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HLA-C*04:09N is expressed at the cell surface and triggers peptide-specific T-cell activation

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All other authors declare no competing financial interests.

Author contributions
CW and LH conceived the project. CW, MLW, SS, JS, HH, AD, TB, and LH designed experiments. AD and TB provided critical material. CW, MLW, and SS performed experiments. CW, MLW, SS, LB, JS, HH, AD, TB, and LH analyzed data. CW, and LH wrote the manuscript with input from all authors. LH coordinated and supervised the project.
ABSTRACT

The null allele HLA-C*04:09N differs from HLA-C*04:01 in a frameshift mutation within its cytoplasmic domain, resulting in translation of 32 additional amino acids that are assumed to prevent cell surface expression. However, we recently identified a multiple myeloma-reactive T-cell receptor (TCR) that appeared to recognize antigen presented on HLA-C*04:09N and encouraged us to ask whether HLA-C*04:09N, albeit not easily detectable at the cell surface, can present antigen sufficient for T-cell activation.

We generated two HLA-class I-deficient cell lines, re-expressed HLA-C*04:09N, detected HLA expression by flow cytometry, and tested for T-cell activation using a cytomegalovirus peptide-specific HLA-C*04:01-restricted TCR. In both cell lines, HLA-C*04:09N expression was detectable at the cell surface and could be enhanced by IFN-γ exposure. Recombinant HLA-C*04:09N expression was sufficient for T-cell activation in vitro, which could be blocked by an HLA-class I-specific antibody, suggesting HLA-TCR interaction at the cell surface. Peripheral blood mononuclear cells isolated from an individual who physiologically expressed HLA-C*04:09N triggered peptide-specific T-cell activation, confirming our results with cells with natural HLA expression levels.

In conclusion, we present peptide-specific HLA-C*04:09N-restricted T-cell activation and suggest consideration of this allele in the appropriate clinical context such as allogeneic stem cell transplantation, or in the setting of cellular therapy.
MAIN TEXT

INTRODUCTION

HLA-C*04:09N (C*04:09N) is one of 344 currently reported null alleles at the HLA-C locus (https://hla.alleles.org, accessed in May 2023). Accounting for frequencies between 0.00002 and 0.0189 in cohorts that include more than 1000 individuals, C*04:09N is considered one of the most frequent null alleles. C*04:09N differs from HLA-C*04:01 (C*04:01) in a point deletion within exon seven, which results in a frameshift and extension of the reading frame 97 base pairs beyond the canonical stop codon, translating into a protein 32 amino acids longer than C*04:01. The additional 32 amino acids do not inhibit intracellular protein expression but were suggested to prevent cell surface expression. Consequently, C*04:09N expression has been reported to be detectable by intracellular staining but not at the cell surface using the pan HLA-class I-specific antibody, clone W6/32, which resulted in its designation as a null allele. The extracellular parts of C*04:09N and C*04:01 are identical, suggesting potential presentation of identical peptide repertoires.

We recently identified a multiple myeloma-reactive T-cell receptor (TCR) that appeared to recognize antigen presented on C*04:09N, calling its status as a null allele into question. Current evidence that defines C*04:09N as a null allele relies on flow cytometry-based detection of HLA cell surface expression using one HLA-class I-specific antibody clone (W6/32) and lacks functional proof of the biological irrelevance of this allele.

Our observations in multiple myeloma encouraged us to study whether C*04:09N, albeit not detectable at the cell surface in previous studies, can present antigen in a form that enables peptide-specific T-cell activation.

We asked experimentally whether C*04:09N expression i) is detectable at the cell surface with current technologies, ii) can be modulated by the cytokine milieu, and iii) is sufficient for peptide presentation and specific T-cell activation.
METHODS

Primary cells
The study was approved by the local institutional review board (protocol EA2/096/15 to LH). All participants provided written informed consent, and all research was conducted in accordance with the Declaration of Helsinki.

Cell lines and culture conditions
HEK293T (RRID: CVCL_0063) and U2OS (RRID: CVCL_0042) were cultivated in DMEM with 10% fetal bovine serum (FBS), 10,000 U/mL penicillin, and 10 mg/mL streptomycin (all Thermo Fisher Scientific) at 37 °C and 5% CO2. If indicated, 1,000 U/ml IFN-γ (PeproTech) were added for 24 hours prior to analysis or co-culture experiments.

TCR expression on human peripheral blood lymphocytes
Recombinant TCR expression in human lymphocytes was done as previously described.8-10 Detailed information on the QYD peptide-specific TCR is part of a previous publication.11

HLA knockout using CRISPR-Cas9
HLA-A, HLA-B, and HLA-C knockout of U2OS was performed as previously described8 and confirmed by flow cytometry.

Recombinant HLA expression
Coding sequences of C*04:09N and C*04:01 (accession numbers HLA01451, HLA00420) were downloaded from IPD-IMGT/HLA (https://www.ebi.ac.uk/ipd/imgt/hla/), synthesized (GeneArt, Life Technologies), and cloned into the pHSE3’ vector under control of an H-2Kb promoter, as previously described.12 Cell lines were transfected with FuGENE® HD
(Promega). For stable expression, cells were selected with 3 mg/ml G418 (Cayman Chem), and single cell clones were generated by limiting dilution.

**Minigene expression**

The amino acid sequence of cytomegalovirus-derived pp65 was downloaded (https://www.ncbi.nlm.nih.gov, Gene ID: 3077579) and two 38 amino acid long sequences containing QYDPVAALF (QYD, underlined: MGQQIFLEVQAIRETVELRQYDPVAALFFFDIDLLLQRG) and KDVALRHVV (KDV, underlined: MTSAFVFPTKDVALRHVVCAHELVCIMENTRATKMQVIG) were reverse translated using EMBOSS. Minigene constructs were synthesized (Integrated DNA Technologies, Inc.), cloned into pcDNA6/V5-His A, and transfected with FuGENE® HD (Promega) 36 hours before co-culture experiments.

**Flow cytometry**

Reagents for determination of HLA expression or T-cell activation upon co-incubation are listed in the supplementary information. For intracellular staining, we used the Cytofix/Cytoperm Fixation/Permeabilization Kit (BD Biosciences). All reagents were used according to the manufacturer’s instructions.

**Magnetic-activated cell separation (MACS)**

For enrichment of CD8⁺ T cells before co-culture experiments, we used MojoSort™ Human CD8 Nanobeads (Biolegend) with minor modifications: CD8 Nanobeads were diluted 1:5 and cells were separated on MS columns (Miltenyi). Enriched populations were on average 71.7% CD8⁺ determined by flow cytometry.
Co-cultures

Co-culture experiments of TCR-transduced T cells with HEK293T or U2OS were performed in 250 μl medium (RPMI-1640 supplemented with 10% FBS, 10,000 U/mL penicillin, and 10 mg/mL streptomycin) in 48-well plates for 18 hours; co-cultures with PBMC were performed in 150 μl medium in 96-well plates for 24 hours at 37 °C and 5% CO₂. Indicated peptides (Genscript) were added at 5 μM final concentration one hour before co-culture. For HLA-blocking, Ultra-LEAF Purified anti-human HLA-class I (clone W6/32, BioLegend, RRID: AB_2561492) or Ultra-LEAF Purified Mouse IgG2aκ isotype control (clone MOPC-173, BioLegend, RRID: AB_11148947) were added at 50 μg/ml final concentration one hour before co-culture. Activation of T cells was detected using the Human IFN-γ ELISA Set BD OptEIA (BD Biosciences, RRID: AB_2869029), the human granzyme B DuoSet ELISA kit (R&D Systems), and by flow cytometry.

HLA typing of the sample MM160

HLA-typing was performed using Holotype HLA-24/7 (Omixon, Budapest, Hungary) in combination with MiSeq (Illumina, San Diego, US) according to manufacturer’s instructions. Sequencing data were analyzed using Twin software version 3.1.1 (Omixon) and IMGT/HLA-Database version 3.32. HLA-C*04:09N was confirmed by sequence-specific primer (SSP) PCR (Olerup SSP® HLA-C*04:09N, Olerup SSP AB, Stockholm, Sweden) according to the manufacturer’s instructions.

Statistical analysis

Statistics were calculated using R, version 4.0.3.¹⁴ Applied tests are stated in the figure legends.
RESULTS

HLA-C*04:09N is expressed at the cell surface

We transfected HEK293T that lack HLA-A, HLA-B and HLA-C expression (HEK_{HLA-KO}) with pHSE3’ expression plasmids that encoded C*04:09N, or C*04:01 as a positive control. HLA expression was determined by flow cytometry after intracellular or cell surface staining with antibodies specific for HLA-BC (clone B1.23.2) or pan HLA-class I (clone W6/32) (Suppl. Tab. 1). As expected, C*04:09N was detectable by intracellular staining (Fig. 1A). However, we also detected C*04:09N cell surface expression after staining with the HLA-BC-specific antibody (Fig. 1A, upper part). In line with previous observations, only residual C*04:09N cell surface expression could be observed with the pan HLA-class I antibody (Fig. 1A, lower part). C*04:01 expression was detectable by intracellular and cell surface staining (Fig. 1A).

Recombinant HLA expression can vary in context of different cell types and due to transfection efficiency. To confirm our findings, we generated an osteosarcoma cell line deficient in HLA-A, HLA-B and HLA-C (U2OS_{HLA-KO}), of which we established a single cell clone-derived cell line that stably expressed C*04:09N as the only HLA-ABC allele (U2OS_{04:09N}). In line with our results from transient HLA expression in HEK_{HLA-KO}, surface expression of C*04:09N was weak but detectable with the HLA-BC-specific, but not with the pan HLA-class I-specific antibody (Fig. 1B).

Microenvironmental influences including, but not limited to the cytokine milieu, can modulate HLA expression. As an example, we determined HLA cell surface expression of U2OS_{04:09N} after exposure to IFN-γ in vitro. While C*04:09N cell surface expression was detectable on average on 21.4% of U2OS_{04:09N}, expression increased to on average 81.8% after IFN-γ treatment (representative example in Fig. 1C). On average, HLA cell surface expression on U2OS_{04:09N}, determined as allophycocyanin (APC) mean fluorescence intensity (MFI), increased 1.9-fold upon IFN-γ exposure (Fig. 1D). IFN-γ exposure did not
substantially affect cell surface staining of C*04:09N with the pan HLA-class I-specific antibody (Suppl. Fig. S1).

Taken together, C*04:09N expression was detectable at the cell surface of two different cell lines and increased upon IFN-γ exposure \textit{in vitro}.

**T cells can be activated by peptides presented on HLA-C*04:09N**

Identical extracellular domains, including the peptide binding grooves, of C*04:09N and C*04:01 suggest binding of a similar peptide repertoire. To determine potential T-cell activation by peptides presented on C*04:09N, we expressed a C*04:01-restricted TCR specific for the cytomegalovirus (CMV)-derived epitope QYDPVAALF (QYD),\textsuperscript{11} which is presented on C*04:01, in human peripheral blood T cells (TQYD) (Suppl. Fig. S2, HLA-class I alleles of the donor used for TCR expression in Tab. 1). IFN-γ and granzyme B secretion were measured to detect T-cell activation. For antigen presentation, HEK\textsubscript{HLA-KO} were co-transfected with expression plasmids encoding C*04:09N and a minigene encoding the target epitope (QYD) or a negative control epitope (KDVALRHVV, called KDV). Only HEK\textsubscript{HLA-KO} co-transfected with C*04:09N and the target epitope QYD activated TQYD, confirming activation of a C*04:01-restricted TCR by a peptide presented on C*04:09N (Fig. 2A). Non-TCR-transduced T cells could not be activated by HEK\textsubscript{04:09N} confirming TCR-dependent T-cell activation (Suppl. Fig. S3). T-cell activation was blocked by pan HLA-class I-specific antibody, which suggested that C*04:09N-T-cell interaction occurred at the cell surface (Fig. 2B).

To confirm our results in a different cell line, U2OS\textsubscript{HLA-KO} and U2OS\textsubscript{04:09N} were transfected with the QYD or KDV minigenes and co-cultured with TQYD. Only U2OS\textsubscript{04:09N} expressing the QYD minigene activated TQYD (Fig. 2C). In line with our results from HEK293T, non-transduced T cells were not activated by U2OS\textsubscript{04:09N} (Suppl. Fig. S4). TQYD activation by U2OS\textsubscript{04:09N} could be blocked by the pan HLA-class I antibody (Fig. 2D).
Our data functionally confirmed i) C*04:09N-dependent, and ii) target epitope-specific T-cell activation.

**PBMC of an HLA-C04:09N+ donor can present QYD and trigger specific T-cell activation**

We obtained peripheral blood mononuclear cells (PBMC) of one multiple myeloma patient (MM160) who physiologically expressed C*04:09N (Tab. 1) and had been part of a previous study of our group. We obtained peripheral blood mononuclear cells (PBMC) of one multiple myeloma patient (MM160) who physiologically expressed C*04:09N (Tab. 1) and had been part of a previous study of our group.8 PBMC of MM160 were rested or incubated with IFN-γ for 24 h, washed, loaded with QYD or the negative control peptide KDV, and incubated with TQYD. CD137 expression on TCR-transduced CD8⁺ T cells (gating strategy in Suppl. Fig. S5, antibody panel in Suppl. Tab. S2) and granzyme B in cell culture supernatants were measured to detect T-cell activation. TQYD were activated by PBMC of patient MM160 in a QYD-specific manner, and activation could be enhanced by pre-incubation of PBMC with IFN-γ (Fig. 3A). Accordingly, increased frequencies of dead cells among target peptide-loaded PBMC after co-culture suggested peptide-specific killing in context of C*04:09N (Fig. 3A and B). Non-TCR-transduced T cells were not activated by QYD loaded onto PBMC of MM160, although granzyme B showed higher background after pre-incubation with IFN-γ (Fig. 3B). To exclude that QYD could potentially be presented by any of the HLA alleles of MM160 other than C*04:09N, we expressed all HLA alleles of MM160 (Tab. 1, one at a time) in HEKHLA-KO, each in combination with the QYD minigene, and tested for activation of TQYD. Only C*04:09N in combination with the QYD minigene activated TQYD (Fig. 3C).

T cells that were used for TCR transduction did neither express C*04:01 nor C*04:09N (Tab. 1); to experimentally exclude antigen presentation by T cells used for TCR transduction, we demonstrated that incubation of non-transduced T cells and TQYD in presence of QYD peptide did not result in T-cell activation (Fig. 3D).
In summary, PBMC of a C*04:09N+ individual can present peptide and trigger T-cell activation in a C*04:09N-restricted manner.
DISCUSSION

C*04:09N was first described in 2002,⁵, ⁷ is considered a null allele due to previously non-detectable cell surface expression, and we are not aware of any functional confirmation that C*04:09N could not trigger peptide-specific T-cell activation. Moreover, we recently identified a multiple myeloma-reactive TCR that appeared to recognize antigen in context of C*04:09N.⁸ This encouraged us to experimentally address whether C*04:09N expression i) can be detected at the cell surface with current technologies, and ii) can trigger peptide-specific T-cell activation in a functional assay.

Accurate detection of C*04:09N cell surface expression requires specific antibodies, which are commercially available against pan HLA-class I (clone W6/32), HLA-BC (clone B1.23.2), and HLA-C (clone DT9). Currently, there is no commercially available antibody specifically against C*04:01 or even C*04:09N. Therefore, we generated two cell lines (HEK293T and U2OS), in which we deleted all endogenous HLA-A, HLA-B, and HLA-C alleles by CRISPR-Cas9. In these cell lines, we expressed single HLA-C alleles of choice, which could subsequently be detected by any of the above-mentioned antibodies (pan HLA-class I, HLA-BC, or HLA-C). We chose HEK293T and U2OS because they are widely available and easy to transflect with high efficiency by standard methodologies. The HLA-C-specific antibody (DT9) delivers only weak staining for flow cytometry compared to the HLA-BC or pan HLA-class I-specific antibodies; furthermore, DT9 can also recognize HLA-E,¹⁵ and was therefore not used in this study.

Recombinant cell surface expression of C*04:09N was weak and only reliably detectable with the HLA-BC-specific antibody (B1.23.2) confirming results of previous studies that only used the pan HLA-class I-specific antibody (W6/32) for C*04:09N detection.⁵, ⁷ A possible explanation, among many others, for the observed differences in sensitivity between the B1.23.2 and W6/32 antibodies could be that W6/32 detects HLA in complex with
β2-microglobulin whereas B1.23.2 recognizes an epitope expressed on both β2-microglobulin-associated and free HLA-class I heavy chains.\(^\text{16, 17}\) C*04:09N surface expression was enhanced by IFN-γ exposure. IFN-γ is known to regulate gene expression beyond HLA-class I genes.\(^\text{18-20}\) \textit{In vitro} application of IFN-γ resulted in substantially increased C*04:09N expression and stronger specific T-cell activation. Based on these observations, we assume relevant effects of the cytokine milieu and tissue-associated modulation of C*04:09N expression \textit{in vivo}.

Independent of whether C*04:09N cell surface expression was detectable by flow cytometry, we asked experimentally whether C*04:09N expression was functionally relevant and could mediate peptide-specific T-cell activation. We demonstrated in two unrelated cell lines that transient or stable C*04:09N expression was sufficient for peptide presentation and specific T-cell activation. Inhibition of T-cell activation by addition of pan HLA-class I antibody to the cell culture indicated that TCR-peptide-HLA interaction occurred at the cell surface. Our results were confirmed with PBMC of a C*04:09N\(^+\) individual. Our data prove functionally that C*04:09N expression, albeit challenging to detect by flow cytometry, is biologically relevant for HLA-restricted T-cell activation.

Our study was entirely carried out \textit{in vitro}; therefore, we cannot draw conclusions on \textit{in vivo} situations. However, strong modulation of C*04:09N expression by the cytokine milieu (IFN-γ), and isolation of a clonally expanded C*04:09-restricted T-cell clone from bone marrow of a multiple myeloma patient\(^8\) strongly suggest immunological relevance of this allele \textit{in vivo}.

We assume our findings to have implications in the fields of hematopoietic stem cell and solid organ transplantation; however, although C*04:09N is considered one of the most abundant null alleles, its overall frequency is relatively low and varies within several orders of magnitude between different populations.\(^2\) Comprehensive analyses of larger cohorts along with C*04:09N peptidome data, especially in comparison with C*04:01, are required to
determine implications for allogeneic transplantation and specific disease associations, as well as in the context of other settings such as cellular therapy.
REFERENCES


TABLES

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Table 1. HLA-class I alleles of the T-cell donor for TCR expression and multiple myeloma patient MM160.

T cells for recombinant TCR expression were isolated from leftover materials of an allogeneic stem cell graft (T-cell donor).
FIGURE LEGENDS

Figure 1. Detection of HLA-C*04:09N cell surface expression by flow cytometry.

(A) HEK_{HLA-KO} were transfected with plasmids encoding C*04:09N (HEK_{04:09N}) or C*04:01 (HEK_{04:01}) and stained with HLA-BC (clone B1.23.2, upper part) or pan HLA-class I (clone W6/32, lower part) antibody for flow cytometry. The plots show one representative example out of n=3 independent experiments. (B) Flow cytometry detection of cell surface C*04:09N expression on U2OS_{04:09N} with HLA-BC (upper part) or pan HLA-class I antibody (lower part). WT indicates wild type. (C) Effects of \textit{in vitro} IFN-\gamma exposure on C*04:09N cell surface expression on U2OS_{04:09N}. If indicated, cells were incubated with 1,000 U/ml IFN-\gamma for 24 h prior to analysis. The flow cytometry plot is representative for n=3 independent experiments. (D) HLA cell surface expression of U2OS_{04:09N} after 24 h IFN-\gamma exposure determined as mean fluorescence intensity (MFI) in n=3 independent experiments with three replicates per experiment. Data points indicate nine individual replicates.

Figure 2. Specific T-cell activation by a peptide presented on HLA-C*04:09N.

(A) T_{QYD} were cultured with HEK_{HLA-KO} which were (co-)transfected with a plasmid encoding C*04:09N and a minigene encoding QYD or KDV. (B) T_{QYD} were incubated with HEK_{HLA-KO} co-transfected with a plasmid encoding C*04:09N and the QYD-minigene in presence of a blocking antibody against pan HLA-class I (clone W6/32) or isotype control (clone MOPC-173). (C) T_{QYD} were incubated with U2OS_{HLA-KO} or U2OS_{04:09N} transfected with the QYD- or KDV-minigene. (D) T_{QYD} were incubated with U2OS_{04:09N} transfected with the QYD-minigene in presence of a blocking antibody against pan HLA-class I (clone W6/32) or isotype control (clone MOPC-173). Bar charts indicate mean ± standard error. In all charts, IFN-\gamma and granzyme B in cell culture supernatants were measured by ELISA. Data are representative of n=3 independent experiments with three technical replicates per experiment.
Statistical significance was determined by paired Student’s t-test and corrected for multiple testing by Bonferroni correction. *** indicates P<0.001.

**Figure 3. QYD peptide-specific T-cell activation in an HLA-C*04:09N+ individual.**

(A) TQYD were labeled with cell trace violet and cultured with PBMC of an HLA-C*04:09N+ individual loaded with 5 μM QYD- or KDV-peptide; PBMC were incubated with 1,000 U/ml IFN-γ for 24 hours before co-culture if indicated. To detect T-cell activation, CD137 expression on TCR-transduced CD8+ T cells was determined by flow cytometry and granzyme B secretion in cell culture supernatants was measured by ELISA. Dead PBMC were determined as zombie NIR+ among all cell trace violet neg. cells. (B) Non-TCR transduced T cells were incubated with PBMC of the same donor as in Figure part A loaded with 5 μM QYD- or KDV-peptide; PBMC were incubated with 1,000 U/ml IFN-γ for 24 h before co-culture if indicated. To detect T-cell activation, CD137 expression on TCR-transduced CD8+ T cells was determined by flow cytometry and granzyme B secretion in cell culture supernatants was measured by ELISA. Dead PBMC were determined as zombie NIR+ among all cell trace violet neg. cells. (C) TQYD were cultured with HEK_{HLA-KO} that recombinantly expressed all HLA alleles of MM160 (one at a time) and the QYD-minigene. IFN-γ and granzyme B in cell culture supernatants were measured by ELISA. Bar charts indicate mean ± standard error. Data are representative of n=3 experiments. (D) To confirm that QYD could not be presented by the T cells used for TCR transduction, TQYD were co-cultured with non-TCR transduced T cells loaded with 5 μM QYD- or KDV-peptide.
Supplementary Table S1

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Supplementary Table S1. Reagents for detection of HLA expression.

All reagents were used according to the manufacturer’s instructions. APC indicates allophycocyanin; PE, phycoerythrin; and AF, Alexa Fluor.
**Supplementary Table S2. Flow cytometry reagents for co-incubation experiments.**

All reagents were used according to the manufacturer’s instructions. APC indicates allophycocyanin; FITC, fluorescein isothiocyanate; and PE, phycoerythrin.

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Supplementary Figure S1

Supplementary Figure S1. Effects of IFN-γ exposure on C*04:09N cell surface expression determined by pan HLA-class I-specific antibody

Effects of *in vitro* IFN-γ exposure on C*04:09N cell surface expression on U2OS04:09N detected by HLA-class I-specific antibody (clone W6/32). If indicated, cells were incubated with 1,000 U/ml IFN-γ for 24 h prior to analysis. The flow cytometry plot is representative for n=3 independent experiments.
Supplementary Figure S2. Confirmation of peptide specificity of \( T_{QYD} \).

\( T_{QYD} \) were incubated with HEK\textsubscript{HLA-KO} that recombinantly expressed HLA-C\*04:01 (HEK\textsubscript{04:01}) and the QYD- or KDV minigene. IFN-\( \gamma \) or granzyme B were detected by ELISA in cell culture supernatants. Bars indicate mean ± standard error of \( n=3 \) experiments.
**Supplementary Figure S3.** Non-TCR-transduced T cells were not activated by QYD on HEK\(_{04:09N}\).

Non-TCR-transduced T cells were incubated with HEK\(_{\text{HLA-KO}}\) which were (co-)transfected with a plasmid encoding C*04:09N and a minigene plasmid encoding QYD or KDV. IFN-\(\gamma\) and granzyme B were detected by ELISA in cell culture supernatants. Bars indicate mean ± standard error of n=3 independent experiments with three replicates per experiment.
Supplementary Figure S4

Supplementary Figure S4. Non-TCR-transduced T cells were not activated by QYD on U2OS_{04:09N}.

Non-TCR-transduced T cells were incubated with U2O_{HLA-KO} or U2O_{04:09N} which were transfected with QYD- or KDV-minigenes. IFN-γ and granzyme B in cell culture supernatants were detected by ELISA. Bars indicate mean ± standard error of n=3 independent experiments with three replicates per experiment.
Supplementary Figure S5

Supplementary Figure S5. Detection of CD137 expression on TCR-transduced T-cells in co-culture experiments.

The figure illustrates the gating strategy of one co-culture as a representative example. TCR-transduced T-cells were labeled with cell trace violet before co-culture with antigen-presenting cells of choice. Pseudo-color plots from left to right show gating on lymphocytes, exclusion of dead cells, gating on cell trace-labeled cells, and identification of CD8+ TCR-transduced cells. The CD137 gate was defined based on non-stimulated controls. APC indicates allophycocyanin; FITC, fluorescein isothiocyanate; and PE, phycoerythrin.