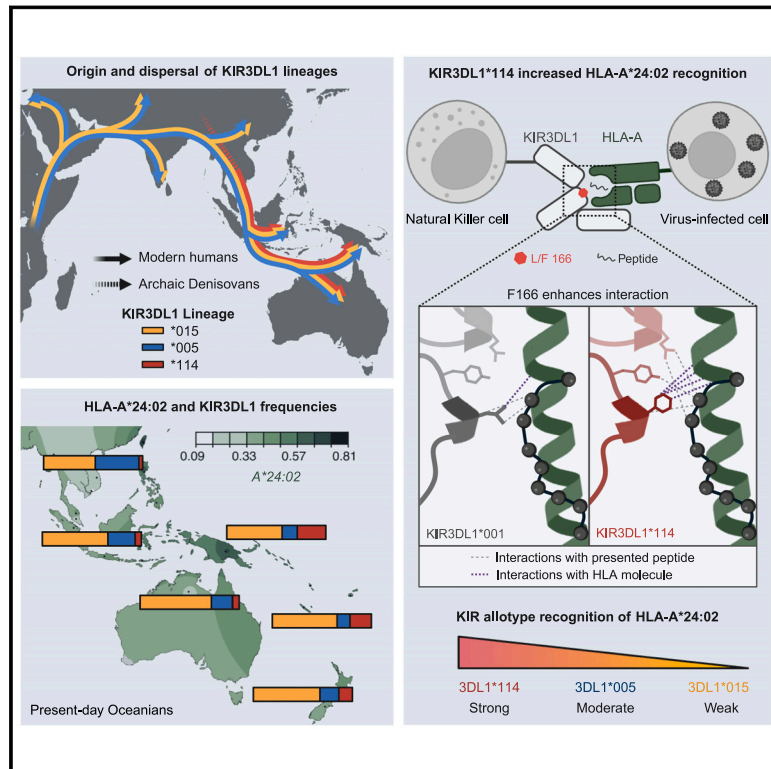


An archaic HLA class I receptor allele diversifies natural killer cell-driven immunity in First Nations peoples of Oceania

Graphical abstract



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In brief

Predominant genetic variants found in First Nations peoples of the Oceania region influence the function of natural killer cells and may explain population-specific susceptibility to diseases, including influenza.

Highlights

- Archaic human natural killer (NK) cell receptor KIR3DL1 allele is frequent across Oceania
- Archaic KIR3DL1 has enhanced binding to modern human HLA class I ligand
- Enhanced binding allowed modern human ligand to expand in frequency
- Archaic admixture has lasting impact, modulating NK cell responses to infection



Article

An archaic HLA class I receptor allele diversifies natural killer cell-driven immunity in First Nations peoples of Oceania

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SUMMARY

Genetic variation in host immunity impacts the disproportionate burden of infectious diseases that can be experienced by First Nations peoples. Polymorphic human leukocyte antigen (HLA) class I and killer cell immunoglobulin-like receptors (KIRs) are key regulators of natural killer (NK) cells, which mediate early infection control. How this variation impacts their responses across populations is unclear. We show that HLA-A*24:02 became the dominant ligand for inhibitory KIR3DL1 in First Nations peoples across Oceania, through positive natural selection. We identify KIR3DL1*114, widespread across and unique to Oceania, as an allele lineage derived from archaic humans. KIR3DL1*114⁺NK cells from First Nations Australian donors are inhibited through binding HLA-A*24:02. The KIR3DL1*114 lineage is defined by phenylalanine at residue 166. Structural and binding studies show phenylalanine 166 forms multiple unique contacts with HLA-peptide complexes, increasing both affinity and specificity. Accordingly, assessing immunogenetic variation and the functional implications for immunity are fundamental toward understanding population-based disease associations.



INTRODUCTION

Host genetics influences immune responses, contributing to the disproportionate morbidity and mortality from infectious diseases experienced by First Nations peoples.^{1–4} During viral infection, HLA class I displays peptides derived from virus proteins on the surface of infected cells, rendering them liable for elimination by cytotoxic cells of immunity. Extensive HLA-A, -B, and -C polymorphisms both define the repertoire of peptides that may be presented by a given individual and impact immune cell receptor binding.^{5,6} For example, whereas HLA-A*02:01 and -B*08:01, which are HLA class I types frequent in populations of European genetic ancestry, mediate protection from severe influenza disease, HLA-A*24:02, which is highly prevalent in First Nations Oceanian and American populations, associates with poor outcome.⁷ Although HLA-A*02:01, A*24:02, and B*08:01 all mediate strong influenza-specific cytotoxic T cell responses,^{8–10} only A*24:02 is also a ligand for inhibitory KIR3DL1, expressed by natural killer (NK) cells.¹¹ NK cells act early in immune responses by killing infected cells, releasing cytokines, and stimulating adaptive immunity.¹² Interaction of inhibitory killer cell immunoglobulin-like receptor (KIR) with HLA class I prevents NK cells from destroying healthy cells while enabling them to sense any virus-induced HLA class I downregulation.^{13–15} Importantly, the ability to respond to missing or altered HLA in this manner is defined by inherited combinations of the highly polymorphic *HLA class I* and *KIR* genes.¹⁶

Not every individual has all the KIR capable of interacting with HLA class I, and not every HLA class I allele can form a KIR ligand.¹⁷ This genetically established combinatorial diversity distinguishes individuals in their infection outcomes.¹⁸ In addition to modulating effector functions, signaling through KIR educates developing NK cells to recognize the specific HLA class I ligands expressed by a given individual.¹⁹ The strength of inhibitory signal transduction, which is dependent both on KIR and HLA allotype, calibrates the effector and killing capacity of the mature NK cells.^{20,21} Among polymorphic receptors shaping diversity of NK cell responses to viral infection, inhibitory KIR3DL1 dominates.^{22,23} Enabling recognition by KIR3DL1, HLA-A*24:02 carries the “Bw4” sequence motif, occurring in amino acid residues 77–83, and common to a subset of HLA-A and -B allotypes.²⁴ There are two ancient lineages of KIR3DL1, which are the 015 lineage that interacts poorly with HLA-A*24, favoring HLA-B allotypes, and the 005 lineage that interacts both with HLA-A and -B allotypes.^{25,26} Although there is a predominance of Bw4⁺ HLA-A allotypes across Oceania, there is also a relative lack of 005-lineage KIR3DL1.²⁷

Likely following emigration out of Africa, present-day First Nations Oceanians diverged from Eurasians ~50–70 kya.^{28,29} Subsequent admixture with archaic humans introduced new genetic material, having major impact on genes of the immune system.^{30–36} Archaic human refers to the extinct hominins, such as Denisovans, who diverged from modern humans in Africa ~600,000 years ago to populate Europe and Asia.³⁷ We examined the immunogenetic composition of First Nations Oceanians and identified a unique and divergent form of KIR3DL1 having characteristics of archaic human sequence. Because such archaic introgression rarely involves protein-coding variation, favoring regulatory elements,^{38,39} we investigated the origin, distribution, and functions of this allele

to determine if it could impact the course of NK cell-driven immunity across Oceania.

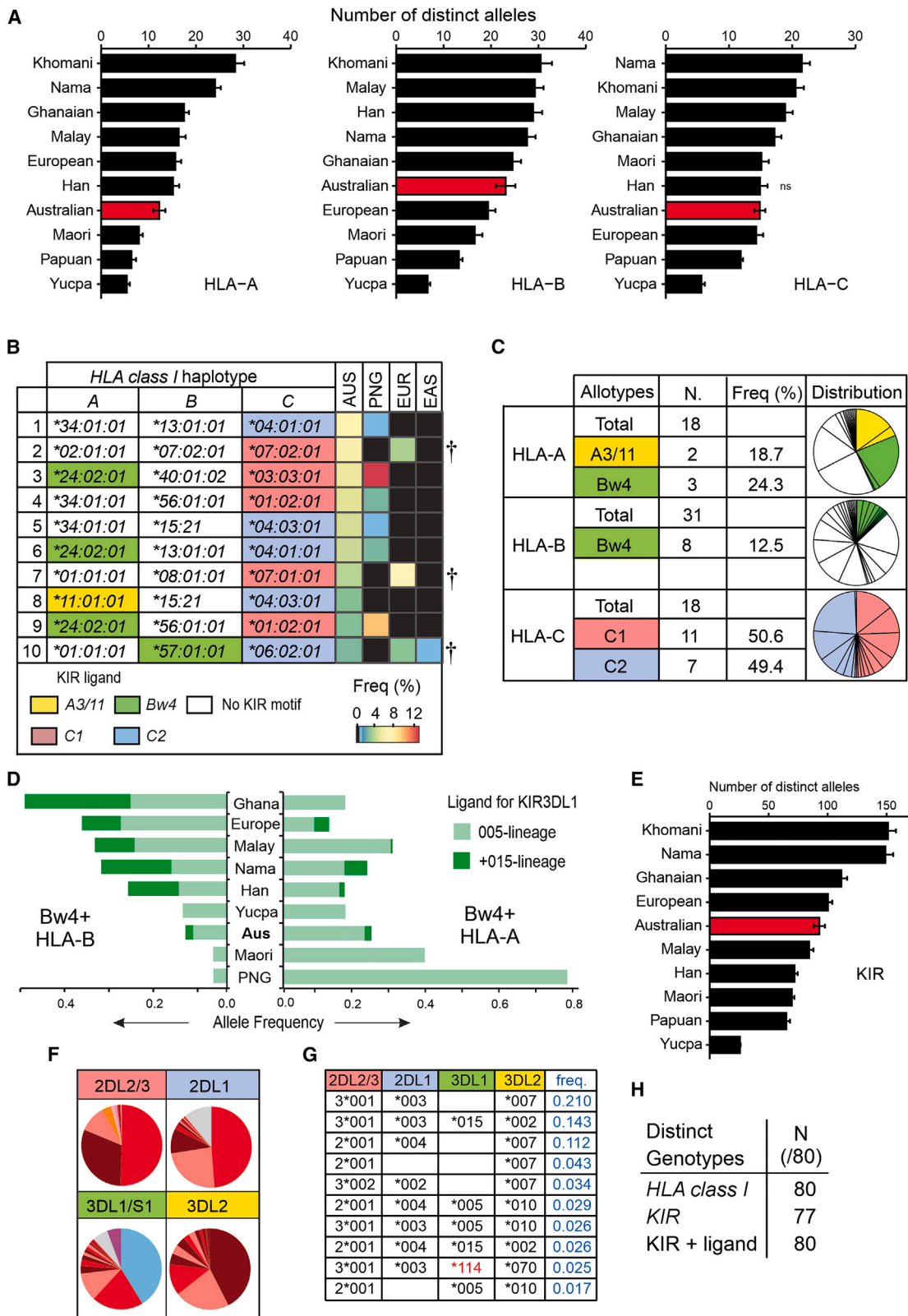
RESULTS

High combinatorial diversity of HLA and KIR in First Nations Australians

Reflecting differential genetic ancestries, environments, and pathogen exposures, human populations have accumulated characteristic *HLA* allele spectra.⁴⁰ In 80 First Nations Australians from northern Australia,⁷ we identified 18, 33, and 25 alleles of HLA-A, -B, and -C, respectively (Table S1). Accounting for sample size, this observed diversity in *HLA class I* alleles is similar to Europeans and West Africans, representing an intermediate between First Nations Papua New Guineans and southern Africans (Nama and Khomani; Figure 1A), who are among the most genetically diverse humans.⁴² A haplotype refers to a specific combination of alleles that are inherited together. The *HLA class I* alleles form 79 distinct haplotypes in the First Nations Australian cohort (Table S1). The most frequent alleles for each locus, HLA-A*34:01 (24%), -B*13:01 (18%), and -C*04:01 (23%), respectively, comprise the most frequent *HLA class I* haplotype (Figure 1B). HLA-A*34:01 and -B*13:01 are characteristic to Oceanian populations,^{43,44} and analysis of genome-wide SNPs shows this haplotype is Australian/Oceanian in origin (Figures 1B and S1). Seven of the ten most frequent *HLA class I* haplotypes have similar Oceanian-related ancestry, whereas three are of likely European descent (Figure 1B).

Together, the 76 unique HLA class I alleles encode 67 distinct protein sequences (allotypes) in First Nations Australians (Figure 1C). Bw4 is the motif that forms the ligand for KIR3DL1 carried by some HLA-A and -B allotypes (ligands and nomenclature are described in STAR Methods). In First Nations Australians, HLA-A*24:02 is the most frequent Bw4⁺ HLA allotype present (21.9%) followed by B*15:22, B*44:02, and B*44:03 (each 2.5%, Table S1). These HLA allotypes bind more strongly to 005 lineage than to 015-lineage KIR3DL1 allotypes.^{23,26} Thus, in First Nations Australians, most Bw4⁺ HLA will interact preferentially with 005-lineage KIR3DL1, and in other Oceanians, all the Bw4⁺ HLA will interact preferentially with 005-lineage KIR (Figure 1D).

KIR polymorphism affects the capacity of NK cells to respond to infection-induced changes in HLA class I expression.^{18,45,46} Of the 13 *KIR* genes, a total of 121 alleles were identified in the cohort, including 27 first characterized here (Figure 1E; Table S1). In addition to KIR3DL1, other KIRs differentially bind A3 and A11, or the C1 or C2 motifs of HLA class I (STAR Methods). In the cohort of First Nations Australians, KIR2DL1 (specific for C2⁺HLA) has nine allotypes, 2DL2/3 (specific for C1 and some C2) has eight, 3DL1 (Bw4) has 13, and 3DL2 (A3/11) has eleven (Figure 1F). The inhibitory KIR alleles are arranged in 55 distinct haplotypes (Figure 1G; Table S1), and every individual in the cohort has a unique combination of KIR and ligands (Figure 1H). The distribution of *KIR* and *HLA class I* alleles is characteristic of a diverse population, due in part to admixture with Europeans, in accordance with genome-wide data.²⁸ First Nations Australians from northern Australia represent a population with substantial genetic variation that has the potential to diversify the NK cell response to viral infections.¹³



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The First Nations *KIR3DL1*114* allotype is divergent and likely introgressed

KIR3DL1 is encoded by the *KIR3DL1/S1* locus, which also encodes the activating *KIR3DS1*, differentiating an additional lineage.⁴⁷ All three lineages of *KIR3DL1/S1* were observed in First Nations Australians, with the 005 lineage represented by three cell-surface expressed allotypes, the 015 lineage by six allotypes, and 3DS1 by a single allotype (Figure 2A). All five *KIR3DL1* alleles we first identified here encode distinct allotypes (Table S1). Of these, *KIR3DL1*114* was observed in five of the 80 individuals. The *KIR3DL1*114* protein sequence is distinguished from *KIR3DL1*015* by three and *005 by five amino acid changes (Figure 2B). Importantly, *KIR3DL1*114* possesses phenylalanine at position 166. Residue 166 is subject to positive natural selection for diversity in hominids,⁴⁷ with three alternative residues found at this site in humans (Figure 2B). Residue 166 is located in the second Ig domain (D1) at the interface with the HLA-peptide complex.^{24,48}

The residues alanine 88 and phenylalanine 166 (Phe-166) that characterize *KIR3DL1*114* are shared with *KIR3DL1*086*, previously identified in Māori²⁷ (Figure 2B). To determine the wider distribution of these alleles, we analyzed genome-wide SNP data for 1,210 unrelated Oceanians, comprising 913 samples from the Oceanian Genomic Variation Project (OGVP),⁴⁹ and an additional 297 Papua New Guinean individuals.⁵⁰ We also sequenced the *KIR* and *HLA* genes from an overlapping cohort of 645 Papuans. Examining their global distribution (STAR Methods) indicates *KIR3DL1*086* and *114 are both specific to Oceanians (Figure 2C). Conservative estimates of the allele frequencies range from 1% in Kinh Vietnamese to 21% in Lowland and 28% in Highland Papuans (Table S3). From individuals having both sequence and SNP data, we inferred the local genetic ancestry and identified 54/56 (92.9%) *KIR3DL1*114*⁺ haplotypes as Papuan, with a positive correlation of *KIR3DL1*114* with Papuan-related ancestry across Oceania and Southeast Asia (Figures S2A–S2D). Thus, *KIR3DL1*114* is characteristic to and widespread throughout Oceania and likely shared by gene flow into southeast Asia.

Throughout Oceania, *KIR3DL1*114* and *086 are each observed in tight linkage with *KIR2DL4*028* and *KIR2DS4*019* (Figure 2D), which, together with *KIR3DL2*070*, characterize

the First Nations Australian *KIR3DL1*114*⁺ haplotypes (Figure 2D). Each of the four alleles is distinguished from the closest known allele⁵¹ by at least two unique single nucleotide substitutions. Analysis of *HLA* genotypes confirmed the five individuals are unlikely to be first- or second-degree relatives. Intrigued by the distinct characteristics and established evidence that archaic admixture is detectible in Oceanians,^{52,53} we further investigated *KIR3DL1*114* carrying haplotypes. Indeed, the D-statistic, which determines allele sharing across lineages,^{33,54} shows statistically significant evidence that the *KIR3DL1*114* haplotype has an allele composition shared with the Denisovan genome (*D* score = 0.95, *Z* score = 40.6, Figure 2E). This analysis also showed the minimum length of the haplotype to be 62,276 bp (Table S3), spanning the *KIR2DL4* through *KIR3DL2* genes. We estimate *KIR3DL1*114* diverged from the *KIR3DL1* 015- and 005-lineages 1.84 mya (95% CI: 0.994–2.996, STAR Methods). This divergence time is older than the split between archaic and modern humans, and the *114 allele group is absent from Africa, suggesting re-introduction from archaic humans to Oceanian ancestors.³² A competing hypothesis is the ancestral lineage present in both modern and archaic humans was lost from Africa either during or shortly after the out-of-Africa migration. However, the probability of a haplotype of 62.3 kbp being shared by modern Oceanians and Denisovans due to incomplete ancestral lineage sorting is extremely low (8.78×10^{-9}),⁵⁵ further supporting transfer to modern humans from archaic humans. The geographical distribution and sequence divergence of *KIR3DL1*114* carrying haplotypes and significant allele sharing with Denisovan therefore make them clear candidates for originating from archaic hominins through introgression.^{32,35}

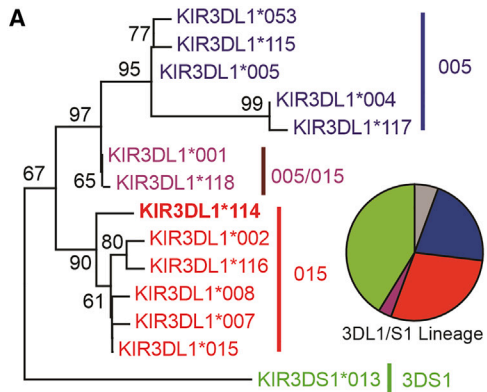
*HLA-A*24:02* is the dominant ligand for *KIR3DL1* in First Nations Australians (Figure 1), and the mean allele frequency of *A*24:02* across Oceania and Southeast Asia is $46.1\% \pm 19.6\%$ (Figure 2C; Table S3). *HLA* is subject to balancing selection, favoring heterozygotes, and promoting diversity in defense against pathogens.^{56–58} By contrast here, homozygosity of *HLA-A* is higher than expected under neutrality, as indicated by the Ewens-Watterson *F*-statistic (e.g., $p < 0.05$ in Australia; Table S3). In Australia and Papua New Guinea, *HLA-A*24:02* drives the reduced *HLA-A* diversity, where the number of *A*24:02* homozygotes is significantly higher than expected

Figure 1. High combinatorial diversity of HLA and KIR in First Nations Australians

- (A) Shown is the number of distinct *HLA-A*, *-B*, and *-C* alleles of 75 random individuals for each indicated population. Error bars represent mean \pm standard deviation from 1,000 resamples with replacement. Unpaired two-sample Wilcoxon tests with Bonferroni multiple test correction were performed between First Nations Australians (shaded red) and each reference population (p for each comparison is <0.001 , except with Han for *HLA-C*, which is not significant, ns).
- (B) The ten most frequent *HLA class I* haplotypes detected in First Nations Australians. The *KIR* ligands are colored: A3/11 (yellow), Bw4 (green), C1 (red), and C2 (blue). At the right are shown their frequencies in First Nations Australians (AUS), Papua New Guineans (PNG), Europeans (EUR), and East Asians (EAS) (key is underneath). †, haplotype of likely European origin (Figure S1; Table S2).
- (C) The distribution of *KIR* ligands among *HLA class I* allotypes. The combined frequencies of each subset are shown.
- (D) Shows the total allele frequencies of Bw4⁺ *HLA-B* (left) and Bw4⁺ *HLA-A* (right) across the representative populations. Bw4⁺ allotypes that preferentially interact with 005 lineage *KIR3DL1* are shown in light green; those that interact with 005 and 015 lineage in dark green.
- (E) The number of distinct *KIR* alleles detected in 75 individuals from each of the populations indicated, obtained as described for (A).
- (F) Frequency spectra of *KIR* alleles encoding inhibitory receptors specific for *HLA class I* (colored by cognate ligand from B). Each segment corresponds to a distinct *KIR* allotype. (Blue) Activating *KIR3DS1*, (purple) allotypes that are not expressed on the cell surface,⁴¹ and (gray) gene absence.
- (G) Ten most frequent haplotypes considering inhibitory *KIR* specific for *HLA class I*.
- (H) The number of distinct *HLA class I* and *KIR* genotypes observed in 80 First Nations Australians. “*KIR* + ligand” excludes non-expressed allotypes and non-functional interactions.

An explanation of *HLA* and *KIR* nomenclature is given in STAR Methods.

See also Figure S1.

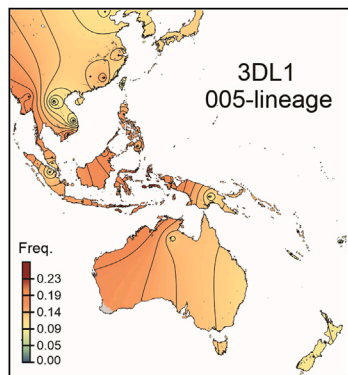
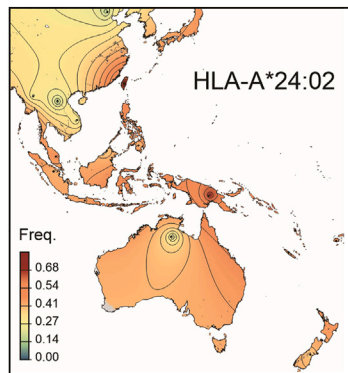
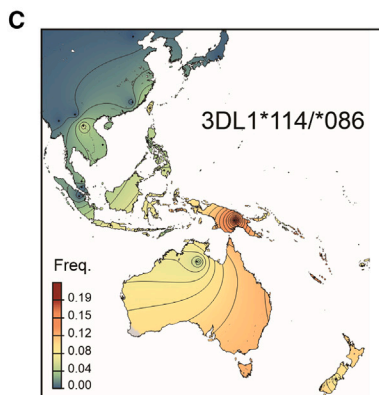
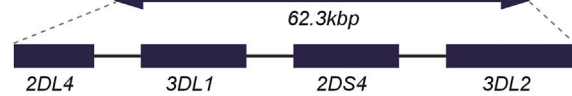


B

	Domain	D0						D1				D2		
		2	20	47	54	58	88	92	138	163	166	182	199	283
KIR3DL1/S1 allotype	L1*015	V	R	V	L	S	P	V	G	P	L	P	P	W
	L1*114	M	-	-	-	A	-	-	-	F	-	-	-	
	L1*086	M	Q	I	I	-	A	-	-	F	-	-	-	
	L1*005	M	-	I	I	-	-	-	-	-	S	-	L	
	S1*013	M	-	-	I	G	-	M	W	S	R	-	L	

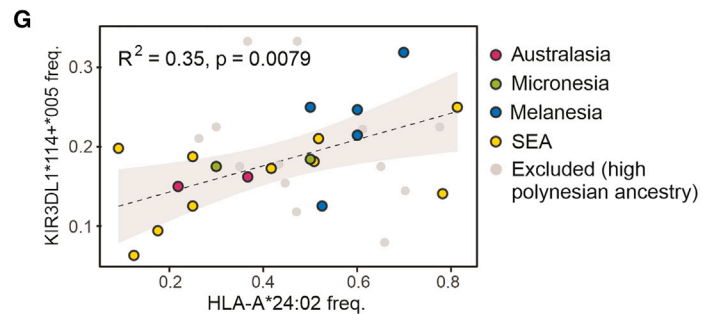
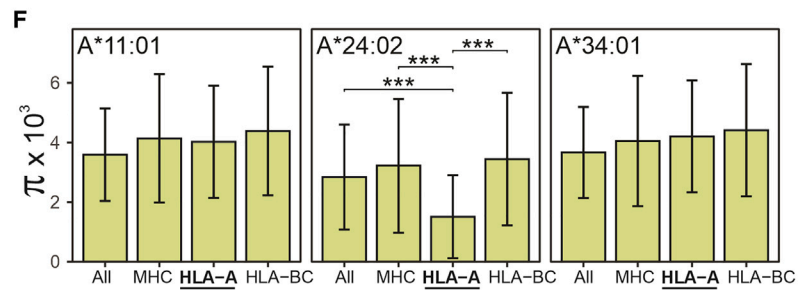
D

Origin	2DL4	3DL1/S1	2DS4	3DL2	N
First Nations	*028	*114	*019	*070	4
Australian (2N=160)	*028	*114	*019	*002	1
Vietnamese (2N=248)	*028	*114	*019	*070	2
Malay (2N=184)	*028	*114	*019	*101	2
Māori (2N=98)	*028	*086	n.d.	*002	4
Polynesian (2N=68)	*028	*086	n.d.	*002	4
Papua New Guinean (2N=1,290)	*028	*114	*019	*002	334
	*028	*086	*019	*002	18
	*028	*114	*019	*111	7
	*028	*114	*019	*070	4
	*028	*114	*019	*010	1



E

	W	D	Z
*114		0.95	40.6
*005		0.39	1.75
*015		-0.52	-2.46



(legend on next page)

under Hardy-Weinberg equilibrium ($p < 0.05$, Table S3). The reduced genetic diversity of *HLA-A*24:02* is not observed in the rest of the genome or in other *HLA-A* alleles (Figure 2F; Table S3), and we found no evidence that *HLA-A*24:02* has archaic human origin (Figure S2E). An earlier study suggested high frequency of *HLA-A*24:02* is due to positive natural selection.⁵⁹ Here, we used the iHS test, which can detect positive selection within 1,000 generations of present, and identified a signal acutely centered on *HLA-A*24:02* (Figure S2F).

Finally, although we observed the high frequency of *HLA-A*24:02* does not correspond with the frequency of 3DL1*005, its strongest-binding KIR partner (Figure 2C), we did observe a statistically significant positive correlation of *HLA-A*24:02* with the combined allele frequencies of *KIR3DL1*005* and **114* (Figure 2G). These findings suggest that the rapid frequency rise of *HLA-A*24:02* across Oceania was permitted by the presence of *KIR3DL1*114* compensating for the scarcity of *KIR3DL1*005*-lineage allotypes.

KIR3DL1*114 has 005 lineage properties

KIR3DL1 polymorphism can determine cell surface expression level, which can impact NK cell function.⁴⁷ With exceptions, 015 lineage allotypes are expressed at high levels on the NK cell surface, and 005 lineage at low levels.⁴⁷ To determine the cell-surface expression of *KIR3DL1*114*, we analyzed NK cells isolated from blood donors selected according to their *KIR3DL1/S1* genotype (Figures S3A and S3B). *KIR3DL1*114* is expressed on the NK cell surface at significantly lower levels than *KIR3DL1*015* or **001* allotypes ($p = 0.0007$ and $p = 0.0013$, respectively) and at a similar level to *KIR3DL1*005* (Figure 3A). Thus, the expression level of this allotype is like the 005 lineage.

By measuring upregulation of CD107a and interferon (IFN)- γ , we tested the response of primary NK cells to target cells expressing *HLA-A*24:02*. Whereas all *KIR3DL1** NK cells responded to *HLA*-deficient targets, NK cells expressing *KIR3DL1*114* or **005* were more inhibited by *HLA-A*24:02* than those expressing *KIR3DL1*001* or **015*-like allotypes (Figure 3B). Showing that the inhibition is specific to *KIR3DL1*, the response was not significantly diminished in NK cells expressing only *KIR2DL2/3* or *KIR2DL1* from the same donors, assessed in parallel (Figure 3C). To determine NK cell responses against a broader

set of target cells transfected with *HLA* class I, we used interleukin-2 (IL-2)-expanded *KIR3DL1** NK cells from donors expressing *KIR3DL1*001*, **005*, **015*, or **114* (Table S4). The targets included all three Bw4⁺ *HLA-A* and six of eight Bw4⁺*HLA-B* allotypes identified in the First Nations Australian cohort (Figure 3D). Also included were B*27:05, which preferentially binds *KIR3DL1*005*, and A*25:01, which does not bind *KIR3DL1*.^{11,26} All four *KIR3DL1* allotypes strongly inhibited NK cells from killing target cells expressing *HLA-A*32:01*, B*44:02, B*51:01, or B*57:01 (Figure 3D). Against the remaining targets, we observed a functional hierarchy, with *KIR3DL1*015* and **001* mediating the least, **005* the greatest, and **114* an intermediate degree of NK cell inhibition (Figure 3D). Within this hierarchy, NK cells expressing *KIR3DL1*114* resemble those expressing *KIR3DL1*001* and **015* in their lack of response to *HLA-B*13:01* and B*27:04/06 targets, yet resemble those expressing *KIR3DL1*005* in being inhibited by *HLA-A*23:01* and *A*24:02* (*A*23:01* is closely related to *A*24:02*, sharing identity in Bw4 motif and surrounding residues⁵¹). The observed differences of *KIR3DL1*114*+NK cell activities are statistically significant (two-way ANOVA; $p < 0.05$ – $p < 0.001$; Figure 3D). The ligand specificity of *KIR3DL1*114* is thus distinct from other allotypes tested in being broader than 015 lineage and narrower than 005-lineage allotypes.

Phenylalanine at residue 166 of KIR3DL1 enhances recognition of HLA-A*24:02

To test the hypothesis that Phe-166 determines the specificity of *KIR3DL1*114*, we performed *in vitro* analyses using reporter cells expressing extracellular domains of individual *KIR3DL1* allotypes (Figures S3D–S3F). Here, *KIR3DL1*114* reporter cells responded less strongly to *HLA-B*51:01* expressing targets than those expressing 3DL1*001, **005*, or **015*, but had enhanced response toward *HLA-B*57:01* targets ($p < 0.01$ – 0.001 ; Figure 3E). Whereas *KIR3DL1*005* and **114* reacted strongly to *HLA-A*24:02* and B*27:05 targets, *KIR3DL1*001* and **015* did not ($p < 0.001$; Figure 3E). We thus observed the same hierarchy of *KIR3DL1* allotype strength as observed using IL-2-expanded NK cells (Figure 3D). Mutating residue 166 of *KIR3DL1*015* (Figure 3F) from leucine to phenylalanine significantly increased activity toward *HLA-A*24:02*, B*27:05, and B*57:01 ($p < 0.01$ – 0.001) but had no effect on the recognition of *HLA-B*13:01* or B*27:06-expressing

Figure 2. KIR3DL1*114 has archaic human origin and coevolved with HLA-A*24:02 in Oceania

- (A) Neighbor-joining phylogenetic analyses of external-domain coding sequences of the *KIR3DL1/S1* allotypes observed in First Nations Australians. Colors represent the three major *KIR3DL1/S1* lineages, 005 (blue), 015 (red), 3DS1 (green), and recombinant 005/015 alleles (purple). *KIR3DL1*114* clusters in the 015 lineage. Bootstrap values for nodes are given when >50%. Lower right pie chart shows relative allele frequencies of the three lineages, according to the same color scheme, and gray indicates gene absence.
- (B) Polymorphic amino acid residues distinguishing the lineages of *KIR3DL1/S1*, alongside *KIR3DL1*086* and **114*. *KIR3DL1*015* is used as the reference sequence; residues identical to **015* are indicated by dashes. Residue 166 is shown in bold.
- (C) Geographic distribution of *KIR3DL1*114/086*, *HLA-A*24:02*, and *KIR3DL1 005*-lineage alleles in Oceania and Southeast Asia (Table S3).
- (D) *KIR* haplotypes carrying *KIR3DL1*114* or **086* and the populations from where they were characterized. (n.d., not determined).
- (E) D-statistics for testing Denisovan introgression of *KIR3DL1*114* ($n = 4$), **005* ($n = 6$), and **015* ($n = 6$). *KIR3DL1*114* only includes homozygous individuals. W, haplotypes contain indicated allele; Z, Z score; D, D score. See STAR Methods and Table S3.
- (F) Nucleotide diversity (π) across distinct regions of the genome in *HLA-A*24:02*, *HLA-A*34:01*, and *HLA-A*11:01* homozygous Papua New Guineans. π in 100 bp windows; *HLA-A* and *-BC* are flanking 500 kbp on each side. (** $p < 0.001$, compared with *HLA-A*; Wilcoxon test; Table S3). Error bars represent mean \pm standard deviation.
- (G) Correlation of *HLA-A*24:02* with *KIR3DL1*114*+*005*-lineage allele frequencies. Each dot represents a population, colored by region (SEA, Southeast Asia). Only populations with less than 50% Polynesian-related ancestry (by RFMix) are included (others are shown in gray). See also Figure S2.

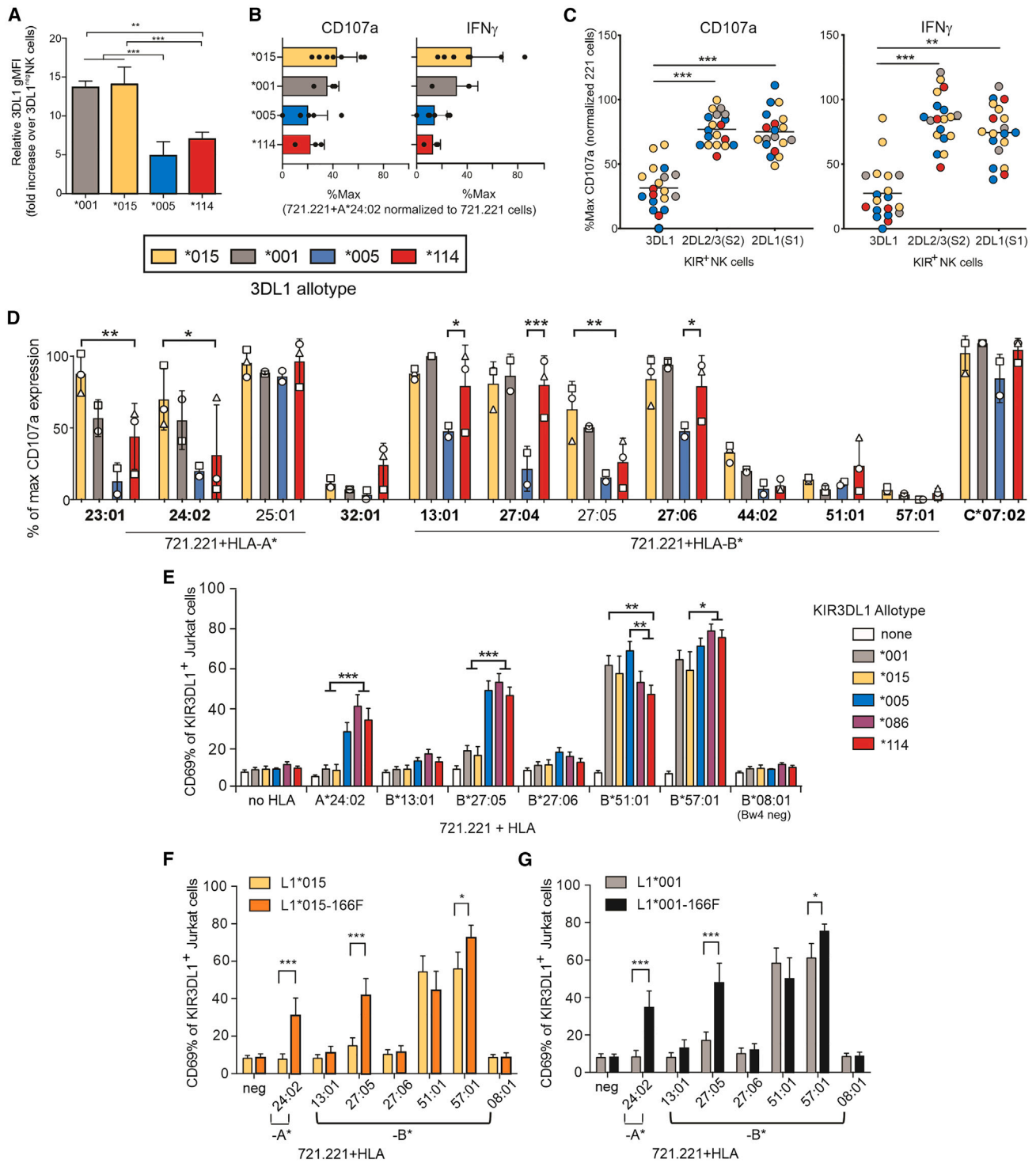


Figure 3. KIR3DL1*114 inhibits NK cells upon recognition of HLA-A*24:02

(A) Shows KIR3DL1 expression levels on the cell surface of primary NK cells isolated from individuals having KIR3DL1*114 ($n = 3$), *001 ($n = 3$), *005 ($n = 4$), or *015-lineage ($n = 4$) allotypes (Figures S3A and S3B; Table S4). The geometric mean fluorescence intensity (gMFI) of KIR3DL1 expression for each allotype was normalized within each donor to KIR3DL1^{neg} NK cells. $**p < 0.01$, $***p < 0.001$; Kruskal-Wallis test. Error bars indicate the standard deviation of the mean. (B) Shows CD107a (left) and IFN- γ (right) expression by *ex vivo* NK cells following incubation with 721.221 cells expressing HLA-A*24:02. NK cells were isolated from donors expressing KIR3DL1*114 ($n = 3$), *001 ($n = 3$), *005 ($n = 6$), or *015-like ($n = 7$). For each donor, values are normalized (% max) to those obtained from

(legend continued on next page)

targets. In these experiments, KIR3DL1*086 had the same recognition pattern as *114 (Figure 3E), which was recapitulated through leucine to phenylalanine substitution of KIR3DL1*001 residue 166 (Figure 3G). Together, these results clearly show the distinct functional profile observed for KIR3DL1*114⁺NK cells isolated from First Nations Australian donors is due to Phe-166.

To understand how substitution at residue 166 affects receptor function, we compared crystal structures of KIR3DL1*001 and KIR3DL1*114, each bound to HLA-A*24:02 complexed with the TW9 peptide. TW9 comprises influenza A virus polymerase basic protein 2, residues 549–557, and induces a CD8⁺T cell response when presented by HLA-A*24:02.⁶⁰ We determined the structures to 1.9 Å and 2.4 Å resolution, respectively (Table S5). Whereas the overall docking geometry of these two KIR3DL1-HLA receptor-ligand complexes^{24,48} remains conserved (Figure 4A), KIR3DL1*001 and *114 differ in their interactions both with the Bw4 motif and the presented peptide (Figures 4B and 4C). Namely, leucine 166 (Leu-166) forms one van der Waals (vdw) interaction with isoleucine 80 of the Bw4 motif, whereas Phe-166 forms nine vdw contacts, interacting with residues 76 and 80 of HLA (Figure S4). The extensive network of hydrophobic contacts with Bw4 thus enhances recognition of the HLA class I molecule by KIR3DL1*114 (Figure 4B). Likewise, KIR3DL1*114 directly contacts residues 7 and 8 of the TW9 peptide (P7 and P8) through three hydrogen bonds and three vdw interactions, whereas KIR3DL1*001 makes only two vdw contacts and no hydrogen bonds with P8 (Figure S4). Thus, KIR3DL1*114 makes more extensive contacts with HLA-A*24:02-TW9 in comparison to KIR3DL1*001.

Included in the peptide contacts made by KIR3DL1*114 are tyrosine 200 (Tyr-200) and glutamic acid 282 (Glu-282), which interact with P8, and P7 and P8 of the peptide, respectively. By comparison, in the KIR3DL1*001-HLA-A*24:02-peptide complex, P8-Asparagine is rotated away from the interface, preventing the formation of hydrogen bonds with Glu-282 and Tyr-200 of KIR3DL1*001 (Figure 4C). Concomitant with the altered orientation of P8, Glu-282 adopts a different conformation compared with the same residue in KIR3DL1*114, increasing the distance from P7 to ~5 Å, precluding the formation of a direct contact between these two residues (Figure 4C). These findings show how Phe-166 enhances interaction with ligand both through direct and indirect

mechanisms. Crystallographic analysis of further receptor-ligand complexes with HLA class I presenting two other distinct epitopes (a viral and a self-peptide) showed this enhancement is observed across disparate ligands for KIR3DL1*114 (Figure 4D; Table S5). Similar results were obtained for KIR3DL1*086, which also carries Phe-166 (Figure 4D). Here, the strongest expected interactions are Phe-166⁺KIR3DL1 with HLA-A*24:02-NEF (HIV peptide) and with B*57:03-AW10 (self-peptide), having four and twelve vdw contacts with peptide, respectively (Figure S4). In summary, polymorphism at residue 166 has a substantive role in determining the functional specificity of KIR3DL1.

KIR3DL1*114 strongly binds influenza peptides presented by HLA-A*24:02

Given that position 166 of KIR3DL1 affects ligand specificity and contacts the HLA-bound peptide, we investigated the peptide specificity of this polymorphism. We selected three influenza A virus peptides known to bind HLA-A*24:02⁶⁰ (Figures 5A and S5) and examined their binding to *001, *015, *005, *086, and *114 allotypes of KIR3DL1. We also selected one HIV and one self-derived peptide, known to bind KIR3DL1 when in complex with HLA-A*24:02 or B*57:03, respectively.^{61,62} In surface plasmon resonance (SPR) experiments, all five HLA class I-peptide complexes bound to each of the KIR3DL1 allotypes (Figure S5). For each given ligand, we observed a clear hierarchy of binding. The KIR3DL1 allotypes showed their greatest distinction in affinity for HLA-B*57:03-AW10 (Figure 5B), where KIR3DL1*001, *005, and *015 bound weakly with a K_D of 40–60 μM, and KIR3DL1*086 and *114 binding affinities were 20- to 30-fold stronger, having a K_D of 2 μM (Figure S5). This notable increase in affinity is consistent with the crystallography data showing multiple interactions with the ligand complex mediated specifically by Phe-166 (Figure 4; Table S5). Although the binding hierarchy was the same with HLA-A*24:02-TW9, KIR3DL1*005 represented an intermediate between the two extremes of low-affinity KIR3DL1*001 or *015, and high-affinity KIR3DL1*086 or *114 (Figure 5C). To confirm the impact of Phe-166, we made KIR3DL1*086-166L and *114-166L constructs and repeated the SPR experiments. Both 3DL1*086-166L and *114-166L showed reduced binding affinities for the HLA-peptide complexes, in each case being like those of KIR3DL1*005

KIR3DL1⁺ NK cells incubated with the parental, HLA class I^{neg} 721.221 cell line. Each dot represents one donor. CD107a $p = 0.07$, IFN- γ $p = 0.02$; Kruskal-Wallis test.

(C) As (B), except NK cells were differentiated into those expressing only KIR3DL1, KIR2DL1/S1, or KIR2DL2/3/S2. Each circle represents one donor. Responses were normalized to the maximal obtained with the parental 221 cell line and compared using a one-way ANOVA with Tukey's multiple comparison test (** $p < 0.01$, *** $p < 0.001$).

(D) Assays using NK cells isolated from ten donors and expanded *in vitro* using IL-2. Each donor was homozygous for one of four expressed KIR3DL1 allotypes. HLA allotype of target cells is indicated on the x axis (allotypes present in the cohort shown in bold). CD107a expression of KIR3DL1⁺ NK cells was normalized to the maximal expression in response to parental 721.221 cells. Background CD107a expression by NK cells was subtracted. For each donor, two–three replicates were performed, and the mean of all replicates from the donors is shown as the bar graph with the standard deviation plotted. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; two-way ANOVA with Bonferroni's multiple comparisons. For each KIR3DL1 allotype, triangles, circles, and squares represent the donors used, whose genotypes are given in Table S4.

(E–G) Shows the result of *in vitro* activation (CD69 upregulation) of reporter cells expressing specific KIR3DL1 allotypes. Jurkat cells were transduced with a given natural (E) or mutated (F and G) KIR3DL1 allotype that was fused to the intracellular domain of CD3 ζ . Colors represent the KIR3DL1 allotypes, as shown in the key. The reporter cells were incubated with a panel of 721.221 target cells for 8 h, and the subsequent upregulation of CD69 was assessed by flow cytometry (Figures S3D and S3E). Three independent experiments were performed, each in triplicate, and statistics performed on the average of the replicates. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$; two-way ANOVA with Tukey's multiple comparisons test.

See also Figure S3.

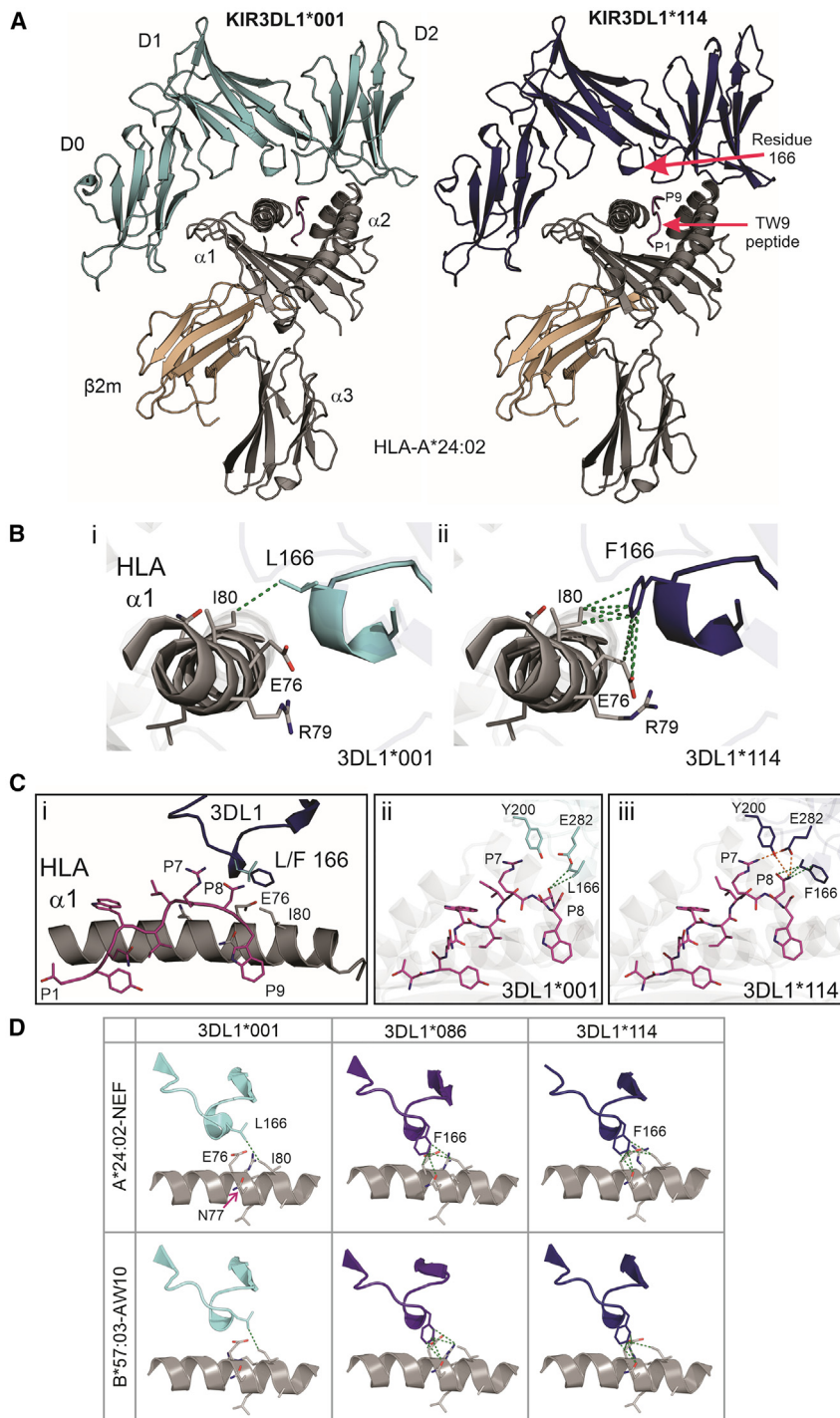


Figure 4. Phenylalanine at residue 166 of KIR3DL1*114 enhances interaction with HLA ligands

(A) Ribbon diagram of the crystal structures solved for KIR3DL1*001 (left, cyan) and KIR3DL1*114 (right, blue) bound to the HLA-A*24:02-TW9 peptide (influenza virus A polymerase basic protein 2 residues 549–557) complex. HLA-A*24:02 in gray, peptide in mauve, and β_2 M in orange. KIR3DL1 and HLA domains are labeled on the left, and positions of the peptide and residue 166 are indicated on the right. (B) Orthogonal view of KIR3DL1 residue 166 interactions with α 1 helix residues of the HLA molecule. (Bi) KIR3DL1*001 (L166), (Bii) KIR3DL1*114 (F166). Dotted lines indicate van der Waals interactions. (C) (Ci) Side view; cut away to show the interactions of KIR3DL1 residue 166 with peptide and HLA molecules. Leucine 166 is represented shaded cyan and phenylalanine dark blue. (Cii) Aerial view of peptide showing contacts with L166 and (Ciii) F166. (D) Comparison of interactions of KIR3DL1 residue 166 from allotypes (left to right) KIR3DL1*001, *086, and *114 with HLA α 1 helix residues of (top) HLA-A*24:02-NEF and (bottom) -B*57:03-AW10. NEF is derived from HIV-1, AW10 from human catenin. See also Figure S4.

which bound to HLA-A*24:02-NEF and B*57:03-AW10, with similar affinity as KIR3DL1*001 and *015, to HLA-A*24:02-FF10 with similar affinity as KIR3DL1*086 and *114, and to the remainder at intermediate affinity (Figure S5). Interestingly, FF10 has the same sequence as RF8, but with two additional residues at the N terminus (Figure 5A). These residues increased the affinity for all KIR3DL1 allotypes, most noticeably for KIR3DL1*005, placing this allotype with similar or greater affinity for the FF10 peptide as KIR3DL1*086 and *114 (Figure 5D). Together, these results suggest that 005-lineage allotypes of KIR3DL1 are highly peptide selective. They also indicate that Phe-166 enhances interaction with HLA-A*24:02 while reducing the sensitivity of KIR3DL1 for the presented peptide.

DISCUSSION

Combinatorial diversity of KIR and HLA class I modulates NK cell-driven control of infectious disease severity.^{63,64} Through killing infected cells, NK cells act before, during, and after development of a memory immune response. Having roles also in conventional T cell-driven immunity, NK cells can lyse specific antigen-presenting cells or kill infected cells through antibody-dependent cytotoxicity (ADCC), and KIR can modulate potentially autoreactive T cells.^{65–68} Optimal interaction of KIR with HLA can therefore

(Figures 5B and 5C). The exception was HLA-A*24:02-FF10, which had similar affinity for each of the KIR3DL1 allotypes tested (Figures S5C and S5D).

Overall, the binding profiles of KIR3DL1*086 and *114 showed statistically significant higher affinity values for HLA-A*24:02 complexes than did KIR3DL1*001 or *015 (Figures 5D and 5E). The greatest variety in binding was seen for KIR3DL1*005,

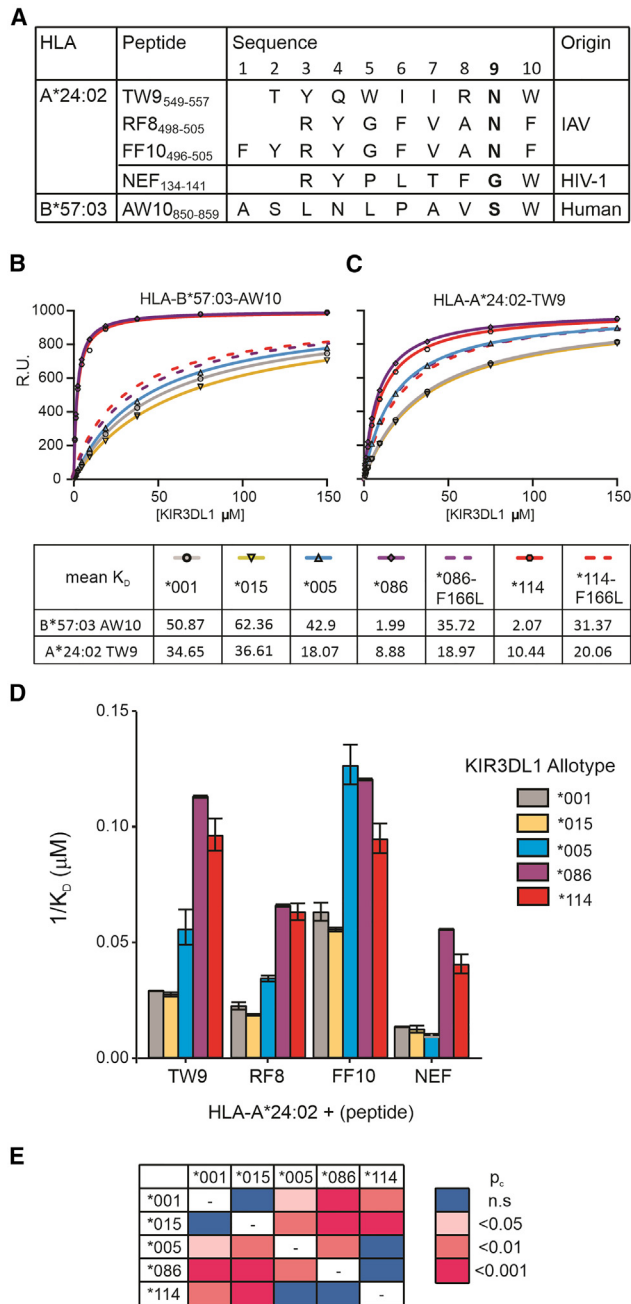


Figure 5. KIR3DL1 allotypes having phenylalanine at residue 166 bind HLA-A*24:02-peptide complexes with greater affinity than do other allotypes

(A) (Left to right) HLA class I allotype, and name, sequence, and origin of bound peptide. Residues in bold correspond to P8 of the 9-mer peptide.

(B and C) Shown are the affinity curves of soluble KIR3DL1 allotypes binding to the immobilized HLA-B*57:03-AW10 (B) or HLA-A*24:02-TW9 complex (C). The KIR3DL1 allotypes are *001, *005, *015, *086, and *114, and mutations *086-F166L, *114-F166L. R.U., response units. Mean K_D values; \pm values and remaining HLA-A*24:02-peptide binding curves are given in Figure S5.

(D) Comparison of K_D values across HLA-A*24:02-peptide complexes. Data are representative of two independent experiments. Error bars are SEM.

determine whether an infection is controlled without incurring collateral damage, both in the short and long term. Consequently, genetically determined diversity of KIR and HLA class I affects the severity of multiple immune-mediated diseases and may also affect the course of specific immunotherapies, including transplantation.¹⁶ Among the KIR/HLA interactions, those of the inhibitory KIR3DL1 with subtypes of HLA-A or -B dominate in diversity and known implications for infectious disease. KIR3DL1 alleles are highly differentiated across populations,²⁵ likely influencing control of region-specific infections.⁶⁹ We identified KIR3DL1*114 to have strong affinity for HLA-A*24:02 and their pairing to be uniquely common across Oceania. KIR3DL1 allotypes that bind most strongly to their ligands drive generation of the most potently acting NK cells. In autoimmunity and some viral infections, the strongly inhibiting KIR3DL1 allotypes can associate with protection from disease severity.^{16,70,71} By contrast, in hematopoietic cell transplantation for acute myeloid leukemia, where donor NK cells can kill host leukemic cells, strongly inhibiting KIR3DL1 allotypes associate with leukemic relapse.⁷² Moreover, these strongly inhibiting allotypes may also reduce success of monoclonal antibody therapy for specific tumors,⁷³ but improve the course of checkpoint inhibition therapy for others.⁷⁴ However, most of the above findings have been established for individuals of European descent, and little is known about the extent or implications of immunogenetic variation in First Nations peoples. Our precise functional characterization of the allotypes we describe here therefore has potential application for multiple aspects of human health across the Oceania region.

We identified *KIR3DL1*114* as an ancient allele group unique to Oceania that is likely introgressed from Denisovans. Having a mean allele frequency of 8.5% across Oceania and 25% in Papua New Guinea, we estimate a minimum of 5 million people to have *KIR3DL1*114*. The polymorphism that defines the specificity of *KIR3DL1*114* occurs at a site previously identified under natural selection for diversity.²⁵ We show HLA-A*24:02, the contemporary ligand for *KIR3DL1*114*, rose to high frequency across Oceania under positive selection during the time since *KIR3DL1*114* was acquired by modern humans. Together, these findings suggest the presence of *KIR3DL1*114* permitted HLA-A*24:02 to rise in frequency and that the interaction of this HLA-A with *114 compensates for the lack both of Bw4 motifs carried by HLA-B and the 005 lineage of *KIR3DL1*. Although the cause and timing of the selection are unknown, one outcome is a unique profile of NK cell receptor diversity for modern-day individuals in this geographic region, defined by a predominance of strongly inhibitory *KIR3DL1* specific for HLA-A. Having distinct evolutionary histories,¹³ HLA-A and -B likely complement each other in function. Although we did not directly study the impact of *KIR3DL1*114* on NK cell education, strong interaction of *KIR3DL1*114* with HLA-A*24:02 implicates this combination as a robust educator of NK cells. Any implications for host immunity of NK cells preferentially interacting with HLA-A remain to be established.

(E) Comparison of K_D values obtained by the *KIR3DL1* allotypes across the four HLA-A*24:02 complexes. t test, Bonferroni corrected (Pc). See also Figure S5.

Although not selected by any health criteria, the initial cohort was assembled to investigate the high prevalence of severe influenza disease in First Nations Australians.^{4,7} Influenza virus remains a major threat to human health,⁷⁵ and treatment for severe disease is limited. One of the most frequent HLA class I alleles in the cohort and the most frequent across Oceania is A*24:02, which presents a risk for developing severe respiratory disease following influenza infection.¹⁰ Peak influenza virus replication occurs within days of infection, prior to the establishment of T cell-driven immune responses. Subsequently, memory T cells ensure repeat challenges by the same or similar virus strain are eliminated. Innate immune responses are therefore necessary to ensure survival when challenged by a strain not previously encountered. NK cells are critical in this early response.^{76,77} NK cells can recognize and kill influenza-infected cells,⁷⁸ and NK cell activation is critical for coordinating and modulating cytotoxic T cell activity to influenza virus infection.⁷⁹ Further highlighting the importance of their role fighting this infection, influenza virus has evolved multiple strategies to evade NK cells.⁸⁰ Accordingly, impaired NK cell function associates with influenza virus susceptibility.⁸¹ Potentially impairing their function, NK cells isolated from lungs express high levels of inhibitory KIR,⁶⁷ and influenza virus infection causes redistribution of HLA class I on the cell surface such that KIR binds more efficiently.⁸² That HLA-A*24:02, uniquely among HLA class I alleles common in Oceania, enables interaction with KIR3DL1 implicates NK cells in the increased susceptibility to severe influenza observed for First Nations Oceanians.

Resembling the functional characteristics of the KIR3DL1*114 interaction with HLA-A*24:02, KIR2DL1 is a stronger and more specific receptor for HLA-C than is KIR2DL3.⁸³ In individuals carrying both KIR2DL1 and KIR2DL3, those NK cells expressing KIR2DL1 had a more inhibited response to influenza-virus-infected cells than those expressing KIR2DL3.⁸⁴ Although the effects of KIR3DL1 polymorphism were not investigated, those findings suggest that the strong specific inhibition of NK cells mediated by KIR3DL1*114 interaction with HLA-A*24:02 will be detrimental to control of influenza virus infection. The influenza virus peptides we studied here had been selected for their ability to stimulate cytotoxic T cell activity when complexed with HLA-A*24:02,⁶⁰ yet we show the same complexes strongly and specifically inhibit NK cell effector activities. Strong inhibition of NK cells may limit their ability to control influenza virus infection. Alternatively, any reduced ability of NK cells to dampen the T cell-driven immune responses may increase the likelihood for collateral damage.¹⁵ The implications of these findings will therefore need further investigation in severe influenza disease settings.

Previous work identifying introgressed immune genes identified single molecules of limited, albeit critical, function in innate immunity and have tended to identify non-coding variants that may affect expression.^{30,39} Exceptions include one rare (HLA-B*73) and one more frequent (HLA-A*11) HLA allele but with no data regarding their functions obtained.³⁵ By contrast, we describe an immune receptor allele characteristic to Oceania and currently representing up to 30% individuals in populations throughout this geographic region. The interaction of NK cell receptors with HLA has wide-ranging implications for innate and

adaptive immunity, not limited to infectious disease, including autoimmunity, cancer, immunotherapy, and neurological disease. As we present direct evidence for functional distinction of the interactions involving KIR3DL1*114, our work thus shows how archaic admixture could have lasting impact on the health of First Nations peoples. We also note that, in addition to archaic introgression, we show that more recent gene flow from Europeans has had a substantial impact on the immunogenetic diversity of First Nations Australians in relation to other First Nations Oceanians.

Limitations of the study

Phe-166 is encoded by a CTT-TTT codon change carried by a haplotype that we show to be highly likely of Denisovan origin. All haplotypes sharing flanking SNPs from the 369 that we sequenced also carried the TTT codon. Although we assume from this observation that Phe-166 is Denisovan in origin, it cannot be ruled out from the data we have that any of the SNPs that define this haplotype could have been derived in modern humans since the Denisovan admixture event took place.

Population-specific co-evolution of KIR and HLA class I ligands is a defining feature of the KIR/HLA class I system.⁸⁵ Here, the prevailing dogma is that the system is subject to balancing selection, whereby populations respond rapidly to any perturbations of the balance through polymorphism that alters specificity or function.¹⁶ KIRs appear to be evolving more rapidly than HLA, able to alter function through single nucleotide substitution, gene deletion, duplication, or fusion, and here we also highlight the use of pre-existing variation.⁸⁶ Although we have focused the study on infectious diseases, combinatorial diversity of KIR and HLA class I may be affected by natural selection through their critical role early in placentation.^{13,16} As HLA-A and -B are not expressed by fetal trophoblast cells, the role of NK cells in reproduction is unlikely to have driven the co-evolution of KIR3DL1*114 with HLA-A*24:02. An assumption we therefore make is that the combination of HLA-A*24:02 with KIR3DL1*114 has been advantageous toward controlling specific pathogen or pathogens that remain unknown. Further work is therefore required to identify the pathogens and whether they remain a threat to health in Oceania.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for the resources should be directed to and will be fulfilled by the lead contact, Dr. Paul J. Norman (paul.norman@cuanschultz.edu).

Materials availability

The reagents generated in this study will be made available upon reasonable request to the [lead contact](#).

Data and code availability

- De-identified human HLA and KIR locus-specific raw sequence data have been deposited in the Harvard Dataverse as <https://doi.org/10.7910/DVN/TRMTCT> and <https://doi.org/10.7910/DVN/JA0U1Z>. As of the date of publication, they are available upon request to [lead contact](#), if access is granted.

- Sequences of the newly characterized HLA and KIR alleles have been deposited in GenBank under accession numbers MH301276-78 and MH938271-297 and are publicly available as of the date of publication.
- Protein structures were deposited in the Research Collaboratory for Structural Bioinformatics Protein Data Bank under accession codes, 9BL2-6, 9BL9, and 9BLA, and are publicly available as of the date of publication.
- This paper does not report any original code.
- This paper analyzes existing, publicly available data, accessible at PDB: 7K80, <https://www.ebi.ac.uk/ipd/>, <https://ftp.ncbi.nlm.nih.gov/pub/mhc/mhc/Final%20Archive/IHWG/Anthropology/>, <https://www.internationalgenome.org/>, <https://www.simonsfoundation.org/simons-genome-diversity-project/>.
- Any additional information required to analyze the data reported in this paper is available from the [lead contact](#) upon request.

CONSORTIA

The members of the Oceanian Genome Variation Project are George Aho, Angela Allen, Stephen Allen, Kathryn Auckland, Andreia Brandao, Ram Gonzalez Buenfil, Carlos D. Bustamante, Phillip Endicott, Francoise Friedlaender, Jonathan S. Friedlaender, Adrian V.S. Hill, Julian R. Homburger, Alexander G. Ioannidis, Carmina Barberena Jonas, Toomas Kivisild, George Koki, Alexander J. Mentzer, Andrés Moreno-Estrada, Stephen Oppenheimer, Tom Parks, Maude Phipps, William Pomat, Eliuel Pretrick, Consuelo D. Quinto-Cortés, Martin Richards, Kathryn Robson, Jean Roux, Sofia Vieyra Sánchez, Alissa L. Severson, Pedro Soares, Abdul Salam M. Sofro, Matthew Spriggs, Mark Stoneking, Tamarua Teariki, Ishwar Verma, Joyce White, and Genevieve L. Wojcik.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.G.B., K.K., S.Y.C.T., A.M., and P.J.N.; investigation, L.L., P.M.S., C.F., N.N.-G., L.H., S.C.W., J.W., S.Z., G.M.-M., K.M.K., E.B.C., L.A.G., and P.J.N.; formal analysis, L.L., P.M.S., C.F., N.F.-P., G.F.H., S.T., S.S., J.P.V., and P.J.N.; resources, S.Y.C.T., J.D., A.M., P.P., M.F.-V., F.Z., A.J.M., S.J.O., W.P., A.G.I., C.B.-J., A.M.-E., P.J.N., J.R., K.K., and A.G.B.; funding acquisition, K.K., A.G.B., L.L., P.J.N., J.R., P.P., S.Y.C.T., and A.M.; writing – original draft, L.L., P.M.S., C.F., N.F.-P., P.P., J.R., K.K., A.G.B., and P.J.N.; writing – review and editing, all authors.

DECLARATION OF INTERESTS

A.G.I. has shares in Galatea Bio, Inc. G.F.H. is currently an employee of Tempus AI. S.C.W. is currently an employee of Miltenyi Biotec Asia Pacific Pte Ltd. L.H. is affiliated with the Department of Internal Medicine II, University Hospital Tübingen, Tübingen, Germany. J.W. is affiliated with The Malignant Hematology, Transplantation, and Cellular Therapy Services, Alfred Health, Melbourne, VIC, Australia. S.Z. is affiliated with the Protein Production Facility (PPF) of the Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia. G.M.-M. is affiliated with the HLA Histocompatibility and Immunogenetics Laboratory, Vitalant, Phoenix, AZ, USA. J.P.V. is affiliated with St. Vincent's Institute of Medical Research, Fitzroy, VIC, Australia.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
pan-HLA Class 1 W6/32	Parham et al. ⁸⁷	N/A
Bw4 RM7.9.63	Zernich et al. ⁸⁸	N/A
HLA-B*27 ME1	Ellis et al. ⁸⁹	N/A
HLA-B*57/58 (B17 Serotype) 3E12	Kostenko et al. ⁹⁰	N/A
Goat anti-mouse IgG-FITC	Millipore	Cat# AP127F, RRID:AB_92468
Mouse IgM-PE	Jackson ImmunoResearch	Cat#115-116-075, RRID: AB_2338628
HLA-C/E DT-9 PE	BD Biosciences	Cat#566372; RRID: AB_2739715
CD107a H4A3 PE-Cy5	BD Biosciences	Cat# 555802, RRID:AB_396136
KIR3DL1(NKB1) DX9 FITC	BD Biosciences	Cat# 340483, RRID:AB_2130832
KIR2DL2/L3/S2 GL183 PE-Cy5.5	Beckman Coulter	Cat# A66900, RRID:AB_2857331
KIR2DL1/S1 EB6B PE-Cy7	Beckman Coulter	Cat# A66899, RRID:AB_3136596
LILRB1 HP-F1 APC	ThermoFisher	Cat# 17-5129-42, RRID:AB_1311214
NKG2A Z199 PE	Beckman Coulter	Cat# IM3291U, RRID:AB_10643228
CD56 NCAM16.2 BV785	BD Biosciences	BD Biosciences Cat# 564058, RRID:AB_2738569
CD3 UCHT1 Pacific Blue	BioLegend	BioLegend Cat# 980004, RRID:AB_3136596
CD3 SK7 APC-Cy7	BD Biosciences	Cat# 341110, RRID:AB_2868766
IFN- γ B27 AF700	BD Biosciences	Cat# 557995, RRID:AB_396977
Purified KIR3DL1 (NKB1) DX9	BD Biosciences	Cat#555964, RRID:AB_396258
CD158e/k 5.133 PE	Miltenyi Biotec	Cat# 130-095-205, RRID:AB_10839570
CD69 FN50 PE	BD Biosciences	Cat#555531, RRID:AB_395916
Bacterial and virus strains		
<i>E. coli</i> BL21 (DE3)	Agilent	Cat#200131
pMIGII	La Gruta et al. ⁹¹	N/A
pPAM-E	La Gruta et al. ⁹¹	N/A
pVSVG	La Gruta et al. ⁹¹	N/A
Biological samples		
Human peripheral blood samples from healthy Australian First Nations Looking Into inFluenza T-cell immunity (LIFT) cohort	Royal Darwin Hospital Outpatient Clinic; Clemens et al. ⁷ (#HREC-2012-1928; #1441452.1; #0931311.5)	N/A
Human peripheral blood samples from healthy individuals	Australian Red Cross LifeBlood (West Melbourne, Australia)	N/A
Human DNA from Papuan Donors	Institute for Medical Research, Papua New Guinea, Oxford Tropical Research Bergström et al. ⁵⁰	N/A
Chemicals, peptides, and recombinant proteins		
Recombinant human IL2	Miltenyi Biotec	Cat#130-097-743
Phytohemagglutinin	ThermoFisher	Cat#10576015
EcoRI-HF	New England Biolabs	Cat#R3101S
BamHI-HF	New England Biolabs	Cat#R3136S
Geneticin (G418 sulfate)	ThermoFisher	Cat#11811098
Golgi Stop	BD Biosciences	Cat#554724
Fixable Live/Dead Aqua Blue viability dye	Life Technologies	Cat#L34957

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Fixable Live/Dead eFluor780 viability dye	Life Technologies	Cat#65-0865-14
Urea	Sigma Aldrich	Cat#57.13.6
Trisma Base	Sigma Aldrich	Cat#77-86-1
Na-EDTA	Astral Scientific	Cat#BIOEB0185
L-arginine-HCl	Sigma Aldrich	Cat#1119-34-2
Oxidized glutathione	Sigma Aldrich	Cat#64376
Reduced glutathione	GoldBio	Cat#64376
PMSF	Sigma Aldrich	Cat#10837091001
Ni-NTA resin	Qiagen	Cat#30410
HEPES-HCl	Sigma Aldrich	Cat#H4034
EndoH	New England Biolabs	Cat#P0702S
PEG 3350	Sigma Aldrich	Cat#202444
Tacsimate	Hampton Research	Cat# HR2-825
Tri-sodium citrate	Merck	Cat#1.06448.0500
P20	Cytiva	Cat#BR100054
Gentle Ag/Ab elution buffer	ThermoScientific	Cat#21027
Qubit Machine	Life Technologies	N/A
MiSeq Instrument	Illumina	N/A

Critical commercial assays

EasySep Human NK Cell Enrichment Kit	Stemcell Technologies	Cat#19055
Cytofix/Cytoperm Plus kit	BD Biosciences	Cat#555028
Fugene6	Promega	Cat#E2691
DNAeasy Blood and Tissue DNA extraction kit	Qiagen	Cat#69506
QBit™ 1X dsDNA HS Assay Kit	ThermoFisher	Cat#Q33230
QBit™ 1X dsDNA BR Assay Kit	ThermoFisher	Cat#Q33265
MIA FORA NGS HLA MFlex 11 high-throughput kit	Immuncor	Cat#SR-800-00534-96

Deposited data

LIFT cohort targeted KIR and HLA sequence reads	This paper	https://doi.org/10.7910/DVN/TRMTCT
PNG targeted KIR and HLA sequence reads	This paper	https://doi.org/10.7910/DVN/JA0U1Z
LIFT cohort HLA class I CDS alleles	This paper	GenBank# MH301276-78
LIFT cohort KIR3DL1 CDS alleles	This paper	GenBank# MH938271-97
KIR3DL1/HLA/peptide complex structural data	This paper	PDB# 9BL2-6, 9BL9, 9BLA
KIR3DL1*001/HLA-A*24:02/NEF structural data	Saunders et al. ⁴⁸	PBD: 7K80
IPD-IMGT/HLA, v3.25.0	Robinson et al. ⁹²	https://www.ebi.ac.uk/ipd/
IPD-KIR v2.13	Robinson et al. ⁵¹	https://www.ebi.ac.uk/ipd/
HLA class I genotypes from the 13 th IHWG	Mack et al. ⁹³	https://ftp.ncbi.nlm.nih.gov/pub/mhc/mhc/Final%20Archive/IHWG/Anthropology/
1000 Genomes Project	Byrska-Bishop et al. ⁹⁴	https://www.internationalgenome.org/
Simons Genome Diversity Project	Mallick et al. ⁹⁵	https://www.simonsfoundation.org/simons-genome-diversity-project/
Oceanian Genome Variation Project	Quinto-Cortes et al. ⁴⁹	N/A
MEGA data from 297 PNG individuals	Bergström et al. ⁵⁰	N/A
Māori KIR3DL1/S1 and HLA class I allele frequencies	Nemat-Gorgani et al. ²⁷	N/A

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<i>Continued</i>		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Ga-Adangbe KIR3DL1/S1 and HLA class I frequencies	Norman et al. ⁹⁶	N/A
Nama and Khomani KIR3DL1/S1 and HLA class I frequencies	Nemat-Gorgani et al. ⁹⁷	N/A
Yucpa KIR3DL1/S1 and HLA class I frequencies	Gendzekhadze et al. ⁹⁸	N/A
Chinese Southern Han KIR3DL1/S1 and HLA class I frequencies	Deng et al. ⁹⁹	N/A
Europeans from USA KIR3DL1/S1 and HLA class I frequencies	Amorim et al. ¹⁰⁰	N/A
Malay KIR3DL1/S1 and HLA class I frequencies	Tao et al. ¹⁰¹	N/A
1000 Genomes Project KIR3DL1/S1 genotypes	Norman et al. ¹⁰²	N/A
KIR3DL1/S1 frequencies, multiple populations	Norman et al. ²⁵	N/A
Native South American KIR3DL1/S1 allele frequencies	Vargas et al. ¹⁰³	N/A
KIR3DL1/S1 allele frequencies from 7 African populations	Nemat-Gorgani et al. ¹⁰⁴	N/A
Experimental models: Cell lines		
Human 721.221 cells	Laboratory of Peter Parham; Shimizu and DeMars ¹⁰⁵	N/A
Human 721.221 cells expressing specified HLA-A, -B or -C allele	Laboratory of Andrew Brooks; Saunders et al. ^{26,48,106} ; Laboratory of Peter Parham; Saunders et al. ¹⁰⁶	N/A
293T cells	ATCC	Cat#CRL-3216
Jurkat cells	Laboratory of James McCluskey; Macdonald et al. ¹⁰⁷	N/A
Expi293F™ cells	ThermoFisher	Cat#A14527
Expi293F™ GnTI cells	ThermoFisher	Cat#A39240
Oligonucleotides		
Mutagenesis primers	This paper (Bioneer Pacific)	See Table S4
Recombinant DNA		
Codon-optimized HLA-B*51:01 construct	GeneArt Strings	N/A
pcDNA3.1(-) Mammalian Expression Vector	Invitrogen	Cat#V79520
KIR3DL1*001/*005 CD3ζ constructs	O'Connor et al. ¹⁰⁸	N/A
RSV.5neo.B2705	Laboratory of James McCluskey; Williams et al. ¹⁰⁹	N/A
pET-30 vector	Studier and Moffatt ¹¹⁰	N/A
pHLSec Mammalian Expression Vector	Novagen	Cat#99845
Software and algorithms		
R version 4.1.1, 4.3.1	r-project.org	N/A
PING	Marin et al. ¹¹¹	https://github.com/Hollenbach-lab/PING
PONG	Harrison et al. ¹¹²	https://github.com/NormanLabUCD/PONG
NGSengine 1.7.0	GenDX	https://www.gendx.com/
STRUCTURE	Falush et al. ¹¹³	https://web.stanford.edu/group/pritchardlab/structure.html
Mega 6.0	Tamura et al. ¹¹⁴	https://www.megasoftware.net/
PLINK2	Chang et al. ¹¹⁵	https://www.cog-genomics.org/plink/2.0/

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
KING version 2.2.2	Manichaikul et al. ¹¹⁶	https://www.kingrelatedness.com/
HIBAG v1.36.4	Zheng et al. ¹¹⁷	https://bioconductor.org/packages/release/bioc/html/HIBAG.html
EAGLE	Taliun et al. ¹¹⁸	https://alkesgroup.broadinstitute.org/Eagle/downloads/
Generic Mapping Tools (GMT)	Wessel and Smith ¹¹⁹	https://www.generic-mapping-tools.org/download/
ADMIXTURE v1.3.0	Alexander et al. ¹²⁰	https://dalexander.github.io/admixture/download.html
pong v1.5	Behr et al. ¹²¹	https://github.com/ramachandran-lab/pong
RFMix v2.03	Maples et al. ¹²²	https://github.com/slowkoni/rfmix
SHAPEIT v4.2.2	Delaneau et al. ¹²³	https://odelaneau.github.io/shapeit4/
Bcftools 1.7	Danecek et al. ¹²⁴	https://samtools.github.io/bcftools/
MEGA v11.0.13	Tamura et al. ¹²⁵	https://www.megasoftware.net/
PAML v4.8	Yang ¹²⁶	http://abacus.gene.ucl.ac.uk/software/paml.html
Picardtools v2.27.5	http://broadinstitute.github.io/picard	N/A
Admixtools v7.0.2	Patterson et al. ⁵⁴	https://github.com/DReichLab/AdmixTools
Arlequin v3.5.2	Excoffier et al. ¹²⁷	https://cmpg.unibe.ch/software/arlequin35/Ar135Downloads.html
VCFtools v0.1.15	Danecek et al. ¹²⁸	https://vcftools.github.io/index.html
Selscan v1.2.0	Szpiech and Hernandez ¹²⁹	https://github.com/szpiech/selscan
XDS	Kabsch ¹³⁰	https://xds.mr.mpg.de/
Aimless (CCP4i program suite)	Collaborative Computational Project ¹³¹	https://www.ccp4.ac.uk/
Phenix	Phenix project - Lawrence Berkeley Laboratory. Adams et al. ¹³²	https://phenix-online.org/
COOT	MRC Laboratory of Molecular Biology. Emsley and Cowtan ¹³³	https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/
PyMOL	Schrödinger, Inc.	https://pymol.org/2/
MX2 Beamline	Australian Synchrotron	N/A
BIAcore T200	GE Healthcare	N/A
GraphPad Prism v9	GraphPad	N/A
BD FACS Diva v8.0.3	BD Biosciences	N/A
BD Fortessa II or BD FACS Canto II	BD Biosciences	N/A
FlowJo v9.9.6 or 10.9.0	BD Biosciences	N/A
bioRender	biorender.com	Graphical abstract
Other		
S200 16/60 column	GE Healthcare	Cat#28989335
HiTrap-Q anion exchange chromatography	GE Healthcare	Cat#17115301
CM5 sensorchip	Cytiva	Cat#BR100530

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human Participants

First Nations Australian Donors

All experiments conformed to the National Health and Medical Research Council (NHMRC) of Australia code of practice and were approved by The University of Melbourne Human Research Ethics Committee (Applications #1441452.1 and #0931311.5) and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (#HREC-2012-1928). Healthy, self-declared First Nations Australian volunteers were recruited from the Royal Darwin Hospital Outpatient clinic as part of the “Looking Into influenza T-cell immunity” (LIFT) cohort.⁷ Written informed consent was obtained from all

donors. Median age was 45 years (interquartile range 30, 55) and 52% were male.⁷ Per individual information on age and gender is not available and unlikely to affect the results of the gene sequencing or NK cell assays performed. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood using Ficoll-Paque (GE Healthcare, Marlborough, MA, USA) density gradient centrifugation and resuspended in fetal calf or bovine serum (FCS/FBS; Gibco/ThermoFisher Scientific, Waltham, MA, USA) containing 10% DMSO for cryopreservation. Samples from other individuals were obtained from Australian Red Cross LifeBlood with approval from the University of Melbourne Human Research Ethics Committee (Project 1238243.2), with PBMC isolated from buffy coats and cryopreserved as above.

Papua New Guinea Donors

DNA was obtained from 645 Papuan individuals as described previously.⁵⁰ Briefly, samples were sourced from a library of samples collected in the 1980s in a series of collaborative projects between the Institute for Medical Research in Papua New Guinea, Liverpool School of Tropical Medicine, and the University of Oxford, UK. The samples were collected, following informed consent, to understand genetic differences between highland and lowland populations in relation to differential disease risk. The median recorded age was 23 years (interquartile range 17, 30) and 42.5% were male. All samples were collected at the time with regulatory approval from the Papua New Guinea Institute of Medical Research, and the Papua New Guinea Medical Research Advisory Council, and endorsed by the Public Health Department. Updated approval for contemporary genetic analysis was provided by the Institute for Medical Research in Papua New Guinea, and the Oxford Tropical Research Ethics Committee (OxTREC).

METHOD DETAILS

KIR and KIR-ligand nomenclature

The *KIR* locus varies in gene content from approximately six to 13 genes per haplotype.¹⁶ Included are up to four genes encoding inhibitory receptors specific for HLA class I; KIR2DL1, 2DL2/3, 3DL1 and 3DL2. In turn a given HLA class I allotype may contain one of four distinct amino acid motifs that enables it to become a KIR ligand. Those HLA allotypes that do not have either of these motifs do not function as KIR ligands. The ligand motifs are A3/11 (carried by some HLA-A), Bw4 (some HLA-A and B), C1 (some HLA-B and C) or C2 (some HLA-C). KIR2DL1 is specific for C2+HLA, 2DL2/3 for C1 and some C2, 3DL1 for Bw4, and 3DL2 for A3/11. KIR are named according to the number of external Ig-like domains (2D or 3D), which can bind to the ligand, and 'L' denotes 'long' cytoplasmic tail that can transmit a signal to inhibit cellular activity upon ligand binding. Throughout this manuscript, any unique DNA sequence that spans a coding region is considered a distinct allele. Those alleles that encode a unique protein sequence define an allotype.

High-resolution KIR and HLA allele genotyping

Genomic DNA from the 80 LIFT donors was prepared from PBMC, or granulocytes isolated after Ficoll separation of PBMC from whole blood, using the DNeasy Blood and Tissue DNA extraction kit (Qiagen, Hilden, Germany). DNA was quantified using a Qubit machine (Life Technologies, Carlsbad, CA, USA). *KIR* and *HLA class I* and *II* genes were analysed concurrently using high throughput sequencing, as described.¹⁰² Sequencing was performed using a MiSeq instrument (Illumina) with V3 chemistry, and the sequencing read length was 2 x 300 bp. To determine the gene and allelic content of the *KIR* locus, sequenced reads were analysed using the Pushing Immunogenetics to the Next Generation (PING) pipeline.^{102,111} The IPD-KIR database v2.13 was used as the source of reference sequences.⁵¹ *HLA* alleles were determined using NGSengine 1.7.0 (GenDX, Utrecht, Holland). *HLA* typing of the additional donors used for NK cell experiments was performed by the Victorian Transplantation and Immunogenetics Service (Australian Red Cross LifeBlood). The KIR3DL1 subtype of these donors was initially identified from gDNA using a multiplex PCR¹³⁴ and confirmed by high resolution KIR typing as above.

Admixture analysis

A previous genome wide study of First Nations Australians identified admixture with Europeans.²⁸ To determine the contribution of this admixture to the high *HLA* diversity in First Nations Australians, we compared the distribution of *HLA-A* and *-B* alleles to those of other populations chosen to represent major world groups. We did not include *HLA-C* due to strong linkage disequilibrium (LD) with *HLA-B*. We examined admixture among the populations using the program STRUCTURE.¹¹³ As additional data we used *HLA class I* genotypes of individuals obtained from the 13th Histocompatibility Workshop⁹³ (<https://ftp.ncbi.nlm.nih.gov/pub/mhc/mhc/Final%20Archive/IHWG/Anthropology/>), as well as Māori,²⁷ Ga-Adangbe,⁹⁶ Hadza,¹⁰⁴ and Khomani⁹⁷ individuals; thus 3,196 individuals were included from 32 populations (Table S2), representing five regions (Europe, Oceania, South East Asia, East Asia, and Sub-Saharan Africa). Using STRUCTURE,¹¹³ the 3,196 individuals were assigned to pre-determined *K* clusters (*K*=2 to *K*=5), where individuals may have contributions from more than one cluster.

The STRUCTURE analysis showed the First Nations Australian *HLA class I* genotypes cluster with other Oceanian populations but are distinguished from them by having a proportion of genetic ancestry shared with Europeans (Figure S1). This finding focused on the *HLA class I* genes is thus consistent with the independent genome-wide study.²⁸ Restricting our analysis to European, Oceanian and Southeast Asian groups revealed that up to 39% of the sampled First Nations Australians have >50% and 33% have >75% European genetic ancestry detected in their *HLA class I* genes (Figure S1). A separate, allele specific, analysis permitted inclusion of *HLA-C*. Analyzing only alleles observed in more than one individual, 9/15 *HLA-A*, 11/28 *HLA-B* alleles, and 6/17 *HLA-C* were

identified to originate from Europeans (Figure S1). One allele of HLA-A (*A*34:01*), six alleles of HLA-B (*B*13:01*, **15:21*, **15:25*, **27:04*, **27:06*, **56:02*) and one allele of HLA-C (**15:09*) are present in Papua New Guineans, but not Europeans, indicating their likely Oceanian origin for First Nations Australians. A further three alleles (*B*40:06*, **48:01*, **55:02*) were identified in East Asians, but not Papua New Guineans or Europeans. As East Asian admixture is not detected in First Nations Australians from Northern Australia,²⁸ these three alleles also likely arrived in Australia during the migration from Papua New Guinea.

Analysis of the ten most frequent First Nations Australian *HLA class I* haplotypes identified six that were observed also in Papua New Guineans and not Europeans, and three in Europeans but not Papua New Guineans (Figure S1). Although this analysis has low sensitivity, the overall admixture proportions and geographic *HLA* allele distributions are consistent with previous studies.^{28,44,135} Together, these results show that although the *HLA class I* allele distribution of First Nations Australians from the North Top End region is closely related to other Oceanian populations, diversity has been increased through admixture with Europeans.

Novel Allele Confirmation

For confirming novel *KIR* alleles, individual exons of the *KIR* genes were amplified from genomic DNA and subjected to Sanger sequencing using primers and protocols as described.⁹⁶ The IPD-KIR database v2.13 was used as the source of reference sequences.⁵¹ For confirming *HLA* allele sequences, HLA-A, -B, -C genes were amplified using long-range PCR reactions from the MIA FORA NGS high-throughput kit (ImmuCor, Inc., Norcross, GA, USA), performed and analyzed according to the manufacturer's protocol. The IPD-IMGT/HLA database v3.25.0 was used as a source of reference sequences.⁹² All novel allele sequences were deposited in GenBank and IPD,⁵¹ and the accession numbers are given in Table S1D.

Phylogenetic analysis

Neighbour-joining phylogenetic analyses of the coding determining sequence (CDS) of the external domains (cytoplasmic tail excluded) were generated, with 500 bootstrap replicates, using the Tamura-Nei model with pairwise deletion, using Mega 6.0.¹¹⁴

Population comparisons

To measure *HLA* and *KIR* diversity across populations (Figure 1) we used representative groups that were previously genotyped to the same high resolution as this study; West Africans from Ghana,⁹⁶ Nama and Khomani Southern Africans,⁹⁷ Yucpa Amerindians,⁹⁸ Chinese Southern Han,⁹⁹ Europeans from USA,¹⁰⁰ Malay,¹⁰¹ and Māori.²⁷ The number of distinct alleles present in 75 randomly selected individuals was counted, and the process was repeated for 1,000 permutations with replacement.

Worldwide distribution of KIR3DL1 ligands

To determine the relative proportions of HLA-A and -B allotypes carrying ligands for 005-lineage and 015 lineage KIR3DL1 we used HLA allele frequency data from the same populations as above. Those allotypes carrying the Bw4 motif of residues 77-83 (N/D77, I/T80, A/L81, L82, R83),¹³ were divided into those that bind preferentially to 005-lineage KIR3DL1 and those that also bind to 015-lineage KIR3DL1. A threshold of 25% maximum binding as previously determined (Figure 2C from Saunders et al.²⁶) was used to distinguish these characteristics. For rare allotypes present in the data set but not previously tested, the binding pattern of the closest aligning allotype was used (e.g. HLA-A*24:03 was given the same value as A*24:02). HLA-A*25 and B*13 have not been shown as effective ligands for KIR3DL1^{23,26,48} and were counted as non-binders for this analysis.

Worldwide distribution of KIR3DL1 alleles

KIR3DL1 allele frequencies were obtained from previously sequenced 1000 Genomes individuals,¹⁰² and other worldwide populations,^{25,99-101,103,104} including Māori and Polynesians²⁷ and First Nations Australians and Papua New Guineans (this study). *KIR3DL1* allele frequencies from additional Oceanian populations were determined through imputation, as described below.

Genome-wide array data

We analyzed previously generated genome-wide array data from 913 Oceanian individuals from the Oceanian Genome Variation Project (OGVP)⁴⁹ and an additional set of 297 Papua New Guinea individuals,⁵⁰ genotyped using the Multi-Ethnic Genotyping Array (MEGA) array. We used PLINK2¹¹⁵ to exclude individuals or SNPs having genotype missingness > 10%, and SNPs having minor allele frequency < 1%. We also removed related individuals (2nd degree or more), as estimated using KING version 2.2.2.¹¹⁶ The final Oceanian dataset after quality control filtering contained 471,417 SNPs and 1,108 unrelated individuals. Of these, 345 individuals overlapped with the set of individuals sequenced as described above. The Oceanian dataset was then merged with reference populations from the phase3 1000 Genomes Project¹³⁶: Utah residents (CEPH) with Northern and Western European ancestry (CEU), Han Chinese in Beijing, China (CHB), Kinh in Ho Chi Minh City, Vietnam (KHV), and Yoruban from Idaban, Nigeria (YRI), available in <http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/>. The final merged dataset contained 463,750 SNPs and 1,517 individuals.

HLA and KIR imputation

HLA alleles were imputed from the genome-wide array data using HIBAG version 1.36.4,¹¹⁷ using default parameters and the pre-trained Asian model for the human genome build GRCh37/hg19, available at https://hibag.s3.amazonaws.com/download/hlares_param/Asian-HLA4-hg19.RData. To assess the accuracy of the imputation, we compared with those individuals who had been

genotyped using other means: Papua New Guinea (345 sequenced as described above), 1000G CHB and KHV (https://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/HLA_types/). Accuracy, defined as the number of correct calls per sample divided by the size of the dataset (2N), was 95.81%.

KIR3DL1/S1 alleles were imputed from the genome-wide array data using PONG.¹¹² We first performed SNP imputation using the Michigan imputation server, where the data was phased using EAGLE.¹¹⁸ Only high-quality imputed variants were kept ($R_{sq} > 0.3$). The published PONG training models did not include the *3DL1*114* allele. We therefore trained a new model using 150 Papua New Guinea, 60 CHB and 40 KHV individuals, and tested the model with 153 Papua New Guineans, 20 CHB, and 24 KHV individuals. This model has an accuracy of 70.05%. Specifically, for *3DL1*114* we prioritized a high specificity (97.72%), to avoid reporting false positives and overestimating its frequency. The estimates of *3DL1*114* allele frequency are thus conservative.

Mapping allele distributions

Relative allele frequencies were plotted using the Generic Mapping Tools (GMT).¹¹⁹ Available point values were used to estimate two-dimensional frequency distributions with *blockmean* and *surface* functions as previously described.⁵⁶ We used the *psmask* function to clip unreliable map areas, considering reliable only those grid cells within a 1,300 km radius of a data point.

Genetic ancestry analyses of Oceanian dataset

To analyze the genetic structure across our Oceanian dataset, we performed ADMIXTURE analysis, using the merged genome-wide array data. LD pruning was applied using PLINK2,¹¹⁵ with a window size of 200kb, step size of 50 SNPs and r^2 threshold of 0.5, resulting in 247,344 unlinked SNPs. ADMIXTURE version 1.3.0¹²⁰ was run in unsupervised mode using ten different iterations with random seeds. The major mode for the ten independent runs was identified using pong version 1.5.¹²¹ As shown in Figure S2A, at $K=5$, there was a component maximized in the YRI population (dark red: “African-related”), others maximized in CEU (orange: “European-related”), CHB (beige: “East Asian – related”), Highlanders from Papua New Guinea (blue: “Papuan-related”), and Polynesian populations (purple: “Polynesian-related”).

After estimating the global genetic ancestries with ADMIXTURE, we used RFMix version 2.03¹²² to perform local ancestry inference. Data was phased using SHAPEIT version 4.2.2,¹²³ with default parameters. RFMix was run with one Expectation-Maximization iteration and four reference panels. As reference, we used the KHV and CHB ($n=202$) for the East Asian ancestry proxy, CEU ($n=99$) for the European proxy, the Papuan reference panel included those individuals with >99% of “Papuan-related” component in the ADMIXTURE analysis ($n=100$), and the Polynesian panel included individuals with >90% of “Papuan-related” component ($n=80$). We only kept windows having a marginal probability >90%. Correlations between global ancestry proportions inferred from ADMIXTURE $K=5$ and RFMIX were higher than 0.94 for all ancestries, and higher than 0.98 for both the Polynesian and Papuan-related components (Figure S2B).

Divergence time estimation

Divergence times between *KIR3DL1*114* and *005 and *015 gene sequences (13,781 bp) were estimated. Bcftools 1.7 consensus¹²⁴ was used to obtain the *KIR3DL1* gene sequences from short-read sequencing data obtained from four *KIR3DL1*114* homozygous Papuan individuals. We used the ClustalW algorithm of MEGA 11.0.13¹²⁵ to align these sequences with one *KIR3DL1*015* and one *KIR3DL1*005* sequence (GenBank ID GU182342.1 and GU182344.1, respectively) and constructed a maximum likelihood phylogenetic tree with default parameters. Divergence time was estimated using mcmctree of PAML v4.8,¹²⁶ using the same parameters as,¹³⁷ except the ‘RootAge’, which was set to a maximum of 6.5mya, corresponding to the split of chimpanzee and human.¹³⁸ The acceptance proportions, times and rates were stable throughout the MCMC runs. Analysis convergence was further explored running the analysis 10 times with different random seeds, showing similar values for the ten sets of posterior times.

Denisovan introgression: D-statistics

To infer Denisovan introgression, we used genome-wide chromosome 19 SNP data from the 1000G Yoruban from Idaban, Nigeria (YRI, *KIR3DS1*⁻ individuals, $N=104$),⁹⁴ the Denisovan genome (<http://cdna.eva.mpg.de/denisova/VCF>),³³ the predicted ancestral sequence for *Homo sapiens* derived from a multiple alignment of seven primate genomes (available in https://ftp.ensembl.org/pub/release-76/fasta/ancestral_alleles), as well as the 14 KHV individuals, and four *KIR3DL1*114*⁺ homozygous individuals described above. The Denisovan genome was lifted over from hg19 to the GRCh38 reference using Picardtools v2.27.5 LiftoverVCF (<http://broadinstitute.github.io/picard>). After keeping only common SNPs from the *KIR2DL4-3DL1* genomic region, 65 SNPs remained in the merged dataset. A similar approach was used for HLA-A alleles, using OGVP array data. Here in each case the allele sequence +/- 500kbp was used.

D-statistics, which measure the degree of allele sharing across populations, were calculated using Admixtools 7.0.2⁵⁴ in the form of D-statistic (W , YRI, Denisovan genomic sequence, Ancestral sequence), where W corresponds to individuals carrying *KIR3DL1*114*, *005, or *015. Significant introgression from Denisovan to *KIR3DL1* haplotypes was considered when D-score > 0 and Z-score > 3.

A recombination hotspot divides the *KIR* locus into two regions, termed ‘centromeric’ and ‘telomeric’ according to their orientation.¹³⁹ All four *KIR3DL1*114* homozygous Papuan individuals we sequenced have the following telomeric *KIR* haplotype: *2DL4*028~3DL1*114~2DS4*019~3DL2*002*, which is the most common haplotype in Papuan *3DL1*114*⁺ individuals (Figure 2D). To infer the length of the introgressed haplotype, we performed the D-statistic test as described above, but progressively including

KIR genes from the telomeric and centromeric regions (*2DL4-3DL1-2DS4-3DL2*, and *2DL1-2DL2/3-2DS2-3DL3*, respectively), until the number of BABA SNPs stopped increasing (SNPs where the allele is shared between the target *3DL1*114* and Denisovan genome).

To test if the *KIR3DL1*114* carrying haplotype is a result of incomplete lineage sorting instead of archaic gene flow into modern humans, we applied the following formula⁵⁵: $P = 1 - \text{gammaCDF}(H, \text{shape} = 2, \text{rate} = \frac{1}{L})$; where P is the probability that a haplotype of a particular length (H) is shared between Oceanians and Denisovans due to incomplete lineage sorting; and $L = 1 / (r \times t)$, where r is recombination rate per generation per base pair (bp) (mean recombination rate around *KIR2DL4* ~ *KIR3DL2* is: 2.91×10^{-8} ; from the HapMap recombination map^{140,141}) and t is the number of generations since the Denisovan and modern human divergence (300×10^3 years ago / 25 years per generation⁵⁵). After applying the formula, we found that the probability of a haplotype of length 62,276 bp present in modern Oceanians due to incomplete ancestral lineage sorting is 8.78×10^{-9} , which supports the alternative hypothesis of being in modern Oceanians due to Denisovan introgression.

Statistical tests for positive selection

To examine the frequency distribution of *HLA* alleles, we used Arlequin version 3.5.2¹²⁷ and R version 4.3.1 to calculate the Ewens-Watterson F -statistic for homozygosity and Hardy Weinberg Equilibrium (HWE) chi-squared tests. We also calculated the nucleotide diversity (π) in 100 bp windows from genome-wide array data using VCFtools version 0.1.15.¹²⁸ Although π may be underestimated using SNP array compared to sequencing data,^{142–144} the results are consistent with *HLA-A*24:02* carrying haplotypes having lower diversity than the two other *HLA-A* alleles common in the geographic region.

To test for evidence of recent positive selection of *HLA-A*24:02*, we computed the iHS statistic (integrated haplotype score¹⁴⁵). We used publicly available whole genome phased sequences of 15 Papua New Guinea individuals from the Simons Genome Diversity Project (SGDP,⁹⁵ available at: https://sharehost.hms.harvard.edu/genetics/reich_lab/sgdp/phased_data2021/). We used these results because SNP coverage within the *HLA-A* region in the MEGA array used for OGVP is insufficient to correctly construct the ancestral and derived haplotypes needed for the iHS calculation. We assigned the ancestral and derived state for each variant based on the ancestral state information from the 1000G phase3 data.¹³⁶ iHS calculation and normalization across frequency bins of the unstandardized iHS were performed using selscan version 1.2.0¹²⁹ with default parameters. To validate that the selection signal observed is due to the *HLA-A*24:02*, we imputed the *HLA-A* alleles in these individuals using HIBAG version 1.36.4,¹¹⁷ and identified 7 and 5 *HLA-A*24:02* homozygotes and heterozygotes, respectively. All the SNPs within the iHS positive peak within the *HLA-A* and flanking region have derived alleles in *HLA-A*24:02*⁺ individuals, confirming the positive selection signal is related to the haplotype of *HLA-A*24:02*.

Purification and expansion of NK cells

PBMC samples were thawed, and NK cells isolated using the EasySep™ Human NK Cell Enrichment Kit, according to the manufacturer's instructions (Stemcell Technologies Vancouver, Canada). Primary NK cells were rested overnight in complete media before use in *ex vivo* analyses. Expanded NK cells were prepared by incubating with irradiated feeder cells and *HLA-G*-expressing 721.221 cells in the presence of 100 U/mL rIL2 (Miltenyi) and 1.5 ng/mL PHA (Gibco/ThermoFisher Scientific) in complete media as previously described.²⁶ All feeder cells were isolated from a single independent donor (donor CM: *HLA-A*32:01*, *A*33:01*, *B*07:05*, *B*44:03*, *C*03:03*, *C*07:01*; *KIR3DL1*004/KIR3DS1*013*). The complete media was RPMI 1640 supplemented with 7.5mM HEPES, 2 mM L-Glutamine, 150 μ M non-essential amino acids, 76 μ M 2-ME, 150 μ g/ml streptomycin and 10% FBS (In Vitro Technologies, Noble Park North, VIC, Australia) and 100 U/mL rIL2 (Miltenyi, Bergisch Gladbach, Germany).

Target cell lines

The B-lymphoblastoid cell line 721.221,¹⁰⁵ which expresses no endogenous *HLA-A*, *-B* or *-C*¹⁰⁵, transfected with *HLA-A*23:01*, *A*24:02*, *A*25:01*, *A*32:01*, *B*08:01*, *B*13:01*, *B*27:05*, *B*44:02*, *B*57:01*, *B*58:01*, *C*04:01* or *C*07:02* have been described previously.^{26,48,106} Codon optimised *HLA-B*51:01* constructs were purchased (GeneArt Strings, ThermoFisher Scientific) and cloned into pcDNA3.1(-) using EcoRI and BamHI. *HLA-B*27:04* and *B*27:06* were generated in the RSV5neo¹⁰⁹ plasmid via sequential site directed mutagenesis from *HLA-B*27:05* using primers listed in Table S4. Plasmid constructs were electroporated into 721.221 cells at 200V and 975 μ F and placed under geneticin selection as previously described.²⁶ Transfected cells were sorted for *HLA* class I expression following staining with pan-*HLA* class I specific W6/32 monoclonal antibody; cells were stained using unconjugated W6/32, and counterstained using FITC conjugated antibody specific for IgG. The geometric mean fluorescence intensity (gMFI) of *HLA* class I expression for each of the cell lines was determined (Table S4). They were maintained in RPMI 1640 media (Media Preparation Unit, University of Melbourne, Australia) supplemented as above, but without IL2. Expression of specific *HLA* alleles was confirmed by staining with mAb specific for the Bw4 epitope (RM7.9.63⁸⁸), *HLA-B27* (ME1⁸⁹) or the B17 (*B*57* and *B*58*) serotype (3E12⁹⁰), followed by anti-mouse IgG-FITC, or *HLA-A24/A2* (One Lambda) followed by anti-mouse IgM-PE. The *HLA-C* controls were stained using a directly conjugated antibody specific for *HLA-C/E* (DT9; PE; BD Pharmingen).

NK cell assays

NK cells were incubated with *HLA* class I-transfected or non-transfected target cells at a 1:2 (*ex vivo* NK cells) or 1:1 (expanded NK cells) effector:target ratio for 1 hour at 37°C in the presence of anti-CD107a-PECy5 (H4A3; BD Biosciences, San Jose, CA, USA). We

used CD107a as a measurement of degranulation and thus a proxy for NK cell killing of target cells¹⁴⁶ and IFN γ as a marker for NK cell activation. Monensin (GolgiStop; BD Biosciences) was added according to the manufacturer's recommendations and following a further four hours of incubation, cells were washed and cell surface stained. The cells were stained for the following markers: KIR3DL1 (NKB1 (DX9), FITC; BD Biosciences), KIR2DL2/L3/S2 (GL183, PE-Cy5.5; Beckman Coulter, Brea, CA, USA), KIR2DL1/S1 (EB6B, PE-Cy7; Beckman Coulter), LILRB1 (HP-F1, APC; eBioscience/ThermoFisher), and NKG2A (Z199, PE; Beckman Coulter). *Ex vivo* NK cells were further stained with Live/Dead Aqua (LifeTechnologies/ThermoFisher), CD56 BV786 (BD Biosciences) and CD3 UCHT1, PacificBlue (BioLegend, San Diego, CA, USA). Expanded NK cells were further stained with eBioscience™ Fixable Viability Dye eFluor™ 780 (Invitrogen/ThermoFisher), CD56 (NCAM16.2, BV421; BD Biosciences) and CD3 (SK7, APC-Cy7, BD Biosciences). Cells were fixed and permeabilized (Cytotix/Cytoperm kit; BD Biosciences), stained for IFN γ (B27, AF700; BD Biosciences) and acquired using a BD LSRFortessa flow cytometer, with FACS DIVA software (Version 8.0.3).

KIR3DL1⁺ NK cells were identified by gating on live NK cells (CD56⁺CD3⁺CD14/19⁻). NK cells expressing other inhibitory receptors for HLA class I were excluded by selecting those negative for NKG2A, KIR2DL2/3 and KIR2DL1/S1. NK cell degranulation (CD107a¹⁴⁶) and IFN γ production was assessed using FlowJo software version 9.9.6 (BD Biosciences, USA). The gating strategy is shown in Figure S3A. To account for any differences in the maximal cytokine production profiles between individuals, the maximum percentage (% of max of IFN γ or CD107a) for each donor was normalized to those values obtained from KIR3DL1⁺ NK cells incubated with the parental 721.221 cell line. Showing that the inhibition is specific to KIR3DL1, upregulation of CD107a or IFN γ was not significantly diminished in NK cells expressing only KIR2DL2/3 or KIR2DL1/S1 from the same donors, assessed in parallel (Figure 3C). Secondly, target cell induced degranulation was restored by blocking the interaction using the KIR3DL1-specific monoclonal antibody DX9 (Figure S3C). Also included in the expanded NK cell assays were HLA-A*25:01 (Bw4⁺), HLA-C*07:02 (C1), neither of which are recognised by KIR3DL1.⁴⁸

Jurkat-KIR cellular reporter and activation assay

Constructs encoding KIR3DL1*001 or *005 linked to CD3 ζ in the GFP⁺ pMIGII⁹¹ vector have been described previously.¹⁰⁸ The KIR3DL1*015+CD3 ζ construct was generated by sequential site directed mutagenesis from the KIR3DL1*001+CD3 ζ construct (Table S4). Further mutagenesis was then performed to generate KIR3DL1*086, *114, *001L166F and *015L166F constructs. Each KIR3DL1+CD3 ζ construct was transduced into the Jurkat CD4⁺ T cell line, using retrovirus.¹⁴⁷ Briefly, 4 μ g pMIGII-KIR3DL1+CD3 ζ along with 4 μ g pPAM-E⁹¹ and 2 μ g pVSVG⁹¹ was transduced into 293T cells using Fugene6 as per manufacturer's instructions (Promega) and cultured in DMEM media with 10 % FBS and supplements. Twenty-four hours after transfection, supernatant was collected, filtered, and used to resuspend freshly pelleted Jurkat¹⁰⁷ cells. Media transfer was performed twice daily for six days and transduced Jurkat cells were then selected by sorting for GFP and KIR3DL1 surface expression using anti-Human CD158e/k-PE, clone 5.133 (Miltenyi Biotec). Stably transduced Jurkat cells were maintained in RPMI (Gibco/ThermoFisher) supplemented as described above for NK cells, but without IL-2. For activation assays, transduced Jurkat cells expressing specific KIR3DL1+CD3 ζ allotypes were incubated with equal numbers of HLA-transfected or non-transfected 721.221 targets for eight hours at 37°C. Cells were then washed, stained for CD69 (clone FN50, PE; BD Biosciences) and fixed. Samples were analysed using a BD FACSCantoII and the expression of CD69 on GFP⁺ transduced Jurkat cells was assessed using FlowJo software Version 10.9.0 (BD Biosciences).

Protein expression and purification

The heavy chains (residues 1-276) of HLA-A*24:02, C*05:01 and B*57:03 were expressed into inclusion bodies in *E. coli* BL21 (DE3) from the pET-30 vector¹¹⁰ and refolded in the presence of full-length β 2-microglobulin (residues 1-99) and the relevant peptides, and purified as described previously.^{24,148} Briefly, the HLA-peptide complexes were refolded by dilution into refolding buffer (5 M Urea, 100 mM Tris-HCl (pH 8), 2 mM Na-EDTA, 400 mM L-arginine-HCl, 0.5 mM oxidized glutathione, 5 mM reduced glutathione, 1 mM PMSF) and stirred for 72 h at 4°C. Then, the refolded solutions were dialyzed in 10 mM Tris-HCl pH 8.0 and purified by anion exchange on a diethylaminoethylcellulose column, followed by gel filtration (S200 16/60 column, GE Healthcare) and HiTrap-Q anion exchange chromatography (GE Healthcare).

Residues spanning from 1 to 299 of KIR3DL1*001, *005, *015 (with an N-terminal 6xHis), and *086, *114, *086-166L and *114-166L (with a C-terminal 6xHis), with secretion tags, were cloned into the pHLSec mammalian expression vector (Novagen) and expressed in Expi293F™ cells (Thermo Fisher) for Surface Plasmon Resonance (SPR) experiments and Expi293F™ GnTI- cells for structural studies. Soluble proteins were purified after 7 days from the culture media using Ni-NTA resin (Qiagen) and further purified by size exclusion chromatography using an S200 16/60 column (GE Healthcare). For SPR studies, purified KIR proteins were concentrated in a buffer composed of 10 mM HEPES-HCl (pH 7.4) and 300 mM NaCl. For crystallization trials, KIR proteins were concentrated to 15 mg/mL in 10 mM Tris-HCl (pH 8) and deglycosylated overnight at 4°C with EndoH (New England Biolabs).

Crystallisation and structure determination

All KIR3DL1/HLA/peptide complexes described were combined at 1:1 molar ratio and crystallized at 294 K using the hanging-drop vapour-diffusion method from a solution comprising 14%-18% PEG 3350, 2% Tacsimate pH 5 and 0.1 M tri-sodium citrate pH 5.6. Crystals were equilibrated in crystallization solution with 35% PEG 3350 added as cryoprotectant and then flash-frozen in liquid nitrogen. Crystallographic diffraction data were collected at the MX2 beamline (Australian Synchrotron). The data were processed with the program XDS¹³⁰ and scaled using Aimless from the CCP4 suite of program.¹³¹ The crystal structures were determined by

molecular replacement using PHASER with KIR3DL1*001 and HLA-A*24:02 (PDB 7K80) as search models. Refinement of the models was carried out with iterative rounds of refinement in Phenix¹³² and manual building in COOT.¹³³ Data collection and refinements statistics are summarised in Table S5. Data were deposited in the Research Collaboratory for Structural Bioinformatics PDB with the accession codes 9BL2-6, 9BL9 and 9BLA. KIR3DL1*001 bound to HLA-A*24:02-NEF was described previously (7K80).⁴⁸

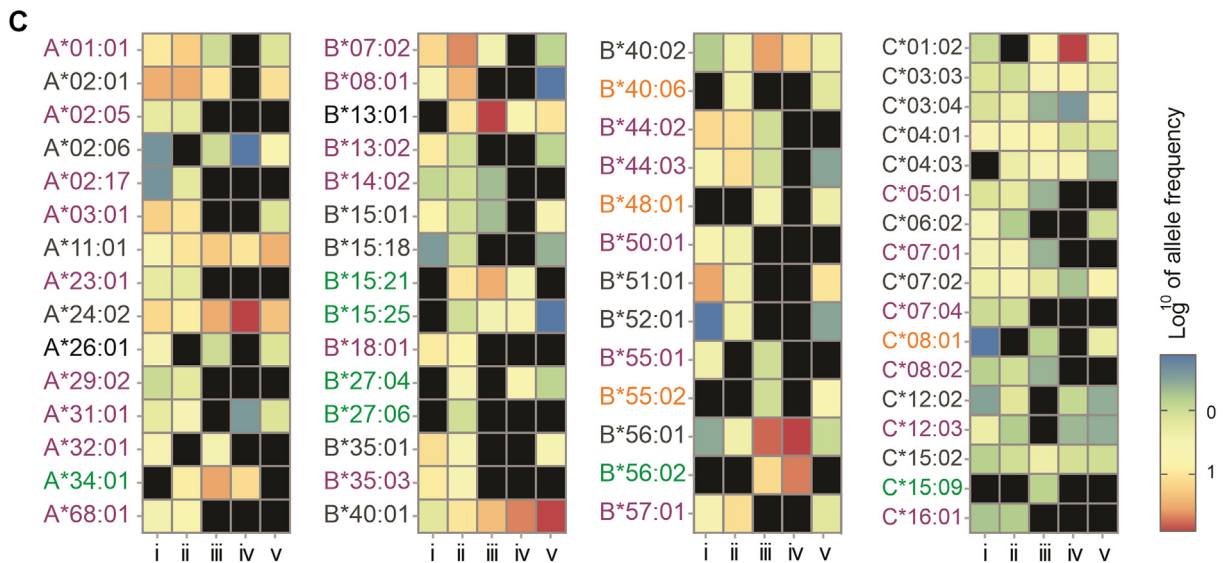
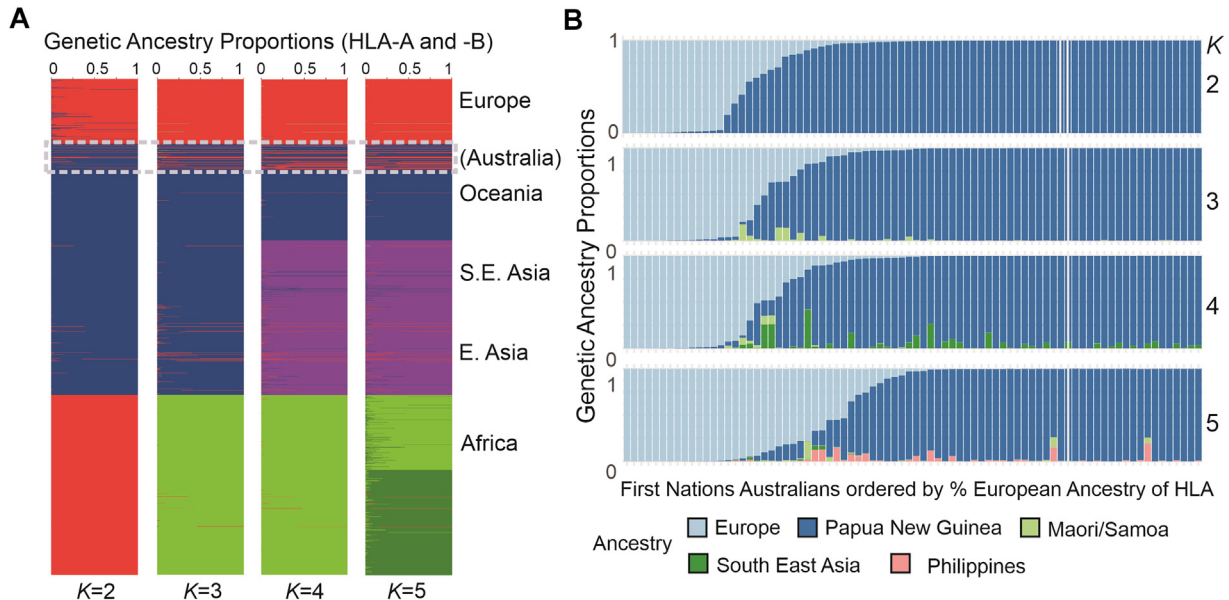
Surface Plasmon Resonance

SPR experiments were conducted using a BIAcore T200 instrument (GE Healthcare Life Sciences) as previously described.⁶¹ Briefly, the HLA-peptide complexes were captured, to a density of ~800 resonance units, on a CM5 sensorchip (Cytiva) by immobilization of the anti-HLA mAb W6/32 (generated in-house).⁸⁷ KIR3DL1*001, *005, *015, *086, *114, *086-166L and *114-166L were serially diluted (0–150 μ M) and injected (60 s association, 180 s dissociation) over the chip at a flow rate of 5 μ l/min at 298 K in a buffer composed of 10 mM HEPES-HCl (pH 7.4), 300 mM NaCl and 0.005% surfactant P20. Chip surfaces were regenerated between each analyte injection using Gentle Ag/Ab elution buffer (Thermo Scientific). Resulting sensograms were subtracted against a reference flow cell coupled with W6/32 in the absence of HLA ligand capture. Binding affinities were determined by equilibrium analysis by implementing the one-site specific binding model using GraphPad Prism v9. All data are representative of two independent experiments, each conducted using duplicate samples. To allow direct comparison, for affinity curves the K_D fold change was normalised based on the B_{max} , where B_{max} was equated to 1000RU for each KIR3DL1/HLA experiment. Data are represented as mean \pm SEM.

QUANTIFICATION AND STATISTICAL ANALYSIS

The description of all statistical analyses is in the corresponding figure legends. For each test, the alternative hypothesis was two-sided. Bar plot error bars represent mean \pm standard deviation. Number of bootstraps, permutation resamples, and independent experimental replicates are indicated in the figure legends. P-values < 0.05 or Z-scores $> |3|$ were considered significant. All multiple test correction was performed using the Bonferroni method. Plots and statistical tests were performed using R version 4.1.1.¹⁴⁹ or GraphPad Prism v9.

Supplemental figures



- i - Europeans
- ii - First Nations Australians (>50% Eur)
- iii - First Nations Australians (<50% Eur)
- iv - Papua New Guinea
- v - East Asians

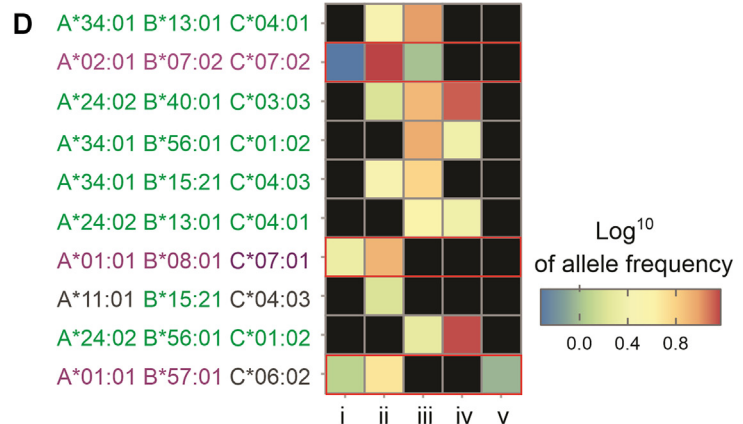


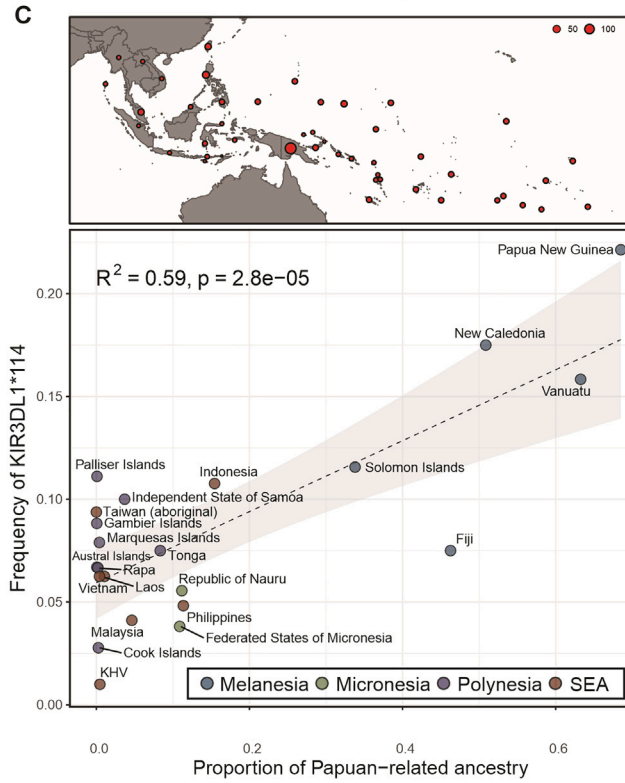
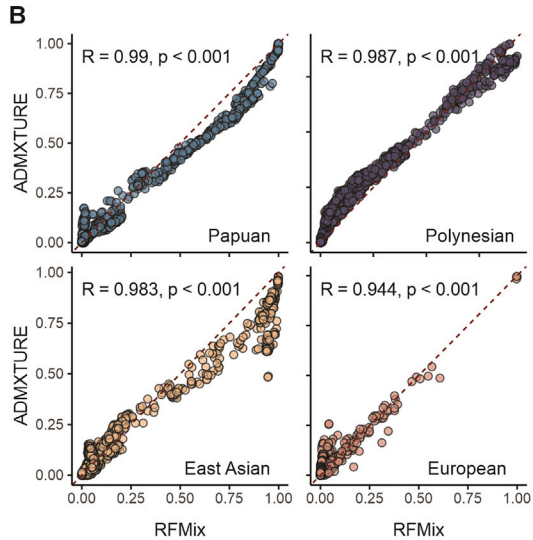
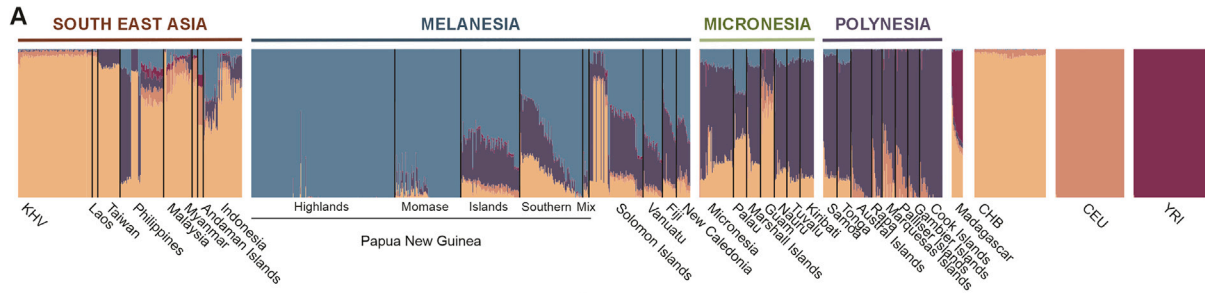
Figure S1. Ancestry proportions of *HLA class I* alleles and haplotypes, related to Figure 1

(A) Ancestry analysis using *HLA-A* and *-B* genotypes of representative populations (Table S2). K values at the bottom indicate the number of population clusters used for that analysis. Each horizontal line represents one individual, with colors indicating relative proportions of the K clusters. First Nations Australians are marked with a box outlined with gray dotted line. The other populations represent Europe, Oceania, Southeast Asia, East Asia, and Sub-Saharan Africa, as indicated at the right.

(B) Shows the genetic ancestry proportions estimated from *HLA-A* and *-B* genotypes of the 80 First Nations Australians. Here, the analysis is restricted to European, Oceanic, and Southeast Asian populations, and each vertical line represents one individual. Colors indicate relative proportions of the K components, and each of them is assigned to a population group (key at the bottom), where each component is maximized.

(C) *HLA-A*, *-B*, and *-C* alleles observed in >1 First Nations Australian are studied. Shown are the frequencies of those alleles observed in (Ci) Europeans, (Cii) First Nations Australians having >50% European-related ancestry in *HLA* (B), (Ciii) First Nations Australians having <50% European-related ancestry in *HLA*, (Civ) Papua New Guineans, and (Cv) East Asians. Colors depict the \log_{10} frequencies as given in the key. Alleles absent from Papuans but present in Europeans and thus of likely European origin are indicated with purple text; those absent from Europeans are indicated with green text. Those alleles absent from Europeans and Papuans but present in East Asians are indicated in orange text. Any alleles present in both Europeans and East Asians are denoted European if frequency <1.5% in East Asians, and vice versa.

(D) For the ten most frequent *HLA class I* haplotypes observed in First Nations Australians (left), this shows the \log_{10} frequency in Europeans (Di), First Nations Australians (Dii and Diii), Papua New Guineans (Div), and East Asians (Dv). Colors depict the frequencies as given in the key at the right. Green text indicates oceanic-descent haplotypes, and purple text indicates likely European-descent haplotypes. For two haplotypes (8 and 10) having inconclusive ancestries from the haplotype frequencies, the genetic ancestries of the component alleles derived in (C) are shown. Haplotypes of likely European origin are marked with a red box.

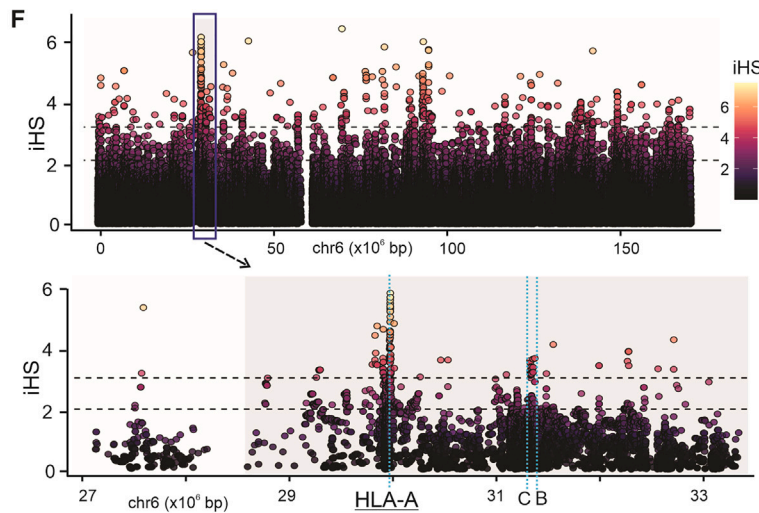


D

Ancestry (%)	KIR3DL1 allele		
	*114	*01502	*00501
Papuan	92.9%	14.0%	40.0%
East Asian	5.4%	58.1%	10.0%
Polynesian	1.8%	32.6%	30.0%
European	0.0%	1.2%	20.0%
N haplotypes	56	86	10

E

	W	D	Z
A*24:02	0.14	1.50	
A*34:01	-0.38	-5.17	
A*11:01	0.49	7.61	



(legend on next page)

Figure S2. Identifying distribution and ancestry of KIR3DL1*114 and HLA-A*24:02, related to Figure 2

(A) Global ancestry proportions estimated using ADMIXTURE at $K = 5$ from SNP array data of the Oceanian and 1000G merged dataset. The major mode of ten independent runs is shown. Each vertical bar represents an individual. These global ancestry proportions were used to identify individuals to be used in the reference panel for the local ancestry inference using RFMIX.

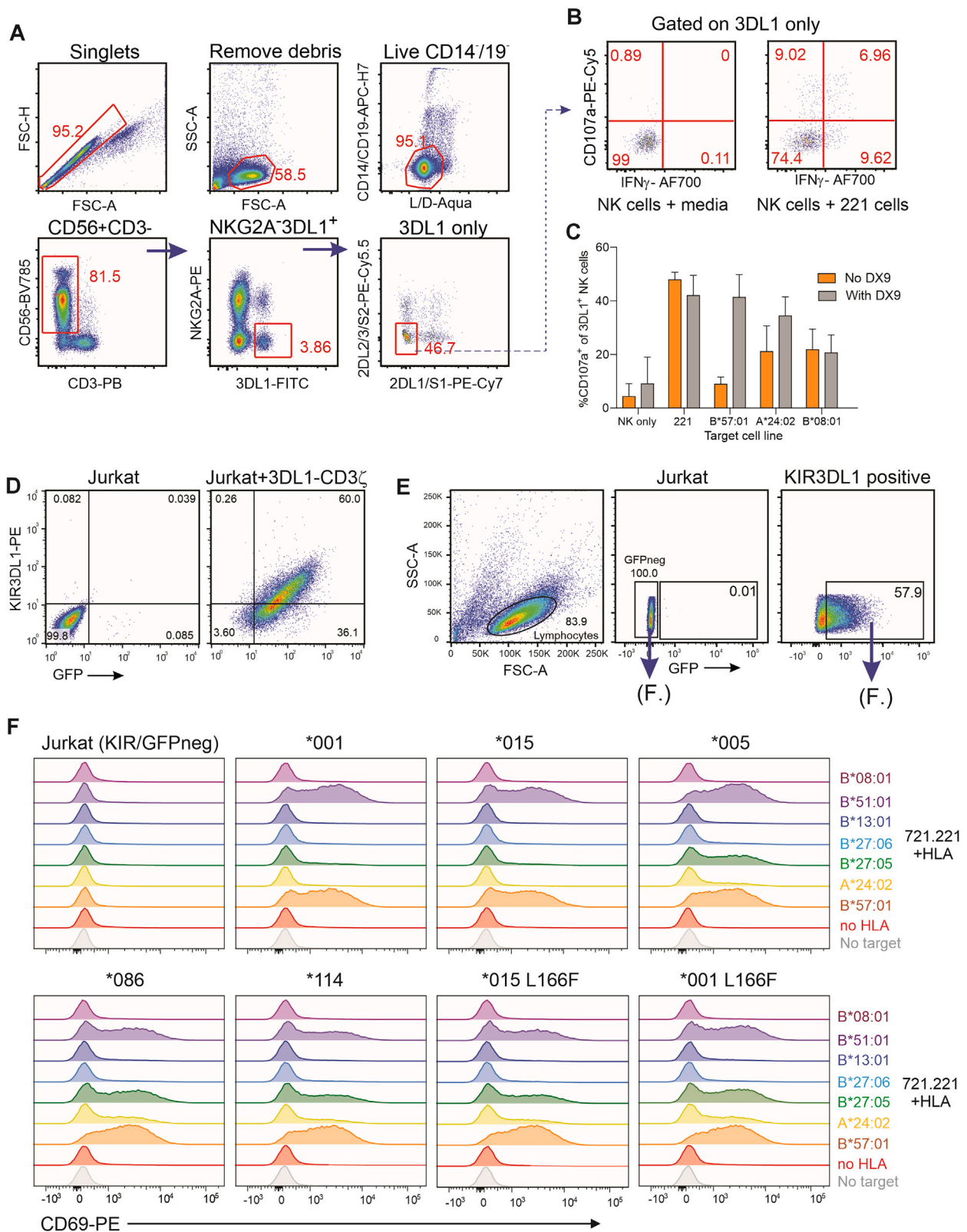
(B) Quality control of the local ancestry analyses performed with RFMIX, showing the correlation between global ancestry proportions inferred from ADMIXTURE $K = 5$ and RFMIX. The high correlations indicate that RFMIX inference does not show a systemic bias in any of the ancestry panels. The results from RFMIX were used to identify the genetic ancestry of the *KIR3DL1*114*, *005-lineage, and *015-lineage haplotypes in our dataset.

(C) Correlation of genome-wide proportion of Papuan-related ancestry with frequency of *KIR3DL1*114*. Top: shows the populations represented by the OGVP. Each dot represents a population, with diameter corresponding to sample size. Bottom: Papuan-related ancestry for each population studies represents the mean genome-wide ancestry inferred from RFMIX. Each dot represents a population group colored by region. SEA, Southeast Asia.

(D) Shows the number of *KIR3DL1*114*, *01502, or *00501 carrying haplotypes having either Papuan, East Asian, Polynesian, or European Ancestry, as described in (B).

(E) Results of Z and D tests for Denisovan introgression of *HLA-A*24:02*, *34:01, and *11:01. An *HLA-A*11* sequence was identified previously to be present in the Denisovan genome.³⁵

(F) Manhattan plot showing the positive iHS scores (representing selection on the derived alleles) performed on the *HLA-A*24:02* homozygous and heterozygous Papuans from SGDP.⁹⁵ Dashed lines represent top 1% and 5% iHS score thresholds.



(legend on next page)

Figure S3. Flow cytometry gating strategies and controls for *ex vivo* and IL-2 expanded KIR3DL1⁺ NK cell inhibition assays, related to Figure 3

(A) Flow cytometry gating strategy used to identify *ex vivo* single positive KIR3DL1⁺ NK cells following incubation with target cells. (Left to right, top to bottom) Purified NK cells were first identified by their forward and side scatter, single cells were selected, and debris removed. Dead cells and those expressing CD14 or CD19 were excluded before gating on CD3⁻ CD56⁺ cells (NK cells). NK cells expressing KIR3DL1 but not NKG2A were next selected, and KIR3DL1⁺ NK cells co-expressing KIR2DL1/S1 or KIR2DL2/3/S2 were excluded.

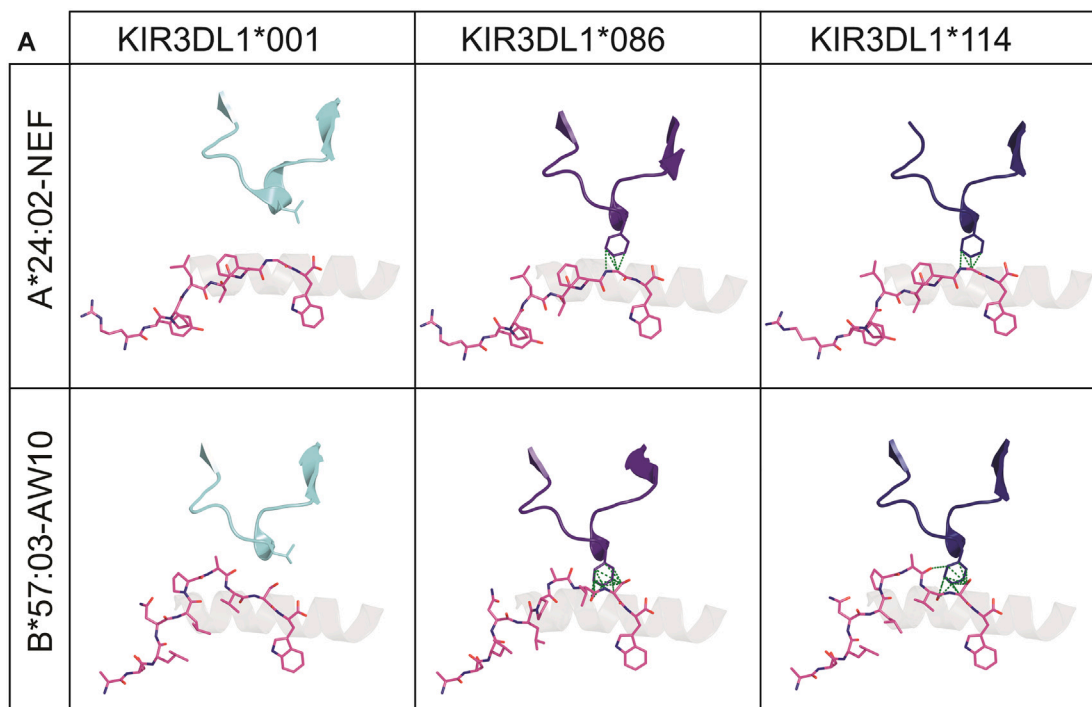
(B) Production of IFN- γ and CD107a by single positive KIR3DL1⁺ NK cells alone or with targets was then assessed.

(C) Shows the results of NK cell experiments from two donors, with and without KIR3DL1 blocking. KIR3DL1⁺ NK cells were sorted and expanded, then incubated with HLA-expressing target cells in the presence (gray) and absence (orange) of 10 μ g/mL DX9 antibody. The resulting expression of CD107a was assessed using flow cytometry (A and B).

(D) Jurkat cells were transduced with KIR3DL1 allotypes fused to the intracellular domain of CD3 ζ .

(E) GFP and KIR3DL1 expression on transduced Jurkat cells were assessed via flow cytometry, staining with anti-KIR3DL1/2-PE (clone 5.133: Miltenyi Biotec).

(F) Representative plots showing GFP expression and CD69 upregulation on reporter cells following 8 h incubation with a panel of HLA-transfected 721.221 target cells. Cells were stained with anti-CD69-PE and gated on GFP⁺ cells (untransfected cells on GFP negative cells), with the percentage of CD69⁺ cells taken as those with expression above that seen with no targets.



B Interactions with HLA molecule

Allotype	Interaction	KIR3DL1	HLA-A*24:02	Distance (Å)
*114	VDW	PHE166 [CD1]	GLU76 [CG]	3.45
		PHE166 [CD1]	GLU76 [OE1]	3.94
		PHE166 [CE1]	GLU76 [CG]	3.67
		PHE166 [CE1]	GLU76 [OE1]	3.74
		PHE166 [CE1]	ILE80 [CD1]	3.51
		PHE166 [CE1]	ILE80 [CG1]	3.63
		PHE166 [CE2]	ILE80 [CD1]	3.88
		PHE166 [CZ]	ILE80 [CG1]	3.43
		PHE166 [CZ]	ILE80 [CD1]	3.41
*001	VDW	LEU166 [CD2]	ILE80 [CD1]	3.92

C. Interactions with presented peptide

Allotype	Interaction	KIR3DL1	TW9	Distance (Å)
*114	H-Bonds	TYR200 [OH]	ASN8 [ND2]	3.75
		GLU282 [OE1]	ASN8 [ND2]	2.82
		GLU282 [OE2]	ARG7 [NH2]	3.49
	VDW	PHE166 [CB]	ASN8 [CG]	3.91
		PHE166 [CB]	ASN8 [OD1]	3.9
		PHE166 [CD2]	ASN8 [OD2]	3.87
*001	H-Bonds	Not present		
	VDW	LEU166 [CB]	ASN8 [OD1]	3.97
		LEU166 [CD2]	ASN8 [CB]	3.75

D Interactions with presented peptide

vdw Interactions	KIR3DL1*114 PHE 166	Peptide	Distance (Å)
NEF	CE1	GLY 7 (N)	3.93
	CE1	GLY 7 (CA)	3.64
	CE2	GLY 7 (CA)	3.77
	C2	GLY 7 (CA)	3.40
AW10	CD1	ALA 7 (O)	3.71
	CE1	VAL 8 (C)	3.78
	CE1	VAL 8 (O)	3.42
	C2	VAL 8 (O)	3.60
	CE1	SER 9 (CA)	3.87
	C2	SER 9 (CA)	3.73
	C2	SER 9 (CB)	3.60
	CG	SER 9 (CB)	3.54
	CD1	SER 9 (CB)	3.67
	CD2	SER 9 (CB)	3.41
CE1	SER 9 (CB)	3.71	
CE2	SER 9 (CB)	3.45	

Figure S4. Contact points of KIR3DL1 with HLA-peptide complexes, related to Figure 4

(A) Compares contact points of KIR3DL1 residue 166 with P8 from HLA-A*24:02-TW9 or B*57:03-AW10, across KIR3DL1 allotypes *001, *086, and *114.

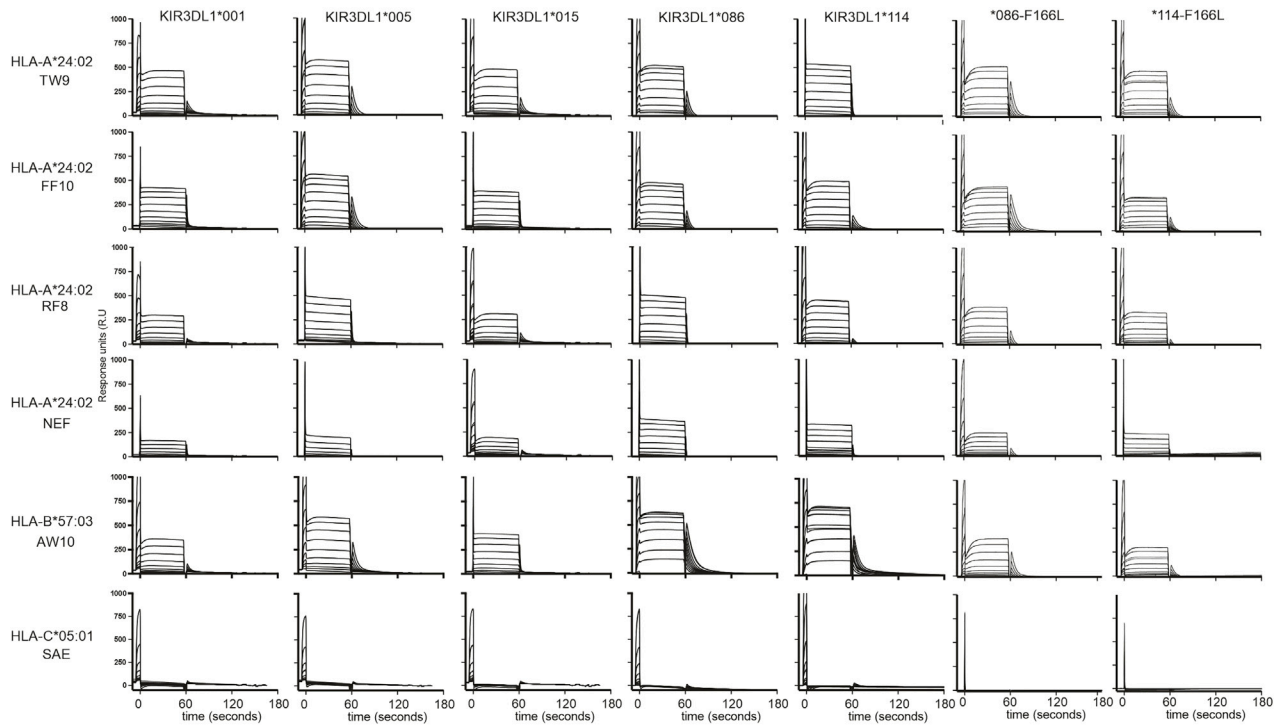
(B and C) Shown are van der Waals (vdw) and hydrogen bonds (H-bonds) identified through crystallography of KIR3DL1*114 or *001 with HLA-A*24:02-TW9 complex. (B) Interactions of KIR3DL1 with HLA molecule. (C) Interactions of KIR3DL1 with TW9 peptide.

(D) Lists the vdw interactions between Phe-166 of KIR3DL1*114 and the peptide residues.

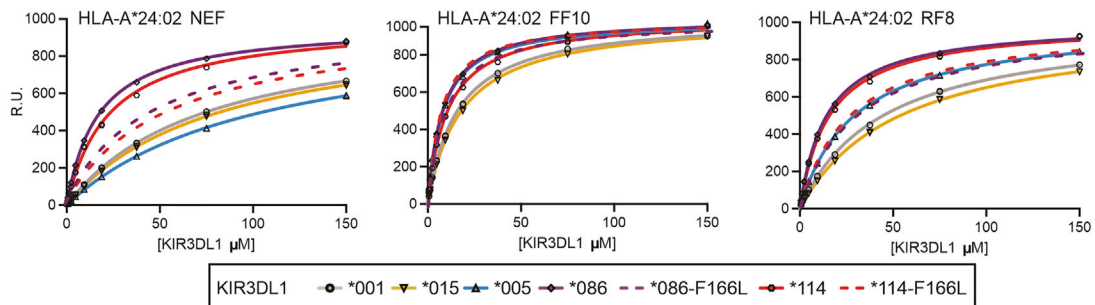
A

HLA	Peptide	Sequence	Organism	Antigen
HLA-A*24:02	TW9 ⁵⁴⁹⁻⁵⁵⁷	TYQWIIRNW	Influenza A virus	Polymerase basic protein 2
	FF10 ⁴⁹⁶⁻⁵⁰⁵	FYRYGFVANF		RNA-directed RNA polymerase catalytic subunit
	RF8 ⁴⁹⁸⁻⁵⁰⁵	RYGFVANF		RNA-directed RNA polymerase catalytic subunit
	NEF ¹³⁴⁻¹⁴¹	RYPLIFGW	HIV-1	Nef
HLA-B*57:03	AW10 ⁸⁵⁰⁻⁸⁵⁹	ASLNLPVSW	Homo sapiens	Catenin
HLA-C*05:01	SAE ⁶⁷⁻⁷⁵	SAEPVPLQL	HIV-1	Rev

B



C



D

HLA-peptide	KIR3DL1*001	KIR3DL1*005	KIR3DL1*015	KIR3DL1*086	*086-F166L	KIR3DL1*114	*114-F166L
A*24:02 TW9	34.65 ± 0.14	18.07 ± 1.20	36.61 ± 2.43	8.88 ± 0.04	18.97 ± 1.95	10.44 ± 0.75	20.06 ± 2.8
A*24:02 FF10	15.94 ± 0.99	7.94 ± 0.28	18.07 ± 0.54	8.33 ± 0.03	9.14 ± 2.06	10.61 ± 0.72	6.67 ± 0.06
A*24:02 RF8	44.89 ± 3.25	29.28 ± 1.08	53.83 ± 1.08	15.28 ± 0.13	31.21 ± 4.81	15.93 ± 0.91	27.08 ± 0.13
A*24:02 NEF	75.01 ± 0.74	104.52 ± 10.1	82.17 ± 7.07	18.06 ± 0.07	45.07 ± 1.37	24.95 ± 2.53	53.27 ± 4.25
B*57:03 AW10	50.87 ± 0.35	42.90 ± 1.07	62.36 ± 1.03	1.99 ± 0.01	35.72 ± 0.05	2.07 ± 0.03	31.37 ± 3.93
C*05:01 SAE	N.B	N.B	N.B	N.B	N.B	N.B	N.B

Figure S5. Surface plasmon resonance: Peptides and data, related to Figure 5

- (A) Sequence and origin of peptides used for HLA class I/peptide complexes. HLA-C*05:01-SAE is used as a negative control, which does not bind KIR3DL1.²⁴
- (B) Sensorgrams of KIR3DL1 allotypes (*001, *005, *015, *086, and *114, and mutations *086-F166L, *114-F166L) binding to refolded HLA-peptide complexes. Experiments were performed in duplicate, and one replicate is shown.
- (C) Affinity curves of KIR3DL1 allotype binding to immobilized HLA-peptide complexes. R.U., response units.
- (D) Steady-state K_D for interactions of KIR3DL1 allotypes with HLA-peptide complexes. N.B., no binding (no detectable interaction). Data are derived from two independent experiments.