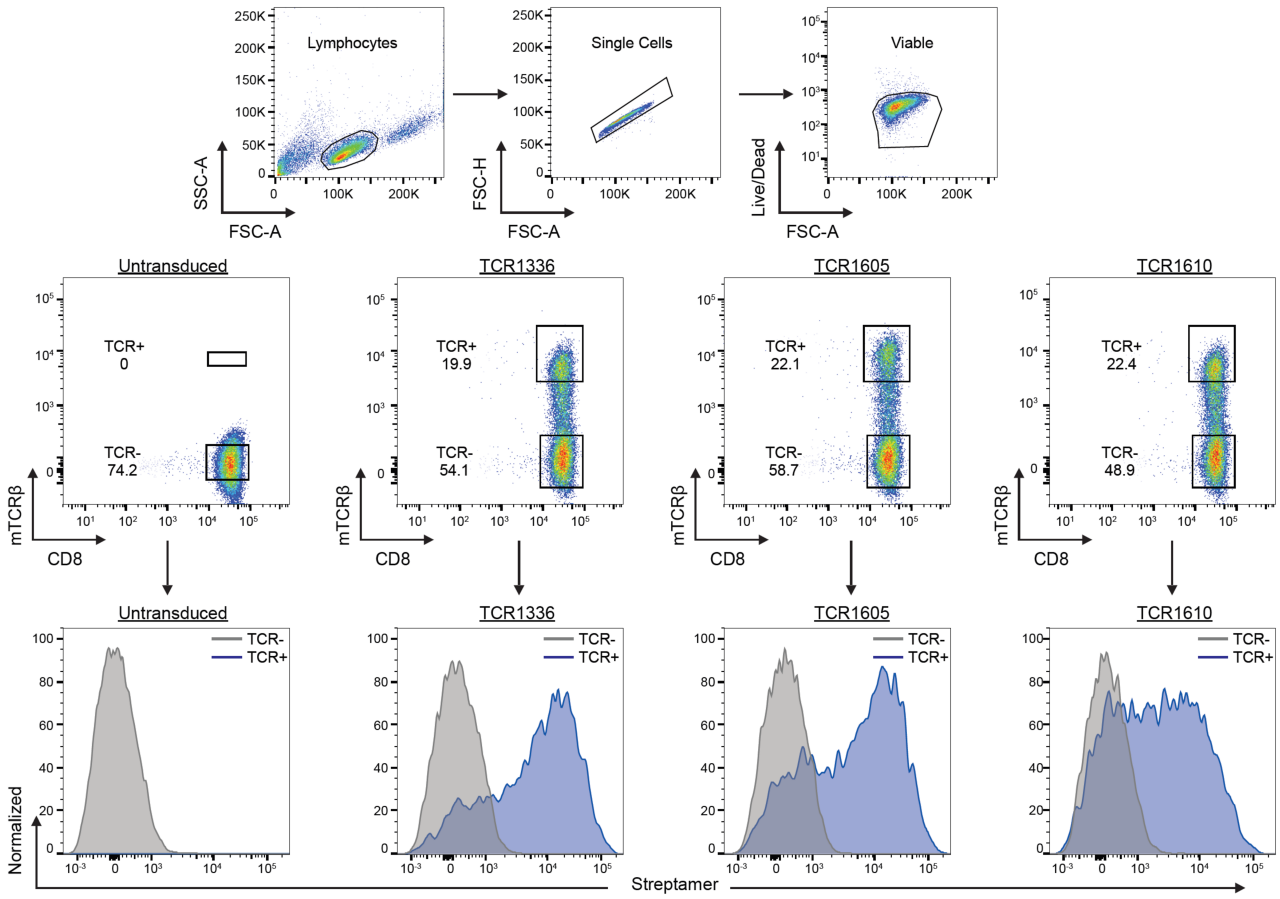


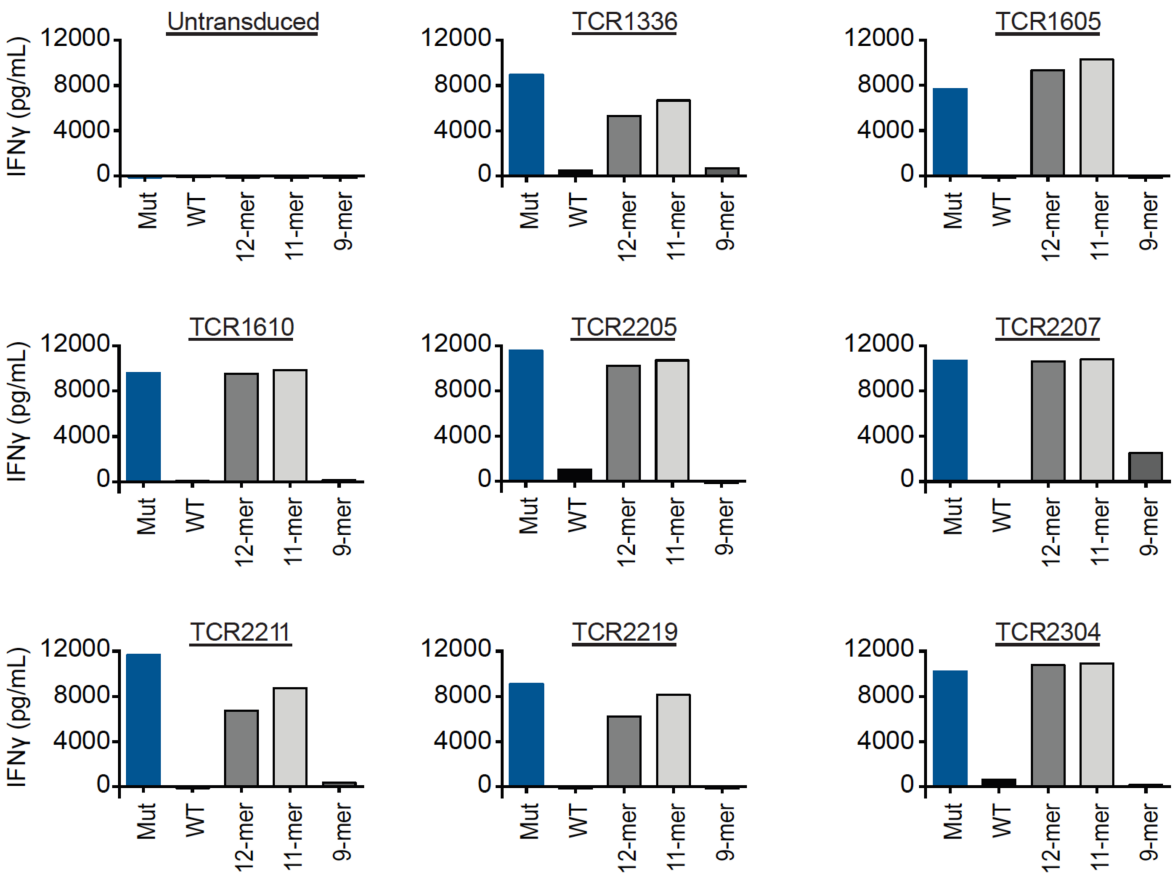
Supplementary Figure 1: Representative streptamer staining of TCR-transduced T cells

CD8+ T cells isolated from healthy donors transduced with the mutation-specific TCRs were stained with anti-mouse TCR β constant domain antibody and streptamer to test the surface expression the TCRs, and functionality of the alpha-beta TCR pairings from the sequencing results.

A

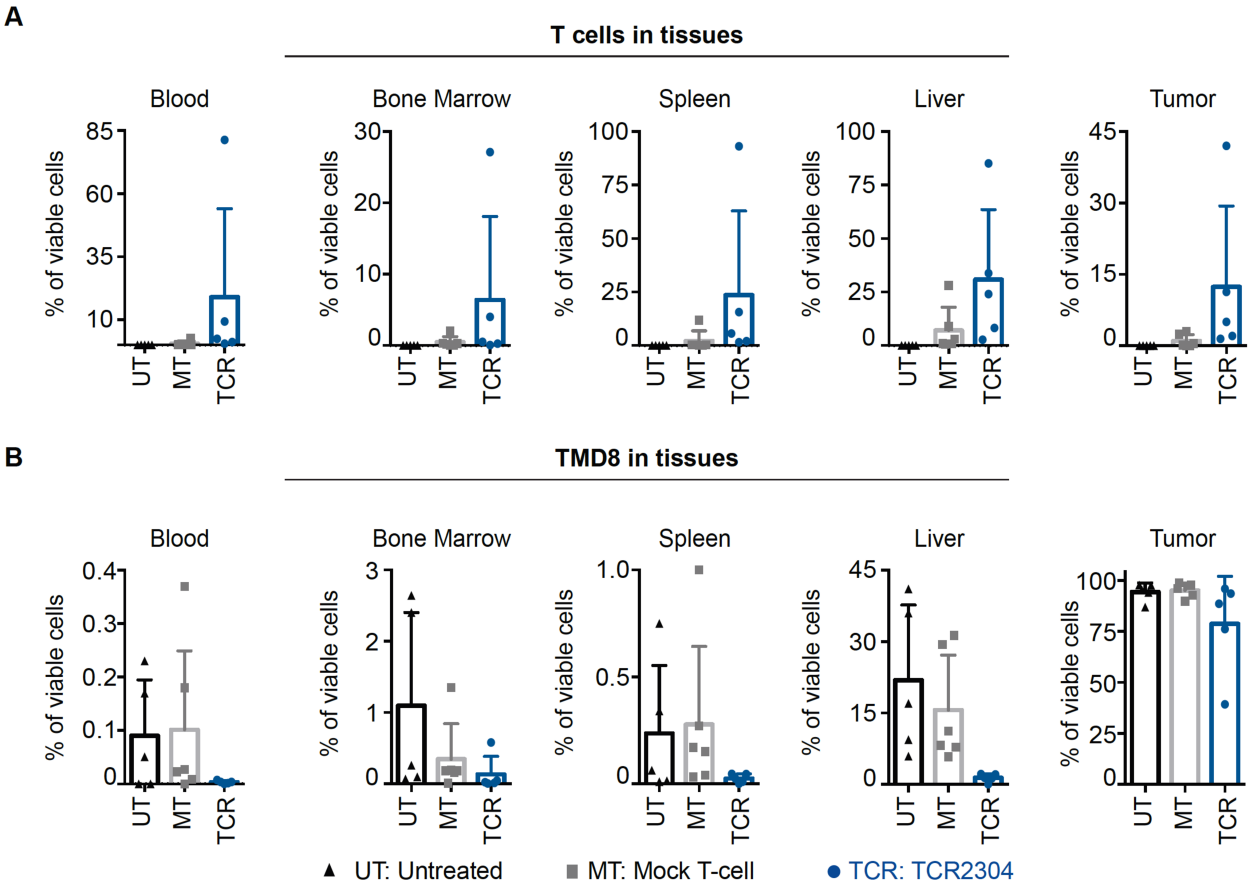
Peptide	HLA type	Sequence	Affinity (nM)	% Rank	Length
9mer	HLA-B07:02	**RPIPIKYKA**	613	1.30 (WB)	9mer
Mut	HLA-B07:02	**RPIPIKYKAM**	12	0.06 (SB)	10mer
Pre-1	HLA-B07:02	**KRPIPIKYKAM**	156	0.60 (SB)	11mer
Pre-2	HLA-B07:02	**QKRPIPIKYKAM**	219	0.70 (SB)	12mer

B



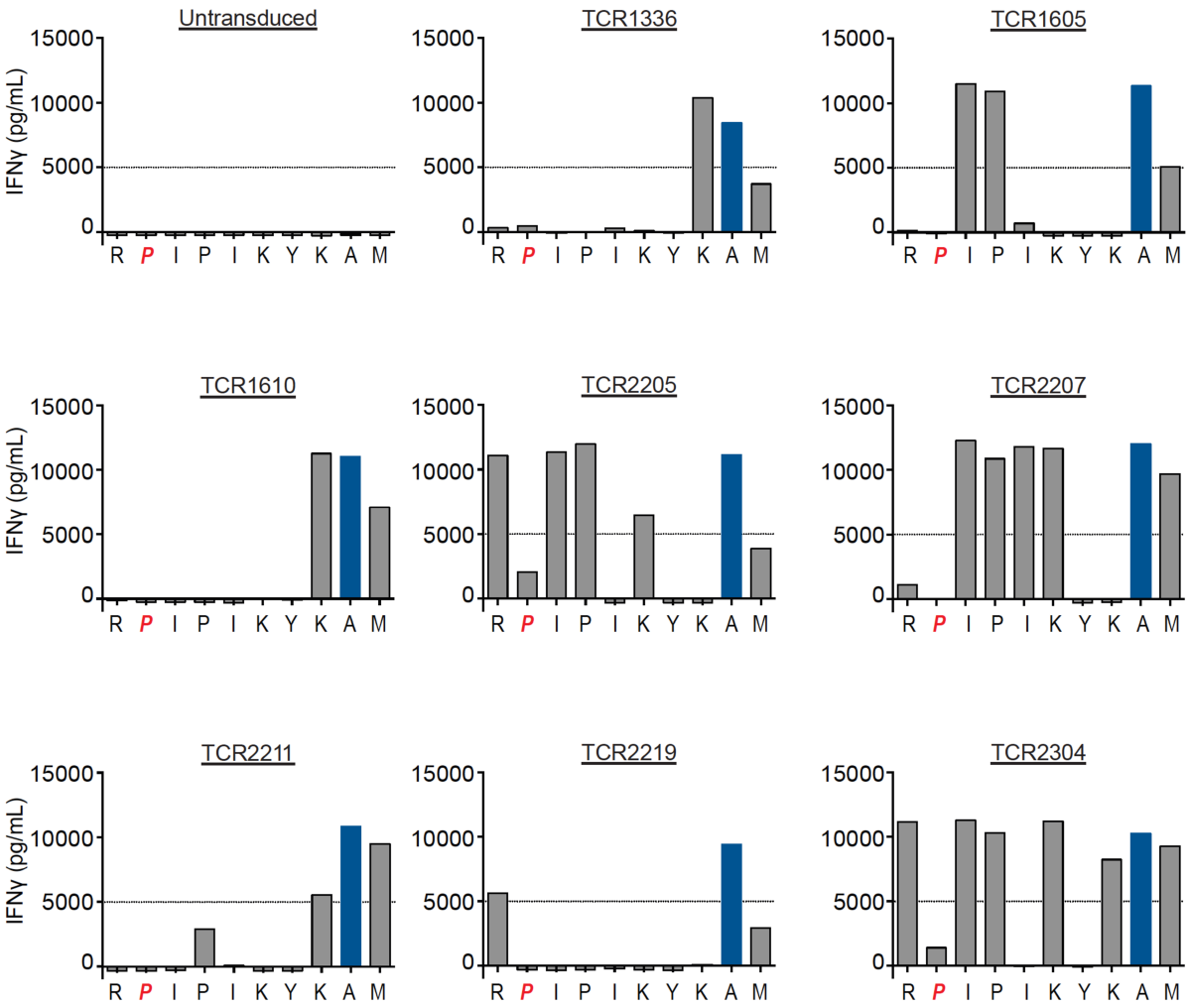
Supplementary Figure 2: Precursor peptides and epitope recognition

A. HLA binding prediction for 9-12mer peptides detected in the mass-spectrometric analysis of the mutant epitope (NetMHC 4.0). *B.* HLA-B*07:02 expressing K562 cells were loaded with peptides and co-cultured with TCR-T cells for 16 hours. IFNγ response was measured by ELISA. Mutation-specific TCRs recognized precursor peptides similarly to the 10mer epitope.



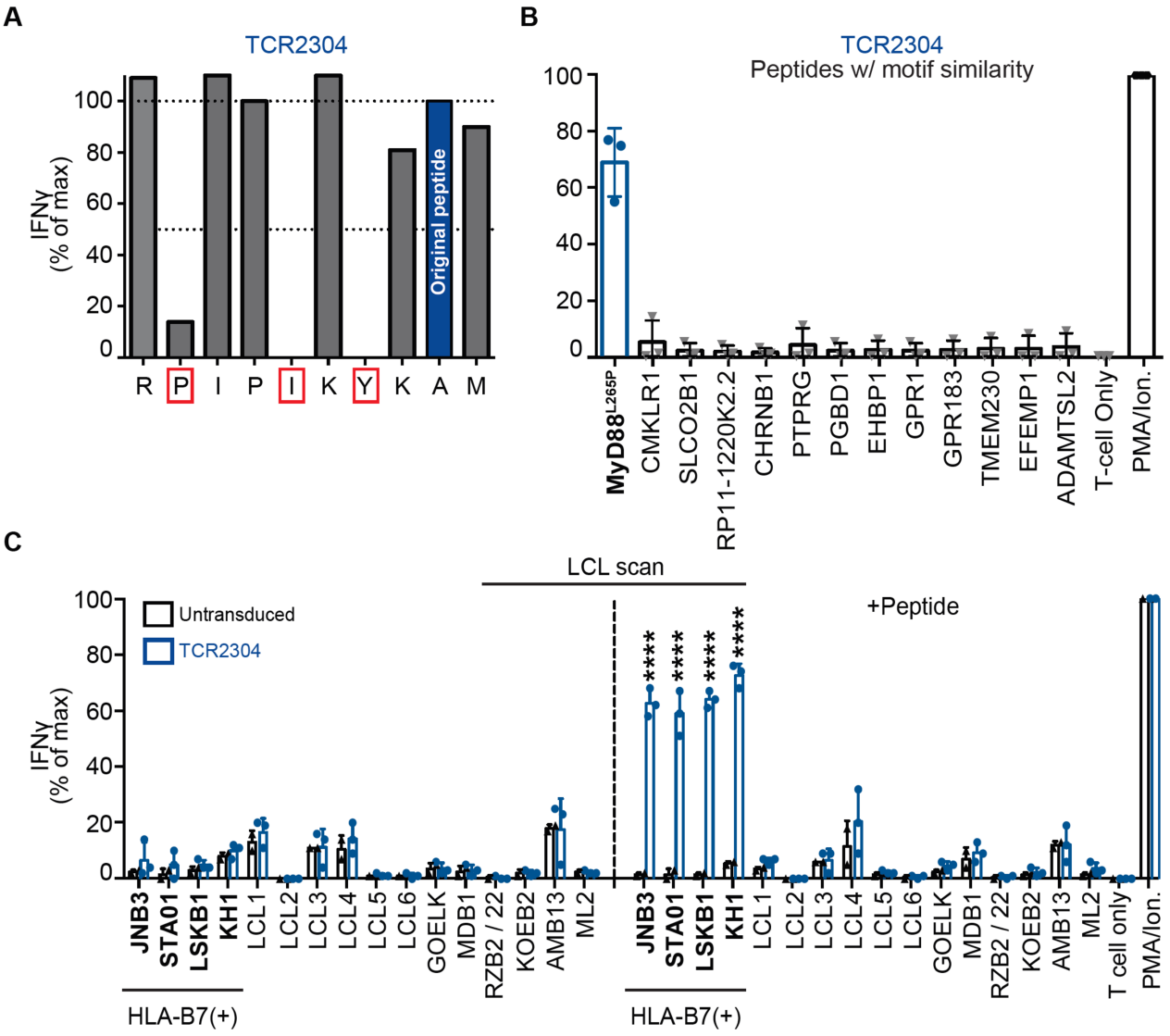
Supplementary Figure 3: Flow cytometric analysis of systemic dissemination of TMD8 cells

NOG mice were s.c. injected with 5x10^6 HLA-B*07:02 positive luciferase expressing TMD8 cells. *A.* Proportion of T cells in tissues of tumor bearing NOG mice. *B.* Proportion of TMD8 cells in tissues of tumor bearing NOG mice. Analysis from single, viable cells. Each symbol represents an individual mouse.



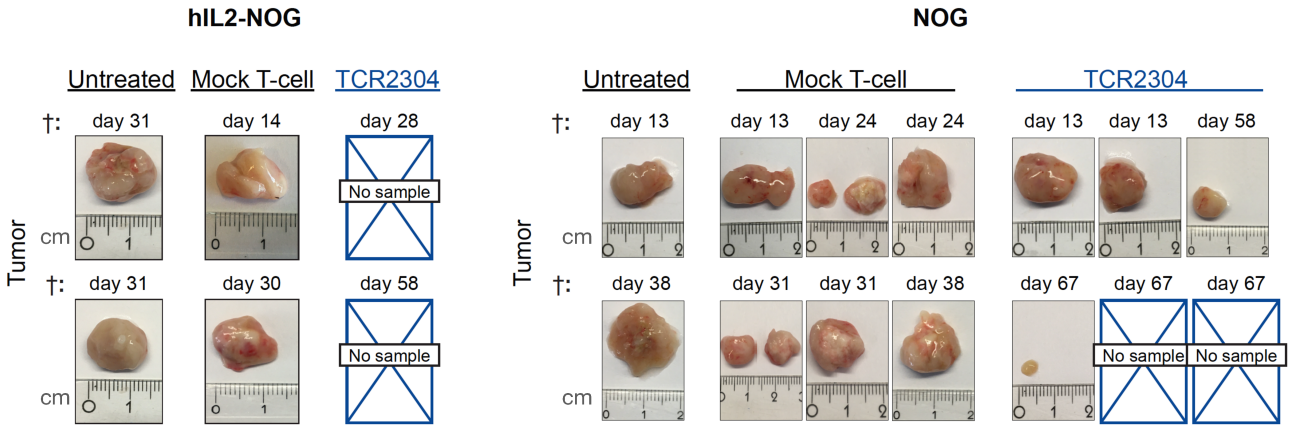
Supplementary Figure 4: Unique alanine scan results of mutation-specific TCRs

HLA-B*07:02 positive K562 cells were loaded either with 10mer mutant epitope or one of the 9 alanine scan peptides in high concentration (10 µg/ml) –to determine the most crucial amino acid positions for recognition, and co-cultured with TCR-T cells for 16 hours. IFNγ response was measured by ELISA.



Supplementary Figure 5: Safety analysis of TCR2304

A. HLA-B*07:02 expressing K562 cells were loaded either with 10mer mutant epitope or one of the 9 alanine scan peptides at the concentration of 10 µg/ml, and co-cultured with TCR2304-T cells for 16 hours. IFNγ response was measured by ELISA. Amino acid positions that negatively affected IFNγ response more than 50% were considered vital for recognition of the mutant epitope by the TCR. *B.* Expitope online tool [19] was used to scan human proteome for peptides with sequence similarity to binding motif of TCR2304 (xPxxIxYxxx) with up to 5 mismatch positions. Resulting peptides with any binding prediction to HLA*B07:02 were loaded on target cells similarly to alanine scan (10 µg/ml), and co-cultured with TCR2304-T cells from 3 different donors for 16 hours. IFNγ response was measured by ELISA. Error bars showing SD. *C.* A library of EBV-immortalized B-LCLs were co-cultured with TCR2304-T cells from 3 different donors for 16 hours, with or without prior peptide loading. IFNγ response was measured by ELISA. Error bars showing SD. Difference between untransduced and TCR-transduced T cells in with or without peptide conditions analyzed by 2-way ANOVA: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.



Supplementary Figure 6: OCI-Ly3 tumors from sacrificed hIL2-NOG and common NOG mice

Mice were s.c. inoculated with $5x10^6$ HLA-B*07:02 positive OCI-Ly3 cells. Once the tumors reached predetermined size of $100mm^3$ (~2weeks after injection), tumor bearing mice were treated with i.v. injection of $1x10^7$ TCR2304-T cells, or mock (untransduced) T cells of the same donor, or PBS as untreated control. Mice were sacrificed with cervical dislocation when tumor size exceeded $1500mm^3$, or or signs of distress was observed.