Immunogenetics (2019) 71:321–333
https://doi.org/10.1007/s00251-018-01100-x

ORIGINAL ARTICLE

Evidence of functional *Cd94* polymorphism in a free-living house mouse population

Linn E. Knutsen[1] · Erik Dissen[2] · Per C. Saether[2] · Elisabeth Gyllensten Bjørnsen[2] · Jaroslav Piálek[3] · Anne K. Storset[1] · Preben Boysen[1]

Received: 11 October 2018 / Accepted: 30 November 2018 / Published online: 10 December 2018

Abstract

The CD94 receptor, expressed on natural killer (NK) and CD8+ T cells, is known as a relatively non-polymorphic receptor with orthologues in humans, other primates, cattle, and rodents. In the house mouse (*Mus musculus*), a single allele is highly conserved among laboratory strains, and reports of allelic variation in lab- or wild-living mice are lacking, except for deficiency in one lab strain (DBA/2J). The non-classical MHC-I molecule Qa-1b is the ligand for mouse CD94/NKG2A, presenting alternative non-americ fragment of leader peptides (Qa-1 determinant modifier (Qdm)) from classical MHC-I molecules. Here, we report a novel allele identified in free-living house mice captured in Norway, living among individuals carrying the canonical *Cd94* allele. The novel $Cd94^{LocA}$ allele encodes 12 amino acid substitutions in the extracellular lectin-like domain. Flow cytometric analysis of primary NK cells and transfected cells indicates that the substitutions prevent binding of CD94 mAb and Qa-1b/Qdm tetramers. Our data further indicate correlation of *Cd94* polymorphism with the two major subspecies of house mice in Europe. Together, these findings suggest that the $Cd94^{LocA}$/NKG2A heterodimeric receptor is widely expressed among *M. musculus* subspecies *musculus,* with ligand-binding properties different from mice of subspecies *domesticus,* such as the C57BL/6 strain.

Keywords NK cell · Inhibitory/activating receptors · MHC · Comparative immunology/evolution · Genomics

Abbreviations

M. m.	*Mus musculus*
B6	C57BL/6
NKC	Natural killer gene complex
Qdm	Qa-1 determinant modifier
KIR	Killer cell immunoglobulin-like receptor

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00251-018-01100-x) contains supplementary material, which is available to authorized users.

✉ Preben Boysen
preben.boysen@nmbu.no

[1] Faculty of Veterinary Medicine, Department of Food Safety and Infection Biology (MatInf), Norwegian University of Life Sciences (NMBU), Oslo, Norway

[2] Division of Anatomy, Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

[3] Research Facility Studenec, Institute of Vertebrate Biology of the Czech Academy of Sciences, Brno, Czech Republic

Introduction

NK cells play an essential role in the defense against transformed and pathogen-infected cells. Their cytotoxic and cytokine-producing activity is a result of the balance of activating and inhibitory stimuli through multiple germline-encoded receptors. The killer cell immunoglobulin-like receptors (KIRs) are the most diverse group of NK cell receptors in humans, whereas the structurally unrelated killer cell lectin-like receptors (KLRs) include the largest family of rodent NK cell receptors, the Ly49 multigene family (Lanier 2008; Natarajan et al. 2002). Both the KIRs and Ly49 receptors are encoded by multigenic families of polymorphic genes that encode both activating and inhibitory receptors. Two other KLR families, CD94 (KLRD) and NKG2 (KLRC), are expressed as heterodimers on NK cells and subsets of T cells (Gunturi et al. 2004; Borrego et al. 2006). In contrast to KIR and Ly49, CD94/NKG2 function and genomic organization are conserved between mammalian species, with orthologues in humans, other primates, cattle, and rodents (Gunturi et al. 2004; Borrego et al. 2006; Vance et al. 1997; Dissen et al. 1997; Shum et al. 2002; Birch and Ellis 2007).

Several inhibitory receptors expressed on NK cells recognize MHC class Ia molecules, which are ubiquitously expressed on nucleated cells, and protect these from killing by NK cells (Pegram et al. 2011). Downregulation of MHC-Ia is common in virally infected and transformed cells, as a way of avoiding recognition and killing by cytotoxic T cells (Mariuzza and Li 2014). When cells deficient in MHC-Ia expression are encountered by NK cells, the lack of inhibitory stimuli may lead to activation and killing by NK cells, according to the "missing self-hypothesis" (Pegram et al. 2011; Ljunggren and Karre 1990). The inhibitory CD94/NKG2A receptor recognizes the non-classical MHC-I-like (MHC-Ib) molecule HLA-E in humans and the orthologue Qa-1b in mice (Lee et al. 1998; Vance et al. 1998; Braud et al. 1998a). Like HLA-E, Qa-1b presents a non-americ peptide derived from the signal sequence of classical MHC-Ia molecules in a TAP-dependent manner (Aldrich et al. 1994; Braud et al. 1998b). In mice, this peptide has the amino acid sequence AMAPRTLLL and is referred to as Qa-1 determinant modifier (Qdm) (Aldrich et al. 1994). Thus, the reportedly conserved and non-polymorphic CD94/NKG2A receptor system allows NK cells to monitor the biosynthesis of highly polymorphic class Ia molecules, as well as TAP function (Vance et al. 1998; Vance et al. 1999). In CD8+ T cells, several studies have indicated that CD94/NKG2A may downregulate T cell responses to viral infections through interaction with Qa-1b/Qdm (Zhou et al. 2008; Moser et al. 2002).

Whereas NKG2A carries a tandem ITIM motif and transmits inhibitory signals via association with the tyrosine phosphatases, SHP-1 and SHP-2, NKG2C, and NKG2E lack ITIMs. In most mammals, NKG2C and -E carry a positively charged residue in their transmembrane regions that form a salt bridge to the activating ITAM-containing adaptor protein DAP12, allowing CD94/NKG2C and CD94/NKG2E heterodimeric receptors to activate NK cell effector functions. In the mouse and rat, however, NKG2C and NKG2E lack the ability to bind DAP12. Instead, in these species, CD94 binds directly to DAP12 and DAP10 via a lysine residue in the transmembrane region of CD94 (Vance et al. 1999; Saether et al. 2011). Mouse NKG2C and NKG2E bind to Qa-1b/Qdm with lower affinity than CD94/NKG2A (Vance et al. 1999). Several studies support that human CD94/NKG2C and CD94/NKG2E play a role in virus infections, including studies indicating that CD94/NKG2C is involved in expansion of NK cells in cytomegalovirus-infected patients (Lopez-Botet et al. 2014). In C57BL/6 (B6) mice, CD94 has been described as essential for resistance to mousepox, a disease caused by the ectromelia orthopox virus. A study investigating CD94-deficient mice found that the susceptibility to mousepox was caused by lack of activating CD94/NKG2E heterodimers on NK cells (Fang et al. 2011). Contrasting to this, a study with NKG2A-deficient mice found that mice deficient in NKGA expression were susceptible to mousepox infection with results indicating that the lack of inhibitory CD94/NKG2A on T cells and resulting activation induced cell death (ACID). Furthermore, results from this study indicated that NKG2A is the only NKG2 receptor expressed on B6 NK cells, with no detectable NKG2C/E (Rapaport et al. 2015).

The *Cd94* gene (also referred to as *Klrd1*) is highly conserved in humans and chimpanzees and no polymorphism has been reported in either species. Moreover, the coding region differs only by two nucleotide substitutions between the two species (Shum et al. 2002). In the house mouse (*Mus musculus*), *Cd94* is located in the natural killer gene complex (NKC) on chromosome 6 and consists of six exons (23, 24). It encodes a 179 residue type II transmembrane protein consisting of a C-type lectin-like domain, a stalk, transmembrane region, and a short cytoplasmic tail. An early study of *CD94* in house mice described five nucleotide substitutions which translated to one amino acid substitution between the *Cd94* alleles of the inbred strains B6 and CB.17 SCID (Vance et al. 1997). This study also indicated limited *Cd94* polymorphism among inbred strains of mice, as Southern blot analysis performed on restriction enzyme digested DNA revealed identical restriction patterns.

The DBA/2J strain is naturally deficient in CD94 surface expression, due to a spontaneous deletion including exon 6 of the *Cd94* gene, which encodes the C-terminal end of the CD94 molecule (Vance et al. 2002; Wilhelm et al. 2003). Another study reported lack of binding of a CD94 monoclonal antibody (mAb) to NK cells from the wild-derived PWK/Pas strain (Adam et al. 2006). To our knowledge, these are the only reports of anomaly in CD94 expression in laboratory strains of the house mouse, while to our knowledge, no reports document the expression or polymorphism of CD94 in natural house mouse populations.

Among the classical laboratory mouse strains, there is limited genetic diversity as they have been inbred in laboratories for decades and are predominantly derived from fancy mice during the twentieth century (Beck et al. 2000). Most of these strains, including B6, derive from complex historical mixtures of three genomes of *M. musculus* subspecies. Their genetic backgrounds are dominated by *Mus musculus* (*M. m.*) *domesticus* origin, with variable contribution by *M. m. musculus* and *Mus musculus castaneus* (Yang et al. 2011). Wild-derived laboratory strains on the other hand are directly derived from wild-caught progenitors of the different subspecies or hybrids of these. These mouse strains represent subspecies that are actively adapting to evolutionary pressure and may serve as a valuable source of polymorphism compared to the inbred strains (Guenet and Bonhomme 2003).

During a previous study where we captured free-living mice for immunophenotyping, we noted that certain individual mice did not stain with a CD94 mAb and thus left these mice and the marker out of the study (Boysen et al. 2011). We here explore this phenomenon further and report the finding of

an aberrant, functionally divergent *Cd94* allele associated with the *M. m. musculus* subspecies of house mice, representing the first documentation of CD94 polymorphism in free-living house mouse populations.

Materials and methods

Animals

All the animals were captured, handled, and euthanized following Norwegian legislation, after approval from the Norwegian Food Safety Authority (FOTS ID 8198) and the Norwegian Environment Agency (ref. 2014/7215). The wild house mice were captured alive using Ugglan traps (Grahnab, Gnosjö, Sweden) from five separate locations at different times in southeastern Norway. Seven individuals were captured at location A (Skiptvet [N: 59° 28′ N; E: 11° 10′]) at two time points, one at location B (Eidsberg [N: 59° 30′; E: 11° 18′]) and three at location C (Maura [N: 60° 15′; E: 11° 01′]), location D (Rakkestad [N: 59° 25′; E: 11° 21′]), and location E (Mysen [N: 59° 33′; E: 11° 19′]) (Fig. 1). The mice were transported to the Norwegian University of Life Sciences campus Adamstuen in plastic cages and kept in these for a maximum of 4 days prior to euthanasia and analysis. Lab mouse material consisting of 10 control C57BL/6J (B6) mice were kept in a specific pathogen-free unit at the Norwegian University of Life Sciences and the University of Oslo. Splenic samples from eight wild mice that have been a priori assigned to either *M. m. musculus* or *M. m. domesticus* subspecies using hybrid index based on five X-linked loci (32) were obtained from a mouse genetic repository in Studenec, Czech Republic. The four *M. m. musculus* individuals originate from Buškovice (Czechia, [N: 50° 13′, E: 13° 23′]), Kaerepere (Estonia, [N: 58° 57′, E: 24° 50′]), Šenkvice (Slovakia, [N: 48° 18′, E: 17° 21′]), and Lindhorst (Germany, [N: 53° 26′, E: 13° 46′]) and the four *M. m. domesticus* individuals from Feldkirch (Austria [N: 47° 15′, E: 9° 35′]), Schweben (Germany, [N: 50° 26′, E: 9° 35′]), Scar, Sunday Island (Scotland, [N: 59° 18′, E: -2° 33′]), and Suckow (Germany, [N: 53° 25′, E: 12° 20′]). The breeding facility at the Institute of Vertebrate Biology in Studenec is approved for keeping and supplying experimental animals (ref. 61974/2017-MZE-17214).

PCR

Total RNA was isolated using QIAzol (Qiagen), chloroform, and RNeasy mini kit (Qiagen), either from pelleted splenocytes or from intact spleen tissue homogenized using

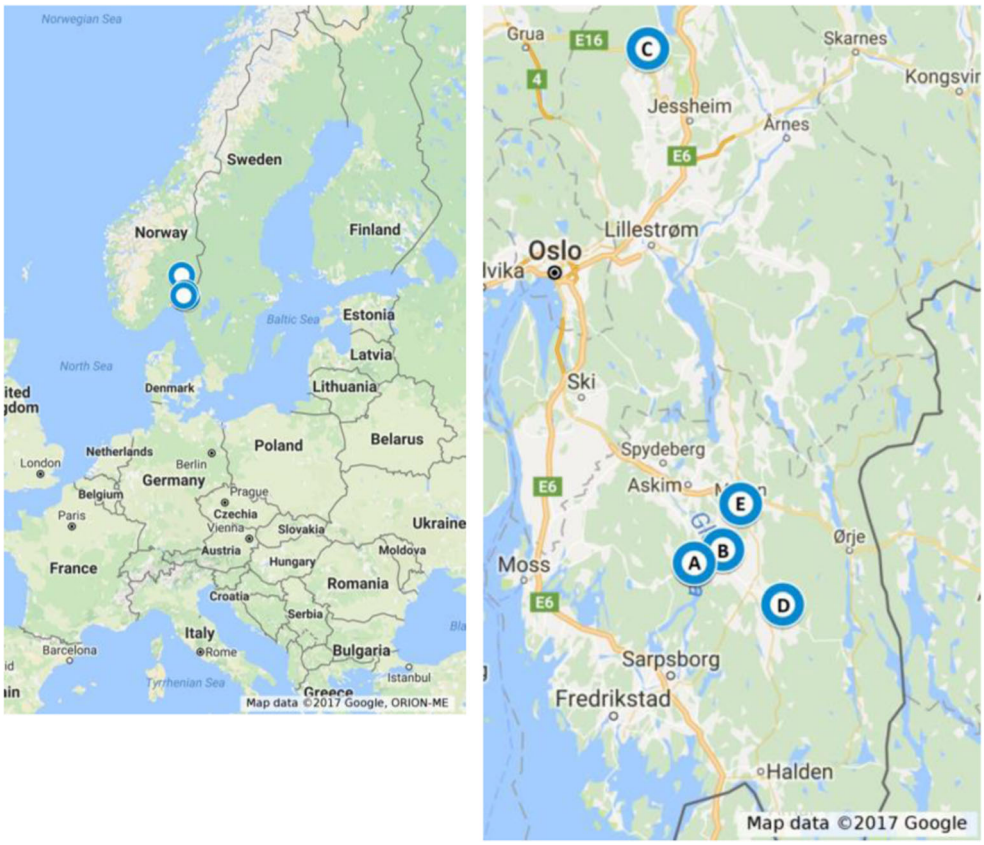


Fig. 1 Locations for collection of free-living mice. Free-living house mice (*Mus musculus*) were collected at five locations in southeastern Norway, indicated as location A–E on the map

Qiagen tissue lyser (Qiagen). cDNA synthesis from total RNA was performed using Superscript III RT following the manufacturer's protocol (Invitrogen). RT-PCR was performed using a forward primer spanning the exon 1-exon 2 junction (5' GGAGACTGATGTCTGTCATCTTTGG 3′), a reverse primer from exon 3 (5' ATTGGGGCTGAAGAAGGCTGG 3′), and Taq polymerase (Invitrogen), with five initial cycles of touchdown followed by 35 cycles with 60 °C annealing temperature. The 276-bp PCR products were analyzed by gel electrophoresis.

Cd94 allele typing was performed by RT-PCR with a common forward primer (5′-AACATCACTTCTCATGGCAG-3′) and reverse primers specific for either the $Cd94^{LocA}$ (5′ CTATTTTTCTTGGACTATAAACAGTGCAGTTC 3′) or $Cd94^{B6}$ (5′ CGCTTTTGCTTGGACTGTAAACAATG CAGTGC 3′) alleles, producing a PCR fragment of 494 bp.

DNA was extracted from manually homogenized liver samples using MasterPure Complete DNA and RNA Purification Kit (Epicentre biotechnologies). To analyze for genetic markers diagnostic for the *M. m. musculus* and *M. m. domesticus* subspecies, PCR genotyping was carried out with Taq polymerase. Primers and PCR conditions were as described for individual loci, *Abpa* on chromosome 7 (Dod et al. 2005), *D11cenB2* on chromosome 11 (Lanneluc et al. 2004), and *Btk* on the X chromosome (Munclinger et al. 2002), and summarized in Searle et al. (Searle et al. 2009). PCR products were analyzed by gel electrophoresis and classified as *musculus*-type or *domesticus*-type alleles.

cDNA cloning and sequencing

The *Cd94* open reading frame (ORF) plus 149 bp in the 3′ untranslated region (UTR) was amplified by RT-PCR using gene-specific primers (forward 5′-AACATCACTTCTCA TGGCAG-3′; reverse 5′ ATTACTTGGGTACAAATGTA TGAG-3′) with PCR conditions as described above and cloned into pCR™ 2.1-TOPO vector (Invitrogen). Sequencing was performed by GATC Biotech and the data analyzed using Vector NTI software (Invitrogen). The nucleotide and putative peptide sequences were deposited in the GenBank database with accession numbers MH337832 and AXK90332, respectively. Figures were generated using T-Coffee (Di Tommaso et al. 2011; Notredame et al. 2000) and Boxshade programs (https://embnet.vital-it.ch/software/BOX_form.html). The genomic sequence of *Cd94* from the PWK/Phj strain has been annotated as a part of the Mouse Genome Project (Keane et al. 2011) and the predicted peptide sequence was downloaded (Ensembl transcript ID: MGP_PWKPhJ_T0080088.1).

Flow cytometry

Splenocyte cell suspension was generated by gently emptying of the splenic capsule in to a petri dish with Dulbecco's PBS with 2 mM EDTA. Erythrocytes were lysed by treatment with NH_4Cl solution for 3 min, followed by washing. Cells were stained with Live/Dead Fixable Yellow Dead Stain Kit (Thermo Fisher Scientific), washed, and resuspended in Dulbecco's PBS with 0.5% BSA and 0.005% NaN_3. Cells ($0.5–1.0 \times 10^6$) were incubated with anti-FcR (CD16/CD32; mAb 93, rat IgG2a, eBioscience) followed by incubation with combinations of fluorochrome-conjugated antibodies for surface staining. Antibodies used were against CD49b (DX5, rat IgM), CD3 (145-2C11, AH IgG), NKG2A (B6 allele specific, 16a11, mIgG2b), CD94 (18d3, rat IgG2a) (all from eBioscience), and NKp46 (29A1.4, rat IgG2a, BioLegend). Qa-1b/Qdm peptide/human β2-microglobulin tetramers labeled with AlexaFluor 647 were obtained through the NIH Tetramer Facility, using Qdm peptide (AMAPRTLLL) from GenScript HK Limited. After incubation, the cells were washed twice, resuspended in BD FACS Lysing solution (BD Biosciences), and analyzed using a Gallios flow cytometer (Beckman Coulter). Data was analyzed using Kaluza software (Beckman Coulter).

3D structural visualization

The predicted spatial positions of the residues that differ between the $Cd94^{LocA}$ and $Cd94^{B6}$ alleles were visualized by marking the homologous positions in the crystal structure of human CD94/NKG2A in complex with HLA-E (PDB ID 3CII), using the Cn3D program (NCBI).

Transfections

Expression plasmid constructs encoding the two allelic versions of mouse CD94 with a C-terminal (extracellular) c-Myc epitope tag were generated and used to transiently transfect non-NK cells alone or in combinations with plasmids encoding mouse NKG2A or NKG2C (both with C-terminal HA tags) and/or rat DAP12 with an extracellular FLAG epitope tag. CHO-K1 and 293T cells were cultured in complete medium (RPMI 1640 supplemented with 10% fetal bovine serum, 1 mM pyruvate, and 1% antibiotic/antimycotic solution (all from Gibco)). Briefly, 0.4 μg of plasmid DNA (per plasmid) was added to 80 μL of PBS, then mixed with 80 μL of 3.2 μM polyethyleneimine (MW 25.000, Polysciences). After 20–30 min incubation, this solution was added to single wells (12-well plates) containing ~ 70% confluent cells in in 1 mL of complete medium. The cells were left to incubate with transfection solution for 48 h, then released as single cells in PBS with 2 mM EDTA and subjected to flow cytometry analysis (FACSCanto II, BD Biosciences) after staining with mAbs (2 μg/mL) towards mouse CD94 (mAb 18d3-eFluor 450, eBiosciences), HA (mAb 16B12-PE, BioLegend), anti-HA), c-Myc (mAb SH1-26E7-FITC, Miltenyi Biotech), and FLAG (rabbit mAb 1042E-Alexa Fluor 405, R&D Systems) epitope tags as described above.

Results

Free-living *M. musculus* specimens presented a negative CD94 phenotype

NK cells, defined as CD3-/NKp46+ lymphocytes (Fig. 2a), did not stain with the anti-CD94 mAb 18d3 in two of three individuals from location A (Fig. 2c), whereas NK cells from all individuals from three other locations in close vicinity (locations B, D, and E) stained with the same antibody (Fig. 2d–f). NK cells from C57BL/6 mice (Fig. 2b) as well as house mice collected from location C (data not shown) also stained with the anti-CD94 mAb. NK cells from all mice tested, including the two CD94-negative individuals from location A, stained with a mAb specific for NKG2A (Fig. 2b–f). Surface expression of NKG2E and NKG2C was not investigated due to unavailability of specific antibodies for these receptors. As NKG2 has previously been reported to be unable to express on murine NK cells without the presence of CD94 (Saether et al. 2011; Vance et al. 2002), this would suggest that CD94 was expressed on the cell surface, even in CD94 mAb-negative individuals.

The *Cd94* gene was transcribed in splenocytes of all free-living *M. musculus* individuals analyzed

To exclude the possibility that the undetectable surface expression of CD94 in some individuals could be caused by lack of *Cd94* gene expression, RT-PCR was performed on the collected *M. musculus* material. The primers used were constructed from the B6 allele to amplify exons 1–3. PCR product was detected in samples of all individuals analyzed (Fig. 3), including the two individuals from location A that had NK cells that did not show CD94 surface staining. Thus, *Cd94* mRNA was present in splenocytes from all individuals tested.

The CD94-negative phenotype mice carried an allelic variant of *Cd94*

Next, to investigate if the lack of CD94 mAb binding could be caused by alterations in the *Cd94* gene, either in the form of a deletion (like in DBA/2J mice) or point mutations, we determined the coding DNA *Cd94* sequence from the collected *M. musculus* individuals. Three individuals from locations

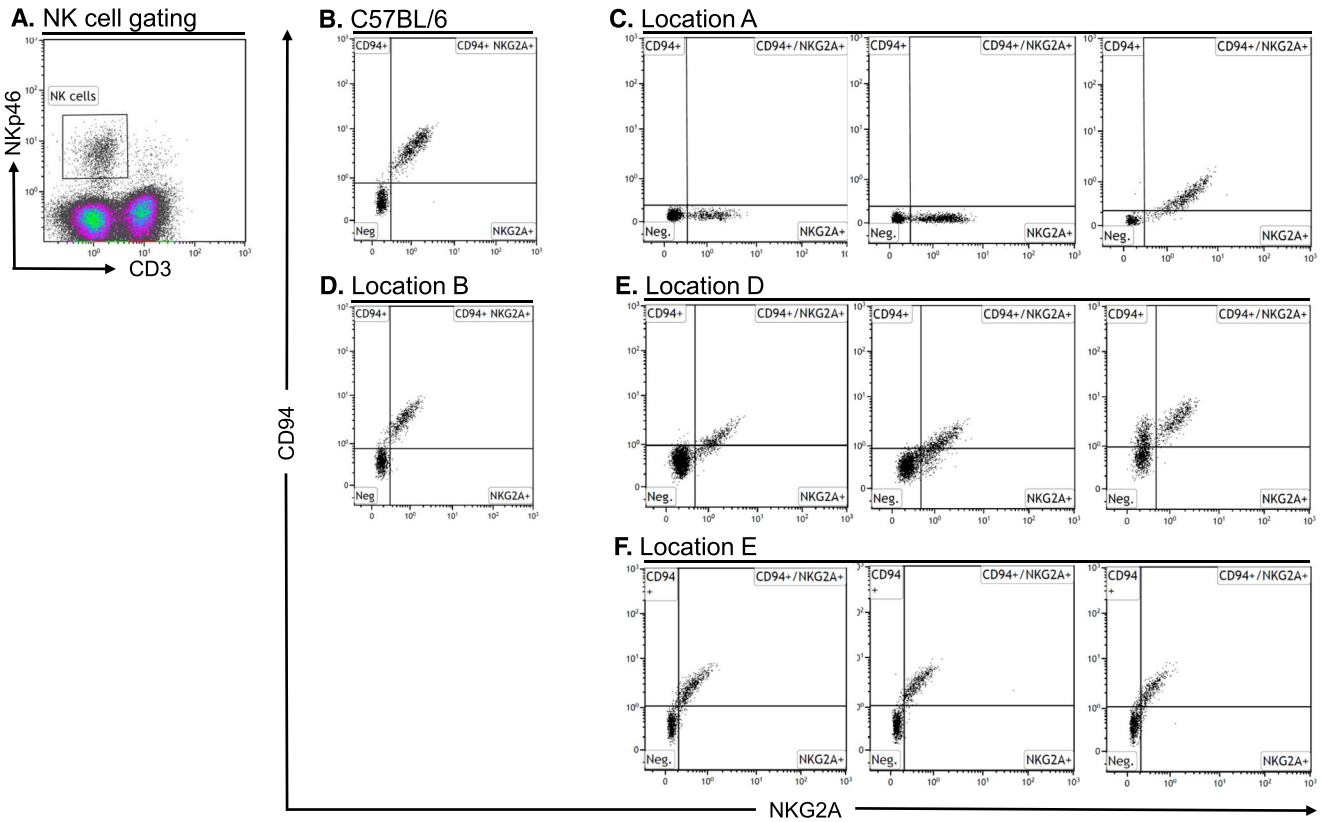


Fig. 2 Identification of wild *Mus musculus* individuals with a CD94^{-}/NKG2^{+} phenotype by flow cytometry. NK cells from free-living house mice were consecutively collected at five different locations (locations A–E) and analyzed by flow cytometry for CD94 expression. Cells were stained with a combination of anti-CD3, anti-NKp46, anti-CD94, and anti-NKG2A mAbs. Each plot represents 100,000 cells from one individual. Plots from each location represent independent flow cytometry analysis following capture. **a** NK cells were gated as NKp46^{+}/CD3^{-} lymphocytes. **b** Samples from three C57BL/6 mice were analyzed as a control, represented by a plot from one individual. **c–f** Three individuals were analyzed from each location, except location D (only one individual was captured and analyzed). Location C is not represented

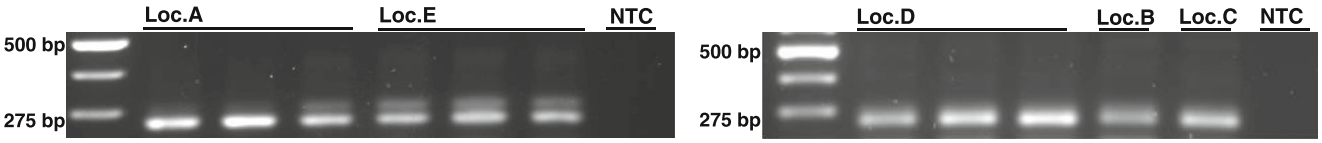


Fig. 3 Investigation of *Cd94* gene expression in splenocytes of wild *Mus musculus*. RT-PCR was performed on total RNA isolated from splenic tissue of collected house mice. The primers were designed to amplify a region spanning exons 1–3 of the B6 *Cd94* mRNA sequence, producing a 275 nucleotide fragment. The origin of the samples is indicated by Loc. A–E and represents one to three of the individuals analyzed from each location. Negative control was performed with no template (NTC)

A, C, and D and two individuals from location E were included. *Cd94* coding cDNA sequences isolated from the three individuals from location A and two individuals from location D revealed 17 nucleotide substitutions when aligned with the B6 allele sequence (Supplementary Fig. 1). Whereas exons 1, 2, 3, and 5 were identical to the B6 allele, four substitutions were located in exon 4 and 13 in exon 6. This novel allele (hereafter $Cd94^{\mathrm{LocA}}$) translated to a predicted peptide of 179 residues with 12 amino acid substitutions compared to the B6 CD94 peptide sequence (hereafter $\mathrm{Cd94^{B6}}$) (Fig. 4a). All amino acid substitutions were located within the extracellular C-type lectin-like domain (Ho et al. 1998). To determine if these substitutions were likely to affect binding to ligand or to the partner chain NKG2A, we placed the substituted residues in a hypothetical 3D model. As the crystal structure of mouse CD94 alone or in complex with NKG2A is not available, we used the human CD94/NKG2A/HLA-E complex structure for visualization of the relative positions of the substitutions in $\mathrm{Cd94^{LocA}}$ (Fig. 4b). In this hypothetical model, several of the substitutions reside on the top of CD94, on the surface that engage ligand. Three substitutions are placed in the β3-β4 "finger" and four others are placed in the loop structure preceding β3. All of these would be expected to affect ligand-binding, and two of the substituted positions are in direct contact with HLA-E in the human structure. The remaining five substitutions are located further away from the ligand-binding surface, in the α1 and β5 regions, but would still likely affect the globular structure. None of the substitutions directly contact NKG2A and none of them would directly affect N-linked glycosylation.

In total, five individuals from two different locations (A and D) carried the $Cd94^{LocA}$ allele, whereas clones from all other wild captured individuals analyzed were identical to $Cd94^{B6}$ (Fig. 4c). A similarity search against genomic sequence from laboratory strains of mice and subsequent alignment revealed that the $Cd94^{LocA}$ is 100% identical to the wild-derived PWK/PhJ mouse strain (hereafter $Cd94^{PWK}$) (Fig. 4c).

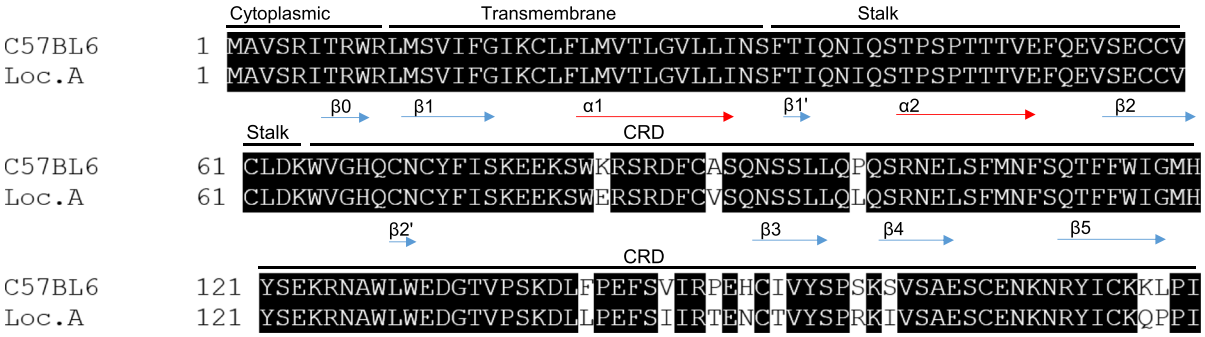
NK cells from two of the individuals from location A carrying the $Cd94^{LocA}$ allele were CD94 negative when analyzed by flow cytometry, but CD94 mAb staining was observed in the third individual. Moreover, the two individuals from location D identified with the $Cd94^{LocA}$ allele were also CD94 positive by flow cytometry. From one of these mice, two clones were identical to $Cd94^{LocA}$, whereas the third clone was identical to $Cd94^{B6}$, suggesting heterozygosity. cDNA samples from each individual were analyzed by PCR using primers specific for either the $Cd94^{LocA}$ or $Cd94^{B6}$ allele. Using the $Cd94^{LocA}$-specific primer, PCR products were detected in samples from the five mice from locations A and D that had yielded the $Cd94^{LocA}$ sequence but not in other individuals, consistent with the cDNA cloning results. PCR with the $Cd94^{B6}$-specific primer produced PCR products from all individuals except the two mice from location A that were CD94 negative by flow cytometry analysis (Fig. 4d). Thus, the CD94-positive individual from location A and two of three individuals from location D were heterozygous for the two *Cd94* alleles, whereas the two CD94 negative individuals were homozygous for the $Cd94^{LocA}$ allele. All other individuals analyzed were homozygous for the $Cd94^{B6}$ allele.

The allelic variant of Cd94 was not recognized by the anti-CD94 mAb 18d3 but was expressed on the surface of transfected cells

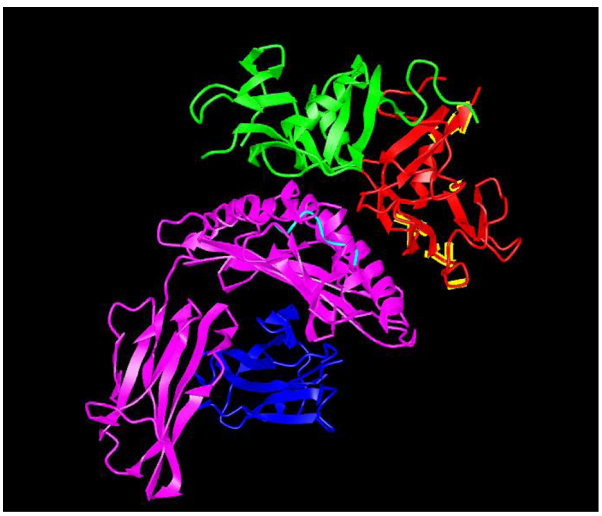
To investigate whether the high number of amino acid substitutions in $\mathrm{Cd94^{LocA}}$ precluded binding to the anti-CD94 mAb 18d3, expression constructs encoding c-Myc-tagged versions

Fig. 4 Wild house mice carry an allelic variant of *Cd94*. **a** Peptide sequence alignment of the *Cd94* allele found in free-living *Mus musculus* from location A (Loc.A) compared to the C57BL/6 allele (AAC28243). Identical amino acids are indicated in black boxes, and white boxes indicate non-identity. Domain boundaries are indicated by black lines above the sequence, and secondary structure elements of α-helices and β-strands are indicated by red and blue arrows, respectively. **b** 3D model of the human CD94/NKG2A in complex with HLA-E, where the homologous positions of the substituted residues of the CD94 allelic variant, aligned to the C57BL/6 sequence, are indicated by yellow color. Red, CD94; green, NKG2A; purple, HLA-E; light blue, Qdm; dark blue, β_2-microglobulin. **c** Peptide sequence alignment of the CD94 lectin-like domain comparing cDNA clones from the *Mus musculus* collected at the indicated locations to C57BL/6 (GenBank accession number AAC28243) and PWK/PhJ (Ensemble Transcript ID MGP_PWKPhJ_T0080088.1). Number of individuals represented per location is indicated in brackets. Three clones were sequenced in both directions from each individual. From location D, clones from one of three individuals yielded both sequences, indicated by 2*. **d** cDNA samples from each individual were analyzed by PCR using a reverse primer specific for either the $\mathrm{Cd94^{LocA}}$ or $\mathrm{Cd94^{B6}}$ sequence. Locations are indicated by Loc.A–E. Negative control was performed with no template (NTC)

a


Cytoplasmic
Transmembrane
Stalk
C57BL6 1 MAVSRITRWRLMSVIFGIKCLFLMVTLGVLLINSFTIQNIQSTPSPTTTVEFQEVSECCV
Loc.A 1 MAVSRITRWRLMSVIFGIKCLFLMVTLGVLLINSFTIQNIQSTPSPTTTVEFQEVSECCV
β0 β1 α1 β1' α2 β2
Stalk
CRD
C57BL6 61 CLDKWVGHQCNCYFISKEEKSWKRSRDFCASQNSSLLQPQSRNELSFMNFSQTFFWIGMH
Loc.A 61 CLDKWVGHQCNCYFISKEEKSWERSRDFCVSQNSSLLQLQSRNELSFMNFSQTFFWIGMH
β2' β3 β4 β5
CRD
C57BL6 121 YSEKRNAWLWEDGTVPSKDLFPEFSVIRPEHCIVYSPSKSVSAESCENKNRYICKKLPI
Loc.A 121 YSEKRNAWLWEDGTVPSKDLLPEFSIIRTENCTVYSPRKIVSAESCENKNRYICKQPPI

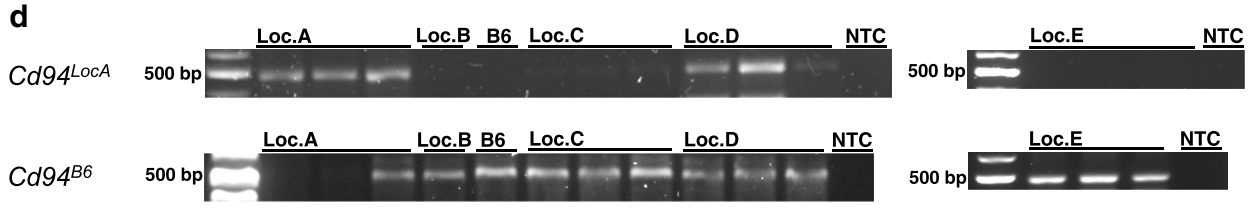
b



c


Loc.A (3) 65 WVGHQCNCYFISKEEKSWERSRDFCVSQNSSLLQLQSRNELSFMNFSQTFFWIGMH
Loc.C (3) 65 WVGHQCNCYFISKEEKSWKRSRDFCASQNSSLLQPQSRNELSFMNFSQTFFWIGMH
Loc.D (2*) 65 WVGHQCNCYFISKEEKSWKRSRDFCASQNSSLLQPQSRNELSFMNFSQTFFWIGMH
Loc.D (2*) 65 WVGHQCNCYFISKEEKSWERSRDFCVSQNSSLLQLQSRNELSFMNFSQTFFWIGMH
Loc.E (2) 65 WVGHQCNCYFISKEEKSWKRSRDFCASQNSSLLQPQSRNELSFMNFSQTFFWIGMH
PWK/PhJ 65 WVGHQCNCYFISKEEKSWERSRDFCVSQNSSLLQLQSRNELSFMNFSQTFFWIGMH
C57BL/6 65 WVGHQCNCYFISKEEKSWKRSRDFCASQNSSLLQPQSRNELSFMNFSQTFFWIGMH
Loc.A (3) 121 YSEKRNAWLWEDGTVPSKDLLPEFSIIRTENCTVYSPRKIVSAESCENKNRYICKQPPI
Loc.C (3) 121 YSEKRNAWLWEDGTVPSKDLFPEFSVIRPEHCIVYSPSKSVSAESCENKNRYICKKLPI
Loc.D (2*) 121 YSEKRNAWLWEDGTVPSKDLLPEFSIIRTENCTVYSPRKIVSAESCENKNRYICKQPPI
Loc.D (2*) 121 YSEKRNAWLWEDGTVPSKDLFPEFSVIRPEHCIVYSPSKSVSAESCENKNRYICKKLPI
Loc.E (2) 121 YSEKRNAWLWEDGTVPSKDLFPEFSVIRPEHCIVYSPSKSVSAESCENKNRYICKKLPI
PWK/PhJ 121 YSEKRNAWLWEDGTVPSKDLLPEFSIIRTENCTVYSPRKIVSAESCENKNRYICKQPPI
C57BL/6 121 YSEKRNAWLWEDGTVPSKDLFPEFSVIRPEHCIVYSPSKSVSAESCENKNRYICKKLPI

d


Cd94LocA
500 bp
Loc.A
Loc.B B6
Loc.C
Loc.D
NTC
500 bp
Loc.E
NTC
Cd94B6
500 bp
Loc.A
Loc.B B6
Loc.C
Loc.D
NTC
500 bp
Loc.E
NTC

of both alleles were used in transfection experiments using non-NK cells. In co-transfection experiments with NKG2A and DAP12, both alleles were expressed on the surface of non-NK cells, but only $CD94^{B6}$ was bound by the 18d3 mAb, thus explaining the negative phenotype observed with this mAb in wild specimens (Fig. 5a). Invariably, the $CD94^{LocA}$ protein was expressed at the cell surface at a lower density than $CD94^{B6}$ with both 293T (human) and CHO-K1 (Chinese hamster) cells (Fig. 5b, Supplementary Fig. 2, and data not shown). The same difference in expression levels was observed in co-transfections with NKG2C plus DAP12 or with DAP12 alone (data not shown), suggesting that the $CD94^{LocA}$ protein could be less efficiently processed through the secretory pathway. Culture at lower temperatures can reduce the stringency of chaperone control for some integral transmembrane proteins. However, surface expression of $CD94^{LocA}$ in transfected CHO-K1 cells was not enhanced after culture at 27 °C (data not shown).

The allelic variant of CD94 on NK cells did not bind the C57BL/6 CD94-NKG2 ligand Qa1-b/Qdm

To investigate the ligand-binding capacity of the allelic variant of CD94 in the free-living mice, four additional individuals were collected from location A and splenocytes were stained with Qa-1b/Qdm tetramers and analyzed by flow cytometry (Fig. 6a). Staining of samples from B6 mice was stained with the tetramer, consistent with previous studies (Salcedo et al. 1998; Zeng et al. 2012). NK cells from one individual from location A stained with both the tetramer and the CD94 mAb. The *Cd94* sequence isolated from this individual was identical to $Cd94^{B6}$ allele and PCR analysis indicated homozygosity for the $Cd94^{B6}$ allele (Fig. 6b). In contrast, the remaining three (out of four) individuals from location A were both Qa-1b/Qdm tetramer and CD94 mAb-negative. PCR analysis indicated that these three individuals were homozygous for the $Cd94^{LocA}$ allele (Fig. 6b) and sequencing resulted in a *Cd94*

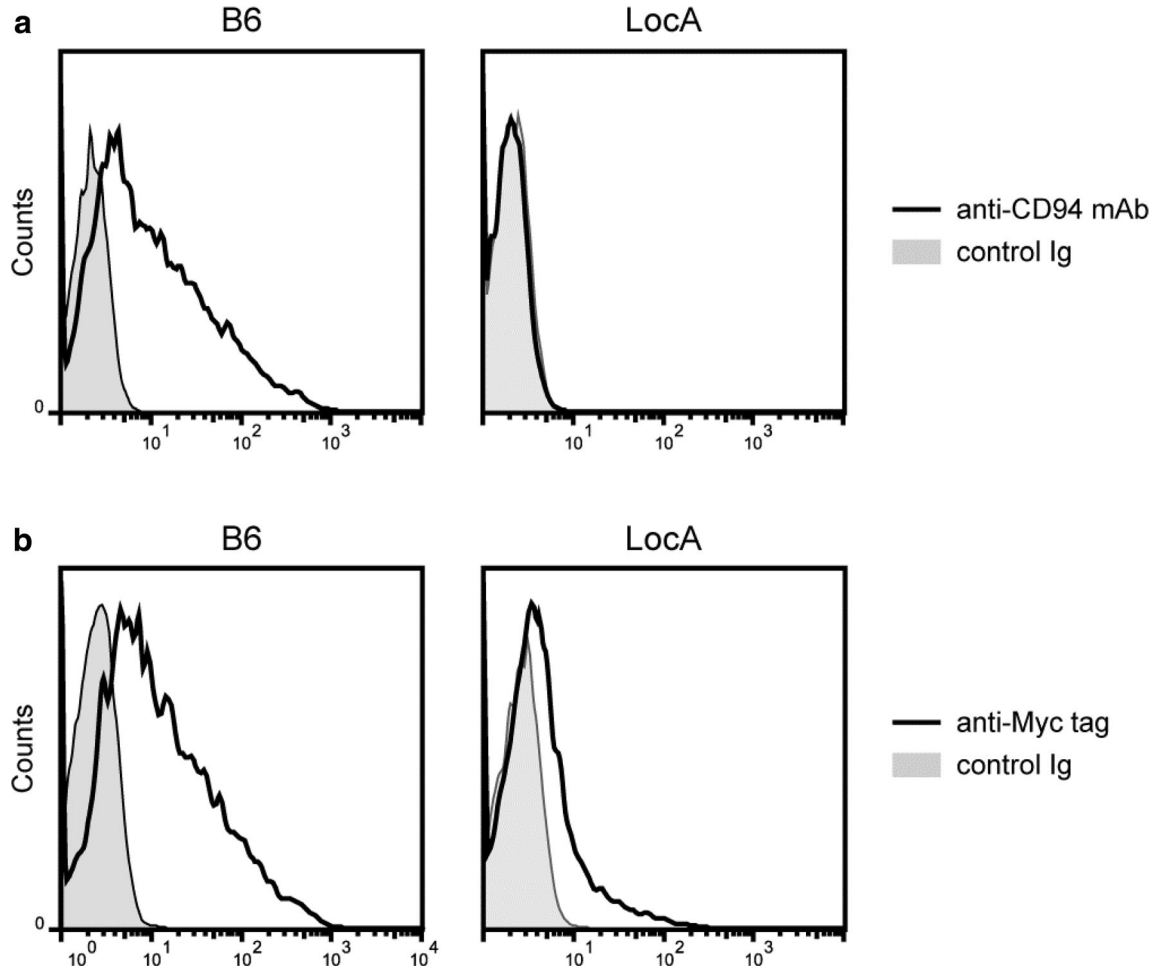


Fig. 5 Surface expression of CD94 allelic variants in transfected cells. Flow cytometry analysis of 293T cells transiently co-transfected with expression constructs encoding mouse NKG2A-HA, DAP12-FLAG, and either the B6 or the LocA allelic versions of CD94 with a C-terminal c-Myc epitope tag. Histograms display staining with **a** the anti-CD94 mAb 18d3 (eFluor 450) or **b** a mAb towards the c-Myc tag (FITC) compared to control antibody

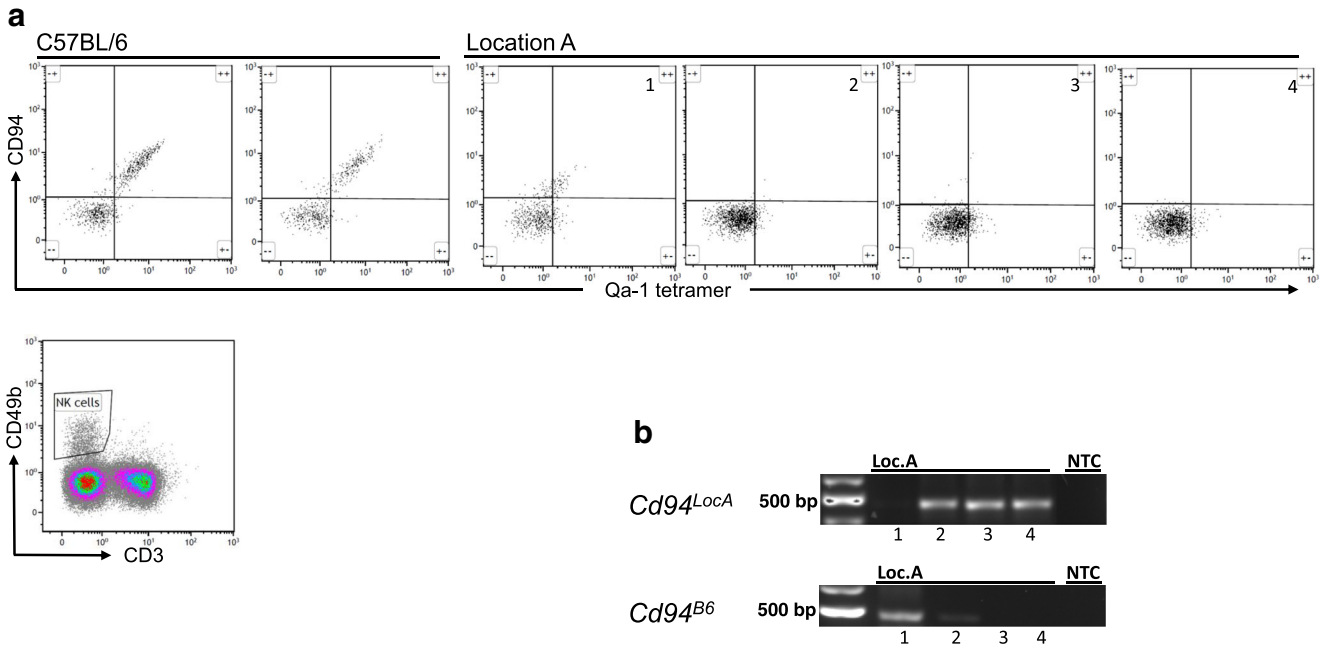


Fig. 6 NK cells from free-living *Mus musculus* with the allelic CD94 variant do not bind Qa-1b/Qdm tetramers. Four free-living *Mus musculus* were collected at location A. **a** Splenocytes from each individual (one to four) were analyzed by flow cytometry for their CD94 phenotype and the ability to bind to the CD94/NKG2-ligand, Qa-1b/Qdm. Two C57BL/6 mice were included as a control. Cells were stained with a combination of mAbs against CD3, CD49b, CD94, NKG2A, and Qa-1b-Qdm tetramers. NK cells were gated as CD49+/CD3-. Each plot represents 200,000 cells from one individual. **b** cDNA from the same individuals from location A (one to four) was analyzed by PCR using a reverse primer specific for either the $Cd94^{LocA}$ or $Cd94^{B6}$ sequence. Negative control was performed with no template (NTC)

cDNA sequence identical to $Cd94^{LocA}$. Together, under the assumption that the $CD94^{LocA}$ allelic variant is expressed on the cell surface of NK cells, these data show that the $CD94^{LocA}$ protein lacks the ability to bind the Qa-1b/Qdm tetramer.

The $Cd94^{LocA}$ allele is associated with *M. musculus musculus* specimens

Multilocus genotyping revealed that all the mice included in this study (except B6 mice) carried genetic markers of *M. m. musculus* (Table 1). Two of three individuals from location D had *D11cenB2* markers associated with both subspecies indicating heterozygosity. These results are consistent with the locations lying within a hybrid zone of the two subspecies (Jones et al. 2010), with genetic markers apparently dominated by *M. m. musculus* origin.

To distinguish if the observed *Cd94* allele is a locally restricted phenomenon or is more widespread, we analyzed splenic samples of confirmed *M. m. domesticus* and *M. m. musculus* origin from different European capture sites. RT-PCR analysis with the $Cd94^{LocA}$-specific primer produced PCR products from samples of three of four individuals of *M. m. musculus* origin (Fig. 7). The *Cd94* cDNA sequences of two of these mice were further analyzed by sequencing, resulting in sequences identical to $Cd94^{LocA}$. One of the *M. m. musculus* individuals was heterozygous for the two *Cd94* alleles, as PCR products also were detected using the $Cd94^{B6}$-specific primer. All four specimens of *M. m. domesticus* origin appeared homozygous for the $Cd94^{B6}$ allele and sequencing of two of the individuals revealed a *Cd94* sequence identical to the $Cd94^{B6}$ allele.

Table 1 Characteristics of house mice typed with the subspecies-specific *Mus musculus* markers

	Genetic marker		
Map reference	*Btk*	*Abpa*	*D11cenB2*
Location A	*m/m (3)*	*m/m (3)*	*m/m (3)*
Location B	*m/m (1)*	*m/m (1)*	*m/m (1)*
Location C	*m/m (3)*	*m/m (3)*	*m/m (3)*
Location D	*m/m (3)*	*m/m (3)*	*m/m (1), m/d (2)*
Location E	–	*m/m (3)*	*m/m (3)*
Control: C57BL/6	*d/d (1)*	*d/d (1)*	*d/d (1)*

Three nuclear genetic markers, *Btk*, *Abpa*, and *D11cenB2*, with allelic differences between *Mus musculus* (*M. m*) *musculus* and *M. m. domesticus*, were analyzed by PCR and gel electrophoresis to classify the mice as either subspecies. C57BL/6 was included as a control. Number of individuals analyzed is in brackets

m/m homozygous for *musculus* allele, *d/d* homogozygous for *domesticus* allele, *m/d* heterozygous for the two alleles

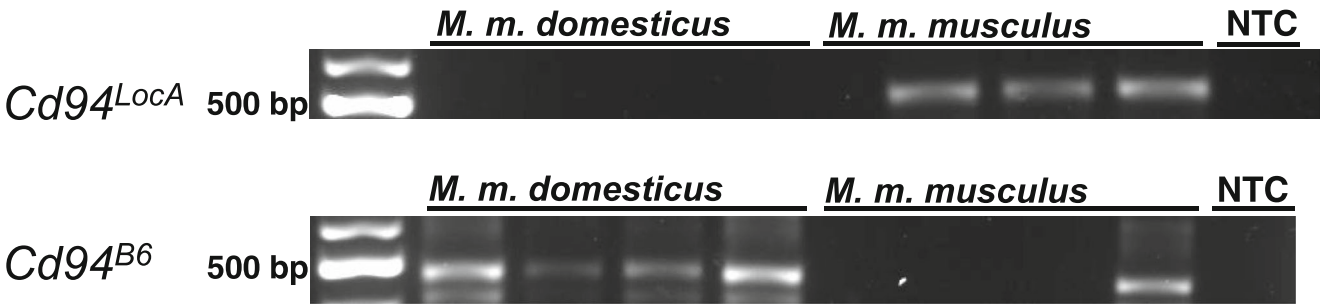


Fig. 7 House mice of *M. m. musculus* origin, but not *M. m. domesticus* origin, carry the new variant *Cd94* allele. cDNA samples from four individuals of confirmed *M. m. domesticus and M. m. musculus origin*, respectively, were analyzed by PCR using a reverse primer specific for the $CD94^{LocA}$ or the $CD94^{B6}$ sequence. Negative control was performed with no template (NTC)

Discussion

In the present study, we report the finding of a new variant *Cd94* allele in a wild population of *M. musculus*. This is, to our knowledge, the first report of CD94 polymorphism in mice, as the CD94/NKG2 receptor complex is generally described as a conserved, relatively non-polymorphic system in both human and mouse (Vance et al. 1997; Shum et al. 2002).

The suspicion of CD94 polymorphism arose from the identification of free-living *M. musculus* individuals with NK cells that did not stain with the anti-CD94 mAb 18d3. Lack of CD94 mAb binding to NK cells has previously been reported in the wild mouse derived PWK/Pas strain, but the cause was not further investigated (Adam et al. 2006). Lack of CD94 surface expression has been reported in the DBA/2J strain due to a deletion in the *Cd94* gene (Vance et al. 2002; Wilhelm et al. 2003). The existence of a laboratory strain naturally deficient in CD94 does not confer much information about the functional importance of this receptor, as laboratory mice are not subject to the natural selective pressure imposed on mice in the wild. In the studies of DBA/2J mice, surface expression of NKG2A was not detectable, and several studies have indicated that NKG2A surface expression by mouse NK cells is dependent on co-expression of CD94 (Saether et al. 2011; Vance et al. 2002; Orr et al. 2010). In all five CD94 mAb-negative individuals analyzed in this study, and in the study of PWK/Pas mice (Adam et al. 2006), NKG2A surface expression was detected, suggesting that CD94 was expressed but not detectable by mAb staining. Detection of *Cd94* mRNA in splenocytes from all individuals included in this study supported this hypothesis. Sequencing of *Cd94* cDNA from the mice from location A and D, and alignment of these against the B6 *Cd94* cDNA sequence, revealed 17 nucleotide substitutions in the protein coding region, of which 12 were non-synonymous. The same substitutions were present in the annotated PWK/Phj *Cd94* sequence. All these substitutions were located in the extracellular lectin-like domain and provide an explanation for lack of reactivity with the anti-CD94 mAb raised against the B6 allele (Vance et al. 1999).

3D modeling using the co-crystal structure of human CD94/NKG2A bound to HLA-E indicated that the substituted positions with a high probability would affect the ligand-binding capacity of $CD94^{LocA}$. In accordance with this, Qa-1b/Qdm tetramer staining was not seen with $CD94^{LocA}$ homozygous mice. In contrast, tetramer staining was observed with NK cells from a $CD94^{B6}$ homozygous wild mouse and from B6 mice, similar to previous studies (Vance et al. 1998; Salcedo et al. 1998; Kraft et al. 2000).

Several studies have indicated that Qa-1b may be the exclusive ligand for the murine CD94/NKG2A receptor (Vance et al. 1999; Kraft et al. 2000). In vitro folding experiments using different peptides have demonstrated that even though Qa-1b can bind other peptides, Qdm is an ideal peptide sequence for binding to Qa-1b (Aldrich et al. 1994; Kraft et al. 2000; Ying et al. 2017). Expression of Qa-1b/Qdm on target cells has been shown to inhibit killing by CD94/NKG2A+ NK cells (Vance et al. 1998; Kraft et al. 2000). The *H2-T23* gene, encoding Qa-1b, has been described as relatively non-polymorphic, similar to other MHC-Ib molecules (Stevens and Flaherty 1996). The *Qa-1b* allele is the most predominant among inbred strains, but three other alleles have been identified, namely *Qa-1a*, *Qa-1c*, and *Qa-1d* (Hermel et al. 1999). Additional *Qa-1* variants have been identified by serology in wild mice, concluding that all mice express Qa-1 molecules (Lindahl 1986; Nakayama et al. 1990). Further, a study determining the putative peptide sequence of the Qa-1 α1 and α2 domains derived from eight wild-derived mice of *M. m. domesticus*, *M. m. castaneus*, and *Mus spretus* origin identified a unique *Qa-1* allele from each (Hermel et al. 2004). These were classified as either *Qa-1a* or *Qa-1b*-like, with no more than 8% amino acid difference in the investigated domains, concluding that there is strong conservation within the *H2–T23* genes. They also found that the *Qa-1* alleles of the wild mice were predominantly *Qa-1a*-like, rather than *Qa-1b*-like, and indicated that other wild mouse populations may contain additional *Qa1-b* or *Qa-1b*-like alleles. We consider the possibility that the $Cd94^{LocA}$ allele has been selected against a different *Qa-1* allele than *Qa-1b*, but this requires further investigation. Another possible explanation for the

substantial sequence difference between $Cd94^{LocA}$ and $Cd94^{B6}$ could be selective pressure against Qa-1 binding, possibly imposed by a virus expressing a putative Qa-1 decoy molecule to achieve protection from NK cell killing. An evolutionary scenario suggesting differences in immunological defense pathways is supported from recent studies documenting that the two house mouse subspecies carry their own co-diverged pathogens, e.g., protozoan *Cryptosporidium* (Kvac et al. 2013), murine cytomegalovirus (Gouy de Bellocq et al. 2015), fungus *Pneumocystic*, and nematode *Syphacia* (Goüy de Bellocq et al. 2018).

In human, chimpanzee, and mice, none or little *CD94* polymorphism has been reported. In contrast, in investigated species of lemurs (*Microcebus murinus* and *Varecia variegata*), which are primates distantly related to humans, the NKC encodes two *Cd94* genes and five to eight *Nkg2* genes (Averdam et al. 2009). The cattle (*Bos taurus*) NKC encodes two *Cd94* genes and up to 12 Nkg2-like genes (Schwartz et al. 2017). A study identifying four different allelic variants found most of the polymorphic residues in the extracellular lectin-like domain (Birch and Ellis 2007). Together, these findings indicate that *Cd94* can indeed be present as both duplicated and polymorphic within mammalian populations. Thus, the previous observations of non-polymorphism of this gene in mice might be due to the limited range of genetic material normally studied, especially with respect to subspecies origin. In the wild, the two European subspecies of *M. musculus* have non-overlapping native ranges that meet along a more than 2500-km-long hybrid zone (Ďureje et al. 2012). It is estimated that these two subspecies diverged between 0.35–1 MY ago (Duvaux et al. 2011). A study of distribution and hybridization patterns of *M. musculus* in Norway describes two sections of such hybrid zones, as well as hybrid formation in areas away from the hybrid zone (Jones et al. 2010). Most laboratory strains including B6 and DBA/2J are dominated by *M. m. domesticus* origin (Yang et al. 2011). Compared to this subspecies of mice, the genetic variation in mice from the *M. m. musculus* side is less understood. The annotated *Cd94* gene from PWK/PhJ was indeed found to be identical to $Cd94^{LocA}$. PWK originates from *M. m. musculus* mice captured in Lhotka near Prague, Czech Republic, far away from the house mouse hybrid zone (Gregorova and Forejt 2000). By investigating the *Cd94* sequence of four mice of confirmed subspecies collected at different sites in Europe, we found *Cd94* sequences identical to $Cd94^{LocA}$ and $Cd94^{B6}$, corresponding to *M. m. musculus* and *M. m. domesticus,* respectively.

In conclusion, we here describe a novel allelic variant of *Cd94*, widely distributed among free-living mice with *M. m. musculus* subspecies origin, which is functionally divergent from the previously studied *Cd94* allele dominating among laboratory strains. Our findings point towards an alternative receptor-ligand interaction for the CD94/NKG2 receptor complex in mice, warranting further investigation.

Author contributions LEK, ED, AKS, and PB conceived the study and designed the experiments. LEK, ED, and PB wrote the manuscript, with contributions from JP and AKS. LEK, JP, and PB caught wild mice. LEK, ED, EGB, PCS, and PB performed lab experiments and analyses. The authors thank Stine Braaen, Hanne M. Haatveit, and Grethe M. Johansen at the Norwegian University of Life Sciences for technical assistance.

Funding This work was funded internally at NMBU.

Compliance with ethical standards

Conflict of interest The authors declare no financial or commercial conflicts of interest to the content of this article.

References

Adam SG, Caraux A, Fodil-Cornu N, Loredo-Osti JC, Lesjean-Pottier S, Jaubert J, Bubic I, Jonjic S, Guenet JL, Vidal SM, Colucci F (2006) Cmv4, a new locus linked to the NK cell gene complex, controls innate resistance to cytomegalovirus in wild-derived mice. J Immunol 176:5478–5485

Aldrich CJ, DeCloux A, Woods AS, Cotter RJ, Soloski MJ, Forman J (1994) Identification of a tap-dependent leader peptide recognized by alloreactive T cells specific for a class Ib antigen. Cell 79:649–658

Averdam A, Petersen B, Rosner C, Neff J, Roos C, Eberle M, Aujard F, Münch C, Schempp W, Carrington M, Shiina T, Inoko H, Knaust F, Coggill P, Sehra H, Beck S, Abi-Rached L, Reinhardt R, Walter L (2009) A novel system of polymorphic and diverse NK cell receptors in primates. PLoS Genet 5:e1000688

Beck JA, Lloyd S, Hafezparast M, Lennon-Pierce M, Eppig JT, Festing MF, Fisher EM (2000) Genealogies of mouse inbred strains. Nat Genet 24:23–25

Birch J, Ellis SA (2007) Complexity in the cattle CD94/NKG2 gene families. Immunogenetics 59:273–280

Borrego F, Masilamani M, Marusina A, Tang X, Coligan J (2006) The CD94/NKG2 family of receptors. Immunol Res 35:263–277

Boysen P, Eide DM, Storset AK (2011) Natural killer cells in free-living Mus musculus have a primed phenotype. Mol Ecol 20:5103–5110

Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS, Lazetic S, Young NT, Bell JI, Phillips JH, Lanier LL, McMichael AJ (1998a) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature 391:795–799

Braud VM, Allan DS, Wilson D, McMichael AJ (1998b) TAP- and tapasin-dependent HLA-E surface expression correlates with the binding of an MHC class I leader peptide. Curr Biol 8:1–10

Di Tommaso P, Moretti S, Xenarios I, Orobitg M, Montanyola A, Chang JM, Taly JF, Notredame C (2011) T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. Nucleic Acids Res 39:W13–W17

Dissen E, Berg SF, Westgaard IH, Fossum S (1997) Molecular characterization of a gene in the rat homologous to human CD94. Eur J Immunol 27:2080–2086

Dod B, Smadja C, Karn RC, Boursot P (2005) Testing for selection on the androgen-binding protein in the Danish mouse hybrid zone. Biol J Linn Soc 84:447–459

Ďureje Ľ, Macholán M, Baird SJE, Piálek J (2012) The mouse hybrid zone in Central Europe: from morphology to molecules. Folia Zool 61:308–318

Duvaux L, Belkhir K, Boulesteix M, Boursot P (2011) Isolation and gene flow: inferring the speciation history of European house mice. Mol Ecol 20:5248–5264

Fang M, Orr MT, Spee P, Egebjerg T, Lanier LL, Sigal LJ (2011) CD94 is essential for NK cell-mediated resistance to a lethal viral disease. Immunity 34:579–589

Gouy de Bellocq J, Baird SJ, Albrechtova J, Sobekova K, Pialek J (2015) Murine cytomegalovirus is not restricted to the house mouse Mus musculus domesticus: prevalence and genetic diversity in the European house mouse hybrid zone. J Virol 89:406–414

Goüy de Bellocq J, Uddin W, Ribas A, Bryja J, Piálek J, Baird SJE (2018) Holobiont suture zones: Parasite evidence across the European house mouse hybrid zone. Mol Ecol. Nov 14. https://doi.org/10.1111/mec.14938

Gregorova S, Forejt J (2000) PWD/Ph and PWK/Ph inbred mouse strains of Mus m. musculus subspecies—a valuable resource of phenotypic variations and genomic polymorphisms. Folia Biol (Praha) 46:31–41

Guenet JL, Bonhomme F (2003) Wild mice: an ever-increasing contribution to a popular mammalian model. Trends Genet 19:24–31

Gunturi A, Berg RE, Forman J (2004) The role of CD94/NKG2 in innate and adaptive immunity. Immunol Res 30:29–34

Hermel E, Hart AJ, Miller R, Aldrich CJ (1999) CTL and sequence analyses of MHC class IB antigens Qa1(c) (H2-T23(r)) and Qa1(d) (H2-T23(f)). Immunogenetics 49:712–717

Hermel E, Hart AJ, Gunduz I, Acton H, Kim C, Wurth M, Uddin S, Smith C, Fischer Lindahl K, Aldrich CJ (2004) Polymorphism and conservation of the genes encoding Qa1 molecules. Immunogenetics 56:639–649

Ho EL, Heusel JW, Brown MG, Matsumoto K, Scalzo AA, Yokoyama WM (1998) Murine Nkg2d and Cd94 are clustered within the natural killer complex and are expressed independently in natural killer cells. Proc Natl Acad Sci U S A 95:6320–6325

Jones EP, Van Der Kooij J, Solheim R, Searle JB (2010) Norwegian house mice (Mus musculus musculus/domesticus): distributions, routes of colonization and patterns of hybridization. Mol Ecol 19:5252–5264

Keane TM, Goodstadt L, Danecek P, White MA, Wong K, Yalcin B, Heger A, Agam A, Slater G, Goodson M, Furlotte NA, Eskin E, Nellaker C, Whitley H, Cleak J, Janowitz D, Hernandez-Pliego P, Edwards A, Belgard TG, Oliver PL, McIntyre RE, Bhomra A, Nicod J, Gan X, Yuan W, van der Weyden L, Steward CA, Bala S, Stalker J, Mott R, Durbin R, Jackson IJ, Czechanski A, Guerra-Assuncao JA, Donahue LR, Reinholdt LG, Payseur BA, Ponting CP, Birney E, Flint J, Adams DJ (2011) Mouse genomic variation and its effect on phenotypes and gene regulation. Nature 477:289–294

Kraft JR, Vance RE, Pohl J, Martin AM, Raulet DH, Jensen PE (2000) Analysis of Qa-1(b)peptide binding specificity and the capacity of Cd94/Nkg2a to discriminate between Qa-1–peptide complexes. J Exp Med 192:613–624

Kvac M, McEvoy J, Loudova M, Stenger B, Sak B, Kvetonova D, Ditrich O, Raskova V, Moriarty E, Rost M, Macholan M, Pialek J (2013) Coevolution of Cryptosporidium tyzzeri and the house mouse (Mus musculus). Int J Parasitol 43:805–817

Lanier LL (2008) Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol 9:495–502

Lanneluc I, Desmarais E, Boursot P, Dod B, Bonhomme F (2004) Characterization of a centromeric marker on mouse chromosome 11 and its introgression in a domesticus/musculus hybrid zone. Mamm Genome 15:924–934

Lee N, Llano M, Carretero M, Ishitani A, Navarro F, Lopez-Botet M, Geraghty DE (1998) HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. Proc Natl Acad Sci U S A 95:5199–5204

Lindahl KF (1986) Genetic variants of histocompatibility antigens from wild mice. Curr Top Microbiol Immunol 127:272–278

Ljunggren HG, Karre K (1990) In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today 11:237–244

Lopez-Botet M, Muntasell A, Vilches C (2014) The CD94/NKG2C+ NK-cell subset on the edge of innate and adaptive immunity to human cytomegalovirus infection. Semin Immunol 26:145–151

Mariuzza R, Li Y (2014) Structural basis for recognition of cellular and viral ligands by NK cell receptors. Front Immunol 5

Moser JM, Gibbs J, Jensen PE, Lukacher AE (2002) CD94-NKG2A receptors regulate antiviral CD8(+) T cell responses. Nat Immunol 3:189–195

Munclinger P, Bozikova E, Sugerkova M, Pialek J, Macholan M (2002) Genetic variation in house mice (Mus, Muridae, Rodentia) from the Czech and Slovak republics. Folia Zool 51:81–92

Nakayama K, Tokito S, Jaulin C, Delarbre C, Kourilsky P, Nakauchi H, Gachelin G (1990) Comparative structure of two duplicated T1a class I genes (T10c and 37) of the murine H-2d MHC. Implications on the evolution of the T1a region. J Immunol 144:2400–2408

Natarajan K, Dimasi N, Wang J, Roy AMDHM (2002) Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. *Annual Review of Immunology* 20:853–885

Notredame C, Higgins DG, Heringa J (2000) T-Coffee: a novel method for fast and accurate multiple sequence alignment. J Mol Biol 302:205–217

Orr MT, Wu J, Fang M, Sigal LJ, Spee P, Egebjerg T, Dissen E, Fossum S, Phillips JH, Lanier LL (2010) Development and function of CD94-deficient natural killer cells. PLoS One 5:e15184

Pegram HJ, Andrews DM, Smyth MJ, Darcy PK, Kershaw MH (2011) Activating and inhibitory receptors of natural killer cells. Immunol Cell Biol 89:216–224

Rapaport AS, Schriewer J, Gilfillan S, Hembrador E, Crump R, Plougastel BF, Wang Y, Le Friec G, Gao J, Cella M, Pircher H, Yokoyama WM, Buller R, Mark L, Colonna M (2015) The inhibitory receptor NKG2A sustains virus-specific CD8+ T cells in response to a lethal poxvirus infection. Immunity 43:1112–1124

Saether PC, Hoelsbrekken SE, Fossum S, Dissen E (2011) Rat and mouse CD94 associate directly with the activating transmembrane adaptor proteins DAP12 and DAP10 and activate NK cell cytotoxicity. J Immunol 187:6365–6373

Salcedo M, Bousso P, Ljunggren HG, Kourilsky P, Abastado JP (1998) The Qa-1b molecule binds to a large subpopulation of murine NK cells. Eur J Immunol 28:4356–4361

Schwartz JC, Gibson MS, Heimeier D, Koren S, Phillippy AM, Bickhart DM, Smith TPL, Medrano JF, Hammond JA (2017) The evolution of the natural killer complex; a comparison between mammals using new high-quality genome assemblies and targeted annotation. Immunogenetics 69:255–269

Searle JB, Jamieson PM, Gunduz I, Stevens MI, Jones EP, Gemmill CE, King CM (2009) The diverse origins of New Zealand house mice. Proc Biol Sci 276:209–217

Shum BP, Flodin LR, Muir DG, Rajalingam R, Khakoo SI, Cleland S, Guethlein LA, Uhrberg M, Parham P (2002) Conservation and variation in human and common chimpanzee CD94 and NKG2 genes. J Immunol 168:240–252

Stevens C, Flaherty L (1996) Evidence foe antigen presentation by the class Ib molecule, Qa-1. Res Immunol 147:286–290

Vance RE, Tanamachi DM, Hanke T, Raulet DH (1997) Cloning of a mouse homolog of CD94 extends the family of C-type lectins on murine natural killer cells. Eur J Immunol 27:3236–3241

Vance RE, Kraft JR, Altman JD, Jensen PE, Raulet DH (1998) Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical

major histocompatibility complex (MHC) class I molecule Qa-1(b). J Exp Med 188:1841–1848

Vance RE, Jamieson AM, Raulet DH (1999) Recognition of the class Ib molecule Qa-1(b) by putative activating receptors Cd94/Nkg2c and Cd94/Nkg2e on mouse natural killer cells. J Exp Med 190:1801–1812

Vance RE, Jamieson AM, Cado D, Raulet DH (2002) Implications of CD94 deficiency and monoallelic NKG2A expression for natural killer cell development and repertoire formation. Proc Natl Acad Sci 99:868–873

Wilhelm BT, Landry J-R, Takei F, Mager DL (2003) Transcriptional control of murine CD94 gene: differential usage of dual promoters by lymphoid cell types. J Immunol 171:4219–4226

Yang, H., Wang, J. R., Didion, J. P., Buus, R. J., Bell, T. A., Welsh, C. E., Bonhomme, F., Yu, A. **H.-T.,** Nachman, M. W., Pialek, J., Tucker, P., Boursot, P., McMillan, L., Churchill, G. A. and Pardo-Manuel de Villena, F., Subspecific origin and haplotype diversity in the laboratory mouse. Nat Genet 2011. 43: 648–655

Ying G, Wang J, Kumar V, Zajonc DM (2017) Crystal structure of Qa-1a with bound Qa-1 determinant modifier peptide. PLoS One 12: e0182296

Zeng L, Sullivan LC, Vivian JP, Walpole NG, Harpur CM, Rossjohn J, Clements CS, Brooks AG (2012) A structural basis for antigen presentation by the MHC class Ib molecule, Qa-1b. J Immunol 188: 302–310

Zhou J, Matsuoka M, Cantor H, Homer R, Enelow RI (2008) Cutting edge: engagement of NKG2A on CD8+ effector T cells limits immunopathology in influenza pneumonia. J Immunol 180:25–29