

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

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**Supplementary Table S1**

<i>Target</i>	<i>Clone</i>	<i>Fluorescent dye</i>	<i>Vendor</i>	<i>RRID/Cat.No.</i>
HLA-BC	B1.23.2	APC	ThermoFisher Scientific	AB_11151509
HLA-class I	W6/32	PE	BioLegend	AB_314875
Live/dead		Zombie violet	BioLegend	423113

**Supplementary Table S1. Reagents for detection of HLA expression.**

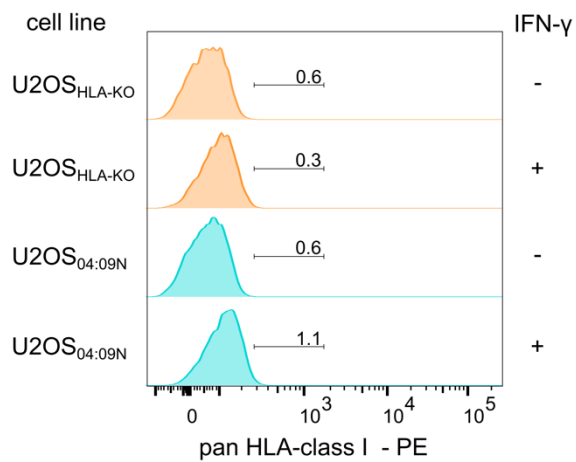
All reagents were used according to the manufacturer's instructions. APC indicates allophycocyanin; PE, phycoerythrin; and AF, Alexa Fluor.

**Supplementary Table S2**

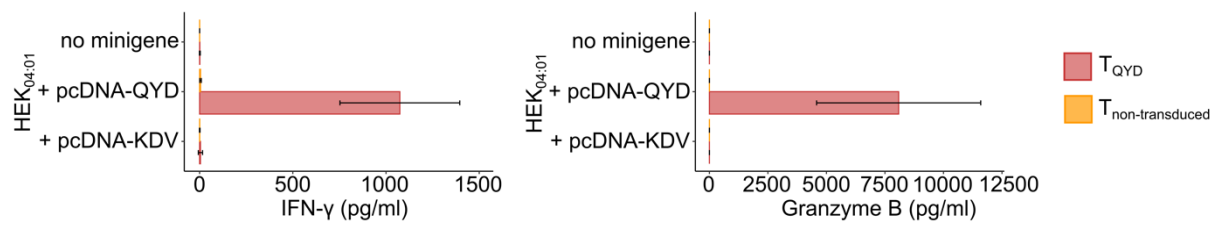
<i>Target</i>	<i>Clone</i>	<i>Fluorescent dye</i>	<i>Vendor</i>	<i>RRID/Cat.No.</i>
CD137	4-1BB	PE	Biolegend	AB_314783
Mouse TCR $\beta$	H57-597	APC	Biolegend	AB_313435
CD8	RPA-T8	FITC	Biolegend	AB_314124
Live/dead		Zombie NIR	Biolegend	423106
Cell trace		Cell trace violet	Invitrogen	C34571

**Supplementary Table S2. Flow cytometry reagents for co-incubation experiments.**

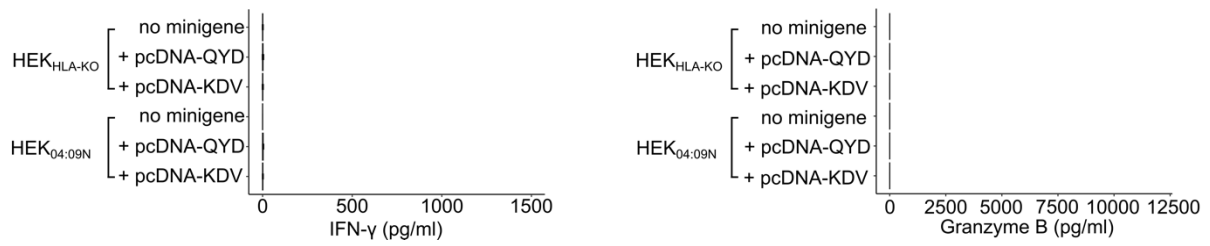
All reagents were used according to the manufacturer's instructions. APC indicates allophycocyanin; FITC, fluorescein isothiocyanate; and PE, phycoerythrin.

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

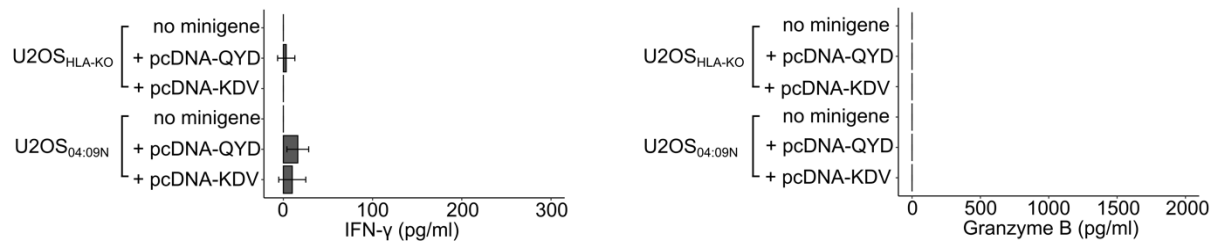
Effects of *in vitro* IFN- $\gamma$  exposure on C\*04:09N cell surface expression on U2OS<sub>04:09N</sub> detected by HLA-class I-specific antibody (clone W6/32). If indicated, cells were incubated with 1,000 U/ml IFN- $\gamma$  for 24 h prior to analysis. The flow cytometry plot is representative for n=3 independent experiments.

**Supplementary Figure S2****Supplementary Figure S2. Confirmation of peptide specificity of T<sub>QYD</sub>.**

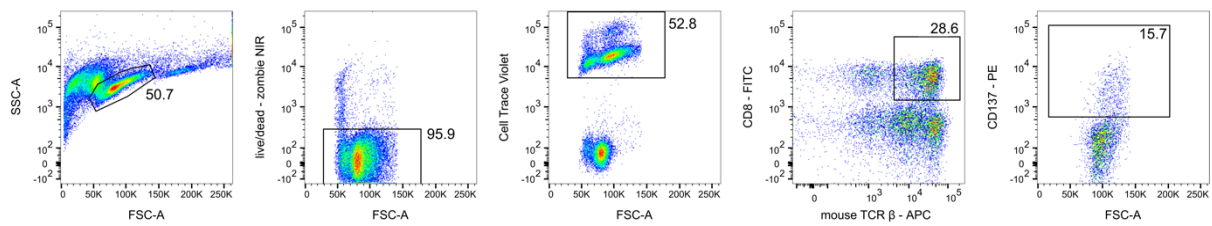
T<sub>QYD</sub> were incubated with HEK<sub>HLA-KO</sub> that recombinantly expressed HLA-C\*04:01 (HEK<sub>04:01</sub>) and the QYD- or KDV minigene. IFN- $\gamma$  or granzyme B were detected by ELISA in cell culture supernatants. Bars indicate mean  $\pm$  standard error of n=3 experiments.

**Supplementary Figure S3****Supplementary Figure S3. Non-TCR-transduced T cells were not activated by QYD on HEK<sub>04:09N</sub>.**

Non-TCR-transduced T cells were incubated with HEK<sub>HLA-KO</sub> 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  $\pm$  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<sub>04:09N</sub>.**

Non-TCR-transduced T cells were incubated with U2OS<sub>HLA-KO</sub> or U2OS<sub>04:09N</sub> which were transfected with QYD- or KDV-minigenes. IFN- $\gamma$  and granzyme B in cell culture supernatants were detected by ELISA. Bars indicate mean  $\pm$  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<sup>+</sup> TCR-transduced cells. The CD137 gate was defined based on non-stimulated controls. APC indicates allophycocyanin; FITC, fluorescein isothiocyanate; and PE, phycoerythrin.