Broad Recognition of Mamu-Bw4 and -A-related MHC class I Ligands by Rhesus Macaque Killer-Cell Immunoglobulin-Like Receptors

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Abstract

Definition of the MHC class I ligands of rhesus macaque KIRs is fundamental to NK cell biology in this species as an animal model for infectious diseases, reproductive biology and transplantation. To provide a more complete foundation for studying NK cell responses in this species, rhesus macaque KIRs representing common allotypes of lineage II *KIR* genes were tested for interactions with MHC class I molecules representing diverse *Mamu-A, -B, -E, -F, -I* and *-AG* alleles. KIR-MHC class I interactions were identified by co-incubating reporter cell lines bearing chimeric KIR-CD3ζ receptors with target cells expressing individual MHC class I molecules and were corroborated by staining with KIR-Ig Fc domain fusion proteins. Ligands for 10 KIRs of previously unknown specificity were identified that fell into two general categories; interactions with multiple Mamu-Bw4 molecules (KIR3DL04*001, KIR3DL10*002, KIR3DL11*001 and KIR3DS04*003) or with Mamu-A-related molecules (KIR3DL07*004, KIR3DS01*003, KIR3DS02*004, KIR3DS06*002, KIR3DSw07*001 and KIR3DSw09*003). Among the latter group, three KIRs (KIR3DL07*004, KIR3DS02*004 and KIR3DSw09*003) also interacted with Mamu-B*045:03, a hybrid molecule with Mamu-A α1-domain sequences. Both groups include inhibitory and activating receptors that vary in the specificity and strength of their MHC class I interactions. However, whereas the majority of KIRs that recognize Mamu-Bw4 ligands are inhibitory, most of the KIRs that recognize Mamu-A-related ligands are activating. These findings more than double the number of rhesus macaque KIRs with defined MHC class I ligands and reveal an overlapping, but nonredundant, pattern of ligand recognition that supports extensive functional diversification of these receptors.

Introduction

Natural killer cell responses in humans and other primate species are regulated in part by interactions between two highly polymorphic sets of molecules; the killer cell Ig-like receptors (KIRs) expressed on the surface of NK cells and their MHC class I ligands on target cells (1, 2). As indicated by their nomenclature, KIRs typically have two or three extracellular Ig-like domains (2D or 3D) and either long (L) or short (S) cytoplasmic tails. KIRs with long cytoplasmic tails contain a pair of ITIMs that transduce inhibitory signals, whereas those with short cytoplasmic tails associate with adaptor molecules such as DAP12 or FcεRIγ to transduce activating signals (2, 3). Inhibitory KIRs normally suppress NK cell responses through interactions with MHC class I ligands on the surface of heathy cells. However, if these interactions are somehow perturbed, for instance as a result of MHC class I downregulation in virus-infected cells or deletion of MHC class I genes in tumor cells, the loss of inhibitory signals can trigger an NK cell response (4-8). Activating KIRs can also interact with MHC class I molecules on unhealthy cells to directly trigger an NK cell response, although their molecular mechanisms are not as well defined (2, 9-11).

Polymorphisms in the *KIR* and *HLA class I* genes have been associated with differences in the course of infection for a number of human viral pathogens and with the outcome of cancer immunotherapy (12-19). *KIR* and *HLA class I* polymorphisms have also been linked to complications during pregnancy and to the success of hematopoietic stem cell and solid organ transplantation (20-23). However, studies to address the immunological mechanisms underlying these observations have been hampered by the lack of a suitable animal model. Mice and other rodents do not have KIRs, but instead express C type lectin-like molecules on their NK cells as polymorphic receptors for MHC class I ligands (2, 24). Moreover, as a consequence of the rapid

pace of KIR and MHC class I evolution (25-27), it is not possible to predict KIR ligands in Old World monkeys on the basis of sequence similarity with human KIRs.

In contrast to humans, which have three classical *HLA class I* genes (*HLA-A*, *-B* and *-C*), Old World monkeys express an expanded array of polymorphic MHC class I genes related to *HLA-A* and *-B*. However, these species do not have an *HLA-C* ortholog, since *MHC-C* arose in hominids as a duplication of an *MHC-B* gene after their divergence from Old World monkeys (28, 29). The *MHC class I* haplotypes of Old World monkeys are polygenic, and in the rhesus macaque typically express two or three *Macaca mulatta (Mamu)-A* genes and four to eleven *Mamu-B* genes (30-32). Depending on their transcriptional abundance, the *MHC class I* genes of macaques can be further classified as "major" or "minor" loci (32). Products of the major genes exhibit the greatest polymorphism and present peptides to virus-specific $CD8^+$ T cells (33), suggesting functional similarities to HLA-A and -B in humans.

Four non-classical *MHC class I* genes have also been identified in Old World monkeys. Orthologs of *HLA-E* and *-F* (*Mamu-E* and *-F* in rhesus macaques) are well conserved and serve similar functions as their human counterparts (34-37). Macaques and other Old World monkeys also have an ortholog of *HLA-G*; however the *MHC-G* genes of these species have accumulated numerous stop-codons and no longer encode functional proteins (38, 39). In lieu of a functional *MHC-G* locus, Old World monkeys have evolved duplicated *MHC-A* genes, designated *MHC-AG*, that appear to serve a similar function (40-43). Like HLA-G, MHC-AG has limited polymorphism, a truncated cytoplasmic tail, and is only expressed on placental trophoblasts that form a barrier between the mother and developing fetus (40, 41, 44). Mamu-AG is therefore thought to contribute to pregnancy success in rhesus macaques through interactions with receptors on NK cells and myeloid cells that stimulate placental vascularization, facilitate fetal

tolerance, and protect the maternal-fetal interface from invading microorganisms (44-46). Rhesus macaques also express Mamu-I, which is the product of a fixed duplication of an *MHC-B* gene with limited sequence variation (47).

In concert with the expansion of their *Mamu-A* and *-B* genes, macaques have evolved an expanded complement of lineage II *KIRs* (26, 48-52). Whereas humans only have two lineage II *KIR* genes that encode KIR3DL1/S1 and KIR3DL2 as receptors for HLA-Bw4 and –A3/A11, respectively, rhesus macaques have at least 19 lineage II *KIRs* that encode inhibitory and activating receptors for Mamu-A and -B molecules (50, 52-56). Furthermore, in contrast to humans, which have multiple lineage III *KIR* genes encoding KIR2DL/S receptors for HLA-C1 and -C2 ligands (1, 2), macaques only have one lineage III *KIR* that expresses a single domain KIR (KIR1D) of uncertain function (52). Thus, while human NK cells are predominantly regulated by KIR2DL/S-HLA-C interactions, macaque NK cells are dependent on KIR3DL/S interactions with Mamu-A and -B ligands (52).

The rhesus macaque has become an increasingly valuable animal model for infectious diseases (57-68), reproductive biology (44, 69), and transplantation (70, 71). Although MHC class I ligands have been identified for a few macaque KIRs (72-75), ligands for the majority of these receptors remain undefined. We therefore performed a systematic survey of MHC class I interactions for common KIR allotypes representing 14 predicted lineage II *KIR* genes in Indian-origin rhesus macaques. By measuring the responses of KIR-CD3ζ-transduced reporter cell lines to target cells expressing individual MHC class I molecules and confirming interactions by staining with KIR-Ig Fc domain (KIR-Fc) fusion proteins, we identified MHC class I ligands for 10 rhesus macaque KIRs of previously undefined specificity. This represents the most

comprehensive survey of ligand recognition by macaque KIRs to date and reveals extensive diversification of the lineage II KIRs that interact with Mamu-Bw4 and -A-related ligands.

Materials and Methods

Jurkat NFAT luciferase reporter cells expressing chimeric KIR-CD3ζ receptors

Complementary DNA sequences encoding the leader peptide of Mamu-KIR3DL05*008 followed by a Flag®-tag (DYKDDDDK) and the D0, D1, D2 and stem domains of rhesus macaque KIRs were synthesized (Integrated DNA Technologies) and cloned into pQCXIP in-frame with sequences encoding the transmembrane and cytoplasmic domains human CD3ζ. Retroviral vectors were packaged into VSV G–pseudotyped murine leukemia virus (MLV) particles by co-transfecting GP2-293 cells (Clontech Laboratories) with pQCXIP constructs and pVSV-G (Clontech Laboratories). Jurkat NFAT luciferase (JNL) cells (Signosis) were transduced via spinoculation for one hour with filtered supernatant collected from transfected GP2-293 cells and 2 d later were placed under selection in RPMI medium supplemented with 10% FBS, L-glutamine, penicillin and streptomycin (R10 medium) plus 0.1 mg/ml hygromycin (Invitrogen) to maintain the luciferase reporter gene and 0.5 µg/ml puromycin (Invitrogen) to select for the KIR -CD3ζ receptor. The puromycin concentration in the medium was gradually increased to 1.0 µg/ml over 3-4 weeks to eliminate untransduced cells and then maintained at a concentration of 0.5 µg/ml. The GenBank accession numbers for rhesus macaque KIR and human CD3ζ sequences are as follows: *Mamu-KIR3DL02*004:01* (LT963635.1), -*KIR3DL04*001:02* (GU299490.1), -*KIR3DL07*004* (EU419060.1), -*KIR3DL10*002:01* (GU112259.1), -*KIR3DL11*001* (GU112271.1), -*KIR3DLw34*002* (previously -*KIR3DLw03*003*; EU419031.1), -*KIR3DS01*003* (GU564161.1), -*KIR3DS02*004:02* (GU014296.1), -*KIR3DS03*003* (EU702454.1), -*KIR3DS04*003:01* (EU419029.1), -*KIR3DS05*002:01* (GU112262.1), -*KIR3DS06*002:02* (GU112298.1), -*KIR3DSw07*001* (GU112272.1), -*KIR3DSw09*003* (MF164924.1) and *CD3ζ* (J04132.1).

Rhesus macaque MHC class I-expressing 721.221 cells

Codon-optimized cDNA sequences encoding rhesus macaque MHC class I molecules were cloned into pQCXIP or pQCXIN. Retroviral vectors were packaged into VSV-G-pseudotyped MLV particles as described above. 721.221 cells were transduced via spinoculation for one hour with filtered supernatant collected from transfected GP2-293 cells and placed under selection in R10 medium containing 0.4 mg/ml puromycin or 0.5 mg/ml G418, depending on the vector, to select for MHC class I expression. The concentrations of puromycin and G418 in the medium were gradually increased to 1.0 µg/ml and 1.0 mg/ml, respectively, over 3-4 weeks to eliminate untransduced cells and then maintained at a concentration of 0.4 µg/ml and 0.5 mg/ml. The GenBank accession numbers for rhesus macaque MHC class I sequences are as follows: *Mamu-A1*001:01:01:01* (LT908117), -*A1*002:01:01:01* (LN899621), -*A1*004:01:01:01* (FN396407.1), -*A1*008:01:01:01* (LN899628), -*A1*011:01:01:01* (AJ542579), -*A1*012:01:01:01* (AF157398.1), -*A2*05:02:01:01* (LN899639), -*A2*05:04:01:01* (LT908121.1), -*A3*13:02:01:01* (LN899654.1), -*A3*13:03:01:01* (AF157401), -*A4*14:03:01:01* (GU080236.1), -*A6*01:03:02:01* (EF602318.1), -*B*001:01:02:01* (LM608018), -*B*002:01:01:01* (LN851852), -*B*005:01:01:01* (LM608023), -*B*007:01:01:01* (U41829.1), -*B*008:01:01:01* (U41830), -*B*015:01* (AM902541.1), -*B*017:01:01:01* (AF199358), -*B*022:01:01:01* (LN899675), -*B*036:01:01:01* (AJ556886.1), -*B*041:01:01:01* (LN899682), -*B*043:01:01:01* (AJ556893), -*B*045:03:01:01* (LM608047), -*B*056:01:01:01* (GQ902079.1), -*B*065:01:01:01* (AJ620416.1), -*I*01:01:01* (LT908864), -*E*02:01:02* (previously E*02:01; NM_001114966.1) -*F*01:01* (LT899414), -*AG2*01:01* (previously AG*01:01; U84783.1), -*AG3*02:01:01:01* (U84785.1), -*AG3*02:02:01* (U84786.1), -*AG3*03:01:01:01* (U84787.1), -*AG3*03:02* (U84789.1), -*AG3*03:03* (KF855159.1), -

*AG3*03:04* (KF855160.1), and -*AG1*05:01* (FJ409466). Only partial-length cDNA sequences were available for *Mamu-AG3*03:03*, -*AG3*03:04*, and -*AG1*05:01*. For constructs expressing these alleles, sequences encoding the α3-, transmembrane and cytoplasmic domains were inferred from the *Mamu-AG* consensus sequence.

KIR-Fc fusion proteins

Complementary DNA sequences encoding the artificial secrecon leader peptide (MWWRLWWLLLLLLLLWPMVWA), the D0, D1, D2 and stem domains of macaque KIRs, and the Fc domain of murine IgG2A were synthesized (Integrated DNA Technologies) and cloned downstream of the CMV promoter in pCIW using unique *Hind* III and *Not* I sites. Subsequent *KIR* sequences were introduced by primer overlap extension. These sequences were PCR amplified to create a product with arms complementary to the insertion site of the vector (5' secrecon, 3' msFc), and then employed as a megaprimer to amplify the entire plasmid with Pfu Ultra II Fusion HS (Agilent). The template vector was eliminated by digestion with Fermentas' FastDigest Dpn I for one hour at 37 °C. After sequence confirmation, plasmid DNA maxipreps were prepared for each construct. Expi-293T cells (7.5 x 10^8) were transfected with the pCIW-KIR-Fc plasmid DNA (250 µg) using the ExpiFectamine 293 Transfection Kit (Life Technologies) according to the manufacturer's recommendations. Five days after transfection, the supernatant was collected, filtered through a 0.45 µM PES filter (Fisher Scientific), and loaded on a Protein A column (GE Healthcare). After washing the columns three times with PBS, KIR-Fc fusion proteins were eluted in gentle Ag/Ab buffer (Thermo Scientific) and buffer exchanged with 50 mM Sodium citrate using an Amicon Ultra-15 Ultracel 50K Centrifugal Filter unit (Millipore). The final concentrations of the purified KIR-Fc fusion proteins were calculated

by dividing the UV absorbance at 280 nm by the theoretical extinction coefficient obtained from ExPASy ProParam (https://web.expasy.org/protparam/). After adding sodium azide (3 mM), the KIR-Fc fusion stocks were snap frozen in liquid nitrogen and stored at -80 °C.

Flow cytometry

KIR-CD3ζ expression on the surface of JNL cells and MHC class I expression on 721.221 cells was confirmed by staining with PE-conjugated anti-Flag® (Miltenyi Biotec) and pan-HLA class I-specific antibodies (clone W6/32; Life Technologies), respectively. These cells were then stained with by Near-IR fluorescent dye (Invitrogen), washed twice in PBS plus 1% FBS (FACS buffer), and fixed in 2% paraformaldehyde PBS. To assess KIR binding, MHC class I-transduced 721.221 cells were stained for 30 minutes at room temperature with Near-IR fluorescent dye, washed twice with FACS buffer and stained for 30 minutes at room temperature with KIR-Fc fusions proteins (10-20 µg). The cells were then washed twice with FACS buffer, stained for 45 minutes with Alex 647-conjugated goat anti-mouse IgG (Jackson ImmunoResearch), washed twice more, and stained for 30 minutes at room temperature with the PE-conjugated pan-MHC class I-specific antibody W6/32. The cells were then washed again and fixed in 2% paraformaldehyde PBS. Data was collected using a FACSymphony™ A3 Cell Analyzer (BD Biosciences) and analyzed using FlowJo version 10.8.0 software (Becton Dickinson). After gating on viable cells, KIR-CD3ζ (Flag-tag) and MHC class I staining was compared to parental JNL and 721.221 cells, respectively. KIR-MHC class I binding was similarly assessed by comparing KIR-Fc versus W6/32 staining on the surface of MHC class I-transduced 721.221 cells to their levels of non-specific staining on parental 721.221 cells.

KIR-CD3ζ⁺ JNL reporter assays

KIR-CD3ζ JNL cells (1x10□) and MHC class I-transduced 721.221 cells (1x10□) were co-cultured overnight at 37°C and 5% CO□ in R10 medium without antibiotics. KIR-CD3ζ□ JNL cells were also incubated with parental 721.221 cells as negative control and with anti-Flag-tag (5 µg/ml) (GenScript) and goat anti-mouse (10 µg/ml) (Poly4053, Biolegend) antibodies as a positive control. Each combination was tested in triplicate wells (100 µl/well). After 12-18 hours, BriteLite Plus luciferase substrate (PerkinElmer) was added to each well (100 µl/well) and the relative light units (RLU) of luciferase activity was measured using a VICTOR X4 multiplate reader (PerkinElmer).

Alignments and Phylogenetic trees

The predicted amino acid sequences of KIR and MHC class I molecules were aligned using Geneious Prime® 2021.2.2 (Biomatters Ltd) and the text format was exported to Microsoft Word for figure construction. Neighbor-joining analysis of CDS nucleotide sequences for the α1-α3 domains of Mamu-A and -B and the D0-D2 domains of KIR molecules was performed using MAFFT (version 7, publicly available at https://mafft.cbrc.jp/alignment/server/) (76, 77). For the analysis of KIR sequences, human lineage II KIRs were included as an outgroup (KIR3DL1*0010101, NP_037421.2 and KIR3DL02*0010101, NP_006728.2). All analyses utilized G-INS-I iterative refinement with 1000 bootstrap replicates. Trees were viewed using Phylo.io (1.0.0) (78).

Statistical analysis

The luciferase induction by KIR-CD3ζ JNL cells (RLU data) was analyzed by one-way ANOVA followed by Dunnett's test for multiple comparisons of mean responses to each of the MHC class I-expressing 721.221 cells with mean responses to the parental 721.221 cells as a negative control. Statistical analyses were performed using GraphPad Prism for Mac OS version 9.2.0.

Results

Extensive sequencing and segregation analyses of *KIR* transcripts support the existence of at least twenty-two *KIR* genes in Indian-origin rhesus macaques (53-56). Although our group and others have identified ligands for a few rhesus macaque KIRs (72-75), ligands for most of the receptors in this species remain undefined. To provide a more complete foundation for studying NK cell biology in the rhesus macaque, we investigated the MHC class I interactions of 14 KIRs representing common alleles of lineage II genes in animals of Indian descent for which ligands have not been previously reported (Fig. 1A). Jurkat cells harboring an NFAT-inducible luciferase reporter gene (JNL cells) were transduced with retroviral vectors expressing KIR-CD3ζ chimeric receptors consisting of the extracellular domains of macaque KIRs (D0, D1, D2 and stem domains) fused to the transmembrane and cytoplasmic domains of human CD3ζ. To confirm surface expression, a Flag-tag was appended to the N-terminus of the D0 domain of each receptor (Fig. 1B). After an overnight incubation with HLA class I-negative 721.221 cells transduced with individual rhesus macaque MHC class I molecules (Supplemental Figs. 1-3), ligand recognition was detected by the MHC class I-dependent upregulation of luciferase by KIR-CD3ζ JNL cells (Supplemental Figs. 4-5). KIR-MHC class I interactions were corroborated by staining MHC class I-expressing 721.221 cells with KIR-Fc fusion proteins consisting of the extracellular domains of macaque KIRs fused to the Fc domain of murine IgG2A (Supplemental Figs. 6-8).

Mamu-Bw4 recognition by multiple rhesus macaque KIRs

KIR3DL04*001- and KIR3DL10*002-CD3ζ JNL cells responded strongly to 721.221 cells expressing Mamu-B*007:01, -B*022:01, -B*041:01, -B*043:01 and -B*065:01 (Fig. 2A). The

magnitude of these responses was generally reflected by the relative intensity of KIR3DL04*001- and KIR3DL10*002-Fc staining on the surface of Mamu-B*007:01+, -B*022:01+, -B*041:01+, -B*043:01+ and -B*065:01+ 721.221 cells (Fig. 2B). Similar responses were also observed for two other KIRs. KIR3DL11*001-CD3ζ JNL cells responded to Mamu-B*007:01, -B*041:01 and -B*065:01, and KIR3DS04*003-CD3ζ JNL cells responded to Mamu-B*22:01, -B*041:01 and -B*065:01 (Fig. 2A). These interactions were further corroborated by KIR-Fc staining of 721.221 cells expressing these molecules (Fig. 2B). KIR-Fc staining affords a comparison of the relative strength of MHC class I interactions across different KIRs that is not possible using independently derived JNL cell lines that may vary in KIR-CD3ζ expression and luciferase induction (Fig. 2B). In the case of KIR3DS04*003, a lower binding affinity for fewer ligands may reflect a role for MHC class I-bound peptide or other cellular factors in reaching a binding threshold necessary to trigger an NK cell response through this activating receptor.

Mamu-B*007:01, -B*022:01, -B*041:01, -B*043:01 and -B*065:01 share a Bw4 motif at residues 77-83 (Supplemental Fig. 2), which was previously shown to contribute to recognition by KIR3DL01 as well as three other rhesus macaque KIRs (73, 75). The identification of Mamu-Bw4 ligands for KIR3DL04*001, KIR3DL10*002, KIR3DL11*001 and KIR3DS04*003 doubles the number of rhesus macaque KIRs known to interact with this group of molecules and reveals an extraordinary expansion of the lineage II KIRs in macaques with Bw4 specificity (Table I). Differences in the particular Mamu-Bw4 molecules recognized by these KIRs and the relative strength of these interactions suggests extensive functional diversification of these receptors.

Multiple KIRs recognize Mamu-AG molecules

Six rhesus macaque KIRs were found to interact with Mamu-AG2*01:01, a non-classical MHC class I molecule expressed by fetal trophoblasts at the maternal-fetal interface of the placenta (40). JNL cells expressing KIR-CD3ζ chimeras for KIR3DL07*004, KIR3DS01*003, KIR3DS02*004, KIR3DS06*002, KIR3DSw07*001 and KIR3DSw09*003 all responded strongly to 721.221 cells expressing Mamu-AG2*01:01 but not other Mamu-AG allotypes (Fig. 3A). The binding of these KIRs to Mamu-AG2*01:01 was further corroborated by KIR-Fc staining of Mamu-AG2*01:01+721.221 cells (Fig. 3B). KIR-Fc staining also revealed low but detectable interactions with Mamu-AG1*05:01 for KIR3DS02*004, KIR3DS06*002 and KIR3DSw09*003 that were not observed in cellular assays with KIR-CD3ζ JNL cells (Fig. 3B). In this case, the detection of weak binding interactions with Mamu-AG*01*05:01 that were not detectable with KIR-CD3ζ JNL cells may reflect somewhat greater sensitivity of KIR-Fc staining. Together with the previous identification of Mamu-AG ligands for KIR3DL05 and KIR3DS02*004 (75, 79), these findings bring the number of rhesus macaque KIRs known to interact with Mamu-AG to seven (Table I).

*Mamu-B*045:03 recognition by KIR3DL07*004, KIR3DS02*004 and KIR3DSw09*003*

In addition to Mamu-AG, JNL cells expressing KIR3DL07*004-, KIR3DS02*004- and KIR3DSw09*003-CD3ζ also responded to Mamu-B*045:03 (Fig. 4A). The binding of these KIRs to Mamu-B*045:03 was also corroborated by KIR-Fc staining (Fig. 4B). Mamu-B*045:03 has not been previously identified as a KIR ligand, and in contrast to Mamu-AG2*01:01, has a Bw6 motif (NLRTLRG) at α1-domain residues 77-83 (Supplemental Fig. 3). To better understand this unusual pattern of responses, we compared the predicted amino acid sequences

for Mamu-B*045:03 and -AG2*01:01. Although Mamu-B*045:03 looks like a typical Mamu-B molecule in its α2-cytoplasmic domains, we noted a patch of sequence similarity with other Mamu-A molecules in residues 13-44 of the α1-domain (Fig. 5A). This included a region of identity at positions 16-19 previously defined as a KIR contact site based on the crystal structure of HLA-B*57 in complex with human KIR3DL1*001 (80). These observations were supported by phylogenetic comparisons. Whereas the full-length amino acid sequence of Mamu-B*045:03 clusters with other Mamu-B molecules (Fig. 5B), the α1-domain of Mamu-B*045:03 is more similar to Mamu-A and -AG molecules (Fig. 5C). A similar pattern of clustering is exhibited by Mamu-B*036, which is hybrid molecule with MHC-A extracellular domains and an MHC-B cytoplasmic tail (Fig. 5C). The recognition of Mamu-B*045:03 by KIR3DL07*004, KIR3DS02*004 and KIR3DSw09*003 may therefore be explained by the origins of this molecule as the product of an ancestral recombination between *MHC-A* and *-B* genes.

Phylogenetic analysis of the D0-D2 of rhesus macaque KIRs reflects ligand recognition

To investigate the relationship between the extracellular domains of rhesus macaque KIRs and their patterns of ligand recognition, we conducted phylogenic analysis of the D0-D2 domains of rhesus KIRs using the human KIR3DL1 and KIR3DL2 as an outgroup. Seven of the eight KIRs that interact with Mamu-Bw4 ligands cluster together on one branch and five of the six KIRs that interact with Mamu-AG and -A-related ligands cluster on separate branch (Fig. 6). Furthermore, within the broader group of receptors that respond to Mamu-AG, the three KIRs that recognize Mamu-B*045:03 all cluster together. However, KIR3DSw07 was an exception. Although KIR3DSw07 recognizes Mamu-AG, its D0-D2 domain sequences group with the Bw4-specific KIRs (Fig. 6). This analysis also revealed that three of the four KIRs for which we were unable

to define a ligand (KIR3DL02*004, KIR3DLw34*002 and KIR3DS05*002) form a distinct phylogenic group (Fig. 6). A common feature of these KIRs is the conspicuous absence of D0 domain residues 34-36 (Fig. 1A), which could explain the inability to detect interactions with any of the MHC class I molecules in our panel. In the case of KIR3DL01*012 and KIR3DL05*008, while these receptors interact respectively with Mamu-Bw4 and -A-related ligands, they exhibit an earlier branching pattern distinct from other macaque KIRs (Fig. 6). It is therefore conceivable that *KIR3DL01* and *KIR3DL05* represent more ancestral lineage II genes in macaques that gave rise to the duplicated genes encoding diverse receptors for Mamu-Bw4 and -A-related ligands.

Discussion

Definition of the MHC class I ligands for rhesus macaques KIRs is fundamental to NK cell biology in this species as a nonhuman model for infectious diseases, transplantation and reproductive biology. As consequence of the rapid pace of KIR evolution in primates, it is not possible to predict the ligands for macaque KIRs on the basis of sequence similarity with their human counterparts. The *KIR* genes of macaques are evolving at a particularly rapid pace characterized by extensive gene duplication and recombination that has generated allelic variation and haplotype diversity that exceeds the diversity of human *KIRs* (55, 56, 81). Although the number of alleles and predicted *KIR* genes varies among geographically distinct populations of macaques, extensive sequencing and segregation analyses support the existence of at least 22 distinct *KIR* genes in rhesus macaques of Indian origin (55, 56, 81). Of these, 19 are predicted to encode lineage II KIRs for Mamu-A and -B ligands. To provide a foundation for studying NK cell responses in rhesus macaques and for basic comparative immunogenetics in primates, we selected common allotypes representing 14 lineage II KIRs for analysis of their MHC class I interactions. By screening 721.221 cells expressing MHC class I molecules corresponding to 37 *Mamu-A, -B, -E, -F, -I* and *-AG* alleles in assays with KIR-CD3ζ JNL reporter cell lines and by staining with KIR-Fc fusion proteins, we identified ligands for 10 previously uncharacterized KIRs.

These analyses revealed two general patterns of ligand recognition; interactions with Mamu-B molecules that contain a Bw4 motif at residues 77-83 and interactions with one or more Mamu-A-related molecules. Of the 14 KIRs tested in this study, four (KIR3DL04*001, KIR3DL10*002, KIR3DL11*001 and KIR3DS04*003) were found to interact with multiple Mamu-Bw4 ligands. Together with our previous studies identifying Mamu-Bw4 ligands for

KIR3DL01*012, KIR3DL06*001, KIR3DL08*002 and KIR3DSw39*002 (73, 75), these findings reveal a remarkable expansion of Bw4 specificity in the rhesus macaque. In contrast to humans, which only have a single *KIR3DL1/S1* gene that encodes receptors for HLA-Bw4, rhesus macaques have at least eight lineage II *KIRs* that encode receptors for Mamu-Bw4 (Table I). KIRs that interact with Mamu-A- or -A-like ligands have also been greatly expanded in rhesus macaques. Six of the KIRs in this study (KIR3DL07*004, KIR3DS01*003, KIR3DS02*004, KIR3DS06*002, KIR3DSw07*001 and KIR3DSw09*003) were found to interact with Mamu-AG2*01:01 alone or in combination with Mamu-B*45:03, a hybrid molecule that contains MHC-A α1-domain sequences. Together with earlier studies defining similar ligands for KIR3DL05 (72, 74, 82), at least seven rhesus macaque *KIRs* are known to encode receptors for Mamu-A or -A-related ligands (Table I).

All of the KIRs that interact with Bw4 ligands recognize Mamu-B*041:01 and -B*065:01 with varying efficiency, suggesting that these molecules may provide a common reference for this specificity (Table I). However, interactions with other Mamu-Bw4 molecules were more variable. Several KIRs, including KIR3DL11*001 and KIR3DS04*003, lack detectable responses to Mamu-B*007:01, -B*022:01 or -B*043:01 (Table I). Differences in the magnitude of KIR-CD3ζ JNL cell responses and KIR-Fc staining reveal additional variation in the strength of these interactions. Although most of the KIRs responding to Mamu-Bw4 ligands are inhibitory, interactions with a couple of activating KIRs (KIR3DS04*003 and KIR3DSw39*002) were identified (Table I). Thus, differences in the preferential recognition of individual Mamu-Bw4 molecules, variation in the strength of these interactions, and the identification of inhibitory and activating KIRs that respond to this group of molecules, suggests extensive functional diversification of this group of receptors.

Rhesus macaque MHC class I haplotypes typically express two or three *Mamu-A* genes and four to eleven *Mamu-B* genes that can be classified as major or minor loci on the basis of their transcriptional abundance (30-32). The Mamu-Bw4 molecules identified as KIR ligands are products of transcriptionally abundant major genes (32). However, phylogenetic and segregation analyses suggest that they are distinct from the *Mamu-B* genes that encode molecules known to present viral peptides to $CD8^+$ T cells (30-33, 83). This is further supported by unique polymorphisms in the cytoplasmic domains of Mamu-B*007:01, -B*022:01, -B*041:01, -B*043:01 and -B*065:01 that are not present in the cytoplasmic tails of molecules such as Mamu-B*008 and -B*017 that present SIV epitopes for recognition by CD8+ T cells (84).

We previously demonstrated that these cytoplasmic domain polymorphisms prevent MHC class I downmodulation by SIV Nef similar to polymorphisms in the cytoplasmic tail of HLA-C that prevent downmodulation by HIV-1 Nef (6, 7, 84, 85). Selective downmodulation of HLA-A and -B (but not HLA-C) by HIV-1 Nef reduces the susceptibility of virus-infected cells to CD8+ T cells (7, 85). While the Vpu proteins of certain HIV-1 isolates may downmodulate HLA-C at later stages of infection (86, 87), HLA-C is initially retained on the cell surface to inhibit NK cells (6). In a similar manner, SIV Nef downmodulates Mamu-A and -B molecules known to present viral peptides to CD8+ T cells, but not the products of duplicated *Mamu-B* genes that serve as ligands for multiple KIRs (75, 84). Hence, parallels in both immunogenetics and virology suggest functional specialization similar to *HLA-B* and *-C* in humans, where the products of certain *Mamu-B* genes have a dominant role in restricting CD8+ T cell responses while others predominantly serve as KIR ligands to regulate NK cells.

Another major group of rhesus macaque KIRs appears to interact exclusively with Mamu-A or A-related molecules. KIR3DL07*004, KIR3DS01*003, KIR3DS02*004,

KIR3DS06*002, KIR3DSw07*001 and KIR3DSw09*003 all recognize Mamu-AG2*01:01 (Table I). Additional interactions with Mamu-AG1*05:01 were detectable for KIR3DS02*004, KIR3DS06*002 and KIR3DSw09*003, and with Mamu-B*045:03 for KIR3DL07*004, KIR3DS02*004 and KIR3DSw09*003 (Table I). Although Mamu-B*045:03 is a Mamu-B molecule, its α1-domain appears to be derived from an *MHC-A* gene (Fig. 5). Thus, the hybrid origins of this molecule as a result of ancestral recombination between *MHC-A* and *-B* genes probably accounts for its interactions with these KIRs. Mamu-B*045:03 recognition may in turn be dependent on sequences shared by these KIRs, since KIR3DL07*004, KIR3DS02*004 and KIR3DSw09*003 have nearly identical D0 domains (Fig. 1). As with Mamu-Bw4 interactions, differences in the preferential recognition of individual Mamu-A-related ligands, the strength of these interactions, and the identification of both inhibitory and activating receptors that recognize these molecules indicates extensive functional diversification of this group of KIRs.

In contrast to the KIRs found to interact with Mamu-Bw4 ligands, the majority of the receptors for Mamu-A and -A-like ligands are activating. Five of the seven rhesus macaque KIRs that interact with Mamu-AG2*01:01 are products of *KIR3DS* genes (Table I). In humans, the ligation of activating lineage III KIRs (KIR2DS) and KIR2DL4 on maternal NK cells of the placental decidua by HLA-C and soluble HLA-G stimulates the release of pro-inflammatory and pro-angiogenic factors that promote placental vascularization to increase blood supply to the developing fetus (20, 21, 45). Since macaques do not express orthologs of HLA-C or -G (28, 29, 38), it is tempting to speculate that Mamu-AG interactions with activating lineage II KIRs may play a similar role in placental development. In contrast to human *KIR* haplotypes, which can be broadly categorized into groups A and B that correspond to inhibitory and activating profiles based largely on fixed differences in the content of activating genes, *KIR* haplotypes in macaques

are much more variable (81). *KIR* haplotypes in rhesus macaques usually contain 4-17 expressed genes, and although the number of activating genes varies, most include at least one activating *KIR* (56, 81). Thus, multiple *KIR3DS* genes that encode receptors for Mamu-AG increases the likelihood that a given animal will express at least one activating KIR capable of engaging this molecule in the placenta. It is therefore conceivable that in the absence of functional orthologs of HLA-C or -G, macaques may depend on KIR3DS interactions with Mamu-AG to promote placental vascularization.

The present study more than doubles the number of rhesus macaque KIRs with defined MHC class I ligands. At least one MHC class I ligand has now been identified for KIRs representing 15 different lineage II *KIR* genes in animals of Indian origin (Table I). These findings reveal a remarkable expansion of two groups of lineage II KIRs with broad specificity for either Mamu-Bw4 or -A-related molecules. The recognition of overlapping, but non-redundant, sets of ligands by both inhibiting and activating KIRs indicates extensive diversification of these receptors in concert with products an expanded complement of *Mamu-A* and *-B* genes. These observations provide a valuable foundation for studying NK cell responses in the rhesus macaque as a pre-clinical model for infectious diseases, transplantation and reproductive biology in addition to advancing our basic understanding of KIR and MHC class I co-evolution in primates.

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Footnotes

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Abbreviations used in this article: KIR, killer-cell Ig-like receptor; *Mamu*, *Macaca mulatta*; JNL, Jurkat NFAT luciferase

Table

Table I. MHC class I ligands of rhesus macaque lineage II KIRs

KIR Gene[a]	Allotype[b]	MHC class I ligands[c]	Group[d]	Refs[e]
KIR3DL01	KIR3DL01*012	Mamu-B*007, -B*022, -B*041, -B*043, -B*065	Bw4	(73)
KIR3DL02	**KIR3DL02*004**	**none detected**		
KIR3DL04	**KIR3DL04*001**	**Mamu-B*007, -B*022, -B*041, -B*043, -B*065**	**Bw4**	
KIR3DL05	KIR3DL05*008	Mamu-A1*002, -A3*13, -B*036, -AG2*01, -AG1*05	A/A-related	(72, 74, 75)
KIR3DL06	KIR3DL06*001	Mamu-B*007, -B*022, -B*041, -B*043, -B*065	Bw4	(75)
KIR3DL07	**KIR3DL07*004**	**Mamu-AG2*01, -B*045**	**A-related**	
KIR3DL08	KIR3DL08*002	Mamu-B*002, -B*007, -B*022, -B*041, -B*065	Bw4	(75)
KIR3DL10	**KIR3DL10*002**	**Mamu-B*007, -B*022, -B*041, -B*043, -B*065**	**Bw4**	
KIR3DL11	**KIR3DL11*001**	**Mamu-B*007, -B*041, -B*065**	**Bw4**	
KIR3DLw34	**KIR3DLw34*002**[f]	**none detected**		
KIR3DS01	**KIR3DS01*003**	**Mamu-AG2*01**	**A-related**	
KIR3DS02	**KIR3DS02*004**	Mamu-AG2*01**, -AG1*05, -B*045**	**A-related**	(82)
KIR3DS03	**KIR3DS03*003**	**none detected**		
KIR3DS04	**KIR3DS04*003**	**Mamu-B*022:01, -B*041, -B*065**	**Bw4**	
KIR3DS05	**KIR3DS05*002**	**none detected**		
KIR3DS06	**KIR3DS06*002**	**Mamu-AG2*01, -AG1*05**	**A-related**	
KIR3DSw07	**KIR3DSw07*001**	**Mamu-AG2*01**	**A-related**	
KIR3DSw09	**KIR3DSw09*003**	**Mamu-AG2*01, -AG1*05, -B*045**	**A-related**	
KIR3DSw39	KIR3DSw39*002[g]	Mamu-B*007, -B*022, -B*041, -B*043, -B*065	Bw4	(75)

[a]Rhesus macaque lineage II KIR genes.

[b]KIR allotypes tested in this study are indicated in bold.

[c]Novel MHC class I ligands defined in this study are indicated in bold.

[d]Group of MHC class I ligands.

[e]References for previously reported KIR ligands.

[f]Previously KIR3DLw03*003 (88).

[g]Previously KIR3DSw08*006 (88).

Figure Legends

FIGURE 1. Rhesus macaque KIRs and KIR-CD3ζ expression on JNL cells. (**A**) An alignment showing the predicted amino acid sequences for the D0, D1 and D2 domains of representative allotypes of rhesus macaque lineage II KIRs. Shaded sequences correspond to predicted MHC class I contact sites (80). Positions of identity are indicated by periods and amino acid differences are identified by their single-letter code. (**B**) Parental and KIR-CD3ζ-transduced JNL cells were stained with a PE-conjugated anti-Flag antibody and Near-IR LIVE/DEAD stain. After excluding dead cells, the fluorescence intensity of KIR-CD3ζ (Flag-tag) staining on KIR-CD3ζ-transduced JNL cells (open) was compared to background staining on parental JNL cells (shaded). Flow cytometry data was analyzed using FlowJo 10.6 for Mac OSX.

FIGURE 2. KIR3D04*001, KIR3DL10*002, KIR3DL11*001 and KIR3DS04*003 interact with multiple Mamu-Bw4 ligands. (**A**) JNL cells expressing the extracellular domains of KIR3DL04*001, KIR3DL10*002, KIR3DL11*001 and KIR3DS04*003 fused to the transmembrane and cytoplasmic domains of human CD3ζ were incubated overnight at a 1:1 E:T ratio with 721.221 cells expressing the indicated Mamu-B molecules. The bar graphs represent the mean and standard deviation (error bars) of luciferase activity (RLU) from triplicate wells of KIR-CD3ζ JNL cells incubated with Mamu-B+721.221 cells (blue), parental 721.221 cells (red) and anti-Flag plus anti-mouse antibodies (gray). Asterisks denote significant differences by one-way ANOVA with Dunnett test (**** $p < 0.0001$). These results are representative of at least three independent experiments. (**B**) Parental and Mamu-B+ 721.221 cells were stained with Near-IR LIVE/DEAD dye, KIR-Fc fusion proteins consisting of the extracellular domains KIR3DL04*001, KIR3DL10*002, KIR3DL11*001 and KIR3DS04*003 fused to the Fc domain

of murine IgG2A followed by AF647-conjugated goat anti-mouse IgG and PE-conjugated MHC class I-specific antibody (W6/32). Data was collected using a FACSymphony™ A3 Cell Analyzer and analyzed using FlowJo 10.8.0 for Mac OSX.

FIGURE 3. KIR3DL07*004, KIR3DS01*003, KIR3DS02*004, KIR3DS06*002, KIR3DSw07*001 and KIR3DSw09*003 interact with Mamu-AG ligands. (**A**) JNL cells expressing the extracellular domains of KIR3DL07*004, KIR3DS01*003, KIR3DS02*004, KIR3DS06*002, KIR3DSw07*001 and KIR3DSw09*003 fused to the transmembrane and cytoplasmic domains of human CD3ζ were incubated overnight at a 1:1 E:T ratio with 721.221 cells expressing the indicated Mamu-AG molecules. The bar graphs represent the mean and standard deviation (error bars) of luciferase activity (RLU) from triplicate wells of KIR-CD3ζ JNL cells incubated with Mamu-AG+ 721.221 cells (purple), parental 721.221 cells (red) and anti-Flag plus anti-mouse antibodies (gray). Asterisks denote significant differences by one-way ANOVA with Dunnett test (**** $p < 0.0001$). These results are representative of at least three independent experiments. (**B**) Parental and Mamu-AG+721.221 cells were stained with Near-IR LIVE/DEAD dye, KIR-Fc fusion proteins consisting of the extracellular domains KIR3DL07*004, KIR3DS01*003, KIR3DS02*004, KIR3DS06*002, KIR3DSw07*001 and KIR3DSw09*003 fused to the Fc domain of murine IgG2A followed by AF647-conjugated goat anti-mouse IgG and PE-conjugated MHC class I-specific antibody (W6/32). Data was collected using a FACSymphony™ A3 Cell Analyzer and analyzed using FlowJo 10.8.0 for Mac OSX.

FIGURE 4. KIR3DL07*004, KIR3DS02*004, and KIR3DSw09*003 interact with Mamu-B*045:03. (**A**) JNL cells expressing the extracellular domains of KIR3DL07*004, KIR3DS02*004, and KIR3DSw09*003 fused to the transmembrane and cytoplasmic domains of

human CD3ζ were incubated overnight at a 1:1 E:T ratio with 721.221 cells expressing the indicated Mamu-B molecules. The bar graphs represent the mean and standard deviation (error bars) of luciferase activity (RLU) from triplicate wells of KIR-CD3ζ JNL cells incubated with Mamu-B+721.221 cells (blue), parental 721.221 cells (red) and anti-Flag plus anti-mouse antibodies (gray). Asterisks denote significant differences by one-way ANOVA with Dunnett test (**** $p < 0.0001$). These results are representative of at least three independent experiments. (**B**) Parental and Mamu-B*045:03+721.221 cells were stained with Near-IR LIVE/DEAD dye, KIR-Fc fusion proteins consisting of the extracellular domains KIR3DL07*004, KIR3DS02*004, and KIR3DSw09*003 fused to the Fc domain of murine IgG2A followed by AF647-conjugated goat anti-mouse IgG and PE-conjugated MHC class I-specific antibody (W6/32). Data was collected using a FACSymphony™ A3 Cell Analyzer and analyzed using FlowJo 10.8.0 for Mac OSX.

FIGURE 5. Mamu-B*045:03 has an α1-domain similar to Mamu-A molecules. **(A)** Amino acid sequence alignment of rhesus macaque MHC class I molecules. α1 domain residues 77-83 corresponding to Bw4 or Bw6 motifs are underlined and shaded sequences correspond to predicted KIR contact sites (80). Positions of identity are indicated by periods and amino acid differences are identified by their single-letter code. Phylogenetic trees of coding nucleotide sequence for the α1-α3 domains of Mamu-A and -B **(B)** and the α1 domains of Mamu-A and -B molecules (**C**). Neighbor-joining analysis was performed using MAFFT (version 7, publicly available at https://mafft.cbrc.jp/alignment/server/). Bootstrap support of greater than 50% is indicated.

FIGURE 6. Phylogeny of the D0-D2 domains of rhesus macaque KIRs reflects ligand recognition. Neighbor-joining analysis of predicted amino acid sequences of the D0-D2 domains of human and rhesus macaque KIRs was performed using MAFFT. Bootstrap support of greater than 50% is indicated. Human KIR3DL1*0010101 and KIR3DL02*0010101 were used as an outgroup. Rhesus macaque KIRs are color coded according to their MHC class I ligands: Mamu-A-related (dark blue), Mamu-AG2*01:01 (blue), Mamu-B*045:03 (light blue), Mamu-Bw4 (green), and no ligands found (gray).

A

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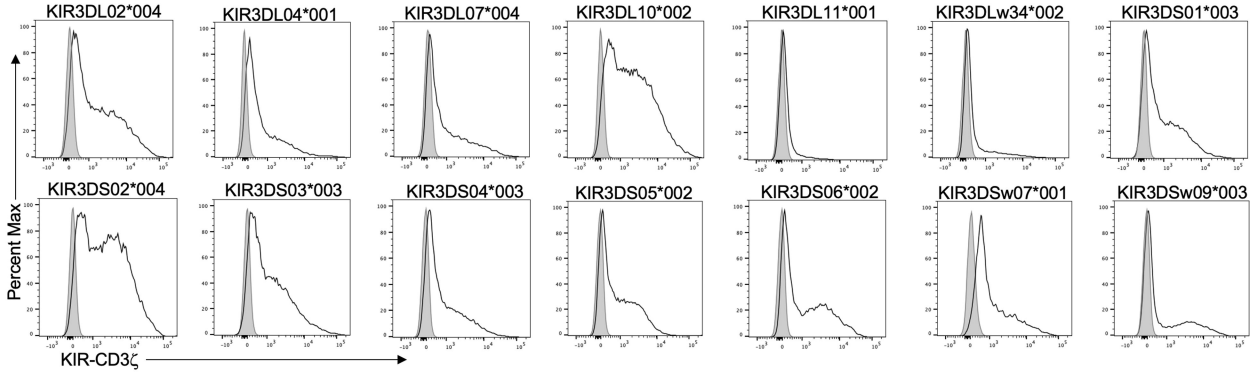
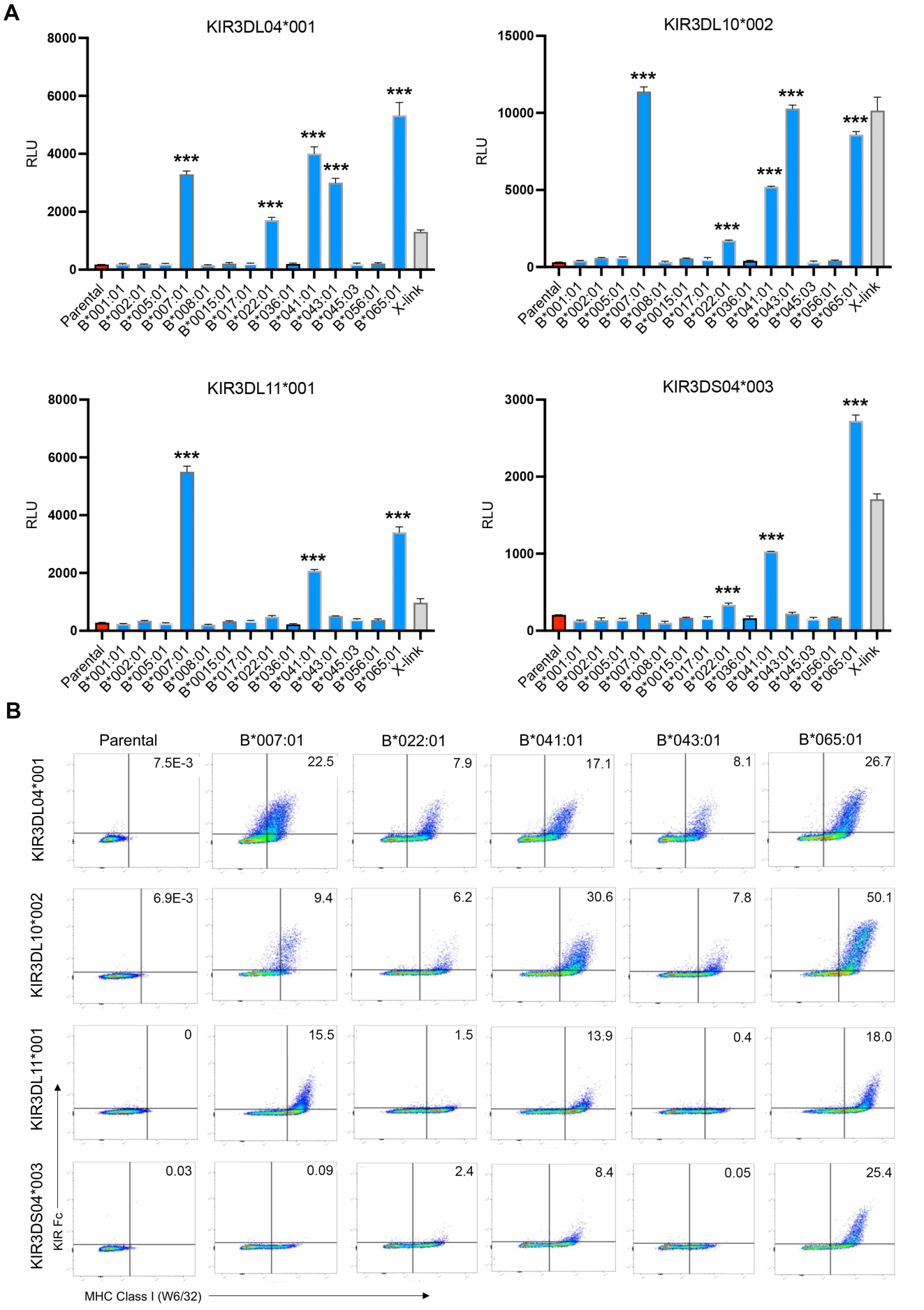
D0 Domain                10        20        30        40        50        60        70        80        90       100
Consensus       HTGGQDKTFLSARPSAVVPQGGHVTLRCYYRRGLNNFTNFTLYKDDRSHVPIFHSRIFQESFLMGPVTPAHAGTYRCRGSYPHSPTEWSALSDPLAIXVT
KIR3DL02*004    Y......N....W...L...........H..G.---.N.........................................L....TP.N....R..
KIR3DL04*001    ................L.................H.................PG...HQ..........D..........Q..........E....M..
KIR3DL07*004    ..........F.....................D.......................................................R..
KIR3DL10*002    Y..........VQ...L................F.................N....Q..........D.........................M..
KIR3DL11*001    ........S...W..P..........Q.H....F.................PG...HQ.....................................M..
KIR3DLw34*002   Y.....N.....W..P............H..G.---.N...........I.............................................R..
KIR3DS01*003    ........................FQ...H.........................Q.....................................R..
KIR3DS02*004    ..........F.....................D.......................................................M..
KIR3DS03*003    .......I...VQ...L......M....H.....Y.................N...............D.........................M..
KIR3DS04*003    ......N.........L.........Q.H....F.................V.......Q.....................................M..
KIR3DS05*002    ......NA....W..P............H..G.---.N.........................................P.N....R..
KIR3DS06*002    ...A...N..F.W..P......L.......H.......................................................M..
KIR3DSw07*001   ................L..............D.................V..N.........................................R..
KIR3DSw09*003   ...D......F.....................D.......................................................R..

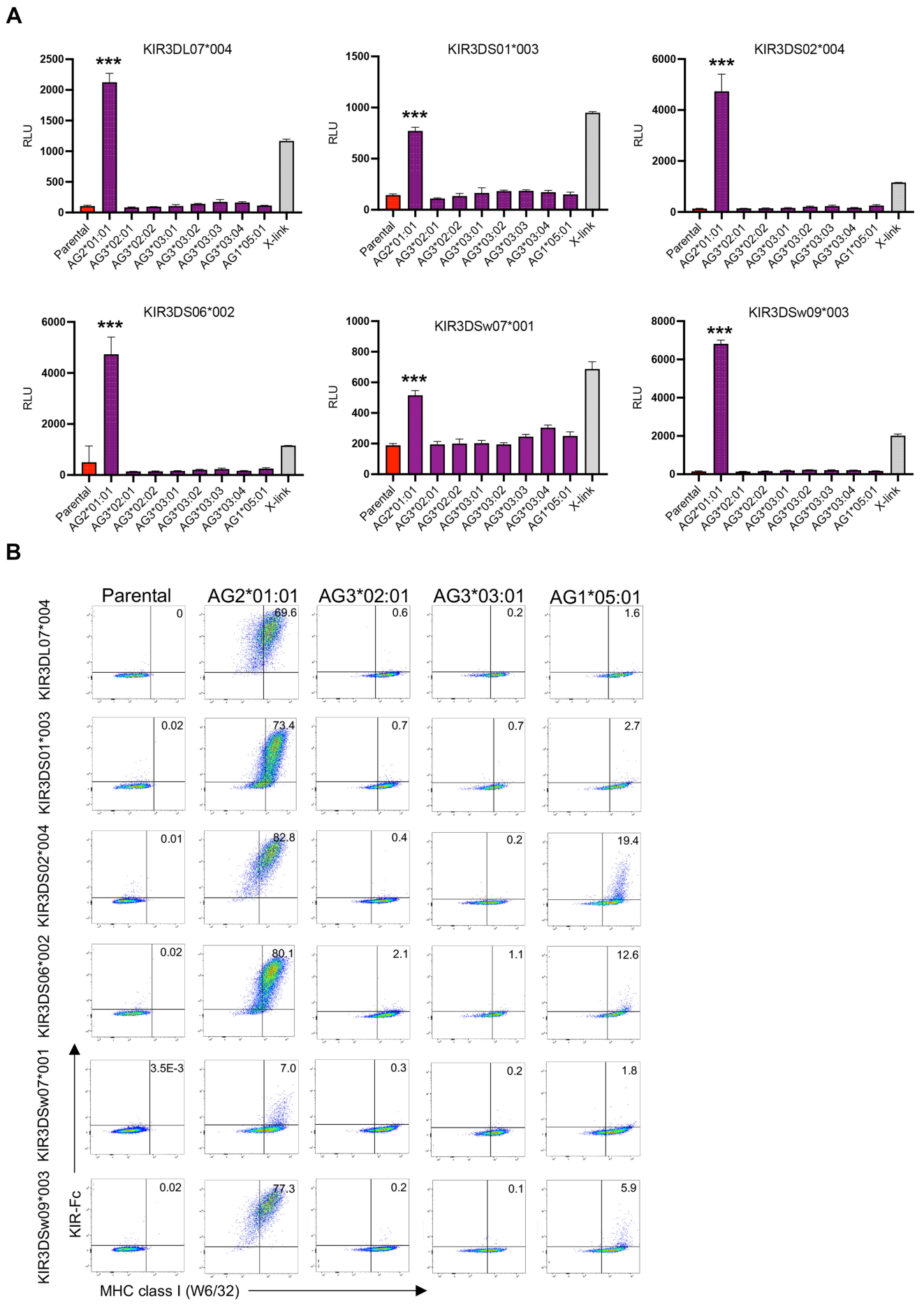
D1 Domain                110       120       130       140       150       160       170       180       190       200
Consensus       GVHRKPSLLALPGPLVKSGETVTLQCSSDTVFXHFFLHSEVTFEKPLHLVGELHGGGSQANYSINSTTSDLAGTYRCYGSVTHSPYVLSAPSDPLDIVIT
KIR3DL02*004    ...K.............................E.....R...L.E.....................MM......F.....F...................
KIR3DL04*001    ..............................I..G...........EL.....................................................
KIR3DL07*004    ..............................E........N.........................................D...............
KIR3DL10*002    ..............................I..E.....................................EE.F....................N....
KIR3DL11*001    ..............................G...........EL.........................E.F....................N....
KIR3DLw34*002   ...K.............................E.....R...L.E..............K.................F...................
KIR3DS01*003    ...K.............................G.......................................................
KIR3DS02*004    ..............................G.................R............KM...............H...............
KIR3DS03*003    ..............................G.................................K.....E.........................
KIR3DS04*003    ......................I......I..G...........EL.....................................T......
KIR3DS05*002    ...K.............................E.....R...L.E..............K.........F...F.....F...................
KIR3DS06*002    ................L..............E........N.......................K..E.......................R.....
KIR3DSw07*001   ..................D............G.................................K.....E.........................
KIR3DSw09*003   ......................I.........E........N.........................................................

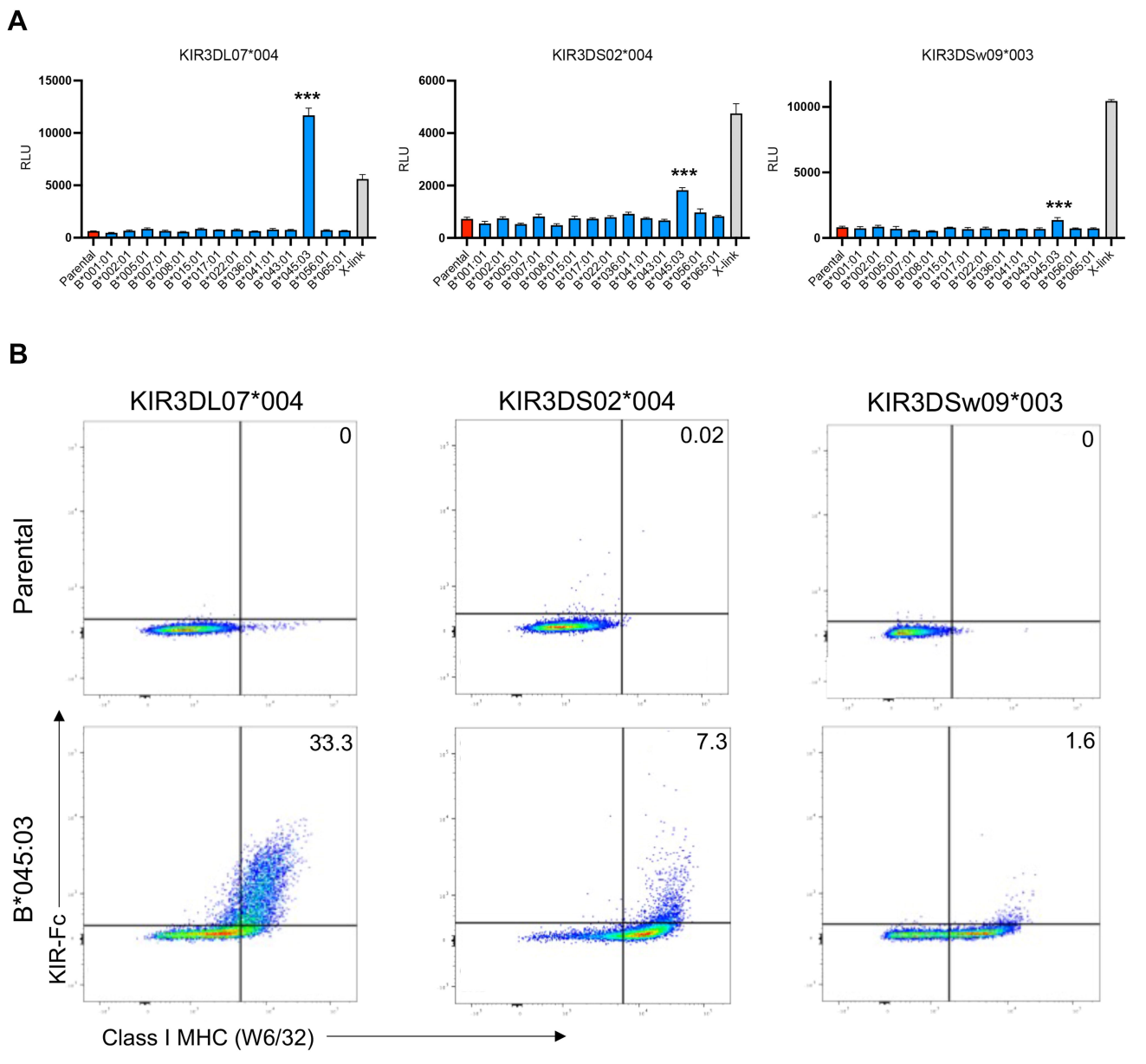
D2 Domain                210       220       230       240       250       260       270       280       290     298
Consensus       GKYEKPSLSAQPGPTVQAGENVTLSCSSXNSFDMYHLSREGEARELSLSAVPSVNGTFQADFPLGPATHGGTYRCFGSFRTAPYKWSDPSDPLPVSVT
KIR3DL02*004    ...............I............RC.......................................................Q.............
KIR3DL04*001    ..............................Q...............................S.......................Q........H....
KIR3DL07*004    ..............................Q.......................................................................
KIR3DL10*002    ..............................Q.............S....V...R.......G...........I........IT.....NS....L....
KIR3DL11*001    ......................I.....RC.......................................................................
KIR3DLw34*002   .L............................RC.................R.......G...................N......H..........
KIR3DS01*003    .L.................D.......QI.......................................................S...Q........HI...
KIR3DS02*004    .L............................RR.................R.......G...................H.T.....H..........
KIR3DS03*003    ..............................Q...............G......Q.......................................
KIR3DS04*003    ..............................RC...............I...................................T...............K
KIR3DS05*002    .L............................RR...............................-....................TS....H..........
KIR3DS06*002    .L............................Q...................A..R.......G...................H.T.....H..........
KIR3DSw07*001   ..............................Q...............G......Q...R.........................Q.............
KIR3DSw09*003   .L............................RC.................R.......G.......................H..........
```

B




A
KIR3DL04*001
KIR3DL10*002
KIR3DL11*001
KIR3DS04*003
RLU
Parental
B*001:01
B*002:01
B*005:01
B*007:01
B*008:01
B*0015:01
B*017:01
B*022:01
B*036:01
B*041:01
B*043:01
B*045:03
B*056:01
B*065:01
X-link
B
Parental
B*007:01
B*022:01
B*041:01
B*043:01
B*065:01
KIR3DL04*001
KIR3DL10*002
KIR3DL11*001
KIR3DS04*003
7.5E-3
22.5
7.9
17.1
8.1
26.7
6.9E-3
9.4
6.2
30.6
7.8
50.1
0
15.5
1.5
13.9
0.4
18.0
0.03
0.09
2.4
8.4
0.05
25.4
KIR Fc
MHC Class I (W6/32)


A
KIR3DL07*004
KIR3DS01*003
KIR3DS02*004
KIR3DS06*002
KIR3DSw07*001
KIR3DSw09*003
RLU
***
Parental
AG2*01:01
AG3*02:01
AG3*02:02
AG3*03:01
AG3*03:02
AG3*03:03
AG3*03:04
AG1*05:01
X-link
B
Parental
AG2*01:01
AG3*02:01
AG3*03:01
AG1*05:01
KIR3DL07*004
KIR3DS01*003
KIR3DS02*004
KIR3DS06*002
KIR3DSw07*001
KIR3DSw09*003
0
69.6
0.6
0.2
1.6
0.02
73.4
0.7
0.7
2.7
0.01
82.8
0.4
0.2
19.4
0.02
80.1
2.1
1.1
12.6
3.5E-3
7.0
0.3
0.2
1.8
0.02
77.3
0.2
0.1
5.9
KIR-Fc
MHC class I (W6/32)


A
KIR3DL07*004
KIR3DS02*004
KIR3DSw09*003
RLU
15000
10000
5000
0
6000
4000
2000
***
Parental
B*001:01
B*002:01
B*005:01
B*007:01
B*008:01
B*015:01
B*017:01
B*022:01
B*036:01
B*041:01
B*043:01
B*045:03
B*056:01
B*065:01
X-link
B
KIR3DL07*004
KIR3DS02*004
KIR3DSw09*003
Parental
B*045:03
0
0.02
0
33.3
7.3
1.6
KIR-Fc
Class I MHC (W6/32)

A

```
α1 Domain          10        20        30        40        50        60        70        80        90
Consensus GSHSMRYFYTSVSRPGRXEPRFIAVGYVDDTQFVRFDSDAESPRMEPRAPWVEQEGPEYWDRETRNMKTXTQTARVNLRTLLRYYNQSEX
A1*002:01 ...........M.....W.......................A.Q.........................AE..N.P....N.RG......A
A1*004:01 .....S....YM.....G....V..................A.Q.......................I...E..N.P.A..N.RG......A
A2*05:02  ....L......M.....GQ....S....................E................QN..IC.AD...L.E.............A
A3*13:03  ...........M.....G.......................A.Q.....................Q....E..N.P.A..N.RG......V
A4*14:03  ...........M.....G................Q.....A.Q.........................A..N.P.A..N.........A
A6*01:03  ........H.CM.....G.......................................E........QK...E..M.P.A..N.RG......A
AG2*01:01 ..................W.............................................Q....P...Y................A
B*045:03  ....L...H.A......W.....S...........Y........E......M........EEQ..IA.ANA..H.G.....RG......G
B*002:01  ...F....S.A......R..WYLE.......................................N...S.VTA..F..G.GN.RG.....KA
B*007:01  ....L...S.A......R..WYFE...........................M........EEA..RA.GNA..H.G....A........G
B*022:01  ....L...T.A......R..WYLE..........W................M..........N..RA.ANA..D.G....A........G
B*036:01  ..................G.............................................Q..Q....A...D.AD.G.........RG
B*041:01  ........S........R..WHLE...........................A........EEQ..IA.GHA..E.G....A........G
B*043:01  V.......S.A......R..WYLE..........W...........................N..RA.GNA..E.GS............G
B*065:01  ....L...S........R..WHLE...........................A........EE...RA.GHA..E.G....A........G
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α2 Domain         100       110       120       130       140       150       160       170       180
Consensus GSHTIQWMYGCDLGPDGRLLRGYEQFAYDGKDYIALNEDLRSWTAADMAAQNTQRKWEAAGEAEQXRAYLEGECLEWLRRYLENGKETLQRA
A1*002:01 ......R................H.S.........................................H.T.........................
A1*004:01 ....Y.V...............................D.........L.............V...H.T.........................
A2*05:02  ....L.T................D.S....R...............................V...W......Q...S...............
A3*13:03  ....F.R.V................Y....R...............................DV..RM........V...P.............
A4*14:03  ....F.R.V..........Q..........R............F...V.............V...D.V....R.V.................
A6*01:03  ......V................S.Y....R.........................TLQT.SA...H......L.V.S...............
AG2*01:01 ......K.......................R...I...........................A...H.T.......................
B*045:03  ......K................D.Y........V..........................RA..RQ.....................M....
B*002:01  ....L..................H..........................................M......T.V.....H...........
B*007:01  ....L.............................................RF........RA...L......K...........QN.S.L..
B*022:01  ......................H..........................................R....Q..............H...........
B*036:01  ......T.......................R................L..................W........V.................
B*041:01  ......................H.L.......................T.RF........R...RW............S.............
B*043:01  ....F.R................D.S.......................RF........R....R......T.V.....H...........
B*065:01  ....L..................D.L.......................T.RF........R....W............S.............
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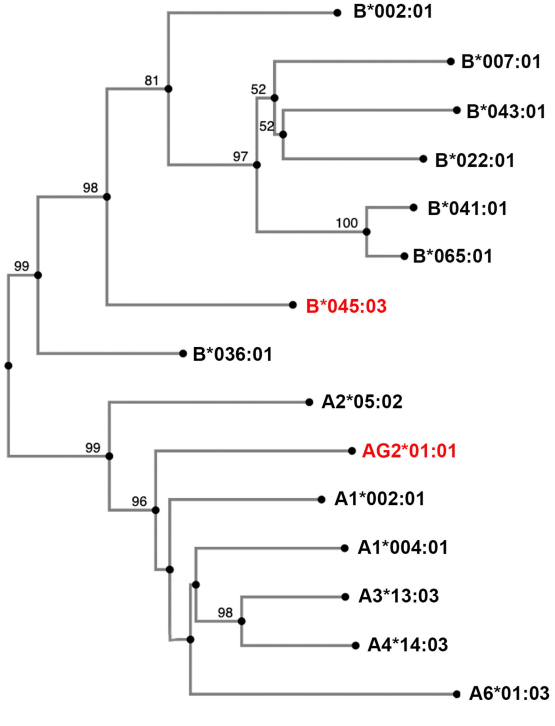
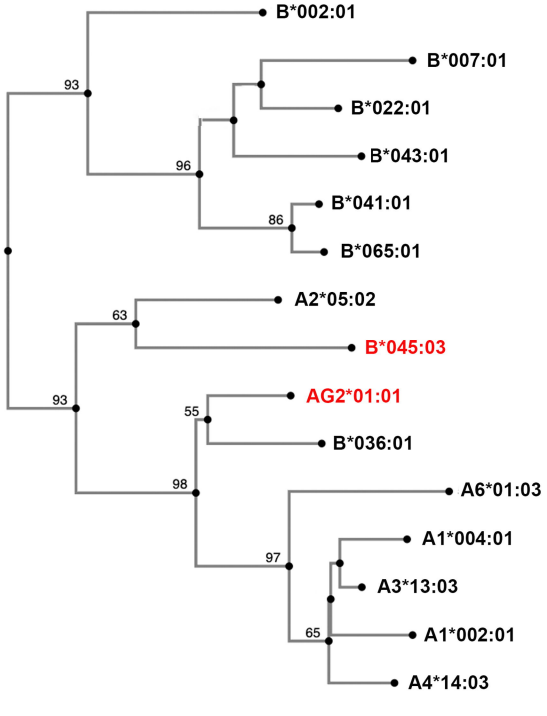
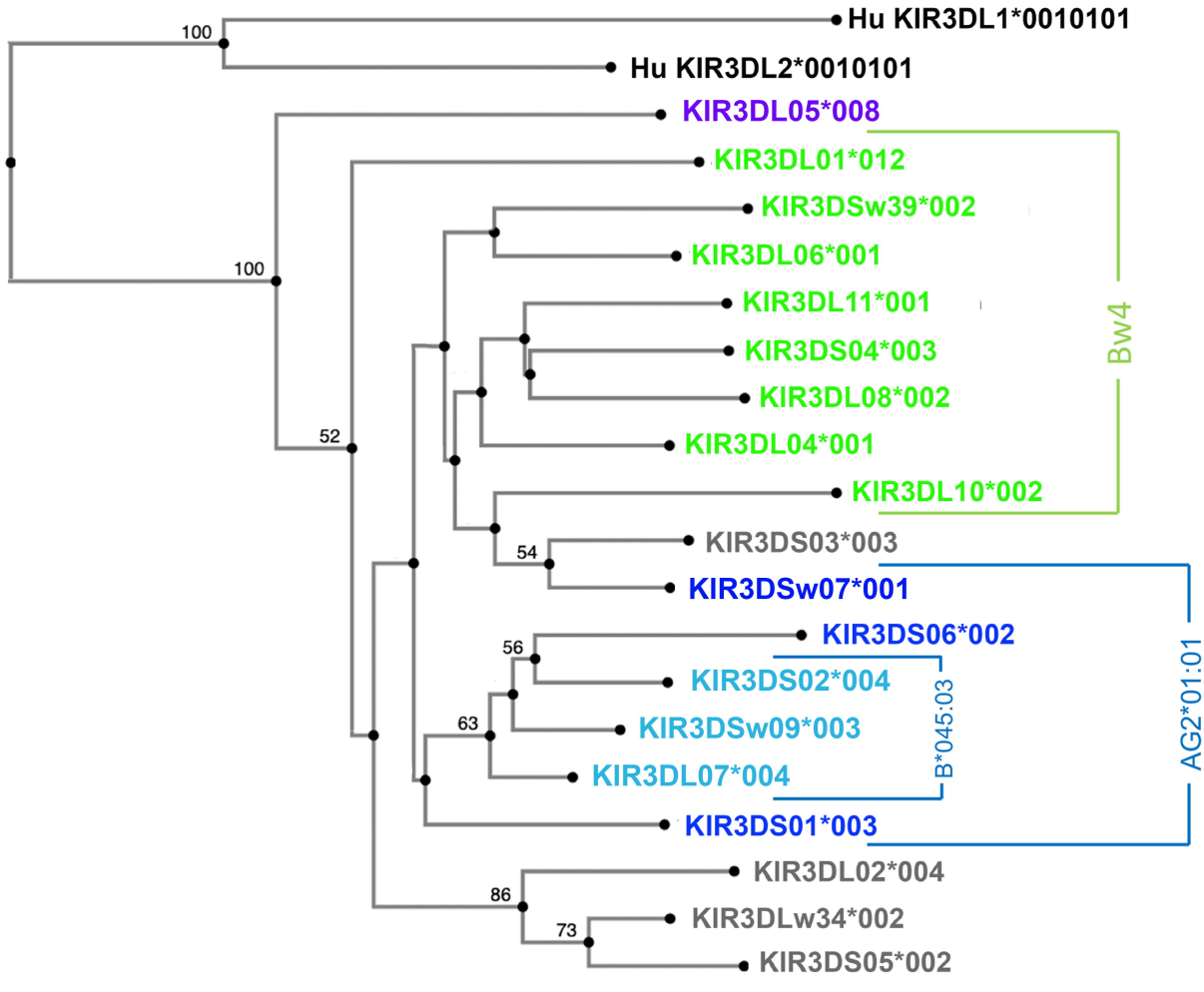
B



C




Hu KIR3DL1*0010101
Hu KIR3DL2*0010101
KIR3DL05*008
KIR3DL01*012
KIR3DSw39*002
KIR3DL06*001
KIR3DL11*001
KIR3DS04*003
KIR3DL08*002
KIR3DL04*001
KIR3DL10*002
KIR3DS03*003
KIR3DSw07*001
KIR3DS06*002
KIR3DS02*004
KIR3DSw09*003
KIR3DL07*004
KIR3DS01*003
KIR3DL02*004
KIR3DLw34*002
KIR3DS05*002
100
100
52
54
56
63
86
73
Bw4
B*045:03
AG2*01:01