

1 **Genetically Determined Strength of Natural Killer Cells is Enhanced by Adaptive HLA
2 class I Admixture in East Asians**

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28

1 Abstract

2 Human natural killer (NK) cells are essential for controlling infection, cancer and fetal
3 development. NK cell functions are modulated by interactions between polymorphic inhibitory
4 killer cell immunoglobulin-like receptors (KIR) and polymorphic HLA-A, -B and -C ligands
5 expressed on tissue cells. All *HLA-C* alleles encode a KIR ligand and contribute to reproduction
6 and immunity. In contrast, only some *HLA-A* and -B alleles encode KIR ligands and they focus
7 on immunity. By high-resolution analysis of *KIR* and *HLA-A*, -B and -C genes, we show that
8 the Chinese Southern Han are significantly enriched for interactions between inhibitory KIR
9 and HLA-A and -B. This enrichment has had substantial input through population admixture
10 with neighboring populations, who contributed *HLA class I* haplotypes expressing the KIR
11 ligands B*46:01 and B*58:01, which subsequently rose to high frequency by natural selection.
12 Consequently, over 80% of Southern Han *HLA* haplotypes encode more than one KIR ligand.
13 Complementing the high number of KIR ligands, the Chinese Southern Han *KIR* locus
14 combines a high frequency of genes expressing potent inhibitory KIR, with a low frequency of
15 those expressing activating KIR. The Southern Han centromeric *KIR* region encodes strong,
16 conserved, inhibitory HLA-C specific receptors, and the telomeric region provides a high
17 number and diversity of inhibitory HLA-A and -B specific receptors. In all these
18 characteristics, the Southern Han represent other East Asians, whose NK cell repertoires are
19 thus enhanced in quantity, diversity and effector strength, likely through natural selection for
20 resistance to endemic viral infections.

21

22

1 **Introduction**

2 Human leukocyte antigen (HLA) class I molecules are critical components of immunity, whose
3 extreme variation associates with resistance and susceptibility to infection, multiple immune-
4 mediated diseases and some cancers (Dendrou et al. 2018). *HLA class I* genes are located in
5 the *major histocompatibility complex (MHC)* of chromosome 6 and encode proteins that bind
6 peptide fragments derived from intracellular protein breakdown and transport them to the cell
7 surface. In doing so they can communicate to the adaptive immune system's T cells whether a
8 tissue cell is healthy, or unhealthy due to infection or cancer. Subsets of HLA class I allotypes
9 additionally contain an externally facing amino acid motif that binds killer cell
10 immunoglobulin-like receptors (KIR), facilitating interaction with natural killer (NK) cells of
11 innate immunity.

12

13 KIR are expressed on the surface of NK cells and regulate their functions through binding to
14 HLA class I ligands on other cells (Cooper et al. 2009; Long et al. 2013). The functions of
15 these interactions are crucial in immunity to aid recognition and elimination of infected or
16 tumorous tissue, and in reproduction to regulate placentation and fetal development (Parham
17 and Moffett 2013). In accordance with these critical and independent roles in human health,
18 KIR and their HLA class I ligands are subject to natural selection, mediating their exceptional
19 diversity across individuals, populations and species (Parham and Moffett 2013; Prugnolle et
20 al. 2005). Indeed, *KIR* and *MHC* are some of the fastest evolving genomic loci in higher
21 primates (Guethlein et al. 2015). Correlating with direct impact on both NK cell development
22 and effector function (Freud et al. 2017; Vivier et al. 2011), numerous studies have implicated
23 combinatorial diversity of *KIR* and *HLA class I* alleles with the course of specific infectious
24 and autoimmune diseases, as well as the success of transplantation (Boudreau and Hsu 2018;
25 Holzemer et al. 2017). Importantly, the quantity as well as quality of these interactions can
26 influence individual responses to infection (Boelen et al. 2018; Pelak et al. 2011). Thus, the

1 polymorphism of *KIR* and *HLA class I* has profound impact on human health. Under-explored
2 are the scale and characteristics of *KIR* and *HLA class I* combinatorial diversity worldwide,
3 and the processes that shape this diversity.

4

5 NK cells express overlapping subsets of *KIR* that are acquired stochastically during their
6 development (Andersson et al. 2009). During this process, the interaction of inhibitory *KIR*
7 with *HLA class I* *KIR* ligands broadens and strengthens subsequent effector functions of the
8 NK cell repertoire (Bjorkstrom et al. 2016; Hoglund and Brodin 2010; Saunders et al. 2015).
9 This education process matures some NK cells, allowing them to respond effectively to specific
10 instances of infection or cancer, and enhances the NK cell repertoire compared to those that
11 develop using other more conserved pairs of ligands and receptors. In this role, and also in
12 pregnancy where *HLA-A* and -B have no function, *HLA-C* is dominant because all expressed
13 *HLA-C* are *KIR* ligands (Guethlein et al. 2015). Four mutually exclusive sequence motifs
14 define the four *HLA class I* epitopes that are *KIR* ligands: C1 is carried by subsets of *HLA-C*
15 and *HLA-B* allotypes. C2 is carried by the other allotypes of *HLA-C*. Bw4 is carried by subsets
16 of *HLA-A* and -B allotypes. The A3/11 motif is carried by a subset of *HLA-A* allotypes (*HLA-*
17 *A*03* and *A*11*). Thus, only some *HLA-A* and -B allotypes are *KIR* ligands and their main
18 role is likely to diversify the NK cell response to pathogens.

19

20 The *KIR* locus on chromosome 19q13.4 varies in gene content, containing up to eight genes
21 encoding inhibitory *KIR* and five encoding activating *KIR* (Wilson et al. 2000). Four of the
22 inhibitory *KIR* and four activating *KIR* have well-characterized *HLA-A*, -B or -C ligands. Two
23 broad groups of *KIR* haplotypes are present in every human population. *KIR A* haplotypes carry
24 all four of the *HLA-class I* specific inhibitory receptors and are associated with resistance to
25 infectious diseases (Bashirova et al. 2006). *KIR B* haplotypes are more variable in their gene
26 number, carrying two or more genes for inhibitory receptors as well as various activating

1 receptor genes, and favor fetal development (Parham and Moffett 2013). A recombination
2 hotspot separates the *KIR* locus into two segments (Wilson et al. 2000). Two inhibitory
3 receptors specific for HLA-C are encoded in the centromeric region, and two HLA-A and -B
4 specific receptors are encoded in the telomeric region. Additional to gene content variation,
5 polymorphism of both receptors and ligands can directly affect NK cell activity (Guethlein et
6 al. 2015). Thus, by varying the number, density, specificity, strength or signaling properties of
7 the receptor-ligand interaction, genetic variation of *KIR* and *HLA class I* can pre-determine
8 functional differences in NK cell repertoires between individuals. This genetic diversity is
9 substantial among populations, as demonstrated with high-resolution studies (Guethlein et al.
10 2015; Nemat-Gorgani et al. 2018). In such detailed analysis, Asian populations are under-
11 represented.

12
13 Comprising 20% of the human population, the Chinese Han are the largest ethnic group in the
14 world (Abdulla et al. 2009). The Han have a complex population history and are presently
15 structured with the Northern and Southern Han forming two main subgroups that are separated
16 geographically by the Yangtze River (Wen et al. 2004). The Southern Han originated through
17 large scale population movement from the north ~1500 years ago, in parallel with admixture
18 with resident and neighboring populations (Hellenthal et al. 2014; Wen et al. 2004).
19 Importantly for the current study, the major genetic distinction between the Northern and
20 Southern Han occurs in the *MHC*, and localizes to the region that spans *HLA-A*, -*B* and -*C*
21 (Chen et al. 2016; Xu et al. 2009). The most significant component of this difference is the
22 *A*33:03-B*58:01-C*03:02 HLA class I* haplotype, which is common in the Southern Han and
23 remains conserved across multiple unrelated individuals (Chen et al. 2016). Such strong
24 linkage disequilibrium is consistent with recent acquisition of this haplotype by admixture
25 (Chen et al. 2016). This haplotype encodes two KIR ligands, HLA-B*58:01 and C*03:02
26 (Guethlein et al. 2015). Although less is known of *KIR* allele diversity in the Han, several

1 studies established that the genes characteristic of *KIR A* haplotypes are common, and
2 demonstrated differences in their distribution among the different Han groups and among other
3 resident populations (Bao et al. 2013; Wang et al. 2012; Yao et al. 2011). These studies also
4 confirmed that *KIR* and *HLA class I* combinatorial diversity is an important factor in pregnancy
5 syndromes, infectious disease, blood cancers and transplantation outcome in the Han. They
6 also uncovered both similarities and differences from the specific disease associations observed
7 in Europeans (Bao et al. 2016; Jiang et al. 2013; Long et al. 2015; Shen et al. 2016; Su et al.
8 2018). To investigate these findings, we have examined how demographic and evolutionary
9 processes have shaped combinatorial diversity of HLA class I and KIR in the Chinese Southern
10 Han.

1 **Materials and methods**

2 ***Study samples***

3 Peripheral blood samples were collected from 306 unrelated healthy volunteer blood donors
4 from Shenzhen, Guangdong, China. All donors self-identified to be of Han ethnicity from
5 southern China. All subjects provided written informed consent for participation in the present
6 research, which was approved by the ethics review board of Shenzhen Blood Center, Shenzhen,
7 Guangdong, China.

8

9 ***Genomic DNA extraction***

10 Genomic DNA was extracted from 400 µl of peripheral blood using a MegCore Nucleic Acid
11 Extractor (MegCore, Taiwan, China). DNA purity and concentration were tested by UV-
12 spectrophotometry using a Biophotometer (Eppendorf, Hamburg, Germany) and adjusted to a
13 concentration of 50-100 ng/µl.

14

15 ***High-resolution HLA-A, -B and -C genotyping***

16 *HLA-A, -B and -C* genotyping was performed using the AlleleSEQR HLA sequencing-based
17 genotyping commercial kit (Atria Genetics, San Francisco, USA). According to the
18 manufacturer's instructions, exons 2-4 for *HLA-A, -B* and *-C* were sequenced in both directions
19 using an ABI 3730XL DNA sequencer (Applied Biosystems, Foster City, CA, USA). HLA
20 genotypes were assigned using the Assign 4.7 software (Conexio Genomics, Fremantle,
21 Australia). Samples giving ambiguous allele combinations by sequencing were further resolved
22 using HLA PCR-SSP (Olerup, Stockholm, Sweden).

23

24 ***High-resolution KIR genotyping***

25 The presence or absence of *KIR2DL1*, *2DL2/3*, *2DL4*, *2DL5*, *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*,
26 *3DL1/S1*, *3DL2* and *3DL3* was first determined for each individual using the 'KIR Ready Gene'

1 PCR-SSP kit (Inno-Train Diagnostik GmbH, Frankfurt, Germany). The *KIR* genes identified
2 using PCR-SSP were then subject to nucleotide sequencing of all exons (Deng et al. 2018).
3 Sequencing reactions were performed using ABI PRISM BigDye Terminator Cycle
4 Sequencing Ready reagents and analyzed using an ABI 3730 DNA Sequencer (Applied
5 Biosystems, Foster City, USA). *KIR* alleles were assigned using Assign 4.7 allele identification
6 software (Conexio Genomics, Fremantle, Australia), and release 2.6.1 (February 2015) of the
7 Immuno-Polymorphism database (IPD) (Robinson et al. 2015). When the sequencing results
8 gave ambiguous allele combinations, we used group-specific PCR primer pairs to amplify and
9 sequence the target alleles separately (Zhang and Deng 2016)

10

11 ***Novel KIR alleles***

12 To confirm and fully characterize any novel allele identified during amplicon sequencing we
13 cloned and sequenced *KIR* transcripts. Further samples of peripheral blood samples were
14 collected, and total RNA isolated using the Maxwell 16 low elution volume simplyRNA Blood
15 Kit (Promega, Madison, USA). Complementary DNA (cDNA) was synthesized using the
16 Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland). *KIR* transcripts
17 were amplified specifically from cDNA using primer pairs described previously (Yawata et al.
18 2006), with addition of KIR3DL3-specific primers (forward 5'-
19 GGTTCTTCTTGCTGGAGGGC-3' and reverse 5'-TTACACGCTGGTATCTGTTGGGG-
20 3'). The amplified transcripts were cloned using the TA cloning kit (Takara, Dalian, China)
21 and at least three clones of any novel allele were sequenced. The sequences of novel *KIR* alleles
22 were submitted to GenBank and the IPD KIR database (Robinson et al. 2015) to obtain official
23 names.

24

25 ***Admixture Estimates***

1 Whole genome SNP genotypes for Japanese (N = 104), Vietnamese (N = 99), Han from Beijing
2 (N = 103), Southern Han (N = 105), and Dai (N = 93) were obtained from the 1000 Genomes
3 Project (Auton et al. 2015). We used any SNPs having minor allele frequency >1% and
4 independent of other SNPs (linkage disequilibrium, $r^2 < 0.3$). Admixture was calculated for
5 chromosome 6 using the ADMIXTURE program (Alexander et al. 2009), with the
6 unsupervised option and $k=3$. Two regions were analyzed, the *MHC* (chr6:28,477,797–
7 33,448,354: 3,541 SNPs) and chromosome 6 excluding the *MHC* (84,898 SNPs). We selected
8 a K of 3 to represent the three primary ancestry groups in the region that are represented in the
9 1000 Genomes data: Japanese, South East Asian, and East Asian (Chen et al. 2016). *HLA class*
10 *I* alleles were obtained from the 1000 Genomes Project data (Gourraud et al. 2014). We
11 analyzed the Hondo Japanese (JPT), Vietnamese (KHV), Chinese Dai (CDX), Chinese
12 Southern Han (CHS), and Beijing Han (CHB) Validating their use for this purpose, the
13 correlation of the *HLA class I* allele frequencies between our study population and the CHS is
14 0.95 ($p=6.65^{-11}$, [Figure S1](#)). Individuals were considered carriers if they had at least one copy
15 of the respective allele. Distributions of ancestry proportions for carriers and non-carriers of
16 specific *HLA* alleles were compared using a Wilcoxon test, using the `wilcox.test` function in R
17 (R Development Core Team 2008).

18

19 ***Estimates of nucleotide diversity***

20 We used π (Nei and Takahata 1993) to measure the nucleotide diversity of haplotypes carrying
21 specific *HLA-B* alleles. We used the phased genomes of the Chinese Southern Han (CHS)
22 population available from the 1000 Genomes Project (Auton et al. 2015), and extracted the
23 genomic region containing the *HLA-B* and -*C* genes, with 500kbp flanking on each side. For
24 each carrier of a given allele, we identified (by sequence) and retained the haplotype
25 representing the allele of interest. For each given allele, we pooled all of the respective
26 haplotypes present in the population and calculated π in 100bp windows using VCFtools

1 (Danecek et al. 2011). Distributions of π values were compared between respective alleles with
2 a Wilcoxon test using the `wilcox.test` function in R.

3

4 ***Tests for positive selection affecting specific HLA class I alleles***

5 We filtered 1,000 Genomes genotyping data of chromosome 6 from the CHS population to
6 remove non-biallelic and duplicated SNPs (Purcell et al. 2007), then phased using the program
7 Eagle (Loh et al. 2016). We used the program Selscan (Voight et al. 2006) to calculate
8 integrated haplotype statistic (iHS). The statistic is a measure of haplotype diversity associated
9 with a given genetic variant, where lower diversity and longer haplotypes correlate with
10 selection of that variant.

11

12 To determine if specific *HLA class I* alleles have been targeted by directional selection in the
13 Chinese Southern Han we again used the 1,000 Genomes SNP data from the CHS population.
14 SNPs within the following hg19 coordinates were used: *HLA-A*, Chr6: 29,910,089 –
15 29,913,770; *HLA-B*, Chr6: 31,321,648 – 31,325,007; *HLA-C*, Chr6: 31,236,517 – 31,239,917.

16 We phased haplotypes from individuals positive for each given *HLA class I* allele and aligned
17 them to reference sequences to identify the haplotype containing that allele. The alignments
18 were then used to identify ‘tagging’ SNPs that could be used to identify each given *HLA class*
19 *I* allele. The criteria for choosing tagging SNP alleles were that they must be present in every
20 individual carrying the corresponding *HLA class I* allele and that they must be absent from the
21 other *HLA class I* alleles in the analysis. We analyzed the alleles present on the 10 most frequent
22 *HLA class I* haplotypes that we observed in the Chinese Southern Han; *HLA-B*15:02* was
23 excluded because we were not able to identify unique tagging alleles on haplotypes carrying
24 this allele. For each tagging SNP, we calculated the integrative haplotype score (iHS) using
25 SelScan. We used the absolute value of iHS since derived alleles under selection will have a
26 negative value and ancestral alleles under selection will have a positive value (Szpiech and

1 Hernandez 2014). Using a Wilcoxon two-sample test, we examined whether the distributions
2 of absolute iHS values differed between tagging SNPs of *HLA* alleles and SNPs of the full
3 chromosome 6.

4

5 ***Haplotype and ligand frequencies***

6 *KIR* and *HLA-A*, *-B* and *-C* allele frequencies were calculated from the observed genotypes.
7 For individuals genotyped as homozygous for an allele of a given *KIR* that has
8 presence/absence polymorphism, the number of copies present was determined by analyzing
9 LD with alleles of the flanking genes. The subsequent genotype distributions for all loci were
10 consistent with Hardy-Weinberg equilibrium. *KIR* haplotype frequencies were determined
11 using PHASE II (Stephens and Donnelly 2003). The following parameters were used; -f1, -x5,
12 and -d1, and from the output, the two haplotypes with highest probability were taken for each
13 individual. Watterson's homozygosity F test was performed using Pypop software (Lancaster
14 et al. 2007), with 10,000 replicates to calculate the normalized deviate F_{nd} test (Salamon et al.
15 1999).

16

17 *HLA class I (A-B-C)* haplotype frequencies were determined using the EM algorithm of
18 'Arlequin software version 3.5 (Excoffier and Lischer 2010). For comparison with other
19 populations, we only used populations for which *HLA class I* genotype data were available
20 from every individual sampled, and for which the resolution of genotyping was the same as the
21 Southern Han described here. We therefore used the subset of populations described in the 13th
22 International Histocompatibility Workshop and Conference report that have 50 or more *HLA-*
23 *A*, *-B* and *-C* genotyped individuals (Meyer et al. 2007). These were supplemented with our
24 own data from the Ga-Adangbe from Ghana in West Africa (Norman et al. 2013), KhoeSan
25 from Southern Africa (Nemat-Gorgani et al. 2018), Yucpa from South America (Gendzkhadze
26 et al. 2009), Europeans from the USA (Norman et al. 2016) and Hondo Japanese (Yawata et

1 al. 2006). We compared the proportion of *HLA class I* haplotypes encoding one KIR ligand to
2 those encoding more than one KIR ligand across populations using a two-proportions Z test,
3 using the prop.test function in R (R Development Core Team 2008).

4

5 ***Comparison of HLA class I and KIR ligand distributions.***

6 Clustering based on allele frequencies: Any *HLA class I* allele occurring in fewer than two of
7 the populations studied was excluded from this analysis. The allele frequencies of all three *HLA*
8 *class I* genes were used for each population. Cluster dendograms were constructed using R
9 3.4.3, hclust with 1,000 bootstrap values. The package used was fpc (Hennig 2020). Cluster
10 dendograms were constructed in the same manner using the frequencies of the *HLA class I*
11 haplotypes encoding one, two or three KIR ligands.

12

13 ***Assessment of receptor/ligand quality and quantity***

14 As described previously (Nemat-Gorgani et al. 2018), experimental data were used to
15 determine the interacting pairs of KIR and HLA class I, which are listed in **Figure S2**. To
16 determine the quantity of receptor/ligand interactions, the number of KIR/HLA allotype pairs
17 that are known to interact were summed for each individual, and homozygous KIR or HLA
18 allotypes were counted twice. To determine the diversity of interactions, the number of
19 different KIR/HLA allotype pairs that are known to interact were summed for each individual
20 (in this case homozygous allotypes were counted once). Populations were compared using
21 unpaired t tests, using GraphPad software.

22

1 **Results**

2 ***High Frequency of KIR ligands in the Southern Han***

3 All HLA-C and subtypes of HLA-A and -B allotypes are ligands for KIR, which are expressed
4 on the surface of NK cells to modulate their functions in immunity and reproduction. Within
5 human populations, *HLA class I* haplotypes tend to form a balance between those that encode
6 HLA-A or -B KIR ligands and those that do not (Guethlein et al. 2015). To determine if this
7 pattern is also observed in the Chinese Southern Han, we analyzed the *HLA-class I* genes of
8 306 healthy individuals. We identified 27 *HLA-A*, 54 *HLA-B* and 29 *HLA-C* alleles (Figure S3).
9 Each of these 110 alleles encodes a different HLA class I allotype, and 58 of them are known
10 KIR ligands (Figure 1A). The majority of 233 *HLA class I* haplotypes, including the ten most
11 frequent (Figure 1B), encode more than one KIR ligand (70.3% of distinct haplotypes; 81.8%
12 by frequency, Figure S4A). This observation is unusual and indicates the balance between
13 having and not having KIR ligands at HLA-A and -B is perturbed in the Chinese Southern Han.

14

15 To investigate the unusually high frequency of KIR ligands, we compared Southern Han *HLA*
16 *class I* haplotypes with those of sub-Saharan African, Oceanian, European and South American
17 populations that represent major modern human groups (Rosenberg et al. 2002; Tishkoff et al.
18 2009). In this data set, rather than the larger and more widely studied Hondo Japanese
19 population, a Ryukyu Japanese population was included because they more closely represent
20 the Japanese population prior to admixture with Han (Takeuchi et al. 2017). Among the eight
21 populations, 1,034 different *HLA class I* haplotypes were observed (Figure S4B). Six
22 populations have a similar distribution of KIR ligands, with each population having an
23 approximately equal frequency of *HLA class I* haplotypes carrying one and two KIR ligands,
24 and a smaller frequency of haplotypes carrying three KIR ligands (Figure 1C). Only Southern
25 Han and South Americans differed from this pattern, with the Han encoding more and the
26 Amerindians encoding less KIR ligands per haplotype than other populations (Figure 1C). The

1 difference in the proportion of *HLA class I* haplotypes encoding one versus more than one KIR
2 ligand between the Southern Han and each of the other seven representative populations is
3 statistically significant, as is that between Amerindians and the other populations (Two-
4 proportions Z-test, Benjamini-Hochberg corrected $p < 0.001$, [Figure 1C](#)). The allele frequency
5 distribution of South American Amerindians was likely influenced by severe population
6 bottlenecks, leading to a reduced genome-wide diversity compared with other populations
7 (Fagundes et al. 2008; Raghavan et al. 2015), whereas the Han were not subject to severe
8 population-specific bottleneck (Henn et al. 2012; Lu et al. 2016; Schiffels and Durbin 2014).
9 To examine if the Chinese Southern Han are representative of other related populations, we
10 examined groups from East Asia (Hondo Japanese and Korean) and Southeast Asia (Thai,
11 Malay and Filipino). This analysis showed these populations also have a high frequency of
12 *HLA class I* haplotypes encoding multiple KIR ligands ([Figure 1D](#)). Our analysis thus shows
13 that East Asian and South East Asian *HLA class I* haplotypes encode more ligands for
14 inhibitory KIR than the haplotypes of any other populations.

15
16 Despite having distinct population histories, the sub-Saharan African, Oceanic, European and
17 Ryukyu Japanese populations all have a similar mean number of KIR ligands per *HLA*
18 haplotype ([Figure 1C](#)). However, very few *HLA class I* haplotypes are shared by any of these
19 populations. For example, only 19 of 369 haplotypes detected in Africans are present in more
20 than one of the three African populations studied, and comparing the disparate Southern
21 African Nama, Indigenous Australian and Ryukyu Japanese populations revealed just five
22 haplotypes in common ([Figure 1E](#)).

23
24
25 ***The Chinese Southern Han acquired HLA haplotypes encoding multiple KIR ligands by***
26 ***admixture***

1 Previous analyses suggested that specific *MHC* region haplotypes (which include the *HLA*
2 genes) present in the Chinese Southern Han were obtained from the Northern Han through
3 admixture (Chen et al. 2016). The most frequent HLA class I allotypes contributing to the
4 enrichment of KIR ligands in the Chinese Southern Han are HLA-A*11, -A*24, -B*46, and -
5 B*58 ([Figure 1B](#)). We therefore examined the relative contributions of admixture to the high
6 frequency of these alleles in the Chinese Southern Han. For this analysis we considered known
7 admixture events (Hellenthal et al. 2014; Wen et al. 2004; Xu et al. 2009) and drew upon the
8 1000 Genomes SNP and *HLA* genotype data (Auton et al. 2015; Gourraud et al. 2014) from
9 Hondo Japanese, Vietnamese, Dai, Beijing Han, and Chinese Southern Han. Consistent with
10 previous work examining whole-genome data (Takeuchi et al. 2017), in analyzing chromosome
11 6 we identified three primary genetic ancestries, corresponding to the Japanese, East Asian
12 (Southern and Beijing Han) and South East Asian (Vietnamese and Dai) population groups
13 ([Figure 2A](#)). That we identify a higher ‘Japanese’ component in the Beijing than Southern Han
14 (36% vs 22%: [Figure 2A](#)) likely reflects the higher proportion in Beijing of Northern Han
15 (Auton et al. 2015), a population from which we have no data for the current study. Supporting
16 this observation, the greatest contribution from China to Japanese ancestry is from the Northern
17 Han (Chen et al. 2016; Takeuchi et al. 2017).

18
19 We compared the relative proportions of the three genetic ancestries in the Chinese Southern
20 Han within the *MHC* region of chromosome 6 to their proportions throughout chromosome 6
21 excluding the *MHC*. This analysis revealed a predominance of East Asian ancestry throughout
22 the length of chromosome 6, including the *MHC* ([Figure 2B](#)). In carriers of *HLA-B*40:01*, the
23 most frequent *HLA-B* allele in Chinese Southern Han, there is also clear East Asian ancestry
24 throughout the length of chromosome 6 ([Figure 2B](#)). The proportion of East Asian ancestry is
25 similar in *B*40:01* carriers than non-carriers (Wilcoxon test, $p=0.98$). By contrast, among
26 *HLA-B*46:01* carriers, the *MHC* is primarily of South East Asian ancestry ([Figure 2B](#)) with

1 carriers having a significantly higher proportion of South East Asian genetic ancestry in the
2 *MHC* than outside the *MHC* ($p = 2.7^{-6}$), or within the *MHC* of non-carriers ($p = 1.9^{-5}$). Similarly,
3 among *HLA-B*58:01* carriers, the *MHC* region is primarily of Japanese ancestry (Figure 2B),
4 with carriers having a significantly higher proportion of Japanese genetic ancestry within the
5 *MHC* than outside the *MHC* ($p = 2.4^{-4}$) and compared with non- *B*58:01* carriers ($p = 6.1^{-7}$).
6 Excluding the *MHC*, carriers of any of these three alleles show East Asian ancestry along
7 chromosome 6 (Figure 2B). Further supporting the observed population structure as specific to
8 the *MHC* region, among the three ancestral groups the F_{ST} values range from 0.098 – 0.161,
9 compared with 0.012 – 0.017 for the remainder of chromosome 6.

10

11 Based on the analysis of *B*46* and *B*58*, we examined the proportions of genetic ancestry of
12 alleles that comprise the 10 most frequent *HLA class I* haplotypes observed in the Chinese
13 Southern Han. The primary genetic ancestry outside of the *MHC* region was determined as East
14 Asian for every allele studied (Figure 2B). Thus, for our comparisons, we determined the
15 primary genetic ancestry in the flanking *MHC* region for each allele and then determined the
16 relative proportion of that ancestry in the remainder of the *MHC*. This analysis identified six
17 haplotypes maintaining strong evidence of East Asian genetic ancestry both within the *MHC*
18 and throughout chromosome 6. These haplotypes include those that carry *A*11:01* and
19 *A*24:02*, as well as *B*40:01* (Figure 2C). It was shown previously that HLA-A*11 and -A*24
20 derive from introgression with archaic humans (Abi-Rached et al. 2011) and our results and
21 others (Gonzalez-Galarza et al. 2015; Solberg et al. 2008) thus suggest these haplotypes are
22 now endemic to East Asia. The analysis also identified four haplotypes having genetic ancestry
23 within the *MHC* that is distinct from the ancestry of the remainder of the chromosome (Figure
24 2C). For three of the haplotypes, which include the two most frequent haplotypes in the
25 population, this distinction is statistically significant ($p_{corr} < 0.01$). Two of these haplotypes
26 contain *B*46:01* and one contains *B*58:01* (Figure 2C). In total, four of five of the *HLA-B*

1 alleles that encode a KIR ligand and are present on these 10 most frequent haplotypes show
2 increased evidence for admixture in the *MHC* region. By contrast, neither of the two *HLA-A*
3 alleles that encode a KIR ligand show a genetic ancestry within the *MHC* that differed from
4 the East Asian ancestry throughout chromosome 6. This finding suggests that the number of
5 *HLA-B* genes encoding KIR ligands was enhanced in Chinese Southern Han by admixture with
6 neighboring or displaced populations. In summary, these findings clearly show that the
7 *B*46:01* and *B*58:01* alleles are present in Chinese Southern Han through admixture.

8

9 ***Positive selection favors HLA haplotypes expressing more than one KIR ligand in Chinese***
10 ***Southern Han***

11 To investigate whether or not the admixed haplotypes were also subject to natural selection we
12 examined further characteristics of their diversity and distribution. We first measured
13 nucleotide diversity (π) of the genomic sequence flanking +/- 500kb of specific *HLA-B* alleles
14 (Figure 3A). We found significantly reduced nucleotide diversity of haplotypes containing
15 *HLA-B*46* compared to haplotypes containing *HLA-B*40* (mean π of *B*40* = 2.2×10^{-3} , *B*46*
16 = 0.6×10^{-3} , Wilcoxon test, $p = 1.24 \times 10^{-12}$). We also observed that haplotypes containing
17 *B*58* have lower diversity than *B*40*, but this reduction was not statistically significant (mean
18 π of *B*58* = 1.6×10^{-3} , Wilcoxon test, $p = 0.12$). This reduced diversity suggests that *B*46*
19 haplotypes have arisen in frequency in the Chinese Southern Han without accumulating
20 mutations. To further explore this finding, we used the iHS statistic, which identifies genomic
21 variants that have increased in frequency recently and rapidly under natural selection, so that
22 their haplotypic background has not yet been diversified by recombination (Voight et al. 2006).
23 We identified a strong signal of recent selection (iHS $\geq 99^{\text{th}}$ percentile) that falls precisely in
24 the *MHC* of the Chinese Southern Han (Figure 3B).

25

1 To investigate the patterns of selection specific to *HLA-B*46:01* and *B*58:01* haplotypes we
2 first identified SNPs that characterize those haplotypes and then compared their distribution of
3 iHS values to the distribution of all the SNPs of chromosome 6 (Figure 3C). For *B*46:01* the
4 mean absolute iHS of 3.38, was significantly higher than the mean for chromosome 6 of 0.785
5 (Wilcoxon two-sample test, $p= 5.77^{-6}$), as was the mean iHS for *B*58:01* (3.43, $p= 1.8^{-3}$).
6 Although the signal for *B*58:01* is weaker, there is a more distinct subset of SNPs having
7 extremely high iHS values ($>99^{\text{th}}$ percentile, Figure 3C), which could indicate recent selection
8 of an older haplotype, although it was not possible from our analysis to determine if the SNP
9 allele was ancestral or derived in each case. Interestingly, the mean iHS for *B*40:01* associated
10 SNPs was also significantly higher than the chromosome average (1.88, $p= 7.7^{-6}$). However,
11 fewer *B*40:01* specific SNPs had an iHS value in the 95th percentile than *B*58:01* or *B*46:01*
12 specific SNPs (30%, 50%, and 100% of SNPs respectively). Because the most frequent
13 *B*40:01* containing haplotypes in the Chinese Southern Han carry either *A*11:01* or *A*24:02*,
14 which are KIR ligands (Figure 1B), we extended the analysis to these alleles (Figure 3C).
15 Again, this analysis showed both *A*11:01* (mean = 2.8, Wilcoxon two sample test $p= 1.45^{-11}$)
16 and *A*24:02* (mean = 1.37, $p= 1.27^{-9}$) associated SNPs have significantly higher iHS values
17 than the chromosome average, with *A*11:01* having a mean iHS that is above the 95th
18 percentile. Haplotypes carrying *HLA-A*11:01*, *A*24:02*, *B*46:01* or *B*58:01* were previously
19 identified to have unusually high LD in this population (Chen et al. 2016). This analysis
20 identified two other *HLA class I* alleles as having highly distinct signatures of directional
21 selection, *A*30* and *C*07*. Whereas *HLA-C*07* is known to interact strongly with KIR to
22 educate NK cells (Hilton et al. 2015a; Yawata et al. 2006), *HLA-A*30* does not possess a KIR
23 ligand. Together, these findings thus illustrate that *HLA class I* in the Chinese Southern Han
24 has been targeted by natural selection and suggest that one major benefit has been to increase
25 the number of KIR ligands present in the population.

26

1 We next examined whether the observed distributions of *HLA class I* encoded KIR ligands
2 were consistent with modern human population dispersal. Cluster analysis shows there are five
3 groups of *HLA class I* frequency spectra that correspond to the broad population groups of
4 African, European, Asian, Oceanian and American origin (Figure 3D). By contrast, three
5 distinct and strongly supported groups cluster according to their proportions of haplotypes
6 encoding one, two or three KIR ligands (Figure 3E). Notable examples are the Ryukyu
7 Japanese and Indigenous Australian populations, who group with Asian populations when
8 analyzed by *HLA class I* haplotype distribution (Figure 3D). By contrast, Ryukyu Japanese and
9 Australians appear more similar to Africans and other groups when analyzed according to the
10 number of KIR ligands encoded by their *HLA class I* haplotypes (Figure 3E). In a counter
11 example, Northern Indian and Tuvan populations (Figure S4C) cluster with Europeans when
12 analyzed by their *HLA class I* alleles, but with East Asians when analyzed by the number of
13 KIR ligands. Thus, *HLA class I* allele frequency distributions are consistent with the origins of
14 the populations studied and with the pattern of human dispersal out of Africa (Henn et al. 2012),
15 whereas the number of KIR ligands encoded by *HLA class I* haplotypes is not. This finding
16 suggests that the similar number of KIR ligands observed across populations is likely due to
17 convergent evolution, because distinct *HLA class I* haplotypes produce similar distributions of
18 KIR ligands. The findings thus also support our assessment that the unusual distribution of KIR
19 ligands in East Asian populations is due to natural selection.

20
21 In summary, these results show that similar quantities of KIR ligands can be obtained using
22 different subsets of *HLA class I* haplotypes, indicating there is pressure to maintain a certain
23 balance of KIR ligands across populations, regardless of the background HLA allotype, and
24 that this balance is perturbed in East Asia. Our observations show that successive rounds of
25 admixture followed by natural selection favouring specific *HLA class I* haplotypes have
26 increased the quantity of KIR/HLA interactions of populations in East Asia. To investigate the

1 characteristics of this receptor and ligand diversity, we next studied the *KIR* locus in the
2 Chinese Southern Han.

3

4 ***High frequency of inhibitory KIR allotypes in Southern Han***

5 The *KIR* locus comprises genes encoding the four inhibitory and six activating KIR known to
6 bind polymorphic HLA class I ligands, and three that do not bind polymorphic HLA class I
7 (Guethlein et al. 2015). In total, we identified 116 *KIR* alleles, representing 101 *KIR* allotypes
8 (Figure S5). A total of 46 novel *KIR* alleles (39.7% of total *KIR* alleles detected) were
9 characterized (Figure S6) and 24.8% of the individual Han carried at least one novel allele.

10 Correcting for the number of individuals tested showed that the Southern Han are more diverse
11 than Amerindians and Oceanians, but less diverse than Europeans and Africans (Figure 4A).

12 *KIR* diversity of the Chinese Southern Han is thus consistent with genome-wide diversity when
13 compared to other populations (Campbell and Tishkoff 2008). The Chinese Southern Han have
14 70 centromeric and 91 telomeric *KIR* haplotype motifs that combine to form a minimum of 199
15 *KIR* haplotypes (Figure S7A-C). The majority are *KIR A* haplotypes (74.7%, Figure 4B),
16 including 8 of the 10 most frequent haplotypes (Figure 4C). This skewing towards *KIR A*
17 haplotypes is more pronounced in the centromeric region (87.9%) than the telomeric (79.7%)
18 region (Figure 4C, Figure S7A-B).

19

20 *KIR A* haplotypes encode all four inhibitory receptors that bind HLA class I ligands and either
21 one or no activating receptors (Wilson et al. 2000). Accordingly, among the *KIR* alleles
22 identified in the Chinese Southern Han, we observed high frequencies of those encoding strong
23 inhibitory receptors. Both KIR2DL1*003, a strongly-inhibiting allotype of KIR2DL1 (Bari et
24 al. 2009; Hilton et al. 2015a), and KIR2DL3*001, a strongly-inhibiting allotype of KIR2DL2/3
25 (Yawata et al. 2006), are common in the Chinese Southern Han, having frequencies of 73.5%
26 and 71.1% respectively (Figure S5). Also frequent are KIR3DL1*015, which is a strong

1 inhibitor on binding to the Bw4 ligand (Yawata et al. 2006), and KIR3DL2*002 that has high
2 expression but unknown functional properties (Figure S5). Noticeably scarce are inhibitory
3 KIR allotypes having mutations that prevent cell surface expression, of which there are many
4 examples (Bari et al. 2009; Hilton et al. 2015b; Pando et al. 2003; VandenBussche et al. 2006).
5 For instance, weakly-expressed KIR3DL1*004 is frequent in many populations (Norman et al.
6 2007), but absent from the Chinese Southern Han (Figure S5, and ref (Tao et al. 2014)). Also
7 rare in the Chinese Southern Han are alleles encoding inhibitory allotypes of reduced function,
8 such as KIR2DL1*004 (3.6%, Figure S5), which is common in other population groups (Bari
9 et al. 2009; Meenagh et al. 2008; Nemat-Gorgani et al. 2014; Norman et al. 2013; Vierra-Green
10 et al. 2012). Moreover, the frequencies of alleles encoding activating receptors are much lower
11 (4.4%-18%) than those encoding inhibitory receptors (91.5%-100%), an effect compounded
12 by presence of multiple non-functional activating KIR allotypes (Figure S5). Exceptional is
13 KIR2DS4, for which the frequencies of functional and non-functional allotypes are balanced
14 (55%:45%, Figure S5). These observations point to a strong requirement in the Southern Han
15 population for retaining high numbers of functional inhibitory KIR, but not activating KIR.
16

17 ***Directional selection reduced centromeric KIR region diversity in the Southern Han***

18 In Chinese Southern Han, the *KIR3DL1/S1* and *KIR3DL2* genes encoding inhibitory NK cell
19 receptors specific for polymorphic HLA class I ligands, have two or three high frequency
20 alleles and multiple less frequent alleles (Figure S5). In contrast, *KIR2DL1* and *2DL2/3* also
21 encode inhibitory receptors but are each dominated by one high frequency allele (Figure 4D).
22 To explore this observation, we compared the observed homozygosity to the expected across
23 populations representing major ancestry groups from Europe, Africa, Asia, South America, and
24 Oceania, using the Ewens-Watterson test (Fnd). *KIR2DL1* and *KIR2DL2/3* are in the
25 centromeric region of the *KIR* locus, whereas *KIR3DL1/S1* and *KIR3DL2* are telomeric *KIR*
26 genes (Wilson et al. 2000). Overall the Southern Han show greater homozygosity compared to

1 other populations, which is more pronounced among centromeric than telomeric *KIR* genes and
2 statistically significant for *KIR2DL2/3* (Fnd = 3.1, P > 0.985, [Figure 4E](#)). This high-resolution
3 analysis of *KIR* alleles, complements recent analysis of genome-wide SNP data that identified
4 directional selection specifically in East Asian centromeric *KIR* (Augusto et al. 2019). The only
5 other population exhibiting directional selection in the centromeric *KIR* region is the Hondo
6 Japanese (Yawata et al. 2006). Thus, the profile observed for East Asian populations is distinct
7 from other populations. Together, these analyses suggest that directional selection reduced
8 sequence diversity of the centromeric *KIR* in the Southern Han, whereas the telomeric *KIR*
9 region retains some diversity. In addition, we observed a minimum of eleven different *KIR*
10 haplotypes having a duplication in the telomeric region ([Figure S7D](#)). The telomeric *KIR* have
11 greater allelic diversity than centromeric *KIR* in Chinese Han ([Figure S7D](#)), and these
12 duplication haplotypes have potential to further diversify the NK cell repertoire because both
13 allotypes of each gene are expressed (Beziat et al. 2013; Norman et al. 2009). We conclude
14 that the centromeric *KIR* region provides consistency to Chinese Southern Han NK cell
15 receptors, whereas the telomeric *KIR* region provides NK cell receptor diversity.

16

17 *Interactions of KIR with HLA class I*

18 NK cell function is modulated by interactions between KIR and their cognate ligands, HLA
19 class I molecules. While all HLA-C molecules are always ligands for KIR, only a sub-set of
20 HLA-A and -B molecules function as KIR ligands. We examined the impact of genetic
21 variation on the diversity and quantity of KIR/HLA class I interactions in the Chinese Southern
22 Han. Individuals have a mean of 6.7 different pairs of interacting KIR and HLA class I ligands.
23 These form a normal distribution in which individuals have from one to twelve interactions
24 (Shapiro -Wilk test, p = 0.147, [Figure 5A](#)). Such normal distributions are seen in other
25 populations (Nemat-Gorgani et al. 2014; Norman et al. 2013). To investigate the distinct HLA-
26 A and -B ligand distribution of the Southern Han we divided this analysis into its major

1 components of KIR interactions with HLA-C, and of KIR interactions with HLA-A and -B
2 (Figure S2). In analyzing only the interactions with HLA-C, we find that functional diversity,
3 as measured by the mean number of different receptor/ligand combinations per individual, is
4 consistent with the overall genetic diversity of the populations studied. At the low end of the
5 range are the Yucpa Amerindians with two different receptor/ligand interactions per individual.
6 At the high end are the Southern African Nama with 4.5 different interactions (Figure 5B).

7

8 When the total number of interacting pairs of inhibitory KIR and HLA-C ligands is analyzed,
9 the ranking remains the same but the difference across populations is reduced, ranging from
10 3.6 to 4.9 viable inhibitory KIR/HLA-C interactions per individual (Figure 5B). On this scale,
11 the Chinese Southern Han are seen to have relatively low diversity and a similar number of
12 interactions between inhibitory KIR and HLA-C to other populations. In sharp contrast, the
13 Chinese Southern Han, together with the Hondo Japanese, have significantly higher number (t-
14 test, $p < 0.0001$) and diversity ($p < 0.001$), of inhibitory KIR interactions with HLA-A and -B
15 than any other population (Figure 5B). Thus, both the quantity and quality of interactions
16 between inhibitory KIR and HLA-A and -B are enhanced in Southern Han and Hondo
17 Japanese. We predict this will also be true for other East Asian populations.

1 **Discussion**

2 Our analysis shows that the geographic distribution of *HLA class I* alleles and haplotypes is
3 consistent with human dispersal out of Africa and the distance of their geographical location
4 from Africa. Despite the significant differences across human populations in the distributions
5 of *HLA class I* alleles (Meyer et al. 2007), the distribution of KIR ligands is very similar. For
6 *HLA-C*, all haplotypes encode one of two alternative KIR ligands, whereas for *HLA-A* and
7 *HLA-B* there is a balance between haplotypes that either encode one or two KIR ligands, and
8 haplotypes that encode no KIR ligand (Guethlein et al. 2015). Thus, a similar balance of KIR
9 ligands is independently maintained in different human populations using very different HLA-
10 A and -B allotypes. In sharp contrast, the Southern Han Chinese and other East Asian
11 populations do not follow these patterns. The high frequencies of the *HLA class I* haplotypes
12 shared by East Asian populations are an indication of their recent shared ancestry (Abdulla et
13 al. 2009). We find a greater abundance of HLA-A and -B KIR ligands in East Asians than other
14 populations, as well as a greater diversity of interactions between inhibitory KIR and HLA-A
15 and -B. That our comparison included Southern African KhoiSan, whose genetic diversity is
16 the highest among modern humans (Henn et al. 2011; Tishkoff et al. 2009), strongly suggests
17 the high frequency and diversity of KIR ligands in East Asia is the result of natural selection.

18

19 The frequency of East Asian *HLA class I* alleles that derive from ancient humans by
20 introgression was previously estimated to be 70-80% (Abi-Rached et al. 2011). The most
21 common of these alleles are *HLA-A*11* and *HLA-A*24*, which encode KIR ligands. We show
22 that more recently, *HLA-B*46:01* and *HLA-B*58:01*, which also encode KIR ligands, were
23 specifically enhanced in frequency in Southern Han following admixture with local
24 populations. HLA-B*46 is a good educator of NK cells (Yawata et al. 2006) and rose in
25 frequency in South East Asia under positive selection (Abi-Rached et al. 2010). The haplotype
26 that encodes HLA-B*58 likely arose in Northern Asia and although the signal is weaker, this

1 may have been selected both in the Northern and Southern Han. Consequently, *HLA-B*46:01*
2 and *HLA-B*58:01* are the most frequent *HLA-B* alleles encoding KIR ligands, and distinguish
3 the most frequent *HLA class I* haplotypes, in the Southern Han. Together with clear
4 demonstration of natural selection recently targeting the *MHC* region, these findings all support
5 the proposition that natural selection in East Asia favors *HLA class I* haplotypes carrying more
6 than one KIR ligand and suggests there were two major waves of adaptive introgression
7 involving these haplotypes. There is evidence for adaptive introgression of *HLA* alleles in other
8 modern human populations (Busby et al. 2017; Rishishwar et al. 2015). For example, Bantu
9 speakers from western central Africa expanded through new habitats and acquired *HLA*
10 haplotypes from rainforest hunter-gatherer pygmies (Patin et al. 2017). Our findings may fit
11 with recent work identifying a second wave of Denisovan-like admixture that is specific to East
12 Asian populations (Browning et al. 2018). Thus, although we show the *HLA-B*46:01* and -
13 *B*58:01* haplotypes were obtained by the Han from neighboring modern human populations,
14 they were likely to have been acquired by those populations as a consequence of admixture
15 with archaic humans.

16
17 Complementing the high number of HLA class I ligands, we find that in the Chinese Southern
18 Han the number of inhibitory KIR is increased relative to other groups. These KIR allotypes
19 are distinguished by their high expression, inhibitory strength and fine specificity for ligand
20 (Boudreau et al. 2017; Hilton et al. 2015a; Saunders et al. 2016). Possessing higher numbers
21 of inhibitory KIR leads to better effector function, and a higher number of inhibitory KIR
22 ligands leads to larger numbers of circulating NK cells, stronger killing and greater diversity
23 of the NK cell repertoire (Beziat et al. 2013; Brodin et al. 2009; Yawata et al. 2006). That the
24 number of receptors (Pelak et al. 2011) and ligands (Thons et al. 2017) correlates with infection
25 control, suggests the diverse NK cell repertoires of the Southern Han have likely evolved to
26 combat infectious diseases common or endemic to East Asia. Although it is difficult to identify

1 the specific pathogen exposure history of the Chinese Southern Han, the most plausible
2 candidates for causing selection pressure are viral infections that have established roles for
3 KIR/HLA interaction during host defense (Abi-Rached et al. 2010; Bashirova et al. 2006). Such
4 pathogens have been shown to be effective drivers of adaptive introgression and natural
5 selection in human populations (Enard and Petrov 2018; Harrison et al. 2019). One example is
6 nasopharyngeal carcinoma (NPC) caused by Epstein-Barr virus. HLA-A*11 offers protection
7 from NPC (Tang et al. 2012), and the interaction of KIR3DL2 with HLA-A*11 is dependent
8 on presentation of peptides derived from EBV (Hansasuta et al. 2004). Influenza is another key
9 candidate, with highly virulent epidemics linked to the combination of dense population,
10 agriculture and industrialization (Cao et al. 2009; Chen et al. 2006). Human specific viral
11 hepatitis infections and arboviruses are also endemic to China and South East Asia, including
12 Japanese encephalitis, Dengue and chikungunya (Bashirova et al. 2006; Khakoo et al. 2004;
13 Naiyer et al. 2017; Petitdemange et al. 2011; Thons et al. 2017; Townsley et al. 2016).
14 Consistent with these observations, *KIR A* has established roles in controlling virus infections
15 (Bashirova et al. 2006; Khakoo et al. 2004), and we recently showed *KIR A* homozygosity
16 protects from leukemia (Deng et al. 2019). Reproduction is also a major driver of selection,
17 where *KIR AA/C2⁺HLA-C* genotype is associated with preeclampsia (Parham and Moffett
18 2013). Thus, the low frequency of C2⁺HLA in East Asia (Figure S3) likely allows the *KIR A*
19 haplotype to reach high frequency (Nemat-Gorgani et al. 2018). High resolution analysis of
20 KIR and HLA diversity will be critical for understanding these and other complex diseases.
21
22 In conclusion, our high-resolution analysis of KIR and HLA class I combinatorial diversity has
23 uncovered a distinctive enhancement of the interactions between inhibitory KIR and HLA-A
24 and -B in East Asians. These genetically determined distinctions likely underlie differences
25 across human populations in their susceptibility to infections and immune-mediated diseases.
26

1 **Supplemental Data**

2 Supplemental Data include **four figures** and **three Excel** spreadsheets.

3

4 **Acknowledgements**

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10

11 **Web Resources and Accession Numbers**

12 The URLs for data, material and programs used herein are as follows:

13 The scripts used in the study are located at https://github.com/n0rmski/Han_Study/
14 ImmunoPolymorphism database (IPD), <http://www.ebi.ac.uk/ipd/>
15 International Histocompatibility Working Group (IHWG), www.ihwg.org/

16

17 The official IPD names (Robinson et al. 2015) and GenBank accession numbers for the *KIR*
18 sequences reported in this paper are:

19 (*KIR* prefix excluded for brevity) *2DL1*00304* (KT438851), *2DL1*00305* (KT438852),
20 *2DL1*030* (KP025959), *2DL1*031* (KP025960), *2DL1*033* (KT438853), *2DL1*034*
21 (KT438854), *2DL2*013* (KM017076), *2DL3*00109* (KF766495), *2DL3*00110* (KF766497),
22 *2DL3*025* (KF766496), *2DL3*026* (KF766498), *2DL3*027* (KF766499), *2DL3*028*
23 (KF766500), *2DL3*029* (KF766501), *2DL3*031* (KF849247), *2DL4*00503* (KT438855),
24 *2DL4*00504* (KT438856), *2DL4*032* (KT438858), *2DL4*033* (KT438859), *2DL4*034*
25 (KT438857), *2DL5A*022* (KT438863), *2DS2*009* (KT438862), *2DS4*00105* (KP025962),
26 *2DS4*017* (KP025961), *2DS4*018* (KP025963), *3DL1*01505* (KF849249), *3DL1*079*

1 (KF849250), *3DL2*00706* (KT899864), *3DL2*00707* (KT899868), *3DL2*083* (KT899867),
2 *3DL2*084* (KT438861), *3DL2*091* (KT438860), *3DL2*093* (KT899866), *3DL2*099*
3 (KT899865), *3DL3*01003* (KU529275), *3DL3*02602* (KU529271), *3DL3*04802*
4 (KU529269), *3DL3*062* (KU529272), *3DL3*063* (KU529270), *3DL3*064* (KU529273),
5 *3DL3*065* (KU529274), *3DSI*078* (KJ001806), *3DSI*082* (KJ001804), *3DSI*083*
6 (KJ001805), *3DSI*084* (KJ001807), *3DSI*085* (KJ365317).

1 **Figure Legends**

2 **Figure 1. Chinese Southern Han *HLA class I* haplotypes express multiple KIR ligands**

3 A. Pie charts show the frequency spectra for HLA-A, -B and -C allotypes of the Southern Han
4 cohort of 306 unrelated individuals (2N=612). Each pie segment represents one allotype.
5 Alternative sequence motifs in the $\alpha 1$ domain of the HLA class I molecule determine the four
6 epitopes recognized by different KIR, and which are also called KIR ligands. The A3/11
7 epitope is carried by HLA-A3 and -A11 (yellow colored pie segments); the Bw4 epitope is
8 carried by subsets of HLA-A and -B allotypes (green colored pie segments). The C1 epitope is
9 carried by a majority of HLA-C allotypes, as well as by HLA-B*46 and HLA-B*73 (red
10 colored pie segments). The C2 epitope is carried by all HLA-C allotypes that do not carry C1
11 (blue-colored pie segments). Grey-colored pie segments correspond to allotypes that are not
12 KIR ligands. Figure S2 lists all the HLA-A, -B and -C allotypes present in the study population
13 and shows which KIR ligand motifs they carry.

14

15 B. Shows the ten most frequent *HLA class I* haplotypes in the Southern Han and their
16 frequencies (2N=612). Colored shading indicates *HLA class I* alleles that encode KIR ligands,
17 as described in panel A.

18

19 C. (left) Bars show the combined frequencies of *HLA class I* haplotypes encoding one (blue),
20 two (gold) or three (green) KIR ligands in eight representative populations worldwide
21 (Southern, Western and Eastern Africa, Europe, Oceania, South America, Japan and Chinese
22 Southern Han). (Right) Heat-plot shows pairwise comparisons between populations of the
23 proportion of *HLA class I* haplotypes encoding one KIR ligand to those carrying two or more
24 KIR ligands. Colors correspond to $-\log^{10}$ of a Benjamini-Hochberg corrected p, as shown in the
25 key.

26

1 D. Heat-plot shows pairwise comparisons of Chinese Southern Han with five East/ South East
2 Asian populations of the proportion of *HLA* haplotypes encoding one KIR ligand to those
3 carrying two or more KIR ligands. Colors correspond to $-\log_{10}$ of a Benjamini-Hochberg
4 corrected p, as shown in the key.

5
6 E. Venn diagrams show the distribution of *HLA class I* haplotypes within representative subsets
7 of populations. The number of haplotypes in each overlapping region is given. The % values
8 indicate the combined frequency of haplotypes unique to a population when compared to the
9 other populations in the diagram.

10
11 **Figure 2. HLA-B*46:01 and -B*58:01 were acquired by admixture into the Chinese
12 Southern Han**

13 A. Shown are the relative proportions of genetic ancestry among Asian populations from the
14 1000 genomes project, plotted by considering three ancestral groups (K = 3: Japanese (green),
15 East Asian (red) and South East Asian (blue)).

16
17 B. Shown are the relative proportions for each of the three genetic ancestries in chromosome 6
18 (left) and within the *MHC* (right) for selected Chinese Southern Han individuals, shown from
19 left to right; all individuals, *B*40:01* carriers, *B*46:01* carriers, *B*58:01* carriers.

20
21 C. Shown for each of the ten most frequent *HLA class I* haplotypes in the Chinese Southern
22 Han is a comparison of mean admixture proportion of the genetic ancestry group that is most
23 abundant in *MHC* compared to the proportion of that ancestry in chromosome 6 with *MHC*
24 excluded. The size of the circle represents the $-\log_{10}$ (Benjamini-Hochberg corrected p) from a
25 Wilcoxon test. The difference in size and shade between the two circles corresponds to extent

1 and direction, respectively, of any shift in the genetic ancestry proportions between the *MHC*
2 and the remainder of the chromosome 6.

3

4 **Figure 3. Positive selection has targeted *HLA class I* genes in the Chinese Southern Han**

5 A. Shown is the nucleotide diversity (π) of genomic sequence +/- 500kbp of the *HLA-B* and -
6 *C* genes for haplotypes containing the specific *HLA-B* alleles, *B**40, *B**46 and *B**58. π was
7 measured in windows of 100bp. *** p <0.001 by Wilcoxon test.

8

9 B. Manhattan plot shows the absolute iHS values above the 95th percentile calculated for
10 independent SNPs throughout chromosome 6 in Chinese Southern Han. The *MHC* region is
11 boxed.

12

13 C. Density plots show the distribution of absolute iHS values for chromosome 6 (top left, grey
14 shading), and for SNPs unique to haplotypes carrying specific *HLA-B* (left, cyan), *HLA-A*
15 (center, purple) and *HLA-C* (right, orange) alleles, as indicated in each plot. The number of
16 SNPs unique to each of the *HLA* alleles is shown in brackets next to the allele name. For each
17 allele, the distribution of iHS values was compared to the distribution on chromosome 6 using
18 a Wilcoxon two-sample test (* p < 0.05, ** < 0.05⁻⁵, *** < 0.05⁻¹⁰). Grey dashed line marks the
19 95th percentile of iHS values for chromosome 6 SNPs (=1.93). The density shown is the kernel
20 density estimate of the SNP counts associated with the distribution of absolute iHS values.

21

22 D. Shows cluster analysis of *HLA class I* allele frequencies from 26 populations. Vertical lines
23 at the left show the clusters identified when five groups were specified in the input parameters
24 (k=5) and the support (%) from 1,000 bootstrap replicates. Population names in green text
25 indicate sub-Saharan African populations, red text – East Asian, brown text – Northeast Asian
26 (Tuva) and South Asian (Indian), blue text – Oceanic, purple text – Amerindian (Yupik are

1 North Amerindian who back-migrated to Siberia (Raghavan et al. 2015), NaDene are North
2 American). J. Hondo are Japanese from the major islands of Japan, J. Ryukyu are Japanese
3 from Okinawa. The *HLA class I* haplotypes detected in each population are described in [Figure](#)
4 [S4D](#).

5

6 E. Shows cluster analysis of the combined frequencies of *HLA class I* haplotypes carrying 1, 2
7 or 3 KIR ligands. Vertical lines at the left show the clusters identified when three groups were
8 specified (k=3) and the support (%) from 1,000 bootstrap replicates.

9

10 **Figure 4. Directional selection on *centromeric KIR* in Southern Han**

11 A. Shows the number of *KIR* alleles present in the Southern Han compared with other
12 populations analyzed at comparable resolution; 75 individuals were selected at random from
13 each population.

14

15 B. Shown are the combined frequencies of *KIR A* (red) and *KIR B* (blue) haplotypes, for the
16 complete haplotypes, and for the *centromeric* and *telomeric* regions.

17

18 C. Shown are the ten most frequent complete, high-resolution, *KIR* haplotypes identified in the
19 Chinese Southern Han population. *KIR A* haplotypes are shaded in red, *KIR B* haplotypes are
20 shaded in blue. At the right is shown for each haplotype the number of individuals carrying the
21 haplotype, and its frequency. All the haplotypes are shown in [Figure S7](#).

22

23 D. Bar graph shows a summary *KIR* allele frequencies. The colors from blue to red correspond
24 to the rank in frequency from highest (blue) to lowest (red). Full frequency distributions are
25 shown in [Figure S5](#).

26

1 E. Shown are normalized deviate values of Ewens-Watterson's F test (F_{nd}) in representative
2 global populations. Positive values of F_{nd} indicate homozygosity, negative values indicate
3 heterozygosity. An asterisk denotes significance ($P < 0.05$ or > 0.95) using the exact test
4 (Salamon et al. 1999).

5

6 **Figure 5. East Asians have a greater diversity of KIR interactions with HLA-A and -B**
7 **than other populations.**

8 A. Plot of the number of different interacting ligand/receptor allotype pairs observed per
9 individual in the Southern Han.

10

11 B. Shows the mean number of different ligand/receptor allotype pairs per individual (left) and
12 the mean total number of ligand/receptor allotype pairs per individual (right) for HLA-C
13 (upper) and HLA-A and -B combined (lower). In the populations shown, *KIR* and *HLA class I*
14 have been analyzed to similar high-resolution as described here for Southern Han. These
15 populations comprise the Yucpa (Gendzehadze et al. 2009), Japanese (Yawata et al. 2006),
16 Māori (Nemat-Gorgani et al. 2014), European (Vierra-Green et al. 2012), Ghanaian (Norman
17 et al. 2013) Nama (Nemat-Gorgani et al. 2018). Error bars are s.e.m. and p values are from a t-
18 test.

19

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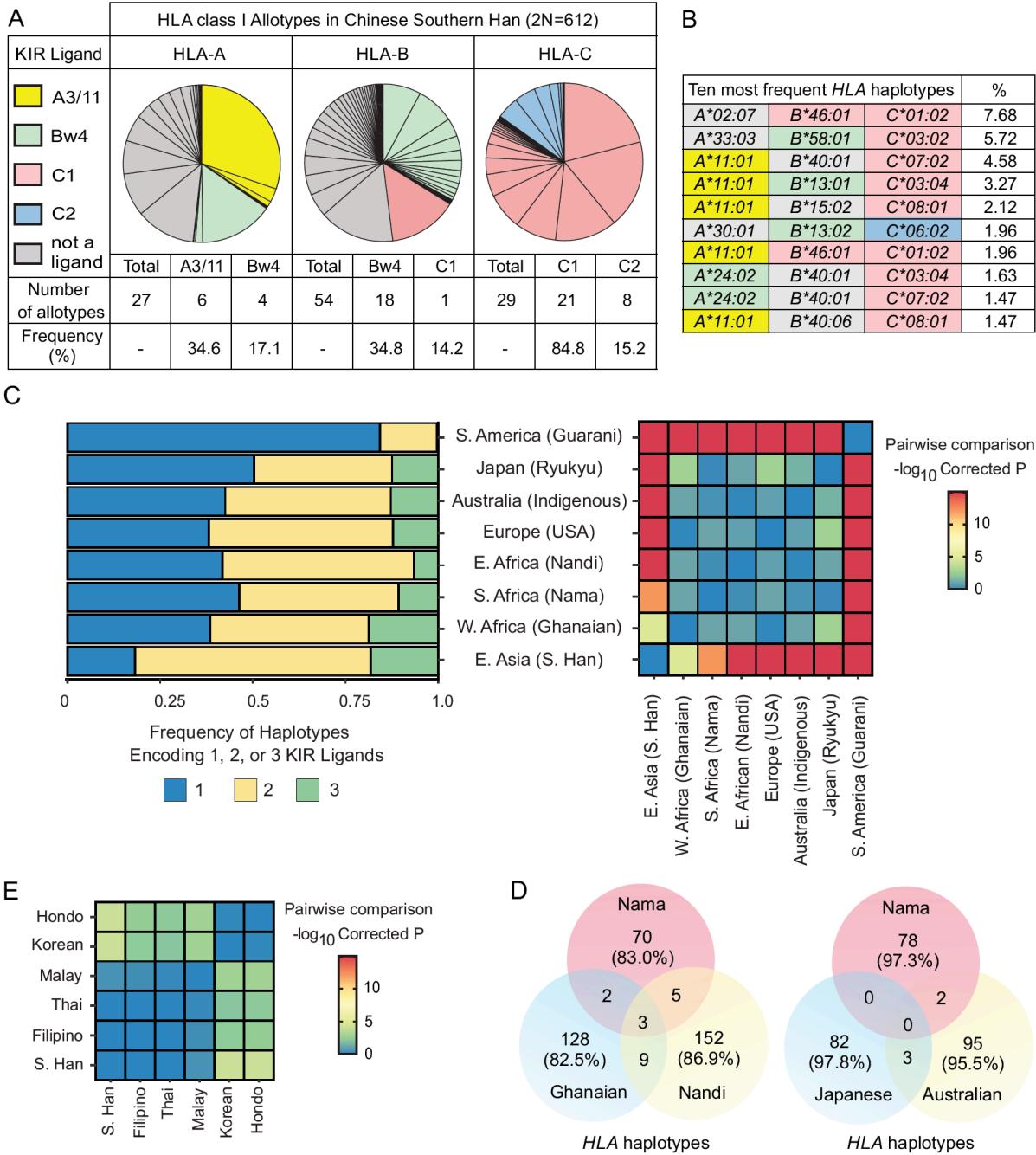
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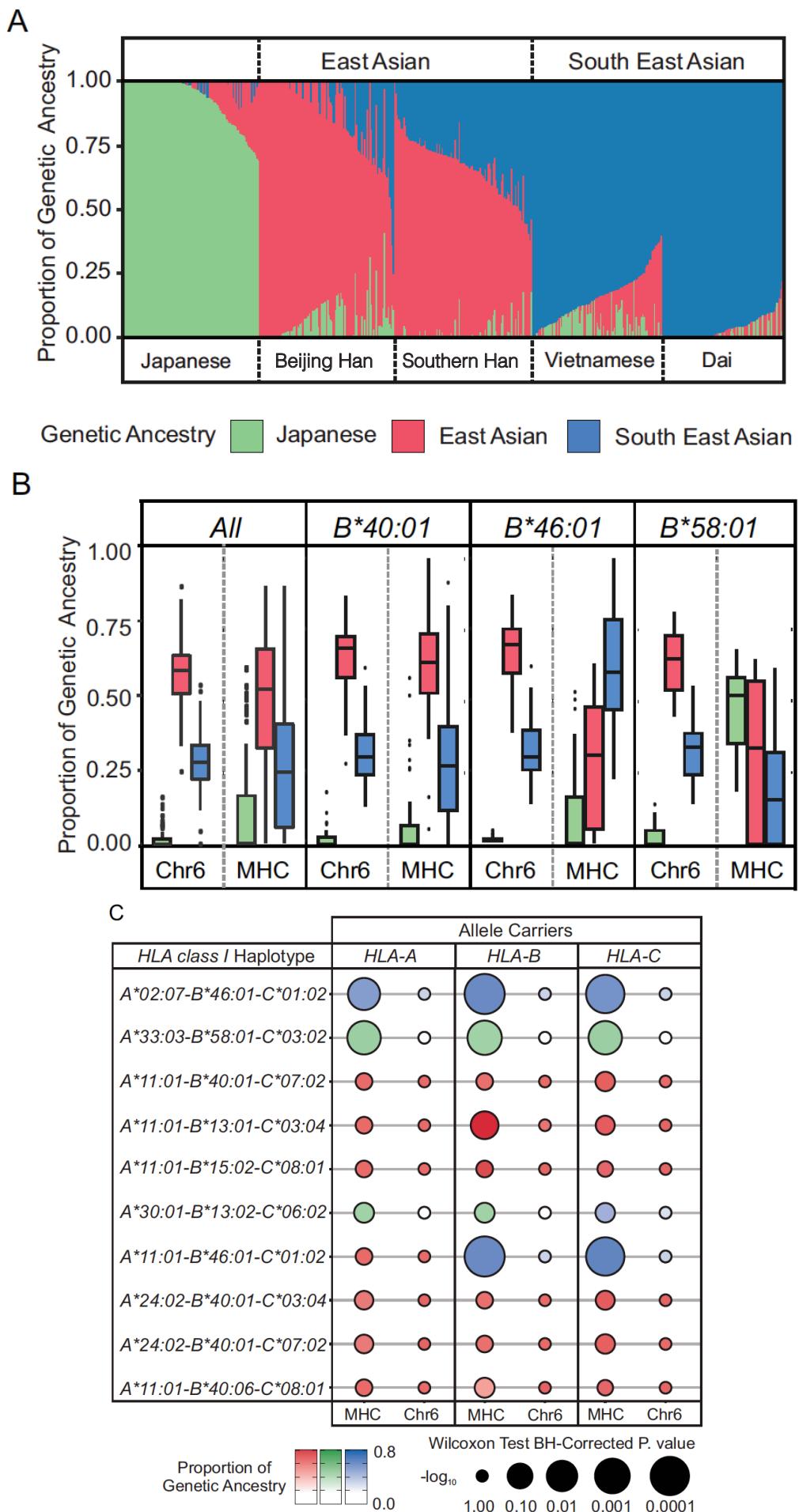
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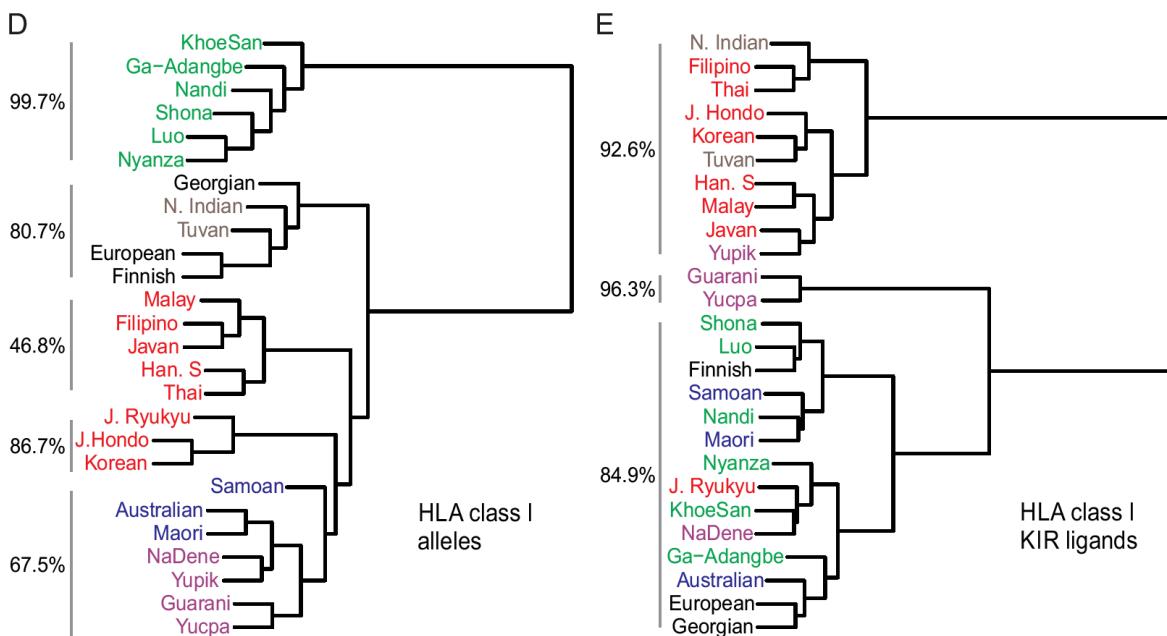
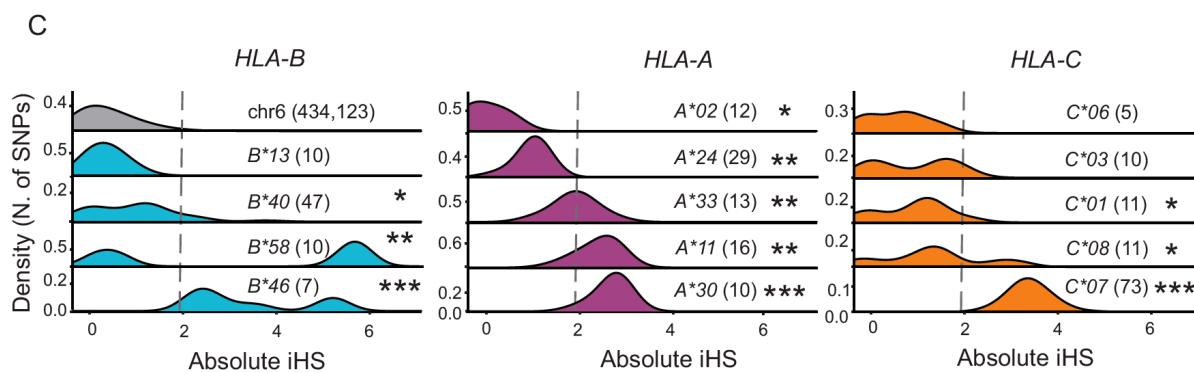
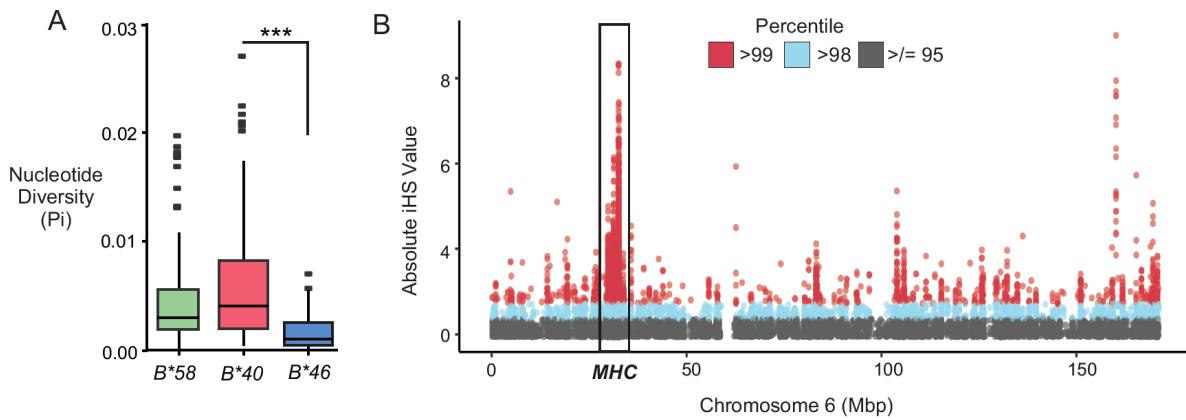
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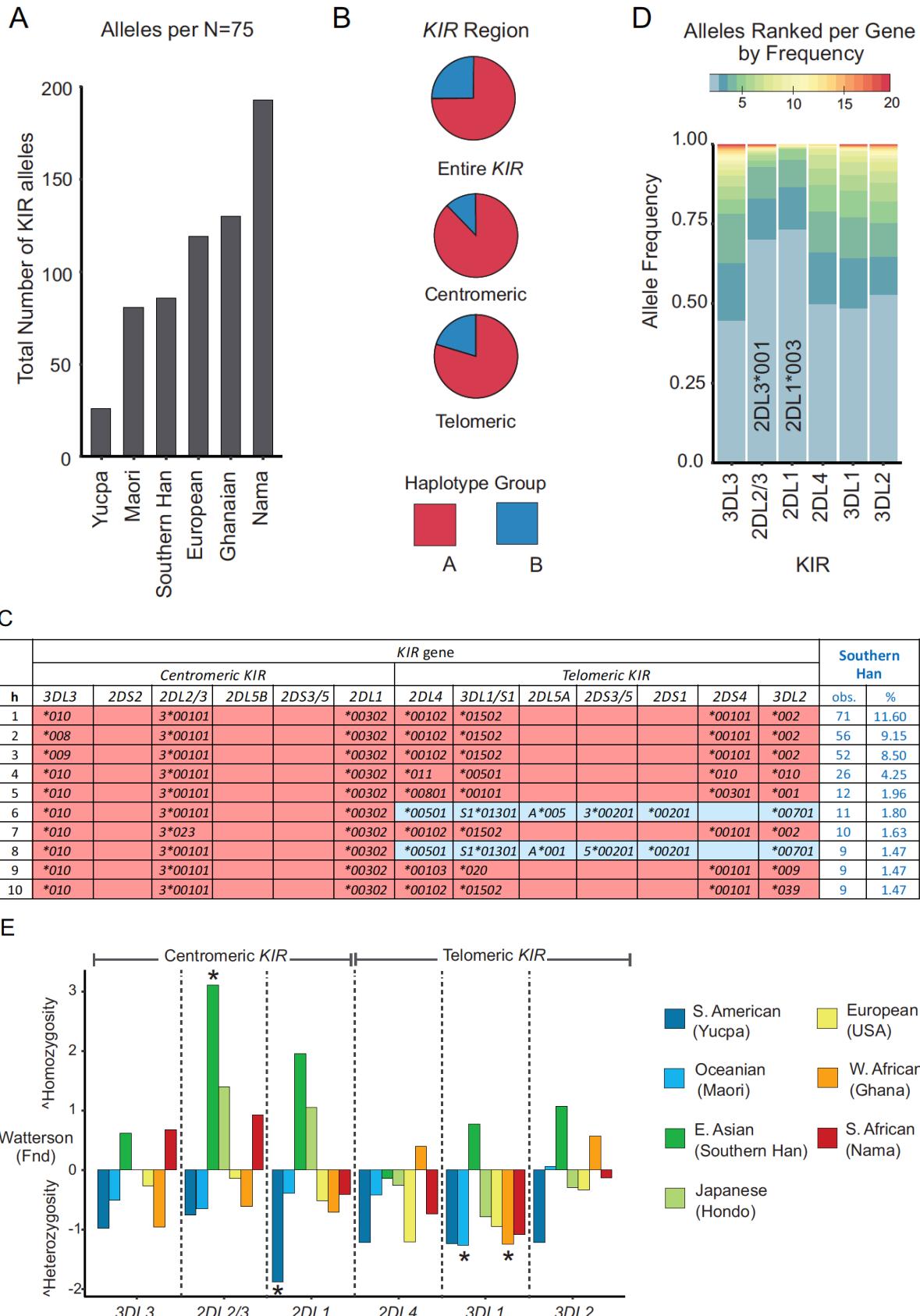
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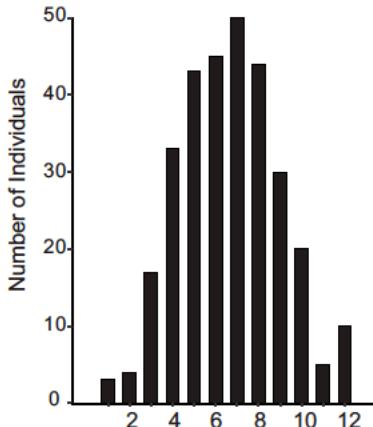




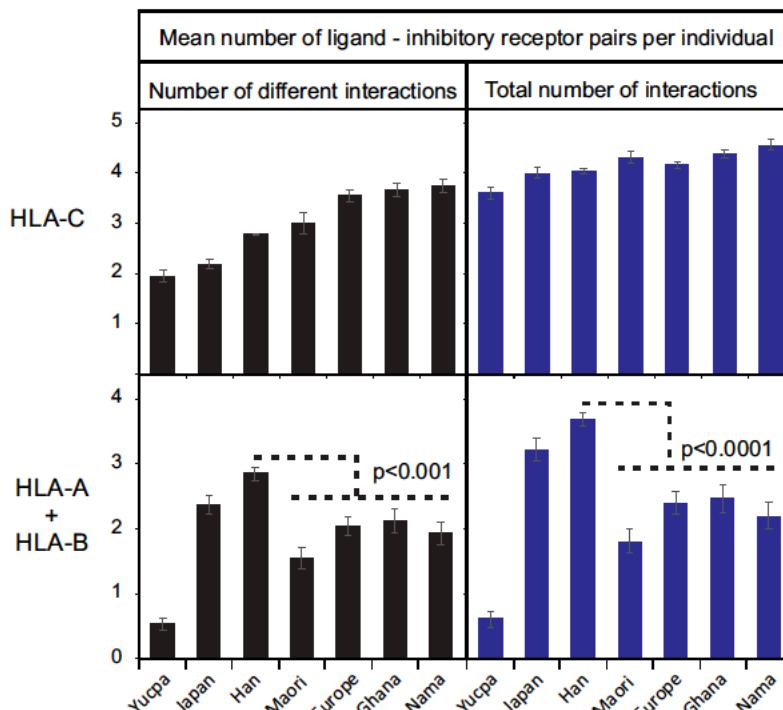


A

Number of Distinct KIR+HLA class I
Interactions per Individual



B



1