducted an immunogenetic analysis of three independent cohorts of HIV-1-infected individuals to investigate the impact of iKIR genes on well-established HLA class I disease associations. We found that genes for iKIRs consistently enhanced the protective effect of *HLA-B*57* and the detrimental effect of *HLA-B*35Px* on viral load and progression to low CD4 count. The result was critically dependent on the presence of the KIR ligand. A reanalysis of two other persistent viral infections, HCV and HTLV-1, found consistent results: all four HLA class I associations studied were enhanced in people with a high inhibitory score. We previously reported this effect as an enhancement associated with one particular iKIR (*KIR2DL2*) (*21*). In HCV infection, enhanced HLA class I associations were detected for individuals with *KIR2DL2* and individuals with a high inhibitory score, but it was impossible to determine which effect was the driver. However, in HTLV-1 infection, new data made it possible to show that, independent of *KIR2DL2,* another iKIR (functional *KIR3DL1*) enhanced both protective and detrimental HLA class I disease associations. The probability of seeing this result by chance is $P = 10^{-16}$; furthermore the HIV-1 findings were replicated in two independent cohorts. Collectively, these data suggest that all iKIRs behave in a similar way and that KIR2DL2 is not functionally unique (though its prevalence and distribution make its effects easier to detect in some cohorts; a hypothesis we suggested previously (*21*)). The iKIRs are closely related, being the product of gene duplication (*46*), so it is gratifying that we find a unifying picture in which all iKIRs enhance HLA class I associations in a similar fashion.

We investigated the underlying mechanism using three approaches: (i) analysis of the duration of HLA class I-mediated effects in the HIV-1-infected cohort; (ii) *in vitro* survival assays combined with *ex vivo* KIR expression analysis and (iii) mathematical modelling. In the HIV-1 cohort, the phenomenon of iKIR-mediated enhancement appears to be related to the longevity of the $CD8^+$ T cell response as both the *HLA-B*57* protective effect and the *HLA-B*35Px* detrimental effect were eroded over time in individuals with a low inhibitory score but maintained in individuals with a high inhibitory score; the difference in rates between these two groups was consistently highly significant. *In vitro*, we found that blocking the KIR:HLA interaction significantly reduced cultured and primary T cell survival and functional $CD107a^+$ T cell survival following activation with superantigen. In addition, presence of the

KIR ligand was associated with a decrease in annexin V binding on $CD4^+$ and $CD8^+$ T cells expressing iKIR directly *ex vivo*. We also found substantial levels of iKIR expression on virus-specific $CD8^+$ T cells among PBMCs isolated from HIV-1 and HTLV-1-seropositive individuals. Finally, in a mathematical model of host-virus dynamics in HIV-1 infection, we predicted that if iKIRs increase $CD8^+$ T cell lifespan (either directly or indirectly) then we would see an enhancement of HLA class I associations, consistent with the immunogenetic data. Further, the model provides an explanation for how increasing $CD8^+$ T cell lifespan can simultaneously have two seemingly contradictory effects: enhancing both protective and detrimental associations. The modelling shows that if $CD8^+$ T cells have longer lifespans (e.g. due to the presences of iKIRs) then the quality of the $CD8^+$ T cell response becomes more important and so differences between protective, average and detrimental HLA alleles become more apparent i.e. both protective and detrimental effects are enhanced.

Our conclusions are that iKIRs enhance HLA class I associations and that this can be explained by an increase in $CD8^+$ T cell survival in the presence of functional iKIRs. A number of ways in which iKIRs can increase $CD8^+$ T cell survival have been described. These can be broadly divided into "direct" (iKIRs on T cells affect T cell lifespan) and "indirect" (iKIRs on other cell populations, e.g. NK cells, affect T cell lifespan); these pathways are summarised in **Fig. S11**. Our finding that, *in vitro*, iKIR expression on $CD8^+$ T cells is directly associated with increased survival does not preclude a role for iKIR on NK cells in indirectly enhancing $CD8^+$ T cell survival. Both effects may be occurring and increasing $CD8^+$ T cell survival and both are consistent with our immunogenetics analysis, the longitudinal study and the mathematical modelling.

The iKIR-ligand binding groups which we use in our definition of "functional iKIR" are simplistic. Incomplete knowledge of how different alleles and different peptides affect binding and signalling precludes a more sophisticated definition. Nevertheless, these simple groupings have proved very powerful in other studies (*23, 27, 29, 31, 47, 48*). With the definition of inhibitory score used we saw clear and reproducible results. This suggests that the inhibitory score is a meaningful metric (indeed the odds of seeing all the results by chance is $<10^{-23}$). It is worth noting that the score is used only to split the cohorts in half, so second order changes to the calculation of the score will not necessarily change the results; indeed

we found that our conclusions were robust to quite dramatic changes to the score (e.g. whether KIR3DL2 with classical class I ligands was included or not). This is because a person's score can change by a large amount and they will still remain in the same strata. Indeed, whether we simply count KIR-ligand pairs or use the inhibitory score has little impact on the conclusions. Another limitation of this work is that the survival assays were only conducted *in vitro*. The strong associations between possession of combinations of iKIR/HLA genes and clinical outcomes such as time to low CD4 count suggest that these effects are important *in vivo* but direct *in vivo* assays were not performed. Murine *in vivo* assays provide limited information since Ly49 receptors differ significantly from KIR (this includes profound differences in structure and in the pattern of expression on T cells). Human *in vivo* studies are limited by ethical considerations but would be an important next step in this work.

HCV, HIV-1 and HTLV-1 are all characterized by continuous high level viral replication and chronic immune activation which has been associated with $CD8^+$ T cell exhaustion and activation-induced T cell death (*49-52*). Chronic immune cell activation is also associated with increased KIR expression on T cells (*5-9, 42, 53*). iKIR 'rescuing' of activated $CD8^+$ T cells may be particularly important in this setting. Most KIR-HLA genetic associations are not replicated across viral infections e.g. *KIR3DS1* with *HLA-Bw4* is protective in the context of HIV-1 but not HTLV-1 (*54*); this is difficult to understand unless a very strong peptide dependence is postulated. In contrast, we report that all iKIRs enhance HLA class I associations in all three persistent virus infections we have studied (HIV-1, HCV and HTLV-1), which suggests a universality that is often missing from KIR studies.

The iKIR-HLA receptor-ligand system also has similarities to the programmed death 1 (PD-1)–programmed death-ligand 1/2 system. Both iKIR and PD-1 are inhibitory receptors which interfere with proximal T cell receptor signalling and are upregulated in the context of chronic viral infection and on tumour infiltrating lymphocytes. PD-1 and iKIR are both expressed on late stage differentiated memory T cells, typically in a mutually exclusive manner (*55*). Blockade or activation of iKIR would thus affect a different population of T cells compared with PD-1 activation/blockade. These observations, combined with the demonstration herein that the iKIR-HLA receptor-ligand system is clinically significant, suggest novel and synergistic targets for therapeutic immune checkpoint blockade.

Materials and Methods

(Full Methods available in **Supplementary Information**)

Study design

The aim of this study was to investigate whether iKIR have a clinically significant impact on the human $CD8^+$ T cell response. We studied the impact of iKIR genotype on 6 well-documented HLA class I disease associations in 3 viral infections (**Table 4**) in 5 independent cohorts. The work was extended and interpreted by mechanistic mathematical modelling, *in vitro* T cell survival assays and *ex vivo* analysis of iKIR expression.

Ethics statement

The immunogenetics study was approved by the NHS Research Ethics Committee (13/WS/0064) and the Imperial College Research Ethics Committee (ICREC_11_1_2). Informed consent was obtained at the study sites from all individuals. For the cellular work, written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Research was conducted under the governance of the Imperial College Healthcare NHS Trust Tissue Bank, approved by the UK National Research Ethics Service (09/H0606/106, 15/SC/0089).

Immunogenetics Cohorts

IAVI is a prospective cohort of treatment-naïve HIV-1 seroconverters from sub-Saharan Africa, identified and followed under Protocol C of the International AIDS Vaccine Initiative (IAVI) (*22, 56*). Individuals who possessed both *KIR3DS1* and its putative ligand *HLA-Bw4-80I* were excluded from the analysis to eliminate the potentially confounding effects of functional *KIR3DS1.* The cohort comprised individuals with time to low CD4 count information (N=491) and individuals with early viral load information (N=461). IAVI partners is an HIV-1 seroprevalent cohort (N=315). US is a cohort of HIV-1 seroconverters (N=548). Kagoshima is a cohort of HTLV-1-infected subjects (N=392). HCV comprises four sub-cohorts of HCV-seropositive subjects (N=782).

Regression

The impact of genotype on outcome was analyzed using multivariate linear, logistic and Cox regression in R v3.0.1 (*57*). Potentially confounding covariates were identified and included in the analysis (listed in **Supplementary Methods**). All reported P values are two tailed.

In vitro T cell survival

CD8$^+$ T Cell Lines. Three iKIR-expressing CD8$^+$ T cell lines were established from the peripheral blood of healthy volunteers (**Fig S4**). The lines were co-cultured with the HLA class I-deficient B cell line 221, either untransfected; or transfected to express a non-cognate HLA molecule (HLA-B57:01 or HLA-C04:01 in the case of KIR2DL3, and HLA-C03:04 or HLA-C04:01 in the case of KIR3DL1); or transfected to express a cognate HLA molecule (HLA-C03:04 in the case of KIR2DL3, and HLA-B57:01 in the case of KIR3DL1). Cells were activated with superantigen staphylococcal enterotoxin E. The count of live T cells at day 5 was enumerated using CountBright Absolute Counting Beads (Invitrogen) in the presence of antibodies to block KIR (GL183, Dx9 (*39*)) or to block HLA class I (Dx17 (*40*)) or as an isotype control. The gating strategy is illustrated in **Fig. S5.**

Primary T cells. PBMC from three *HLA-B*27*$^+$ individuals with ankylosing spondylitis were stimulated with staphylococcal enterotoxin B. Survival of KIR3DL2$^+$ CD4$^-$ T cells was quantified in the presence of isotype controls or antibodies that block KIR3DL2 (DX31) or homodimeric HLA-B27 (HD6 (*41*)).

Pentamer staining

Cryopreserved PBMC samples from *HLA-A*02*$^+$ HIV-1$^+$ (N=16) and *HLA-A*02*$^+$ HTLV-I$^+$ (N=9) subjects were thawed and stained sequentially with a fixable amine dye to exclude dead cells, fluorochrome-labelled peptide-HLA class I pentamers (ILKEPVHGV-A02:01, SLYNTVATL-A02:01 or LTFGWCFKL-A02:01 for HIV-1 and LLFGYPVYP-A02:01 for HTLV-1) and directly conjugated monoclonal antibodies specific for CD3, CD8, CD28, CD45RA, KIR2DL1, KIR2L2/L3, KIR3DL1 and KIR3DL2. The fraction of iKIR$^+$ pentamer$^+$ CD8$^+$ T cells was enumerated within each phenotypic compartment defined by the expression of CD28 and CD45RA.

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References and Notes (ref 59 onwards appear only in SI)

1. P. Parham, Immunogenetics of killer cell immunoglobulin-like receptors. *Mol. Immunol.* **42**, 459-462 (2005).
2. N. Wagtmann, S. Rajagopalan, C. C. Winter, M. Peruzzi, E. O. Long, Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer. *Immunity* **3**, 801-809 (1995).
3. L. B. Cook, M. Elemans, A. G. Rowan, B. Asquith, HTLV-1: persistence and pathogenesis. *Virology* **435**, 131-140 (2013).
4. J. Crouse, H. C. Xu, P. A. Lang, A. Oxenius, NK cells regulating T cell responses: mechanisms and outcome. *Trends Immunol.* **36**, 49-58 (2015).
5. N. Anfossi, V. Pascal, E. Vivier, S. Ugolini, Biology of T memory type 1 cells. *Immunol. Rev.* **181**, 269-278 (2001).
6. P. Bowness, A. Ridley, J. Shaw, A. T. Chan, I. Wong-Baeza, M. Fleming, F. Cummings, A. McMichael, S. Kollnberger, Th17 cells expressing KIR3DL2+ and responsive to HLA-B27 homodimers are increased in ankylosing spondylitis. *J. Immunol.* **186**, 2672-2680 (2011).
7. P. Bonorino, V. Leroy, T. Dufeu-Duchesne, S. Tongiani-Dashan, N. Sturm, M. Pernollet, E. Vivier, J. P. Zarski, P. N. Marche, E. Jouvin-Marche, Features and distribution of CD8 T cells with human leukocyte antigen class I-specific receptor expression in chronic hepatitis C. *Hepatology* **46**, 1375-1386 (2007).
8. K. Poon, D. Montamat-Sicotte, N. Cumberbatch, A. J. McMichael, M. F. Callan, Expression of leukocyte immunoglobulin-like receptors and natural killer receptors on virus-specific CD8+ T cells during the evolution of Epstein-Barr virus-specific immune responses in vivo. *Viral Immunol.* **18**, 513-522 (2005).
9. N. K. Bjorkstrom, V. Beziat, F. Cichocki, L. L. Liu, J. Levine, S. Larsson, R. A. Koup, S. K. Anderson, H. G. Ljunggren, K. J. Malmberg, CD8 T cells express randomly selected KIRs with distinct specificities compared to NK cells. *Blood* **120**, 3455-3465 (2012).
10. J. Roger, A. Chalifour, S. Lemieux, P. Duplay, Cutting edge: Ly49A inhibits TCR/CD3-induced apoptosis and IL-2 secretion. *J. Immunol.* **167**, 6-10 (2001).
11. S. Ugolini, C. Arpin, N. Anfossi, T. Walzer, A. Cambiaggi, R. Forster, M. Lipp, R. E. Toes, C. J. Melief, J. Marvel, E. Vivier, Involvement of inhibitory NKRs in the survival of a subset of memory-phenotype CD8+ T cells. *Nat. Immunol.* **2**, 430-435 (2001).
12. S. Ugolini, E. Vivier, Regulation of T cell function by NK cell receptors for classical MHC class I molecules. *Curr. Opin. Immunol.* **12**, 295-300 (2000).
13. N. T. Young, M. Uhrberg, J. H. Phillips, L. L. Lanier, P. Parham, Differential expression of leukocyte receptor complex-encoded Ig-like receptors correlates with the transition from effector to memory CTL. *J. Immunol.* **166**, 3933-3941 (2001).
14. G. Alter, S. Rihn, H. Streeck, N. Teigen, A. Piechocka-Trocha, K. Moss, K. Cohen, A. Meier, F. Pereyra, B. Walker, M. Altfeld, Ligand-independent exhaustion of killer immunoglobulin-like receptor-positive CD8+ T cells in human immunodeficiency virus type 1 infection. *J. Virol.* **82**, 9668-9677 (2008).
15. A. Gati, N. Guerra, C. Gaudin, S. Da Rocha, B. Escudier, Y. Lecluse, A. Bettaieb, S. Chouaib, A. Caignard, CD158 Receptor Controls Cytotoxic T-Lymphocyte Susceptibility to Tumor-Mediated Activation-Induced Cell Death by Interfering with Fas Signaling. *Cancer Res.* **63**, 7475-7482 (2003).
16. C. Cerboni, A. Zingoni, M. Cippitelli, M. Piccoli, L. Frati, A. Santoni, Antigen-activated human T lymphocytes express cell-surface NKG2D ligands via an ATM/ATR-dependent mechanism and become susceptible to autologous NK- cell lysis. *Blood* **110**, 606-615 (2007).
17. K. Soderquest, T. Walzer, B. Zafirova, L. S. Klavinskis, B. Polic, E. Vivier, G. M. Lord, A. Martin-Fontecha, Cutting edge: CD8+ T cell priming in the absence of NK cells leads to enhanced memory responses. *J. Immunol.* **186**, 3304-3308 (2011).

18. S. N. Waggoner, M. Cornberg, L. K. Selin, R. M. Welsh, Natural killer cells act as rheostats modulating antiviral T cells. *Nature* **481**, 394-398 (2011).
19. S. H. Lee, K. S. Kim, N. Fodil-Cornu, S. M. Vidal, C. A. Biron, Activating receptors promote NK cell expansion for maintenance, IL-10 production, and CD8 T cell regulation during viral infection. *J Exp Med* **206**, 2235-2251 (2009).
20. D. Peppa, U. S. Gill, G. Reynolds, N. J. Easom, L. J. Pallett, A. Schurich, L. Micco, G. Nebbia, H. D. Singh, D. H. Adams, P. T. Kennedy, M. K. Maini, Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. *J Exp Med* **210**, 99-114 (2013).
21. N. K. Seich Al Basatena, A. Macnamara, A. M. Vine, C. L. Thio, J. Astemborski, K. Usuku, M. Osame, G. D. Kirk, S. M. Donfield, J. J. Goedert, C. R. Bangham, M. Carrington, S. I. Khakoo, B. Asquith, KIR2DL2 enhances protective and detrimental HLA class I-mediated immunity in chronic viral infection. *PLOS Pathog.* **7**, e1002270 (2011).
22. https://www.iavi.org/.
23. M. P. Martin, Y. Qi, X. Gao, E. Yamada, J. N. Martin, F. Pereyra, S. Colombo, E. E. Brown, W. L. Shupert, J. Phair, J. J. Goedert, S. Buchbinder, G. D. Kirk, A. Telenti, M. Connors, S. J. O'Brien, B. D. Walker, P. Parham, S. G. Deeks, D. W. McVicar, M. Carrington, Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat. Genet.* **39**, 733-740 (2007).
24. A. A. Bashirova, E. Martin-Gayo, D. C. Jones, Y. Qi, R. Apps, X. Gao, P. S. Burke, C. J. Taylor, J. Rogich, S. Wolinsky, J. H. Bream, P. Duggal, S. Hussain, J. Martinson, A. Weintrob, G. D. Kirk, J. Fellay, S. P. Buchbinder, J. J. Goedert, S. G. Deeks, F. Pereyra, J. Trowsdale, M. Lichterfeld, A. Telenti, B. D. Walker, R. L. Allen, M. Carrington, X. G. Yu, LILRB2 interaction with HLA class I correlates with control of HIV-1 infection. *PLOS Genet* **10**, e1004196 (2014).
25. J. P. Goodridge, A. Burian, N. Lee, D. E. Geraghty, HLA-F and MHC class I open conformers are ligands for NK cell Ig-like receptors. *J. Immunol.* **191**, 3553-3562 (2013).
26. M. Carrington, G. W. Nelson, M. P. Martin, T. Kissner, D. Vlahov, J. J. Goedert, R. Kaslow, S. Buchbinder, K. Hoots, S. J. O'Brien, HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science* **283**, 1748-1752 (1999).
27. S. I. Khakoo, C. L. Thio, M. P. Martin, C. R. Brooks, X. Gao, J. Astemborski, J. Cheng, J. J. Goedert, D. Vlahov, M. Hilgartner, S. Cox, A.-M. Little, G. J. Alexander, M. E. Cramp, S. J. O'Brien, W. M. C. Rosenberg, D. L. Thomas, M. Carrington, HLA and NK Cell Inhibitory Receptor Genes in Resolving Hepatitis C Virus Infection. *Science* **305**, 872-874 (2004).
28. K. Hirayasu, J. Ohashi, K. Kashiwase, H. Hananantachai, I. Naka, A. Ogawa, M. Takanashi, M. Satake, K. Nakajima, P. Parham, H. Arase, K. Tokunaga, J. Patarapotikul, T. Yabe, Significant association of KIR2DL3-HLA-C1 combination with cerebral malaria and implications for co-evolution of KIR and HLA. *PLOS Pathog.* **8**, e1002565 (2012).
29. N. Vince, A. A. Bashirova, A. Lied, X. Gao, L. Dorrell, P. J. McLaren, J. Fellay, M. Carrington, HLA class I and KIR genes do not protect against HIV type 1 infection in highly exposed uninfected individuals with hemophilia A. *J. Infect. Dis.* **210**, 1047-1051 (2014).
30. C. M. Ayo, F. B. Frederico, R. C. Siqueira, C. C. Brandao de Mattos, M. Previato, A. P. Barbosa, F. H. Murata, A. P. Silveira-Carvalho, L. C. de Mattos, Ocular toxoplasmosis: susceptibility in respect to the genes encoding the KIR receptors and their HLA class I ligands. *Sci. Rep.* **6**, 36632 (2016).
31. M. P. Martin, X. Gao, J.-H. Lee, G. W. Nelson, R. Detels, J. J. Goedert, S. Buchbinder, K. Hoots, D. Vlahov, J. Trowsdale, M. Wilson, S. J. O'Brien, M. Carrington, Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat. Genet.* **31**, 429-434 (2002).
32. C. R. Bangham, CTL quality and the control of human retroviral infections. *Eur. J. Immunol.* **39**, 1700-1712 (2009).
33. M. Altfeld, E. T. Kalife, Y. Qi, H. Streeck, M. Lichterfeld, M. N. Johnston, N. Burgett, M. E. Swartz, A. Yang, G. Alter, X. G. Yu, A. Meier, J. K. Rockstroh, T. M. Allen, H. Jessen, E. S. Rosenberg, M. Carrington, B. D. Walker, HLA Alleles Associated with Delayed Progression to

AIDS Contribute Strongly to the Initial CD8(+) T Cell Response against HIV-1. *PLOS Med.* **3**, e403 (2006).

34. J. A. M. Borghans, A. Mølgaard, R. J. de Boer, C. Keşmir, HLA Alleles Associated with Slow Progression to AIDS Truly Prefer to Present HIV-1 p24. *PLOS One* **2**, e920-e920 (2007).
35. H. Chen, Z. M. Ndhlovu, D. Liu, L. C. Porter, J. W. Fang, S. Darko, M. A. Brockman, T. Miura, Z. L. Brumme, A. Schneidewind, A. Piechocka-Trocha, K. T. Cesa, J. Sela, T. D. Cung, I. Toth, F. Pereyra, X. G. Yu, D. C. Douek, D. E. Kaufmann, T. M. Allen, B. D. Walker, TCR clonotypes modulate the protective effect of HLA class I molecules in HIV-1 infection. *Nat. Immunol.* **13**, 691-700 (2012).
36. A. Kosmrlj, E. L. Read, Y. Qi, T. M. Allen, M. Altfeld, S. G. Deeks, F. Pereyra, M. Carrington, B. D. Walker, A. K. Chakraborty, Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection. *Nature* **465**, 350-354 (2010).
37. A. Y. Kim, T. Kuntzen, J. Timm, B. E. Nolan, M. A. Baca, L. L. Reyor, A. C. Berical, A. J. Feller, K. L. Johnson, J. Schulze zur Wiesch, G. K. Robbins, R. T. Chung, B. D. Walker, M. Carrington, T. M. Allen, G. M. Lauer, Spontaneous control of HCV is associated with expression of HLA-B 57 and preservation of targeted epitopes. *Gastroenterology* **140**, 686-696.e681 (2011).
38. Y.-J. Chwae, M. J. Chang, S. M. Park, H. Yoon, H.-J. Park, S. J. Kim, J. Kim, Molecular Mechanism of the Activation-Induced Cell Death Inhibition Mediated by a p70 Inhibitory Killer Cell Ig-Like Receptor in Jurkat T Cells. *J. Immunol.* **169**, 3726-3735 (2002).
39. L. Binyamin, R. K. Alpaugh, T. L. Hughes, C. T. Lutz, K. S. Campbell, L. M. Weiner, Blocking NK Cell Inhibitory Self-Recognition Promotes Antibody-Dependent Cellular Cytotoxicity in a Model of Anti-Lymphoma Therapy. *J. Immunol.* **180**, 6392-6401 (2008).
40. A. D'Andrea, C. Chang, J. H. Phillips, L. L. Lanier, Regulation of T cell lymphokine production by killer cell inhibitory receptor recognition of self HLA class I alleles. *J Exp Med* **184**, 789-794 (1996).
41. S. K. Payeli, S. Kollnberger, O. Marroquin Belaunzaran, M. Thiel, K. McHugh, J. Giles, J. Shaw, S. Kleber, A. Ridley, I. Wong-Baeza, S. Keidel, K. Kuroki, K. Maenaka, A. Wadle, C. Renner, P. Bowness, Inhibiting HLA-B27 homodimer-driven immune cell inflammation in spondylarthritis. *Arthritis Rheum.* **64**, 3139-3149 (2012).
42. L. T. van der Veken, M. D. Campelo, M. A. van der Hoorn, R. S. Hagedoorn, H. M. van Egmond, J. van Bergen, R. Willemze, J. H. Falkenburg, M. H. Heemskerk, Functional analysis of killer Ig-like receptor-expressing cytomegalovirus-specific CD8+ T cells. *J. Immunol.* **182**, 92-101 (2009).
43. C. Willberg, E. Barnes, P. Klenerman, HCV immunology-Death and the maiden T cell. *Cell Death Differ.* **10**, S39-S47 (2003).
44. N. K. Björkström, V. D. Gonzalez, K.-J. Malmberg, K. Falconer, A. Alaeus, G. Nowak, C. Jorns, B.-G. Ericzon, O. Weiland, J. K. Sandberg, H.-G. Ljunggren, Elevated Numbers of FcγRIIIA+ (CD16+) Effector CD8 T Cells with NK Cell-Like Function in Chronic Hepatitis C Virus Infection. *The Journal of Immunology* **181**, 4219-4228 (2008).
45. P. S. Sung, V. Racanelli, E.-C. Shin, CD8(+) T-Cell Responses in Acute Hepatitis C Virus Infection. *Front. Immunol.* **5**, 266 (2014).
46. P. J. Norman, P. Parham, Complex interactions: The immunogenetics of human leukocyte antigen and killer cell immunoglobulin-like receptors. *Semin. Hematol.* **42**, 65-75 (2005).
47. G. Ahlenstiel, M. P. Martin, X. Gao, M. Carrington, B. Rehermann, Distinct KIR/HLA compound genotypes affect the kinetics of human antiviral natural killer cell responses. *J. Clin. Invest.* **118**, 1017-1026 (2008).
48. A. Nakimuli, O. Chazara, S. E. Hiby, L. Farrell, S. Tukwasibwe, J. Jayaraman, J. A. Traherne, J. Trowsdale, F. Colucci, E. Lougee, R. W. Vaughan, A. M. Elliott, J. Byamugisha, P. Kaleebu, F. Mirembe, N. Nemat-Gorgani, P. Parham, P. J. Norman, A. Moffett, A KIR B centromeric region present in Africans but not Europeans protects pregnant women from pre-eclampsia. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 845-850 (2015).

49. C. L. Day, D. E. Kaufmann, P. Kiepiela, J. A. Brown, E. S. Moodley, S. Reddy, E. W. Mackey, J. D. Miller, A. J. Leslie, C. DePierres, Z. Mncube, J. Duraiswamy, B. Zhu, Q. Eichbaum, M. Altfeld, E. J. Wherry, H. M. Coovadia, P. J. Goulder, P. Klenerman, R. Ahmed, G. J. Freeman, B. D. Walker, PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* **443**, 350-354 (2006).
50. B. Bengsch, B. Seigel, M. Ruhl, J. Timm, M. Kuntz, H. E. Blum, H. Pircher, R. Thimme, Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. *PLOS Pathog.* **6**, e1000947 (2010).
51. T. Kozako, M. Yoshimitsu, H. Fujiwara, I. Masamoto, S. Horai, Y. White, M. Akimoto, S. Suzuki, K. Matsushita, K. Uozumi, C. Tei, N. Arima, PD-1/PD-L1 expression in human T-cell leukemia virus type 1 carriers and adult T-cell leukemia/lymphoma patients. *Leukemia* **23**, 375-382 (2009).
52. B. Asquith, Y. Zhang, A. J. Mosley, C. M. de Lara, D. L. Wallace, A. Worth, L. Kaftantzi, K. Meekings, G. E. Griffin, Y. Tanaka, D. F. Tough, P. C. Beverley, G. P. Taylor, D. C. Macallan, C. R. Bangham, In vivo T lymphocyte dynamics in humans and the impact of human T-lymphotropic virus 1 infection. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 8035-8040 (2007).
53. B. Huard, L. Karlsson, KIR expression on self-reactive CD8+ T cells is controlled by T-cell receptor engagement. *Nature* **403**, 325-328 (2000).
54. G. M. O'Connor, N. K. Seich Al Basatena, V. Olavarria, A. MacNamara, A. Vine, Q. Ying, M. Hisada, B. Galvao-Castro, B. Asquith, D. W. McVicar, In contrast to HIV, KIR3DS1 does not influence outcome in HTLV-1 retroviral infection. *Hum. Immunol.* **73**, 783-787 (2012).
55. J. Duraiswamy, C. C. Ibegbu, D. Masopust, J. D. Miller, K. Araki, G. H. Doho, P. Tata, S. Gupta, M. J. Zilliox, H. I. Nakaya, B. Pulendran, W. N. Haining, G. J. Freeman, R. Ahmed, Phenotype, function, and gene expression profiles of programmed death-1(hi) CD8 T cells in healthy human adults. *J. Immunol.* **186**, 4200-4212 (2011).
56. A. Kamali, M. A. Price, S. Lakhi, E. Karita, M. Inambao, E. J. Sanders, O. Anzala, M. H. Latka, L. G. Bekker, P. Kaleebu, G. Asiki, A. Ssetaala, E. Ruzagira, S. Allen, P. Farmer, E. Hunter, G. Mutua, H. Makkan, A. Tichacek, I. K. Brill, P. Fast, G. Stevens, P. Chetty, P. N. Amornkul, J. Gilmour, I. A. H. P. Partnership, Creating an African HIV clinical research and prevention trials network: HIV prevalence, incidence and transmission. *PLOS One* **10**, e0116100 (2015).
57. R: A language and environment for statistical computing. URL http://www.R-project.org/. (2014).
58. M. Stern, L. Ruggeri, M. Capanni, A. Mancusi, A. Velardi, Human leukocyte antigens A23, A24, and A32 but not A25 are ligands for KIR3DL1. *Blood* **112**, 708-710 (2008).

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59. J. J. Goedert, R. J. Biggar, D. M. Winn, D. L. Mann, D. P. Byar, D. M. Strong, R. A. DiGioia, R. J. Grossman, W. C. Sanchez, R. G. Kase, et al., Decreased helper T lymphocytes in homosexual men. I. Sexual contact in high-incidence areas for the acquired immunodeficiency syndrome. *Am. J. Epidemiol.* **121**, 629-636 (1985).
60. J. J. Goedert, C. M. Kessler, L. M. Aledort, R. J. Biggar, W. A. Andes, G. C. White, J. E. Drummond, K. Vaidya, D. L. Mann, M. E. Eyster, A prospective study of Human Immunodeficiency Virus type 1 infection and the development of AIDS in subjects with hemophilia. *N. Engl. J. Med.* **321**, 1141-1148 (1989).
61. S. P. Buchbinder, M. H. Katz, N. A. Hessol, P. M. O'Malley, S. D. Holmberg, Long-term HIV-1 infection without immunologic progression. *AIDS* **8**, 1123-1128 (1994).
62. D. Vlahov, N. Graham, D. Hoover, C. Flynn, J. G. Bartlett, J. B. Margolick, C. M. Lyles, K. E. Nelson, D. Smith, S. Holmberg, H. Farzadegan, Prognostic indicators for AIDS and infectious disease death in HIV-infected injection drug users: plasma viral load and CD4+ cell count. *JAMA* **279**, 35-40 (1998).
63. K. J. Jeffery, K. Usuku, S. E. Hall, W. Matsumoto, G. P. Taylor, J. Procter, M. Bunce, G. S. Ogg, K. I. Welsh, J. N. Weber, A. L. Lloyd, M. A. Nowak, M. Nagai, D. Kodama, S. Izumo, M. Osame,

C. R. Bangham, HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 3848-3853 (1999).
64. D. Vlahov, J. C. Anthony, A. Munoz, J. Margolick, K. E. Nelson, D. D. Celentano, L. Solomon, B. F. Polk, The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. *NIDA Res. Monogr.* **109**, 75-100 (1991).
65. M. W. Hilgartner, S. M. Donfield, A. Willoughby, C. F. Contant, B. L. Evatt, E. D. Gomperts, W. K. Hoots, J. Jason, K. A. Loveland, S. M. McKinlay, Hemophilia growth and development study. Design, methods, and entry data. *The American Journal of Pediatric Hematology/Oncology* **15**, 208-218 (1993).
66. C. L. Thio, X. Gao, J. J. Goedert, D. Vlahov, K. E. Nelson, M. W. Hilgartner, S. J. O'Brien, P. Karacki, J. Astemborski, M. Carrington, D. L. Thomas, HLA-Cw*04 and hepatitis C virus persistence. *J. Virol.* **76**, 4792-4797 (2002).
67. H. A. Prentice, M. A. Price, T. R. Porter, E. Cormier, M. J. Mugavero, A. Kamali, E. Karita, S. Lakhi, E. J. Sanders, O. Anzala, P. N. Amornkul, S. Allen, E. Hunter, R. A. Kaslow, J. Gilmour, J. Tang, Dynamics of viremia in primary HIV-1 infection in Africans: insights from analyses of host and viral correlates. *Virology* **449**, 254-262 (2014).
68. W. Jiang, C. Johnson, N. Simecek, M. R. Lopez-Alvarez, D. Di, J. Trowsdale, J. A. Traherne, qKAT: a high-throughput qPCR method for KIR gene copy number and haplotype determination. *Genome Med.* **8**, 99 (2016).
69. T. M. Therneau, A Package for Survival Analysis. *http://CRAN.R-project.org/package=survival*, (2015).
70. T. M. Therneau, P. M. Grambsch, *Modeling Survival Data: Extending the Cox Model*. (Springer, New York, 2000).
71. S. van der Linden, H. A. Valkenburg, A. Cats, Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum.* **27**, 361-368 (1984).
72. Y. Shimizu, R. DeMars, Production of human cells expressing individual transferred HLA-A,-B,-C genes using an HLA-A,-B,-C null human cell line. *J. Immunol.* **142**, 3320-3328 (1989).
73. M. Y. Li, H. Shu, Multiple stable periodic oscillations in a mathematical model of CTL response to HTLV-I infection. *Bull. Math. Biol.* **73**, 1774-1793 (2011).
74. A. G. Lim, P. K. Maini, HTLV-I infection: a dynamic struggle between viral persistence and host immunity. *J. Theor. Biol.* **352**, 92-108 (2014).
75. H. Gomez-Acevedo, M. Y. Li, S. Jacobson, Multistability in a model for CTL response to HTLV-I infection and its implications to HAM/TSP development and prevention. *Bull. Math. Biol.* **72**, 681-696 (2010).
76. K. Pelak, A. C. Need, J. Fellay, K. V. Shianna, S. Feng, T. J. Urban, D. Ge, A. De Luca, J. Martinez-Picado, S. M. Wolinsky, J. J. Martinson, B. D. Jamieson, J. H. Bream, M. P. Martin, P. Borrow, N. L. Letvin, A. J. McMichael, B. F. Haynes, A. Telenti, M. Carrington, D. B. Goldstein, G. Alter, Copy number variation of KIR genes influences HIV-1 control. *PLOS Biol.* **9**, e1001208 (2011).
77. M. P. Martin, V. Naranbhai, P. R. Shea, Y. Qi, V. Ramsuran, N. Vince, X. Gao, R. Thomas, Z. L. Brumme, J. M. Carlson, S. M. Wolinsky, J. J. Goedert, B. D. Walker, F. P. Segal, S. G. Deeks, D. W. Haas, S. A. Migueles, M. Connors, N. Michael, J. Fellay, E. Gostick, S. Llewellyn-Lacey, D. A. Price, B. A. Lafont, P. Pymm, P. M. Saunders, J. Widjaja, S. C. Wong, J. P. Vivian, J. Rossjohn, A. G. Brooks, M. Carrington, Killer cell immunoglobulin-like receptor 3DL1 variation modifies HLA-B*57 protection against HIV-1. *J. Clin. Invest.*, (2018).
78. P. M. Saunders, P. Pymm, G. Pietra, V. A. Hughes, C. Hitchen, G. M. O'Connor, F. Loiacono, J. Widjaja, D. A. Price, M. Falco, M. C. Mingari, L. Moretta, D. W. McVicar, J. Rossjohn, A. G. Brooks, J. P. Vivian, Killer cell immunoglobulin-like receptor 3DL1 polymorphism defines distinct hierarchies of HLA class I recognition. *J Exp Med* **213**, 791-807 (2016).

79. G. M. O'Connor, J. P. Vivian, E. Gostick, P. Pymm, B. A. Lafont, D. A. Price, J. Rossjohn, A. G. Brooks, D. W. McVicar, Peptide-Dependent Recognition of HLA-B*57:01 by KIR3DS1. *J. Virol.* **89**, 5213-5221 (2015).
80. S. B. Taner, M. J. Pando, A. Roberts, J. Schellekens, S. G. E. Marsh, K.-J. Malmberg, P. Parham, F. M. Brodsky, Interactions of NK Cell Receptor KIR3DL1*004 with Chaperones and Conformation-Specific Antibody Reveal a Functional Folded State As Well As Predominant Intracellular Retention. *J. Immunol.* **186**, 62-72 (2011).
81. P. J. Norman, J. A. Hollenbach, N. Nemat-Gorgani, L. A. Guethlein, H. G. Hilton, M. J. Pando, K. A. Koram, E. M. Riley, L. Abi-Rached, P. Parham, Co-evolution of human leukocyte antigen (HLA) class I ligands with killer-cell immunoglobulin-like receptors (KIR) in a genetically diverse population of sub-Saharan Africans. *PLOS Genet* **9**, e1003938 (2013).
82. C. L. Althaus, R. J. De Boer, Dynamics of immune escape during HIV/SIV infection. *PLOS Comput. Biol.* **4**, e1000103 (2008).
83. J. M. Conway, A. S. Perelson, Post-treatment control of HIV infection. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 5467-5472 (2015).
84. F. Li, W. Ma, Z. Jiang, D. Li, Stability and Hopf Bifurcation in a Delayed HIV Infection Model with General Incidence Rate and Immune Impairment. *Comput. Math. Methods Med.* **2015**, 206205 (2015).
85. H. W. van Deutekom, G. Wijnker, R. J. de Boer, The rate of immune escape vanishes when multiple immune responses control an HIV infection. *J. Immunol.* **191**, 3277-3286 (2013).
86. R. Batorsky, R. A. Sergeev, I. M. Rouzine, The route of HIV escape from immune response targeting multiple sites is determined by the cost-benefit tradeoff of escape mutations. *PLOS Comput. Biol.* **10**, e1003878 (2014).
87. V. Muller, A. F. Maree, R. J. De Boer, Small variations in multiple parameters account for wide variations in HIV-1 set-points: a novel modelling approach. *Proc. Biol. Sci.* **268**, 235-242 (2001).
88. R. A. Kaslow, M. Carrington, R. Apple, L. Park, A. Munoz, A. J. Saah, J. J. Goedert, C. Winkler, S. J. O'Brien, C. Rinaldo, R. Detels, W. Blattner, J. Phair, H. Erlich, D. L. Mann, Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. *Nat. Med.* **2**, 405-411 (1996).
89. M. Carrington, S. J. O'Brien, The influence of HLA genotype on AIDS. *Annu. Rev. Med.* **54**, 535-551 (2003).
90. A. S. Perelson, Modelling viral and immune system dynamics. *Nat. Rev. Immunol.* **2**, 28-36 (2002).

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Competing Interests

The authors declare that they have no competing interests.

Figure Legends

Figure 1: Inhibitory KIR genes enhance the protective effect of *HLA-B*57* on early set-point viral load and time to low CD4 count.

A. The IAVI cohort (N=461) was stratified into individuals with or without the functional iKIR of interest, into individuals with different inhibitory scores and into individuals with a low or high count of functional iKIRs. In each stratum, the protective effect of *HLA-B*57* on early set-point viral load was analyzed using multivariate linear regression. The coefficients (change in early set point viral load associated with *HLA-B*57*) are plotted. It can be seen that the protective effect of *HLA-B*57* was enhanced by all 3 iKIR, by high inhibitory score and by high count of iKIRs with a dose effect with inhibitory score. Definitions. Func *2DL1*$^{+}$: *KIR2DL1*$^{+}$*C2*$^{+}$. Func *2DL2*$^{++}$: *KIR2DL2*$^{+}$*C1*$^{+}$. Func *2DL2*$^{+}$: *KIR2DL2*$^{+}$*C2*$^{+}$. Func *2DL3*$^{+}$: *KIR2DL3*$^{+}$*C1*$^{+}$. Func *2DL1*$^{-}$: *KIR2DL1*$^{-}$ or *KIR2DL1*$^{+}$*C2*$^{-}$. Func *2DL2*$^{-}$: *KIR2DL2*$^{-}$. Func *2DL3*$^{-}$: *KIR2DL3*$^{-}$ or *KIR2DL3*$^{+}$*C1*$^{-}$. Inhibitory score: +++: >2.5, ++: >2.0, +: ≤2.5. -: ≤2.0.

B. Cox survival curves are plotted for *HLA-B*57*$^{-}$ individuals (black dashed line), *HLA-B*57*$^{+}$ individuals with a low inhibitory score (red dotted line) and *HLA-B*57*$^{+}$ individuals with a high inhibitory score (green solid line). It can be seen that individuals with *HLA-B*57* and a high inhibitory score (>2.5) progressed to low CD4 count (<350 cells per mm^{3}) at the slowest rate (N = 491).

C. The cohort (N=461) was stratified into individuals with or without *HLA-B*57* and the relationship between early set-point viral load and the count of functional iKIRs was analyzed. Early viral load is shown in grey boxes, and the corresponding linear regression is shown as a line. The number of functional iKIRs was significantly associated with decreasing viral load in *HLA-B*57*$^{+}$ individuals ($P = 0.018$) but not *HLA-B*57*$^{-}$ individuals ($P = 0.59$). This indicates that iKIRs enhance the *HLA-B*57*-associated reduction in early viral load in a dose-dependent manner; and that iKIRs in the absence of *HLA-B*57* are not in themselves significantly protective (see also **Supplementary Table S2**).

Figure 2: The *HLA-B*57* protective effect on viral load is maintained in the presence of a strong inhibitory KIR signal but lost in its absence.

A. The IAVI cohort was stratified into individuals with a low inhibitory score (≤2.5, left) and individuals with a high inhibitory score (>2.5, right). In each stratum, the local nonparametric

regression lines (loess) were calculated from the longitudinal viral load measurements (5197 person-visits). Pink circles denote viral load measurements in *HLA-B*57*$^{-}$ individuals; summarised with the pink loess line. Blue circles denote viral load measurements in *HLA-B*57*$^{+}$ individuals; summarised with the blue loess line. Dashed lines are the 95% confidence intervals around the loess lines. The vertical lines denote the time at which the number of individuals in either arm fell below 10 (loess lines to the right of this time point, i.e. within the grey shaded area, are unreliable and should be ignored). Viral load is residualized for gender; plots of raw viral load are indistinguishable.

B. Bootstrap estimation of the correlation between the *HLA-B*57* protective effect and time in the low (≤2.5) and high (>2.5) inhibitory score strata. Analysis was restricted to timepoints <500 days to ensure large cohort size. The protective effect of *HLA-B*57* decreased significantly more rapidly in people with a low inhibitory score (*P*=0.00002, Wilcoxon Mann Whitney two-tailed).

Figure 3: Impact of iKIR-HLA interaction on CD8^{+} T cell survival *in vitro* & iKIR expression *ex vivo*

A. CD8^{+} T cell lines (plots from left ED, KIR2DL3^{+}; VTE1 KIR2DL3^{+}; SK1 KIR3DL1^{+}) were cocultured with superantigen either without adding ligand for KIR i.e. with 221 cells or with 221 cells transfected to express irrelevant, non-cognate HLA (open bars); with ligand i.e. 221 transfected to express cognate HLA with or without isotype control antibody (black bars) and with KIR-HLA blocking i.e. 221 transfected to express cognate HLA with addition of antibody to block KIR (GL183, DX9) or to block HLA (DX17) (red bars). The count of live CD8^{+} T cells after 5 days is shown (mean± SE). ED: 5 experiments each consisting of 3 replicates; VTE1: 1 experiment, 4 replicates; SK1: 1 experiment, 2 replicates. It is consistently seen that there are greater numbers of viable T cells with isotype control than when the KIR-HLA interaction is blocked or when cognate ligand is absent.

B. Metaanalysis of survival of the 3 KIR-expressing CD8^{+} T cell lines. **Block HLA**. Coculture with 221 cells transfected to express cognate HLA with isotype control antibody compared to coculture with 221 cells transfected to express cognate HLA with anti-HLA antibody. Median difference = 48% *P* = 0.001. **Block KIR**. Coculture with 221 cells transfected to express cognate HLA with isotype control antibody compared to coculture with 221 cells transfected to express cognate HLA with anti-KIR antibody. Median difference = 40% *P* = 0.001. **Irrelevant**

HLA. Coculture with 221 cells transfected to express cognate HLA compared to coculture with 221 cells transfected to express irrelevant, non-cognate HLA. Median difference = 57% *P* =1.5x10^{-5}. **221 only.** Coculture with 221 cells transfected to express cognate HLA compared to coculture with 221 cells. Median difference = 53% *P* = 0.002. Survival of VTE1 plotted on left hand y axis (for all conditions), ED and SK1 plotted on right hand y axis (for all conditions). We consistently found that absence of KIR:HLA ligation (either by blocking HLA, blocking KIR, adding a non-ligating HLA or not adding HLA) resulted in significantly decreased CD8$^+$ T cell survival.

C. PBMCs from 3 *HLA-B27+* individuals with ankylosing spondylitis were left untreated (open bars); cultured with superantigen (SEB) with or without isotype control antibody (black bars) or cultured with SEB with antibodies that block KIR3DL2 or homodimeric HLA-B27 (red bars). The count of live KIR3DL2$^+$ CD4$^-$ T cells after 5 days is shown, (mean± SE). Blocking the iKIR-HLA interaction with specific antibodies decreased the count of live KIR3DL2$^+$ T cells compared to isotype control (block KIR: median difference=63%; block HLA: median difference=50%).

D. The KIR2DL3-expressing CD8$^+$ T cell line VTE1 was cultured as in A. The number of live CD8$^+$ CD107a$^+$ T cells was quantified by flow cytometry at day 5.

E. The fraction of pentamer$^+$ CD8$^+$ T cells expressing iKIRs (KIR2DL1, KIR2DL2/L3, KIR3DL1 or KIR3DL2) directly *ex vivo* is plotted for *HLA-A2$^+$* individuals infected with HIV-1 (N = 16) or HTLV-1 (N = 9).

Figure 4: Simulations of iKIR enhancement of HLA class I disease associations in HIV-1.

A. Dynamics of HIV-1-specific CD8$^+$ T cells, infected and uninfected CD4$^+$ T cells and viral load during HIV-1 infection, for a representative hypothetical *in silico* subject with average (dashed black line) or protective (green solid line) HLA class I alleles.

B&C. Fraction of runs that result in an iKIR-mediated enhancement of the protective (B) or detrimental (C) HLA effect as a function of virus transmissibility (β) and CD8$^+$ T cell proliferation rate (s_2) (left); as a function of CD8$^+$ T cell lysis rate (d_2) and proliferation rate (s_2) (center) and as a function of CD8$^+$ T cell death rate (μ_4) and proliferation rate (s_2) (right).

D&E. Distribution (D) and frequency (E) of runs with enhancement when we consider an impairment in the CD8$^+$ T cell lysis rate due to iKIRs (percentage reduction from left to right:

0%, 5% , 15% and 30%).

F. Viral load as a function of $CD8^+$ T cell lifespan ($1/\mu_4$) for a hypothetical person with detrimental (magenta dotted), average (black dashed) or protective (green solid) HLA class I alleles. It can be seen that as the survival of $CD8^+$ T cells increases both the effect of protective HLA (difference in viral load between average and protective HLA) and the effect of detrimental HLA (difference in viral load between average and detrimental HLA) increases. Compare for example, a hypothetical person with a high number of iKIR-HLA pairs (blue bar marked KIR hi) and a hypothetical person with a low number of iKIR-HLA pairs (blue bar marked KIR low).

Tables

A. Outcome: Early viral load setpoint								
	B57⁺ KIR⁻		*B57⁺ KIR⁺*		Cohort numbers			
	Coeff	P value	Coeff	P value	*B57⁺ KIR⁻*	*B57⁻ KIR⁻*	*B57⁺ KIR⁺*	*B57⁻ KIR⁺*
Functional *2DL1*	-0.14	0.6	-0.53	0.003 **	10	125	25	301
Functional *2DL2* (strong)	-0.31	0.1	-0.63	0.02 *	17	153	10	178
Functional *2DL2* (weak)			-0.59	0.01 *			15	208
Functional *2DL3*	-0.26	0.2	-0.61	0.002 **	18	180	17	246
Inhibitory score (cut-off=2.0)	-0.20	0.4	-0.56	0.004 **	14	195	21	231
Inhibitory score (cut-off=2.5)	-0.31	0.1	-0.59	0.0096 **	21	258	14	168
Count (cut-off=2.0)	-0.20	0.4	-0.56	0.004 **	14	195	21	231
B. Outcome: Time to low CD4 count (<350 cells/mm³)								
	B57⁺ KIR⁻		*B57⁺ KIR⁺*		Cohort numbers			
	HR	P value	HR	P value	*B57⁺ KIR⁻*	*B57⁻ KIR⁻*	*B57⁺ KIR⁺*	*B57⁻ KIR⁺*
Functional *2DL1*	0.73	0.6	0.36	0.01 *	10	132	25	324
Functional *2DL2* (strong)	0.36	0.09 .	0.19	0.03 *	17	168	10	187
Functional *2DL2* (weak)			0.71	0.40			15	221
Functional *2DL3*	0.56	0.18	0.32	0.05 .	18	195	17	261
Inhibitory score (cut-off=2.0)	0.49	0.17	0.42	0.06 .	14	214	21	242
Inhibitory score (cut-off=2.5)	0.57	0.15	0.22	0.04 *	21	280	14	176
Count (cut-off=2.0)	0.49	0.17	0.42	0.06 .	14	214	21	242

Table 1. Functional iKIRs enhance the protective effect of *HLA-B*57* on early set-point viral load and time to low CD4 count.

The IAVI cohort was stratified into individuals with or without the functional iKIR of interest, with or without a high inhibitory score (two cut-offs considered) and with or without a high

count of functional iKIRs (only one cut-off possible). In each stratum, the protective effect of *HLA-B*57* on early set-point viral load (A) and time to low CD4 (B) was analyzed using multivariate linear regression and the Cox proportional hazards model, respectively. The coefficients (Coeff), hazard ratios (HR), P values and cohort sizes are reported. The coefficient (Coeff) is the change in $\log_{10}$ viral load associated with possession of the HLA allele (a Coeff>0 represents a detrimental effect). The hazard ratio (HR) is the relative risk of progression associated with possession of the HLA allele (HR >1 represents a detrimental effect).

It can be seen that *HLA-B*57* was consistently and significantly protective in the presence but not in the absence of functional iKIRs.

Definitions: Functional *2DL1*$^{+}$: *KIR2DL1*$^{+}$*C2*$^{+}$. Functional *2DL2+* (strong): *KIR2DL2*$^{+}$*C1*$^{+}$. Functional *KIR2DL2+* (weak): *KIR2DL2*$^{+}$*C2*$^{+}$. Functional *2DL3+*: *KIR2DL3*$^{+}$*C1*$^{+}$. Functional *2DL1*$^{-}$: *KIR2DL1*$^{-}$ or *KIR2DL1*$^{+}$*C2*$^{-}$. Functional *2DL2*$^{-}$: *KIR2DL2*$^{-}$. Functional *2DL3*$^{-}$: *KIR2DL3*$^{-}$ or *KIR2DL3*$^{+}$*C1*$^{-}$. Inhibitory score (cut-off=2.0): inhibitory score>2.0. Inhibitory score (cut-off=2.5): inhibitory score>2.5. Count (cut-off=2.0): count of functional iKIRs>2.0. The column headings, "*KIR+*" and "*KIR-*" refer to the high and low strata. E.g. for Functional *2DL1*, "*KIR+*" denotes the Functional *KIR2DL1*+ strata, "*KIR-*" the Functional *KIR2DL1*- strata; for inhibitory score (cut off=2.0), "*KIR+*" denotes the strata of individuals with inhibitory score>2.0, "*KIR-*" denotes the strata of individuals with inhibitory score<=2.0 etc.

Note the stratification provided by the count of iKIRs is the same as one of the stratifications provided by inhibitory score. We report both because the count benefits from being a more objective measure (independent of the choice of weight) whilst the inhibitory score benefits from a greater diversity of values, typically allowing for stratification at more than one cut-off.

All cut-offs that gave a sufficiently balanced analysis were considered.

A. IAVI partners *HLA-B*57*								**Outcome: log viral load**
	B57⁺ KIR-		*B57⁺ KIR⁺*		Cohort numbers			
	Coeff	P value	Coeff	P value	*B57⁺ KIR⁻*	*B57⁻ KIR⁻*	*B57⁺ KIR⁺*	*B57⁻ KIR⁺*
Inhibitory score (cut-off=2.0)	-0.37	0.3	-0.33	0.1	10	122	31	152
Inhibitory score (cut-off=2.5)	-0.21	0.4	-0.46	0.06	18	159	23	115
Count iKIR (cut-off=2.0)	-0.37	0.3	-0.33	0.1	10	122	31	152
B. Pooled IAVI & IAVI partners *HLA-B*57*								**Outcome: log viral load**
	B57⁺ KIR⁻		*B57⁺ KIR⁺*		Cohort numbers			
	Coeff	P value	Coeff	P value	*B57⁺ KIR⁻*	*B57⁻ KIR⁻*	*B57⁺ KIR⁺*	*B57⁻ KIR⁺*
Inhibitory score (cut-off=2.0)	-0.33	0.08 .	-0.47	0.0002 ***	27	330	64	420
Inhibitory score (cut-off=2.5)	-0.35	0.01 *	-0.51	0.0007 ***	47	439	44	311
Count iKIR (cut-off=2.0)	-0.33	0.08 .	-0.47	0.0002 ***	27	330	64	420
C. Pooled IAVI & IAVI partners *HLA-B*35Px*								**Outcome: log viral load**
	B35Px⁺ KIR⁻		*B35Px⁺ KIR⁺*		Cohort numbers			
	Coeff	P value	Coeff	P value	*B35Px⁺ KIR⁻*	*B35Px⁻ KIR⁻*	*B35Px⁺ KIR⁺*	*B35Px⁻ KIR⁺*
Inhibitory score (cut-off=2.0)	-0.12	0.4	+0.31	0.003 **	49	308	104	380
Inhibitory score (cut-off=2.5)	+0.08	0.5	+0.27	0.04 *	87	399	66	289
Count iKIR (cut-off=2.0)	-0.12	0.4	+0.31	0.003 **	49	308	104	380
D. US *HLA-B*57* African Americans Outcome: time to low CD4 count (<200 cells/mm³)								
	B57⁺ KIR⁻		*B57⁺ KIR⁺*		Cohort numbers			
	HR	P value	HR	P value	*B57⁺ KIR⁻*	*B57⁻ KIR⁻*	*B57⁺ KIR⁺*	*B57⁻ KIR⁺*
Inhibitory score (cut-off=2.0)	0.54	0.42	0.21	0.04 *	8	92	23	89
Inhibitory score (cut-off=2.5)	0.54	0.41	0.22	0.04 *	10	110	21	71
Count iKIR (cut-off=2.0)	0.51	0.37	0.21	0.04 *	8	92	23	88
E. US *HLA-B*57* All ethnicities Outcome: time to low CD4 count (<200 cells/mm³)								
	B57⁺ KIR⁻		*B57⁺ KIR⁺*		Cohort numbers			
	HR	P value	HR	P value	*B57⁺ KIR⁻*	*B57⁻ KIR⁻*	*B57⁺ KIR⁺*	*B57⁻ KIR⁺*
Inhibitory score (cut-off=2.0)	0.29	0.24	0.25	0.004 **	8	236	36	210
Inhibitory score (cut-off=2.5)	0.34	0.15	0.25	0.01 *	11	271	33	175
Count iKIR (cut-off=2.0)	0.36	0.32	0.27	0.006 **	8	235	36	211

F. US *HLA-B*35Px* African Americans Outcome: time to low CD4 count (<200 cells/mm^3)								
	B35Px$^+$ KIR$^-$		*B35Px$^+$ KIR$^+$*		Cohort numbers			
	HR	P value	HR	P value	*B35Px$^+$ KIR$^-$*	*B35Px$^-$ KIR$^-$*	*B35Px$^+$ KIR$^+$*	*B35Px$^-$ KIR$^+$*
Inhibitory score (cut-off=2.0)	0.22	0.15	2.06	0.05 .	11	89	34	78
Inhibitory score (cut-off=2.5)	0.82	0.68	2.08	0.08 .	24	96	21	71
Count iKIR (cut-off=2.0)	0.29	0.13	2.04	0.06 .	11	89	34	77
G. US *HLA-B*35Px* All ethnicities Outcome: time to low CD4 count (<200 cells/mm^3)								
	B35Px$^+$ KIR$^-$		*B35Px$^+$ KIR$^+$*		Cohort numbers			
	HR	P value	HR	P value	*B35Px$^+$ KIR$^-$*	*B35Px$^-$ KIR$^-$*	*B35Px$^+$ KIR$^+$*	*B35Px$^-$ KIR$^+$*
Inhibitory score (cut-off=2.0)	1.54	0.22	2.57	0.001 **	20	224	44	202
Inhibitory score (cut-off=2.5)	1.89	0.02 *	2.42	0.01 *	37	245	27	181
Count iKIR (cut-off=2.0)	1.81	0.09 .	2.74	0.0004 ***	20	223	44	203

Table 2. Functional iKIRs enhance the protective effect of *HLA-B*57* and the detrimental effect of *HLA-B*35Px* in two independent replication cohorts.

Two independent replication cohorts were considered (IAVI-partners and US). In a separate analysis IAVI and IAVI-partners were pooled to increase cohort size. Cohorts were stratified into individuals with a low or high inhibitory score (two cut-offs considered) and into individuals with a low or high count of functional iKIR (only one cut-off possible). In each stratum the effects of *HLA-B*57* and *HLA-B*35Px* were analyzed. *HLA-B*35Px* was not analyzed in IAVI partners as the HLA gene alone had no detectable impact (Coeff=+0.04 P=0.8).

Definitions as in **Table 1**. In all cohorts, all variables which had a significant impact on outcome were included in the model as covariates. A: linear regression, covariates: gender. B&C: linear regression, covariates: gender, cohort. D&F: Cox regression, covariates: age, subcohort. E&G: Cox regression, covariates: *KIR3DL1$^+$Bw4-80I$^+$*, age, ethnicity, subcohort. In all cohorts where functional *KIR3DS1* (*KIR3DS1$^+$Bw4-80I$^+$*) was protective individuals with functional *KIR3DS1* were removed prior to analysis to give a clean background (cohorts reported in B, C, E, G).

Note, the only balanced stratification provided by the “count of functional iKIRs” (namely ≤2 *vs.* >2) is the same as the stratification provided by the inhibitory score with a cut-off of 2.0. The inhibitory score has more diversity than the count and so typically more stratifications by inhibitory score than by count are possible. All cut-offs that gave a sufficiently balanced analysis were considered.

KAGOSHIMA COHORT. OUTCOME: ODDS OF DISEASE (HAM/TSP)								
A. *HLA-B*54*								
	B54⁺ KIR⁻		*B54⁺ KIR⁺*		Cohort numbers			
	OR	P value	OR	P value	*B54⁺ KIR⁻*	*B54⁻ KIR⁻*	*B54⁺ KIR⁺*	*B54⁻ KIR⁺*
Functional *2DL2* (strong)	1.69	0.17	12.14	0.004 **	62	231	20	79
Functional *3DL1*	1.98	0.13	4.45	0.007 **	48	147	34	163
Inhibitory score (cut-off=1.5)	1.29	0.7	5.33	0.0007 ***	36	94	46	216
Inhibitory score (cut-off=1.75)	2.19	0.03 *	9.35	0.06 .	73	253	9	57
Count functional iKIR (cut-off=1)	1.31	0.6	5.31	0.0007 ***	36	93	46	217
B. *HLA-A*02:07*								
	A0207⁺ KIR⁻		*A0207⁺ KIR⁺*		Cohort numbers			
	OR	P value	OR	P value	*A0207⁺ KIR⁻*	*A0207⁻ KIR⁻*	*A0207⁺ KIR⁺*	*A0207⁻ KIR⁺*
Functional *2DL2* (strong)	0.33	0.04 *	0.15	0.1	23	270	7	92
Functional *3DL1*	0.54	0.3	0.07	0.006 **	21	174	9	188
Inhibitory score (cut-off=1.5)	0.81	0.8	0.10	0.003 **	16	114	14	248
Inhibitory score (cut-off=1.75)	Insufficient number of *A02:07⁺KIR⁺* individuals							
Count functional iKIR (cut-off=1)	0.83	0.8	0.10	0.003 **	16	113	14	249
C. *HLA-C*08*								
	C08⁺ KIR⁻		*C08⁺ KIR⁺*		Cohort numbers			
	OR	P value	OR	P value	*C08⁺ KIR⁻*	*C08⁻ KIR⁻*	*C08⁺ KIR⁺*	*C08⁻ KIR⁺*
Functional *2DL2* (strong)	0.66	0.4	0.16	0.02 *	43	250	13	86
Functional *3DL1*	0.53	0.19	0.48	0.3	39	156	17	180
Inhibitory score (cut-off=1.5)	1.09	0.9	0.32	0.02 *	28	102	28	234
Inhibitory score (cut-off=1.75)	0.62	0.2	0.08	0.04 *	49	277	7	59
Count functional iKIR (cut-off=1)	1.11	0.9	0.32	0.02 *	28	101	28	235

Table 3. Functional iKIRs enhance HLA class I associations in HTLV-1 infection.

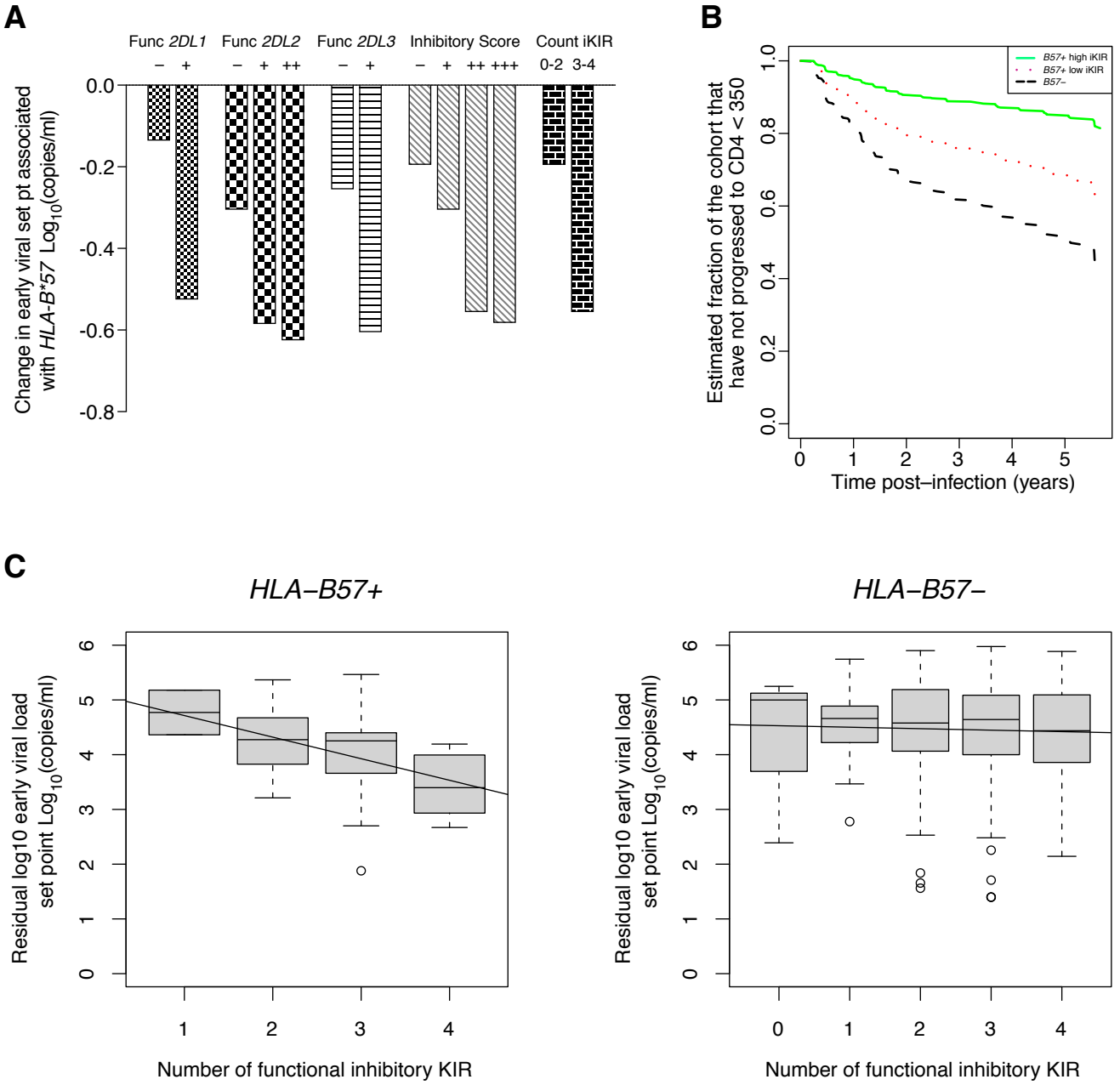
The Kagoshima cohort was stratified into individuals with and without the functional iKIR of interest. In each stratum the effect of (A) *HLA-B*54*, (B) *HLA-A*02:07* and (C) *HLA-C*08* on HAM/TSP status was analyzed using multivariate logistic regression (with covariates age & gender). The odds ratio (OR), P values and cohort sizes are reported. An OR <1 represents a protective effect (reduced odds of HAM/TSP).

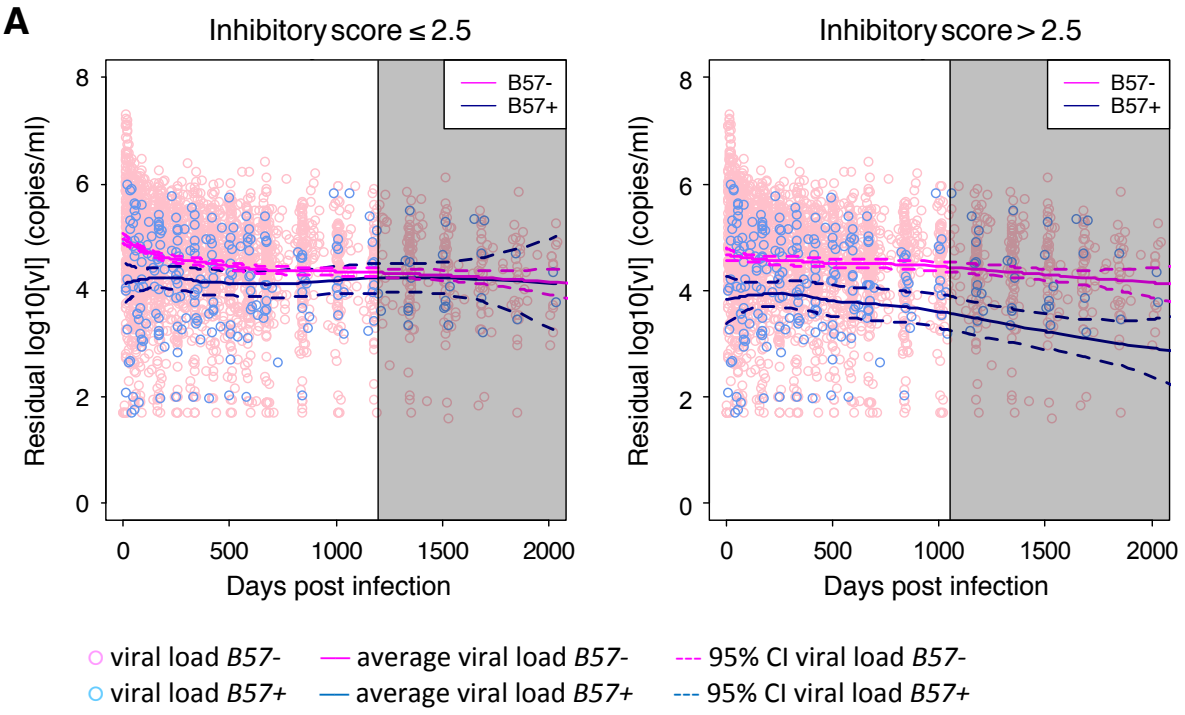
The detrimental effect of *HLA-B*54* and the protective effects of *HLA-A*02:07* and *HLA-C*08* on HAM/TSP status were enhanced in people with strong functional *KIR2DL2*, functional *KIR3DL1* and a high inhibitory score. There was an insufficient number of people with *HLA-C2* to analyse functional *KIR2DL1*, weak functional *KIR2DL2* and functional *KIR2DL3*. Similar results were obtained if we use factors instead of stratification. The ligands of KIR3DL1 are contentious with one paper reporting that KIR3DL1 binds HLA-A*23, -A*24 and –A*32 as well as HLA-Bw4 molecules (*58*). If we amended our definition of functional *KIR3DL1* to include these *HLA-A* alleles then the conclusions were unchanged with the exception of *HLA-C*08* where our conclusion was strengthened (magnitude of enhancement of protective effect of *HLA-C*08* in the functional *KIR3DL1*-positive stratum is greater).

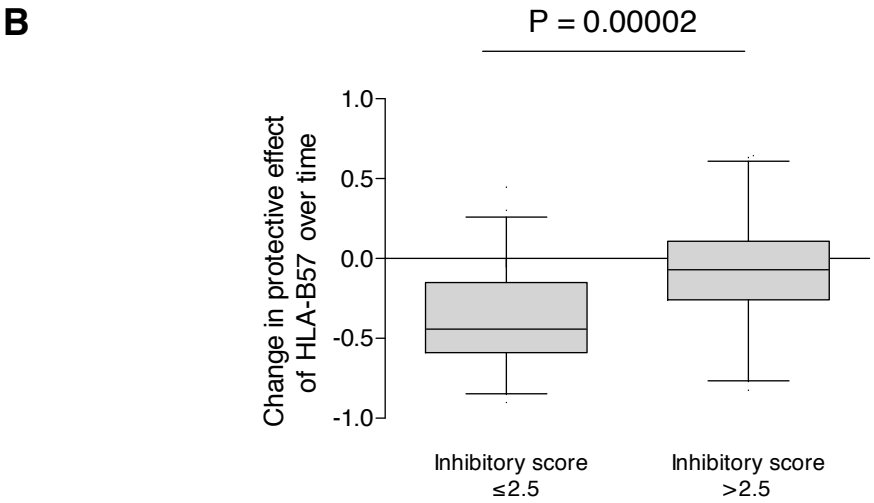
All cut-offs that gave a sufficiently balanced analysis were considered. The definitions of 'low' and 'high' iKIR counts and scores were reduced compared with other cohorts in this study to compensate for lower overall iKIR counts and scores in the Japanese cohort.

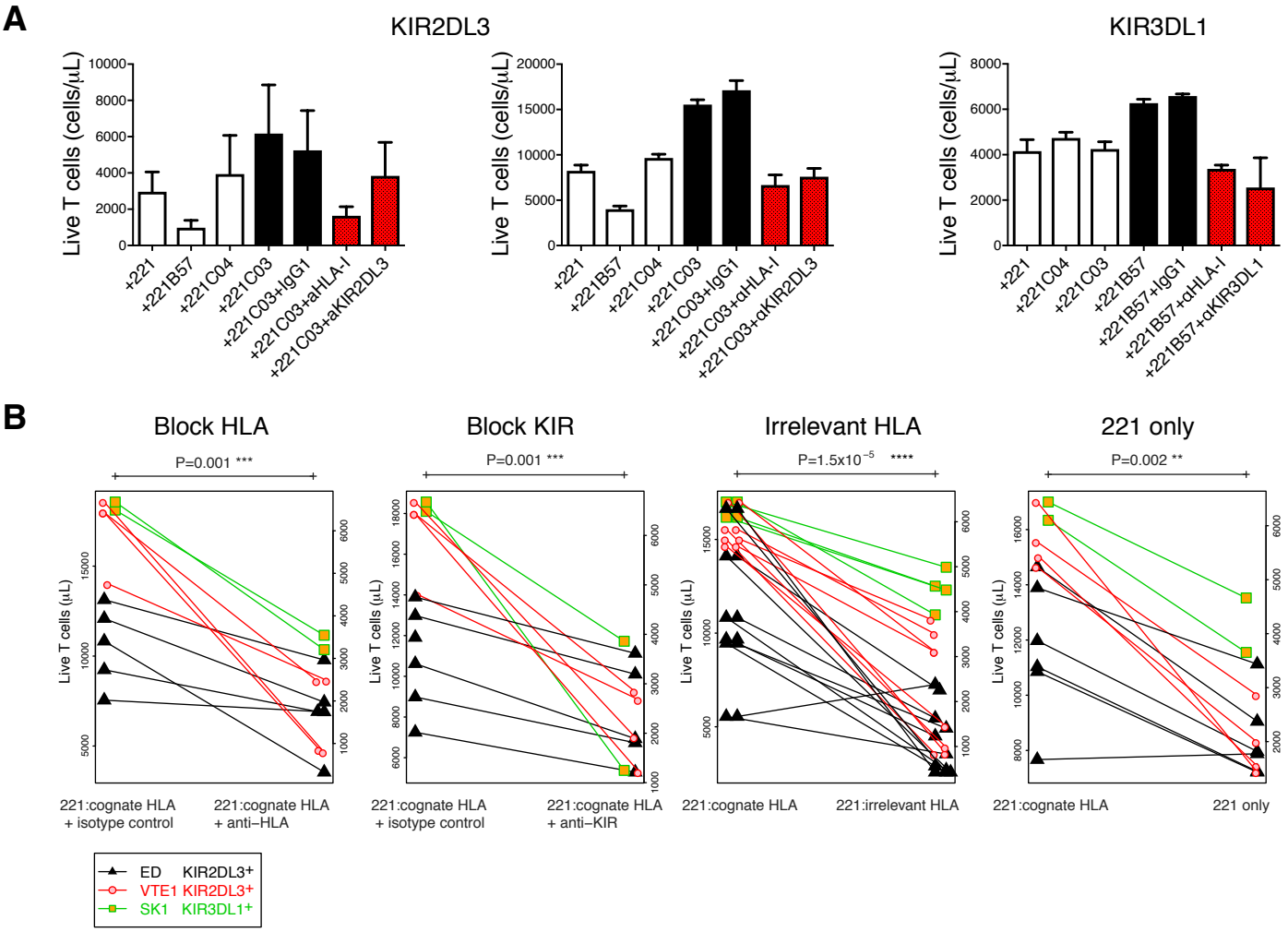
	Protective	**Detrimental**
HIV-1	*HLA-B*57*	*HLA-B*35Px*
HTLV-1	*HLA-A*0207, HLA-C*08*	*HLA-B*54*
HCV	*HLA-B*57*	

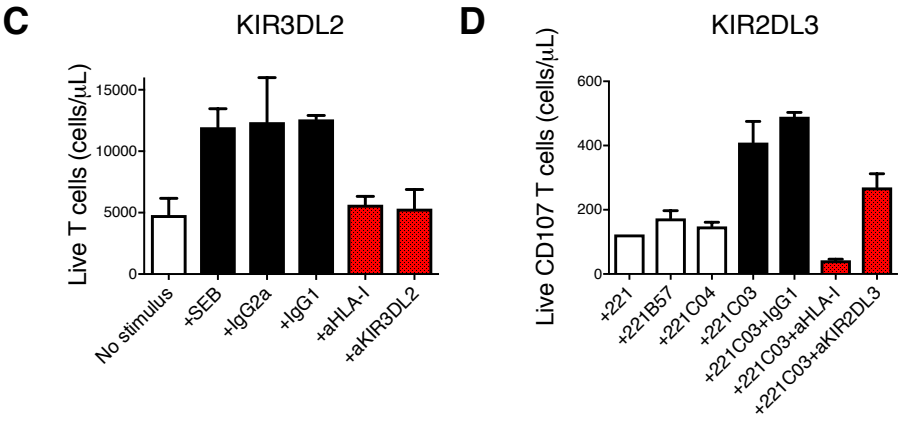
Table 4. HLA class I disease associations studied.

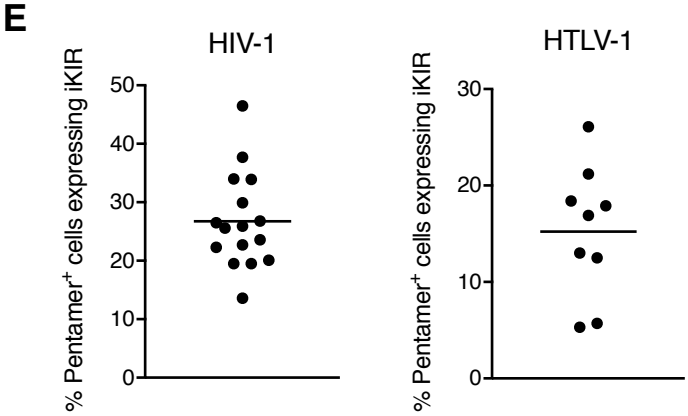

A
Func 2DL1
Func 2DL2
Func 2DL3
Inhibitory Score
Count iKIR
− +
− + ++
− +
− + ++ +++
0-2 3-4
Change in early viral set pt associated with HLA-B*57 Log10(copies/ml)
0.0
-0.2
-0.4
-0.6
-0.8
B
B57+ high iKIR
B57+ low iKIR
B57−
Estimated fraction of the cohort that have not progressed to CD4 < 350
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
4
5
Time post−infection (years)
C
HLA−B57+
HLA−B57−
Residual log10 early viral load set point Log10(copies/ml)
0
1
2
3
4
5
6
1
2
3
4
Number of functional inhibitory KIR

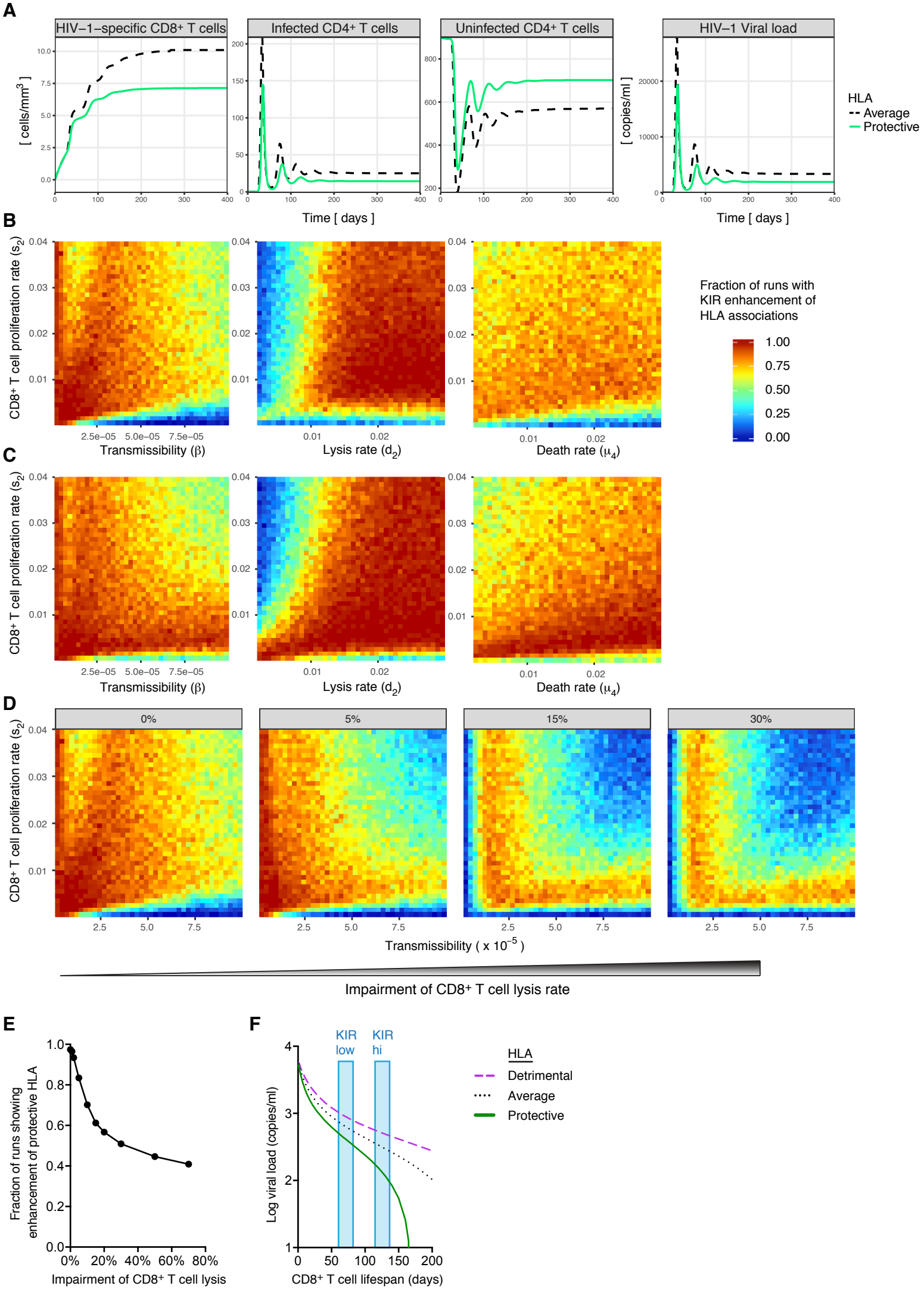

A
Inhibitory score ≤ 2.5
Inhibitory score > 2.5
B57-
B57+
Residual log10[vl] (copies/ml)
Days post infection
viral load B57-
viral load B57+
average viral load B57-
average viral load B57+
95% CI viral load B57-
95% CI viral load B57+


B
P = 0.00002
Change in protective effect of HLA-B57 over time
Inhibitory score ≤2.5
Inhibitory score >2.5


A
KIR2DL3
KIR3DL1
Live T cells (cells/µL)
+221
+221B57
+221C04
+221C03
+221C03+IgG1
+221C03+aHLA-I
+221C03+aKIR2DL3
+221C03+αKIR2DL3
+221B57+IgG1
+221B57+aHLA-I
+221B57+aKIR3DL1
B
Block HLA
Block KIR
Irrelevant HLA
221 only
P=0.001 ***
P=0.001 ***
P=1.5x10^-5 ****
P=0.002 **
Live T cells (µL)
221:cognate HLA + isotype control
221:cognate HLA + anti−HLA
221:cognate HLA + isotype control
221:cognate HLA + anti−KIR
221:cognate HLA
221:irrelevant HLA
221:cognate HLA
221 only
ED KIR2DL3+
VTE1 KIR2DL3+
SK1 KIR3DL1+


C
KIR3DL2
Live T cells (cells/µL)
No stimulus
+SEB
+IgG2a
+IgG1
+aHLA-I
+aKIR3DL2
D
KIR2DL3
Live CD107 T cells (cells/µL)
+221
+221B57
+221C04
+221C03
+221C03+IgG1
+221C03+aHLA-I
+221C03+aKIR2DL3


E
HIV-1
HTLV-1
% Pentamer+ cells expressing iKIR


A
HIV−1−specific CD8+ T cells
Infected CD4+ T cells
Uninfected CD4+ T cells
HIV−1 Viral load
[cells/mm3]
[copies/ml]
Time [days]
HLA
Average
Protective
B
C
D
CD8+ T cell proliferation rate (s2)
Transmissibility (β)
Lysis rate (d2)
Death rate (μ4)
Fraction of runs with KIR enhancement of HLA associations
1.00
0.75
0.50
0.25
0.00
0%
5%
15%
30%
Transmissibility (x 10−5)
Impairment of CD8+ T cell lysis rate
E
Fraction of runs showing enhancement of protective HLA
Impairment of CD8+ T cell lysis
F
KIR low
KIR hi
Log viral load (copies/ml)
CD8+ T cell lifespan (days)
HLA
Detrimental
Average
Protective