PJ Norman et al. 2008 Supplemental Material

Supplemental Figure S1

3DL1/2v +

donor	origin	genotype *3DL1/S1*	*3DL1/S1*	*3DL2*	*3DL2*
1	African American	*001	*059	*001	-
2	African American	*033	*059	*008	-
3	African American	*020	*060	*009	-
4	African American	*01502	*059	*001	-
5	African American	*01501	*059	*013	-
6	African American	*002	*059	*002	-
7	African American	*031	*059	*001	-
8	African American	3DS1*01301	*059	*007	-
9	African American	*040	*061	*010	-
10	African American	*01501	*059	*013	-
11	Ghana	*01502	*060	*002	-
12	Ghana	*00401	*059	*003	-
13	Ghana	*028	*060	*001	-
14	Ghana	*01501	*060	*013	-
15	Ghana	*01501	*059	*013	-
16	Ghana	*01501	*059	*013	-
17	Ghana	*01502	*059	*001	-
18	Ghana	*031	*060	*001	-
19	Ghana	*01502	*059	*002	-
20	Ghana	*00401	*059	*001	-
21	Ghana	*001	*059	*001	-
22	Ghana	*01701	*060	*010	-
23	Ghana	*022	*059	*001	-
24	Ghana	*022	*060	*001	-
25	Ghana	*001	*059	*001	-
26	Ghana	*01701	*059	*010	-
27	Ghana	*007	*059	*008	-
28	Ghana	*01501	*059	*013	-
29	Ghana	*031	*059	*001	-
30	Ghana	*01502	*059	*001	-
31	Nigeria	*01501	*060	*013	-
32	Nigeria	*00401	*059	*003	-
33	Nigeria	*028	*059	*001	-
34	Nigeria	*031	*059	*001	-
35	Nigeria	*01501	*060	*013	-
36	Nigeria	*001	*059	*001	-
37	Nigeria	*00401	*059	*003	-
38	Nigeria	*01501	*059	*013	-
39	Nigeria	-	*059	*006	-
40	Nigeria	*020	*059	*001	-
41	Nigeria	*033	*059	*008	-
42	Nigeria	*022	*059	*008	-
43	Nigeria	*01502	*059	*001	-
44	Nigeria	*01502	*059	*001	-
45	Nigeria	*01501	*060	*013	-
46	Nigeria	*035	*060	*001	-
47	Tanzania	*040	*061	*002	-
48	Tanzania	*028	*059	*001	-
49	Tanzania	*020	*061	*001	-
50	Tanzania	3DS1*01301	*059	*007	-
51	Tanzania	*001	*059	*001	-
52	Tanzania	*01501	*059	*013	-
53	Tanzania	*00401	*059	*003	-
54	Tanzania	*006	*059	*001	-
55	Zimbabwe	*01501	*059	*013	-
56	Zimbabwe	*01501	*059	*013	-
57	Zimbabwe	*031	*059	*001	-
58	Zimbabwe	*00501	*059	*010	-
59	Zimbabwe	*00401	*059	*003	-
60	Zimbabwe	*01501	*059	*013	-
61	Zimbabwe	*001	*059	*003	-
62	Zimbabwe	*00401	*059	*013	-
63	Zimbabwe	*01501	*059	*019	-
64	Zimbabwe	*00501	*059	*010	-
65	Zimbabwe	3DS1*01301	*059	*007	-

3DL1/2v -

donor	origin	genotype *3DL1/S1*	*3DL1/S1*	*3DL2*	*3DL2*
1	African American	*01502	*00501	*002	*010
2	African American	*00401	*017	*003	*010
3	African American	*00501	*019	*001	-
4	African American	*01502	*00501	*002	*003
5	African American	*01502	*007	*002	*008
6	African American	*030	*008	*009	*013
7	African American	*00401	*031	*001	-
8	African American	*007	*008	*008	*009
9	African American	*01701	*008	*009	*023
10	African American	3DS1*01301	-	*007	-
11	African American	*01501	3DS1*01301	*007	*024
12	Ghana	*01501	*022	*009	*013
13	Ghana	*01501	*01502	*013	*025
14	Ghana	*00401	*022	*001	*003
15	Ghana	*01501	-	*001	*009
16	Ghana	*01502	*00501	*001	*010
17	Ghana	*001	3DS1*01301	*001	*010
18	Ghana	*001	*01501	*001	*009
19	Ghana	*01701	*041	*002	*010
20	Ghana	*001	-	*001	-
21	Ghana	*040	-	*001	*002
22	Ghana	*01502	*040	*001	*002
23	Ghana	*01501	*022	*001	*013
24	Ghana	*031	*028	*001	-
25	Ghana	*001	*041	*001	*032
26	Ghana	*00401	*01502	*003	*029
27	Ghana	*001	*035	*001	-
28	Ghana	*01501	*033	*008	*013
29	Nigeria	*041	3DS1*01301	*001	-
30	Nigeria	*00401	*01501	*011	*013
31	Nigeria	*01501	*028	*010	*013
32	Nigeria	*01502	-	*001	*002
33	Nigeria	*01501	*01502	*001	-
34	Nigeria	*01502	*007	*001	-
35	Nigeria	*01502	3DS1*01301	*013	*023
36	Nigeria	*022	*022	*001	-
37	Nigeria	*031	-	*001	*006
38	Nigeria	*031	3DS1*01301	*001	*007
39	Nigeria	*01501	*01502	*002	*013
40	Nigeria	*01501	*031	*001	*013
41	Nigeria	*00401	*033	*003	*008
42	Nigeria	*019	*00401	*001	*003
43	Nigeria	*001	3DS1*01301	*001	*006
44	Nigeria	*01501	*007	*008	*014
45	Nigeria	*01501	*007	*008	*014
46	Nigeria	*031	-	*001	*009
47	Nigeria	*01501	*01502	*013	*029
48	Nigeria	*01502	*020	*001	-
49	Tanzania	*00401	*01502	*001	*005
50	Tanzania	*001	*01501	*001	-
51	Tanzania	*01502	*031	*023	*001
52	Tanzania	*001	*00101	*001	-
53	Tanzania	*001	*01502	*001	*002
54	Tanzania	*00401	*01502	*002	*003
55	Tanzania	*00501	*017	*001	-
56	Tanzania	*00401	*033	*003	*008
57	Zimbabwe	*031	-	*001	*006
58	Zimbabwe	*041	*017	*009	*011
59	Zimbabwe	*041	*01501	*003	*026
60	Zimbabwe	*031	3DS1*01301	*001	*006
61	Zimbabwe	*01501	*033	*008	*010
62	Zimbabwe	*00401	*01501	*011	*013
63	Zimbabwe	*001	*027	*001	-
64	Zimbabwe	*001	*00401	*001	*003
65	Zimbabwe	3DS1*01301	-	*001	*010

Fig. S1. *KIR3DL1* and *3DL2* genotypes from individuals with and without *3DL1/2v*.

Shown are the *KIR3DL1* and *3DL2* genotypes obtained by pyrosequencing from 65 individuals with *3DL1/2v* (*3DL1* alleles **059-61*) and 65 individuals without *3DL1/2v* randomly selected from the same populations. The population of origin for each individual is shown at the left.

Supplemental Figure S2

A

Long Range PCR	Forward (5' - 3')	Reverse (5' - 3')	size (kb)
A *2DL4-3DL1i3**	ATGCTGAGCCCAGAGCGTT	ACAGTGAGAAGCCCAGACG	4.5
B *3DL1e1*-i5**	GAGTTTAAATCATTTGAACTGGTTCTG	GGAAGCTCCTTAGCTAAGGATT	5.9
C *3DL1e5*-i6*	CGTCACTCTCCCTACGAGTT	AACGTCTCAGAACAGCCTGT	6.9
D *3DL2i6-i7*	CACAGGAGGACAGGTGGTTT	AGCACCAGCGATAAAGGAAGA	3.8
E *3DL2i7*† *-i9*†	TTGTCCTAAGGAGATGTTCCA	GTGATTGCAGCCTCAAGTAGG	1.3
F *3DL2i8 -FCAR*	GACCCTCAGGAGGTGACG	ACAGGAGGGTGGTCTGTTTG	7.7

B

Band	Number of amplifications per donor	3DL1/2v donor genotype *3DL1 /S1* *3DL2*	Number of clones end sequenced and (fully sequenced)							
			C229		N158		C338		N118	
			allele 1	allele 2	allele 1	allele 2	allele 1	allele 2	allele 1	allele 2
			*059 -	*031 *001	*060 -	*035 *001	*061 -	*040 *010	*059 -	- *006
A	3		16 (3)	0	6 (3)	0	3 (3)	3 (3)	4 (4)	0
B	2		6 (3)	9 (3)	8 (3)	7 (3)	6 (6)	6 (3)	12 (4)	0
C	3		16 (3)	0	17 (3)	0	7 (3)	0	46 (8)	0
D	2		3 (3)	4 (4)	3 (3)	5 (5)	3 (3)	2 (2)	5 (5)	3 (3)
E	3		3 (3)	3 (3)	3 (3)	4 (4)	5 (5)	3 (3)	8 (5)	8 (3)
F	3		8 (3)	4 (2)	3 (3)	7 (3)	5 (5)	0	5 (5)	3 (3)
GenBank Accession			EU267269		EU267271		EU267270		FJ459734	

C

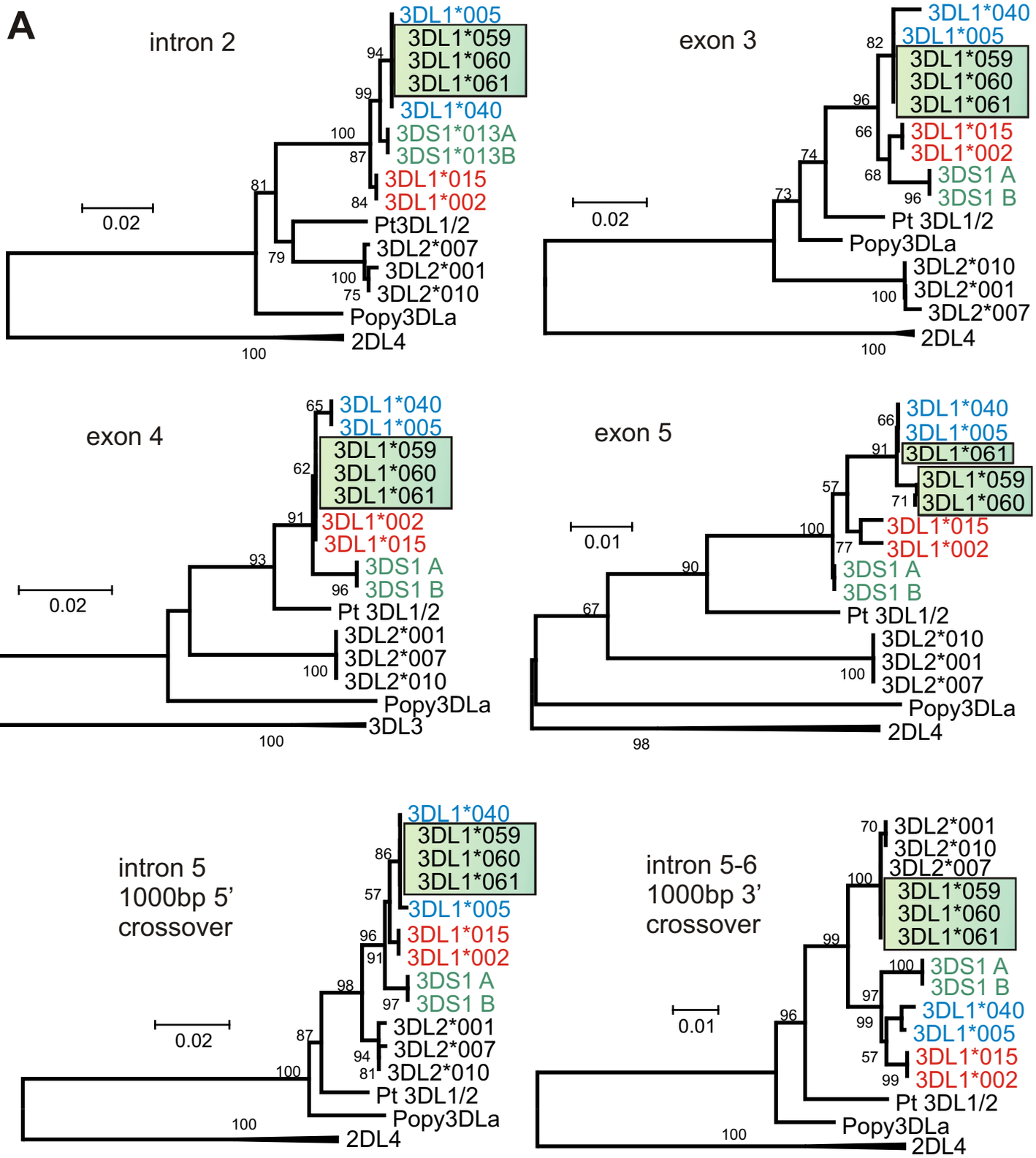
Fig. S2. Sequencing the *KIR3DL1/2v* haplotype

(A) Shown are PCR primers used to generate amplicons that overlap and span the interval from *2DL4* to *FCAR* on haplotypes that contain *3DL1/2v*. At the right are the amplicon sizes. This protocol was optimized in one donor from a series of shorter reactions, as shown in Supplemental Fig. S7, and then repeated using three further donors shown in panel (B). * denotes primers specific for *3DL1/S1* (Norman et al. 2007). † denotes primers specific for *3DL2* (Artavanis-Tsakonas et al. 2003).

(B) A *3DL1/2v* haplotype with identical structure was characterized from multiple amplifications and multiple donors. Summarized are data from four donors, representing three *3DL1/2v* alleles, from whom the *3DL1/2v* haplotype was amplified, cloned and sequenced. The column at the left shows that each amplicon from every donor was generated in at least two independent reactions. The number of clones that were sequenced at the 5' and 3' ends are indicated at the right, those fully sequenced using internal primers are shown in parenthesis. Below each donor number is shown their *3DL1/S1* and *3DL2* genotype as determined by pyrosequencing; **059*, **060* and **061* are the three *3DL1/2v* alleles. Donor N118 is assigned as hemizygous for *3DL1*059*, because the genotype comprises one copy of the *3DL1/2v* haplotype and one copy of the *3DL1/S1* negative haplotype, which contains *3DL2* (Norman et al. 2002). GenBank accession numbers for the four independently-generated *3DL1/2v* genomic sequences are indicated at the bottom.

(C) Shown is the central 200bp from a continuous sequence trace that spans the *3DL1 – 3DL2* crossover point in intron 5. The chromatograph is aligned with *3DL1* and *3DL2*, the informative sites are shown in red and with a dagger below. The horizontal bar at the center indicates the 30bp region where *3DL1/2v* switches identity from *3DL1* to *3DL2*. All four donors gave identical *3DL1/2v* sequences; the sequence shown was generated from donor C229 and the alignment was performed using DNAStar. The trace was cut into three segments to fit the page.

Supplemental Figure S3



B

5' crossover

Divergence	mean mya	sd	95% CI
3DL1/2v from 3DL1	**0.74**	0.35	0.27 - 1.55
3DL1 from pt3DL12v	**6.88**	1.69	4.11 - 10.9

3' crossover

Divergence	mean mya	sd	95% CI
3DL1/2v from 3DL2	**2.51**	1.44	0.72 - 6.50
3DL2 from pt3DL12v	**12.59**	1.70	9.12 - 15.8

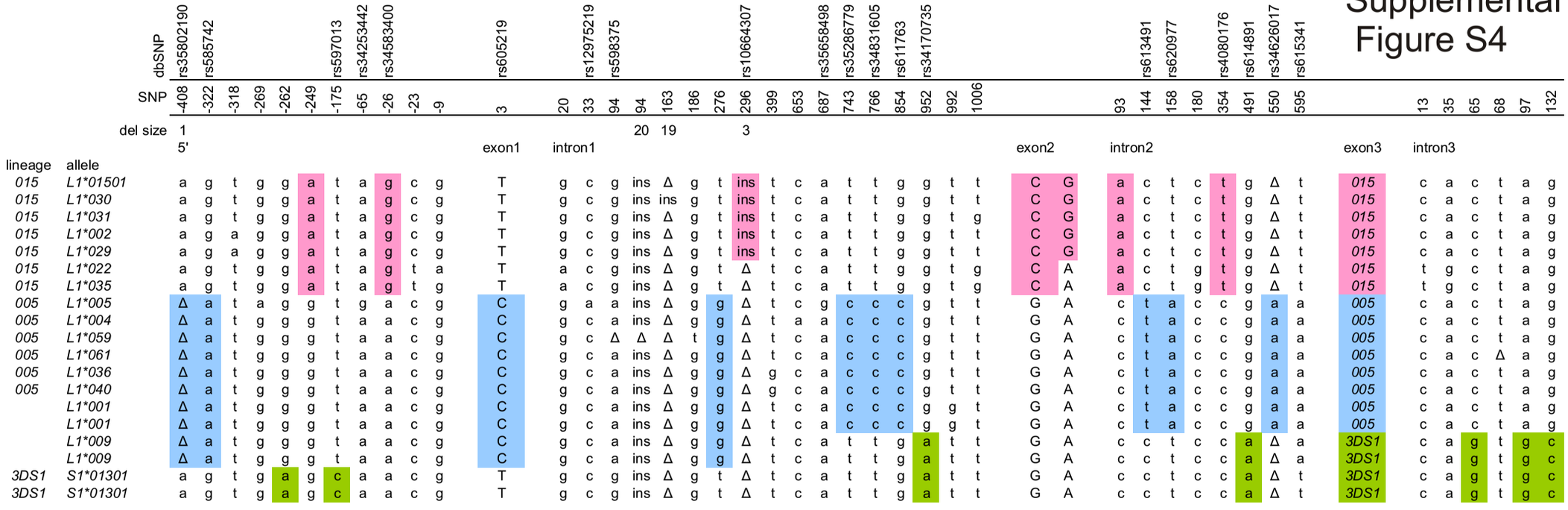
Fig. S3. Phylogenetic analysis of *3DL1/2v* alleles.

(A) Shows the phylogenetic analyses of intron 2 and exons 3, 4 and 5 of *3DL1/2v (3DL1*059, *060* and **061), 3DL1/S1, 3DL2, Pt3DL1/2* and *Popy3DLA*. Two sequences from each *3DL1/S1* allelic lineage were used in the analyses and are colored according to lineage, red – *015*, blue – *005* and green – *3DS1*. The *3DL1/2v* alleles (*3DL1*059*, **060* & **061*) are boxed and highlighted. *3DL1/2v* groups with alleles of the *3DL1-005* lineage for all three exons. *3DL1/2v* also grouped with *005*-lineage alleles in the introns that are not shown. Phylogenetic trees from the 1000bp intervals either side of the recombination point where *3DL1/2v* switches identity from *3DL1* to *3DL2* are shown at the bottom. The three *3DL1/2v* alleles group with the *3DL1-005* lineage in the intron sequence upstream from the crossover, then cluster with *3DL2* downstream from the crossover.

Neighbor-joining trees are shown with support from 500 bootstraps.

(B) Shown are divergence time estimates performed using the dataset that was used to generate the trees in panel B.

Supplemental Figure S4



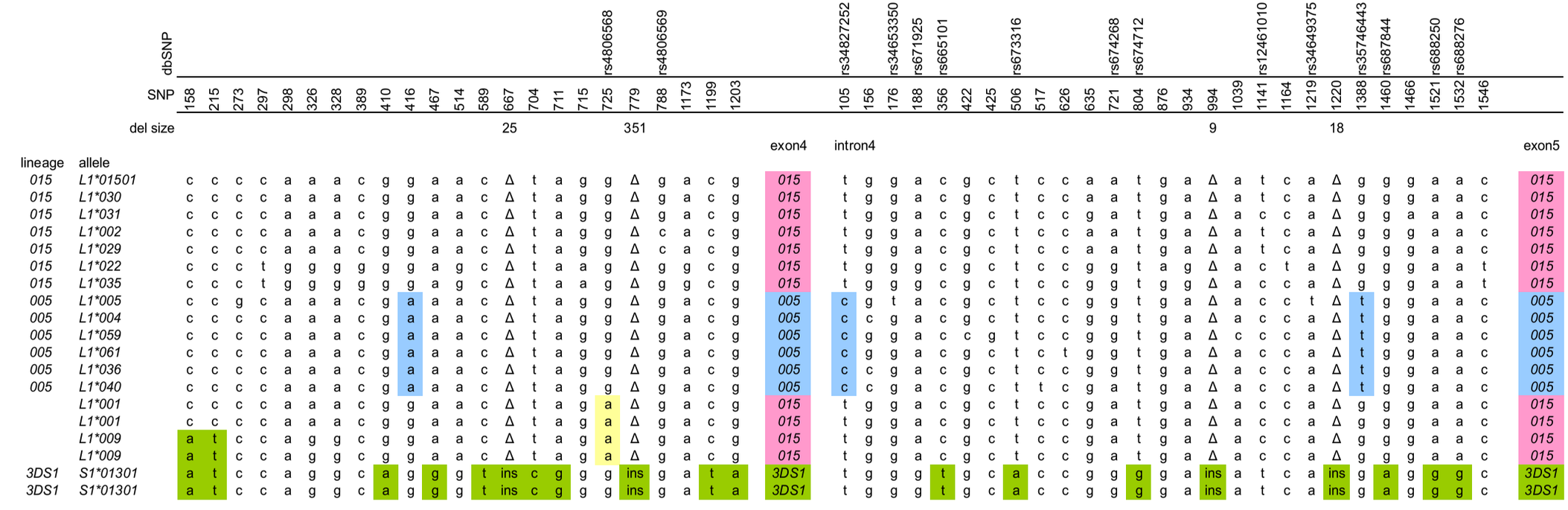


Fig. S4. Alignment of *3DL1/S1* intron markers

Shown are the nucleotide differences in the introns of *3DL1/S1* alleles. dbSNP reference numbers are indicated at the top. The numbers shown are the alignment position relative to the longest sequence for each intron. Markers that are characteristic of the three lineages are indicated (blue -005, red -015 and green -3DS1). Δ- deletion/insertion.

Supplemental Figure S5

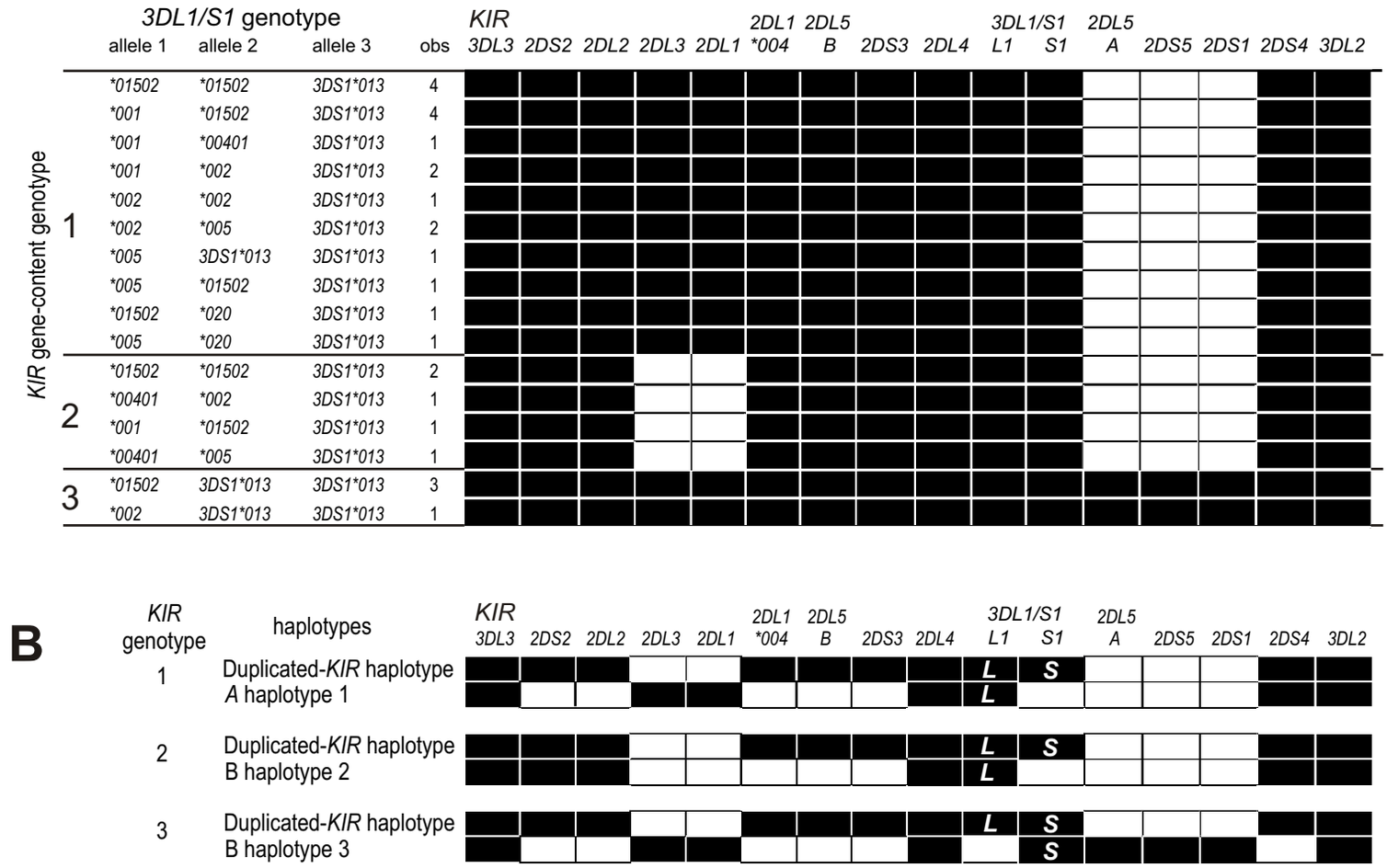


Fig. S5. Three common *KIR* gene-content genotypes that harbor duplicated-locus haplotypes.

Human *KIR* haplotypes vary in gene content. The most common is the *A* haplotype, which has seven loci, and the less-frequent *KIR B* haplotypes have from 7-12 loci (Uhrberg et al. 1997). Presence/absence typing has revealed many different *KIR* genotypes, the most frequent worldwide represents individuals who are homozygous for the *A* haplotype (Norman et al. 2001).

(A) Shown are three *KIR* gene-content genotypes (1, 2 & 3) that were determined by presence/absence typing, and which account for 50% of individuals in this study that have a duplicated-locus haplotype. Four columns at the left show, for each of the gene-content haplotypes, the different *3DL1/S1* genotypes (allele 1, 2, 3) and the number of individuals observed (obs). On the right, black boxes indicate presence of the *KIR* gene, white boxes indicate absence.

(B) Shown are the *KIR* haplotypes that form genotypes 1-3 from panel A. Individuals with genotype 1 are heterozygous for the *A* haplotype plus a duplication-haplotype. Genotypes 2 and 3 have common *B* haplotypes plus the duplication-haplotype.

KIR-locus presence/absence genotypes were determined by PCR-SSP (Uhrberg et al. 1997).

Supplemental Figure S6

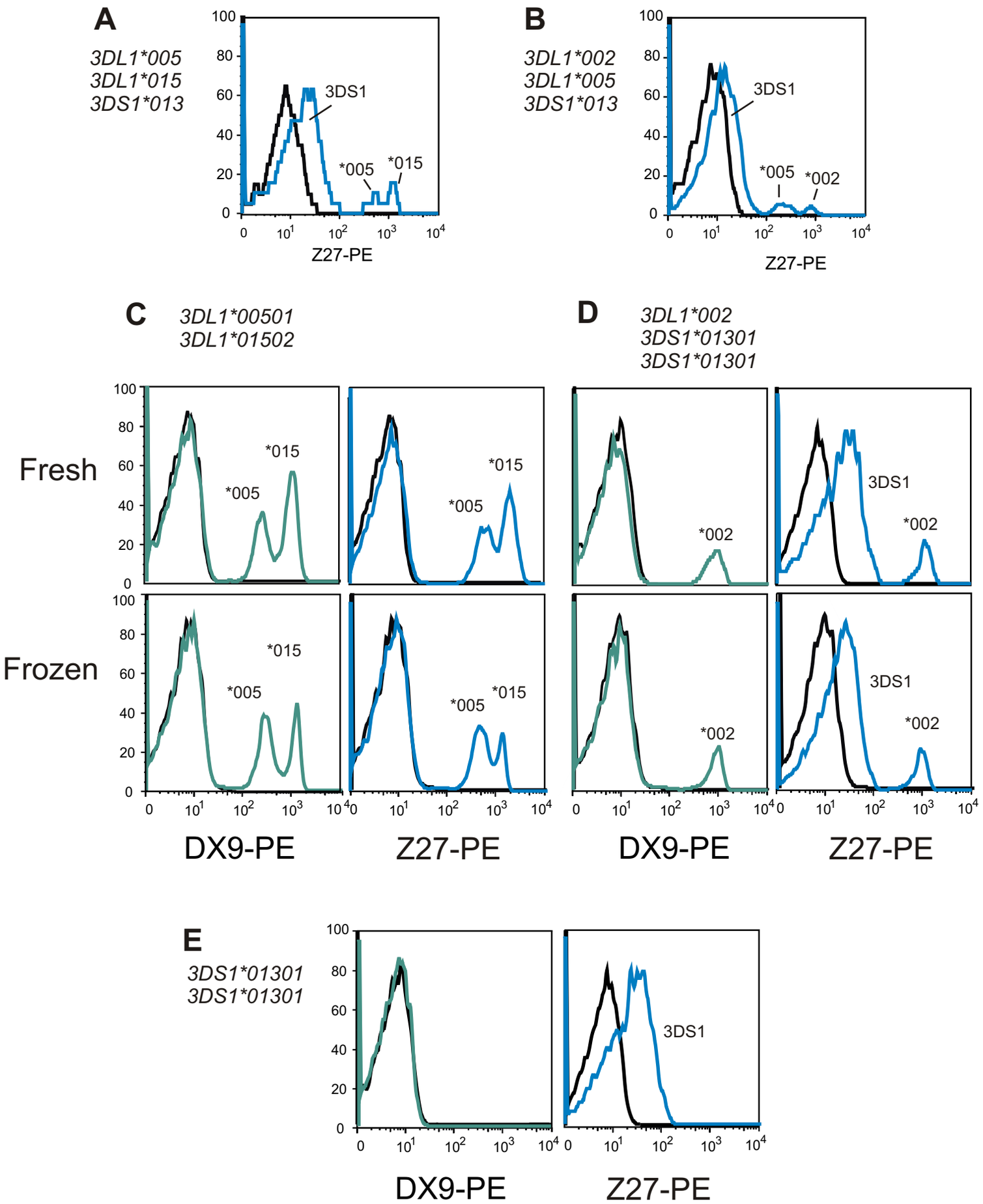


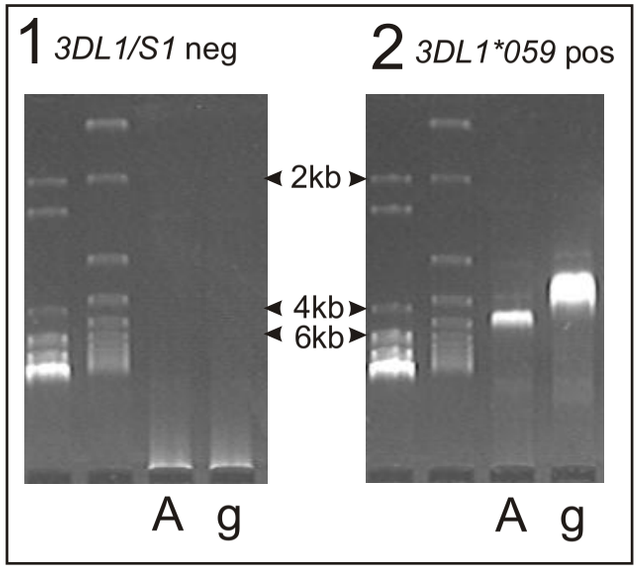
Fig. S6. Monoclonal antibodies DX9 and Z27 discriminate 3DS1 from 3DL1 on fresh and frozen-thawed NK cells.

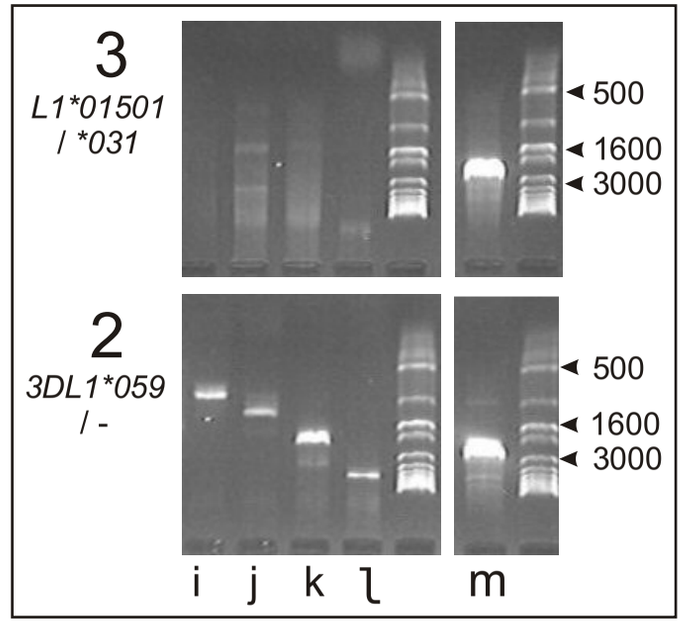
Shown are *3DL1/S1* genotypes and flow cytometer plots of NK cells from five donors; A-E.

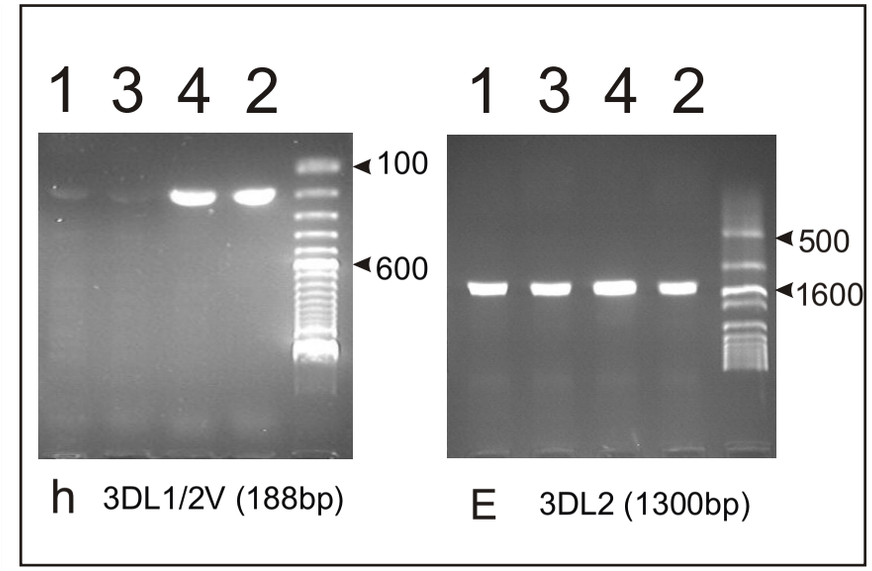
Donors A and B are two unrelated individuals who both express all three lineages of 3DL1/S1 receptors; these FACS analyses are from NK cells that had been stored frozen for >5 years and then thawed prior to analysis.

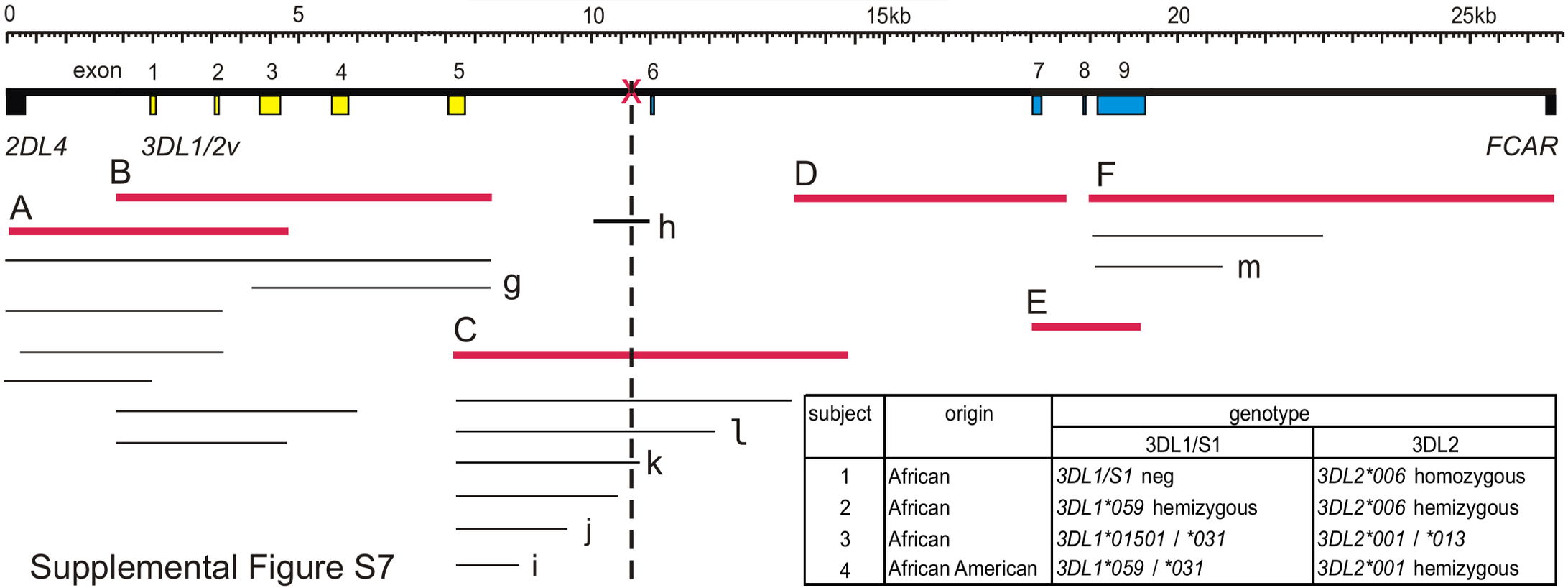
(C) and (D) show that freezing does not affect the ability to detect KIR expression. Shown at the top are the staining profiles from fresh cells, underneath are frozen-thawed cells from the same donors stained in parallel. Lymphocytes were stored frozen for 3 years (C) and six months (D). Donor C is heterozygous for *3DL1*015* and **005* and is negative for the 3DS1*013 peak. Donor D is son 1 from Figure 6C and has two expressed copies of *3DS1*01301* and one of *3DL1*002*.

Donor E is homozygous for *3DS1*01301*, and is shown to illustrate the differential staining of 3DS1*013 by DX9 and Z27 monoclonal antibodies (Carr et al. 2007; Trundley et al. 2007).









subject	origin	genotype	
		3DL1/S1	3DL2
1	African	*3DL1/S1* neg	*3DL2*006* homozygous
2	African	*3DL1*059* hemizygous	*3DL2*006* hemizygous
3	African	*3DL1*01501 / *031*	*3DL2*001 / *013*
4	African American	*3DL1*059 / *031*	*3DL2*001* hemizygous

Supplemental Figure S7

Fig. S7 Haplotype amplification strategy.

Shown are the amplicons used to characterize the interval from *2DL4* to *FCAR* in four different *3DL1/2v* haplotypes. All of the amplicons, which are shown to scale as horizontal lines below the schematic haplotype, were generated from one *3DL1/2v*$^+$ donor during development of the procedure. Six of the amplicons were generated from all four *3DL1/2v*$^+$ donors, and are shown in red and labeled A-F. At the top of the figure are examples of control reactions; the numbers 1-4 shown in each panel refer to four control DNA samples for which genotypes are shown in the lower right of the figure. The amplicons generated in the control reactions are labeled g-m. At the top left, donor 1, who is homozygous for a *3DL1/S1* negative haplotype (Norman et al. 2002), produced no band from reactions A and g (and no others up until D; not shown) but, due to the presence of *3DL2*, has a normal band from reaction E (right panel). The center panel shows allele-specific reactions up to and surrounding the crossover (marked by red X and dotted line). The panel at the top right shows reaction h, which is a *3DL1/2v*-specific PCR with a 188bp product, having forward primer specific for *3DL1* and the reverse specific for *3DL2*. Shown alongside in the right panel is band E, which was generated using primers specific for *3DL2* and is a positive control for all four donors.

All of the amplicons shown in black and red were characterized from donor number 4 and those shown in red were characterized from donors 2, 4 and two further *3DL1/2v*$^+$ donors that are described in Fig. S2. The primers for A-E are shown in Fig. S2, and all other primers are shown in Table S1.

A

Supplemental Table S1

Exon/cDNA	Forward primer	dbSNP	Reverse primer (5'-3')
KIR2DL4 Pyrosequencing			
exon 3	TGTTGTAGGGAGACGCCAC		AGTTGGGGCCTGGATGATC
	nnnnTGCCTCAAGGAGGACACGT		o-GGGACCCCATCTTTCTTGTA
exon 5	CTAGGCCATAGAGCAGGGC		CTCAGCTAAGGCTCTAGGAC
	nnnnCTCTTCTCCTTCCAGGTCTA		o-GACATGGGACAGACATTGG
exon 7*	TCGCCAGACACCTGCATGCTG		TGTTCACTGTTCTGTGTCCC
	nnnnTGTGATTAGGTACTCAGTGG		o-CAGGGGACGTGAGGATACA
158R	TGACTCTTCGGTGTCA	**rs618835**	
395Y	CCTTCGCTTACAGCC	**rs2075769**	
412R	GGGCCCCACGGTTCG	**rs1051454**	
480R	ACCATCTATCCAGGGA	**rs1051455**	
506M	GAAGCCCATGAACTTAG		
615R, **625S**	GCTCTTTCCATGGATCTC	**rs1051456**	
762Y	GGCCATCATCCTCTT		
i7R (812 A/-)	TGCCTTCAGCTCACA		
KIR3DL2 Pyrosequencing			
exon 3	GGGAGACGCCATGTCTATGT		TAGTCACAGGCTCCAGGGT
	nnnnCCTCCTCTCTAAGGCAGTG		o-GAAGCCCAGACAGAAAGCC
exon 4	GAGATTGATTCAGGCTGCTG		GTTGGTACAGACCTCACCG
	nnnnCATTCCAGGTGCCATGGATG		o-TGTGTCCCAATGACAATGAGA
exon 5	AGGAAATAGACATGAAGAGAGT		CATGCTTCTCCCCATCATCG
	nnnnACATTCCAGGCAGACTTTCC		o-AAGCAGTGGGTCACTTGAGT
exons 7-9	TTGTCCTAAGGAGATGTTCCA		GTGATTGCAGCCTCAAGTAGG
	nnnnAGCCTCACGGATACAGTCT		o-GTTTTGAGACAGGGCTGTTG
100Y, 110S, **122R**	ACCCTTCCTGTCTGC	**rs3745893**	
332R, 337S	CTCACTGGGTGGTCG	**rs654686**	
337S	CGCCACCCAGCAACC		
394M	TCCCTCCTGGCCCAC	**rs3745894**	
456Y, **474K**	CAGATGTCATGTTTGAGC	**rs1048270**	
497R	CGGGATCTCTGAGGA	**rs1048271**	
892Y, 893R, 896M	ACAGATGCTTCGGCT		
1190Y	GACCCTCAGGAGGTG	**rs3745902**	
1244S	ACAGAGAAAAATCAGTCG		
1265Y	GAGGCCCAAGACACC		

B

alignment (Fig S6)	Band	primer (5'-3')	alignment (Fig S6)	Band	primer (5'-3')
90		GCCCTGTCTCAAACCCAGC	9000	i	ACCAGAGGCCAGGAAGGG
2000		GAGTTTAAATCATTTGAACTGGTTCTG	9400	j	GTTTATATCCAAAGGAAAGGACA
3000		GGGAATCGAGGGAGGGAGT	10500		GAGGCTGGGCATAGTGGC
3500		GGCCCTCTGGACCAAGAAC	11000	k	AGAAATGTAAATTACAATCGCGA
4000	g	CTTCTGGGCACTGGGAGT	12000	l	CACTCTGTTATCTAATGTTGGA
6000		GTTCTCATTGTCACTGGGACA	13500		AAGARCAGAGGCCAAATGCA
10500	h	AAATTAGCTGAGCATGGTGAC	20750	m	GAAATTAGAGGATTCAGGCTG
10700	h	GCATTTACATCCAATGGCTTTC	22500		CTACGGCCCAAGGAATTACA

Supplemental Table S1 Oligonucleotide primers for PCR and pyrosequencing

(A) Pyrosequncing: o- = biotin. dbSNP numbers correspond to the SNP shown in bold.

* as described by (Witt et al. 2000)

(B) PCR primers used to generate the amplicons shown in Fig. S7, the alignment refers to the scale at the center of Fig. S7, the band letters refer to the amplicons labeled in Fig. S7.

References for Supplemental Material

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