Supplementary information

Supplementary Tables

Supplementary Table 1

		peptide-specific T cells (total number)		
peptide	*graft*	*before expansion*	*after expansion*	*expansion (fold)*
HPV	G1	$1.49x10^3$	$4.61x10^4$	31
	G2	$1.06x10^4$	$2.86x10^5$	27
	G3	$2.49x10^4$	$1.45x10^6$	58
	G4	$3.60x10^3$	$2.36x10^4$	7
	G5	$8.42x10^3$	$1.14x10^4$	1
YPL	G1	$1.02x10^3$	$1.49x10^4$	15
	G2	$1.67x10^5$	$4.48x10^6$	27
	G3	$1.58x10^4$	$2.36x10^4$	1
	G4	$1.06x10^4$	$1.53x10^4$	1
	G5	$2.73x10^3$	$2.23x10^4$	8
EPL	G1	$4.09x10^4$	$1.67x10^6$	41
	G2	$1.24x10^5$	$1.11x10^7$	89
	G3	$1.13x10^5$	$8.67x10^6$	77
	G4	$3.70x10^5$	$1.75x10^7$	47
	G5	$4.10x10^4$	$1.18x10^7$	288
GLC	G6	$3.55x10^5$	$4.92x10^6$	14
CLG	G6	$1.10x10^6$	$9.01x10^6$	8
FLY	G6	$2.67x10^5$	$6.87x10^6$	26
YVL	G6	$1.16x10^6$	$3.10x10^7$	27

Supplementary Table 1 Peptide-specific $CD8^+$ T cell expansion in individual grafts

Total numbers of leukocytes were determined by automatic cell counting (Sysmex Hematology Analyzer XN-350, Sysmex Corporation, Kōbe, Japan). Numbers of peptide-specific T cells were calculated by flow cytometry-determined frequencies of peptide-specific T cells among total leukocytes.

Supplementary Table 2

reagent/specificity	*clone*	*fluorochrome*	*vendor*
viability dye		7AAD	Biolegend
fixable viability dye		Zombie Red	Biolegend
fixable viability dye		Zombie Yellow	Biolegend
CD3	UCHT1	AF700	Biolegend
CD4	SK3	Pacific Blue	Biolegend
CD8a	RPA-T8	FITC	Biolegend
CD8a	RPA-T8	APC	Biolegend
CD8a	RPA-T8	BV510	Biolegend
CD14	M5E2	APC/Fire750	Biolegend
CD19	SJ25C1	APC/Fire750	Biolegend
CD20	2H7	PerCP/Cy5.5	Biolegend
CD45	J33	Krome Orange	Beckman Coulter
CD56	5.1H11	APC/Fire750	Biolegend
CD137	4B4-1	PE	Biolegend
TCRαβ	IP26	AF700	Biolegend
murine CD3	17A2	PE/Cy7	Biolegend
murine CD3	17A2	BV421	Biolegend
murine TCRβ	H57-597	PE	Biolegend
HPV-specific HLA-B*35:01 tetramer		PE	NIH TCF
YPL-specific HLA-B*35:01 tetramer		APC	NIH TCF
EPL-specific HLA-B*35:01 tetramer		APC	NIH TCF
GLC-specific HLA-A*02:01 tetramer		PE	NIH TCF
CLG-specific HLA-A*02:01 tetramer		APC	NIH TCF
FLY-specific HLA-A*02:01 tetramer		PE	NIH TCF
YVL-specific HLA-A*02:01 tetramer		APC	NIH TCF

Supplementary Table 2 Flow cytometry staining reagents

All reagents were reactive against human species-reactive unless otherwise stated. AF: Alexa Fluor, FITC: fluorescein isothiocyanate, APC: allophycocyanin, BV: Brilliant Violet, PerCP: peridinin chlorophyll, Cy: cyanine, TCF: Tetramer Core Facility

Supplementary Table 3

G	*peptide*	*sorted cells (total number)*	*Successfully TCRαβ–sequenced cells (total number)*	*clonal T cells (total number)*	*number of T cell clones*
G1	HPV	92	87	81	6
	EPL	184	176	156	13
G2	HPV	184	144	104	11
	YPL	184	126	98	7
	EPL	184	124	81	17
G3	HPV	184	99	44	9
	EPL	184	128	85	21
G4	EPL	92	92	90	2
G5	EPL	184	158	107	24
G6	GLC	184	49	24	8
	CLG	184	36	32	1
	FLY	184	70	47	11
	YVL	184	27	14	6

Supplementary Table 3 Frequencies of TCR-recombinant CD8$^+$ human T cells

G: stem cell graft, clonal T cells: cells were determined clonal if we detected at least two cells with identical TCRαβ CDR3 amino acid sequences.

Supplementary Table 4

							cf (%)				
peptide	*TRAV*	*CDR3 α AA sequence*	*TRAJ*	*TRBV*	*CDR3 β AA sequence*	*TRBJ*	*G1*	*G2*	*G3*	*G4*	*G5*
HPV	5*01	CAESYTGGFKTIF	9*01	6-1*01	CASGSEAFF	1-1*01	6		nc		
HPV	19*01	CALSEAGGFGNEKLTF	48*01	10-3*01	CAISDPRDSYEQYF	2-7*01		nc	nc		
EPL	1-2*01	CAVRGSGGSYIPTF	6*01	10-3*01	CATGTGDSNQPQHF	1-5*01		2	11		
EPL	25*01	CAGRFMFSGGYNKLIF	4*01	28*01	CASSLPGANVLTF	2-6*01			4	2	
EPL	2*01	CAVEDMNSGGYQKVTF	13*01	28*01	CASKRTATYEQYF	2-7*01			nc		8

Supplementary Table 4 Overlap of epitope-specific TCRs between different stem cell grafts

TRAV: TCRα V-gene and allele, TRAJ: TCRα J-gene and allele, TRBV: TCRβ V-gene and allele, TRBJ: TCRβ J-gene and allele, AA: amino acid, cf: clone frequency among clonally expanded cells specific for the respective epitope, G: stem cell graft, nc: detectable but not clonally expanded

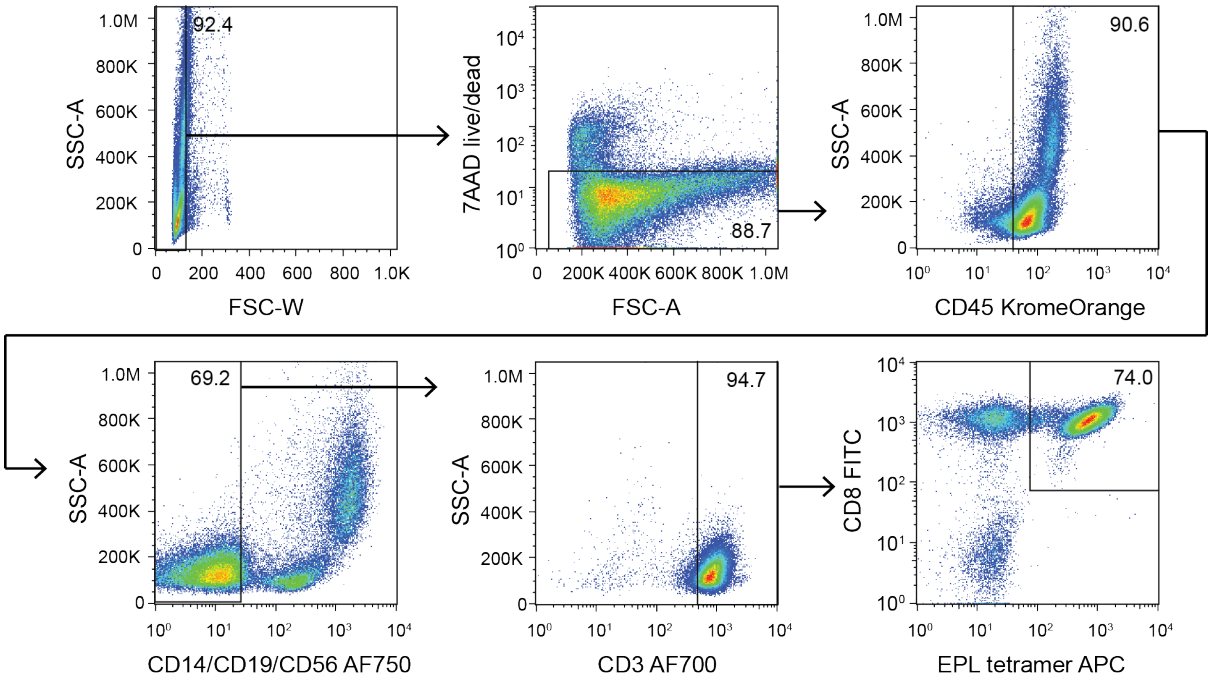
Supplementary Table 5

cell type and name	*HLA-A*		*HLA-B*		*HLA-C*	
miniLCL	01:01	26:01	35:01	57:01	04:01	06:02
miniLCL	02:01	29:02	44:02	45:01	06:02	-
LCL B01	03:01	24:02	15:01	35:01	03:03	04:01
LCL B03	02:01	23:01	15:01	58:01	03:04	07:01
LCL DJS	02:01	03:01	35:01	37:02	04:01	06:02
LCL JY	02:01	-	07:02	-	07:02	-

Supplementary Table 5 HLA class-I data of all LCL and miniLCL

Supplementary Figures

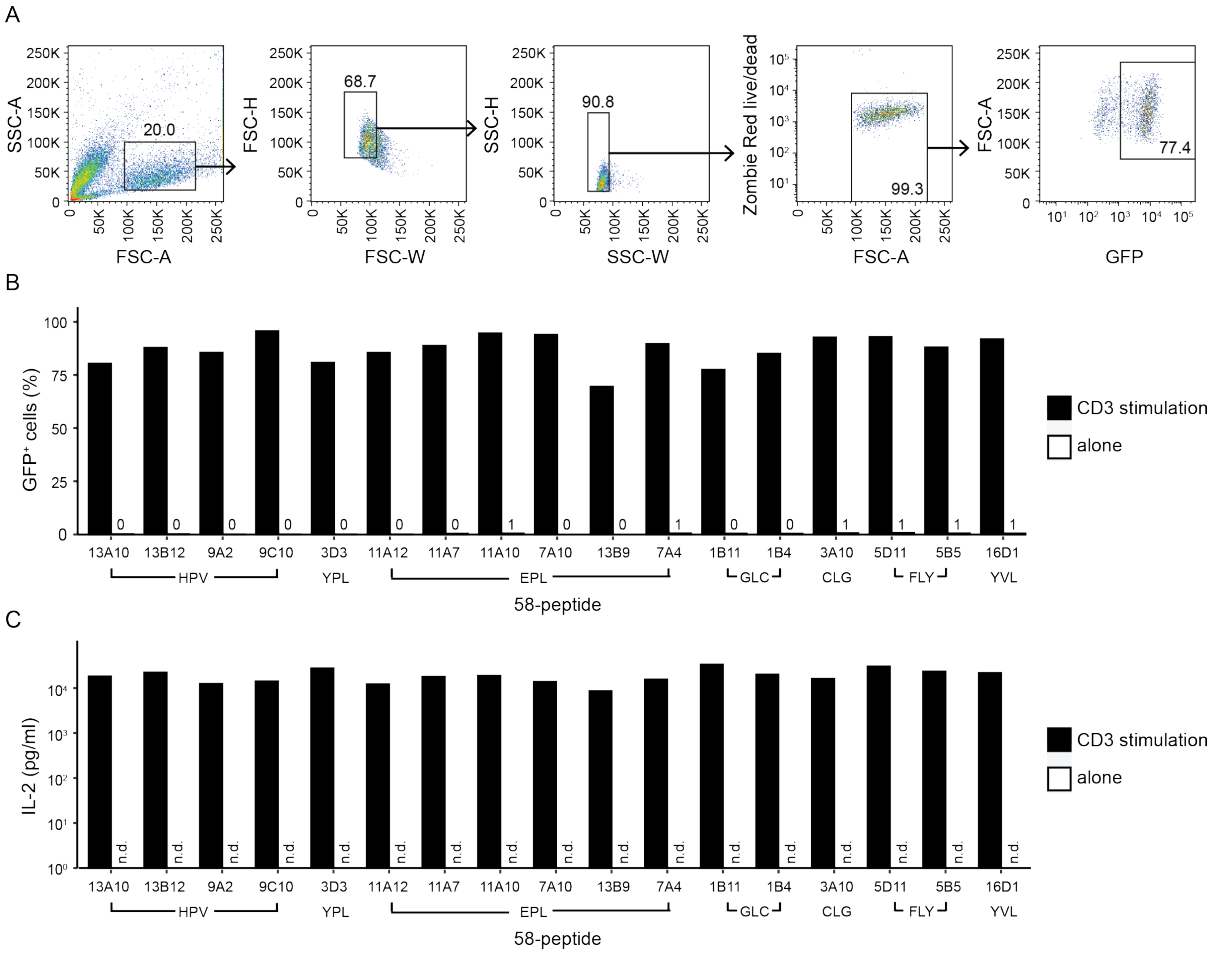
Supplementary Figure 1



Supplementary Figure 1 Identification of epitope-specific T cells by pMHC tetramer staining

Gating strategy for peptide-specific T cells before (day 0) and after (day 9) peptide stimulation. Gating on single, live, $CD45^+$, $CD14^-CD19^-CD56^-$ cells. After selection of $CD3^+$ T cells, the tetramer gate was set based on staining of cells that were expanded in presence of a different peptide. Numbers within or adjacent to gates indicate percentages.

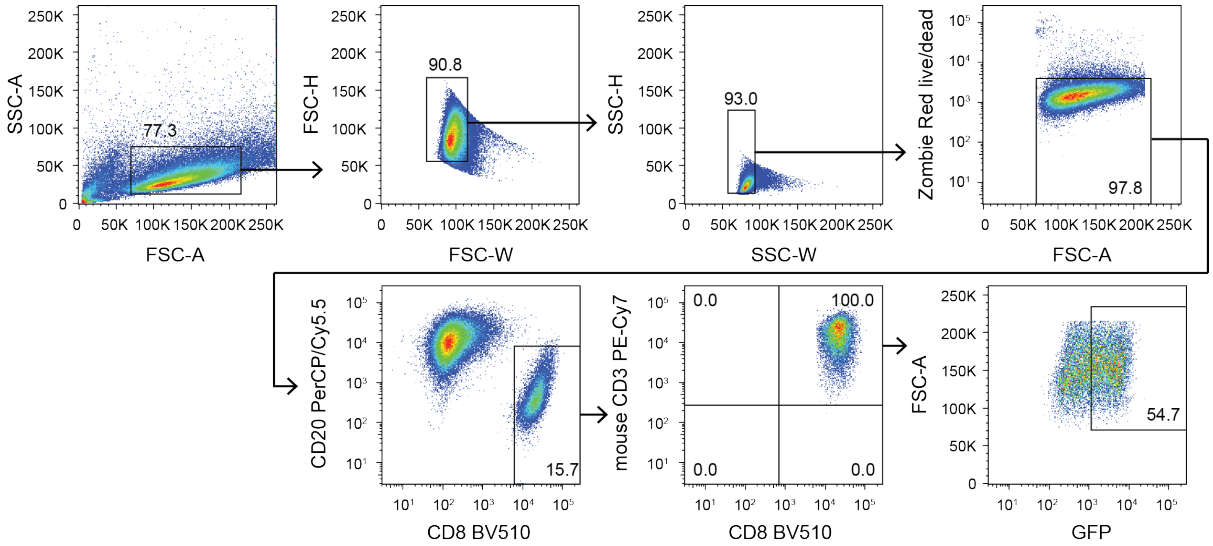
Supplementary Figure 2



Supplementary Figure 2 Stimulation of TCR-recombinant 58α⁻β⁻ cells with plate-bound anti-CD3

(A) Gating strategy for the identification of GFP⁺ TCR-recombinant cells. 58-GLC1B11 stimulated with CD3 is shown as an example. GFP gates were set based on non-stimulated controls. (B) Summarizes GFP expression of all TCR-recombinant 58α⁻β⁻ cell lines upon CD3 stimulation following the gating strategy presented in figure part A. "Alone" refers to TCR-recombinant 58α⁻β⁻ reporter cell lines alone. Numbers in places of bars indicate percentages. (C) IL-2 production measured by ELISA in cell culture supernatants corresponding to data in figure part B. n.d. = not detectable.

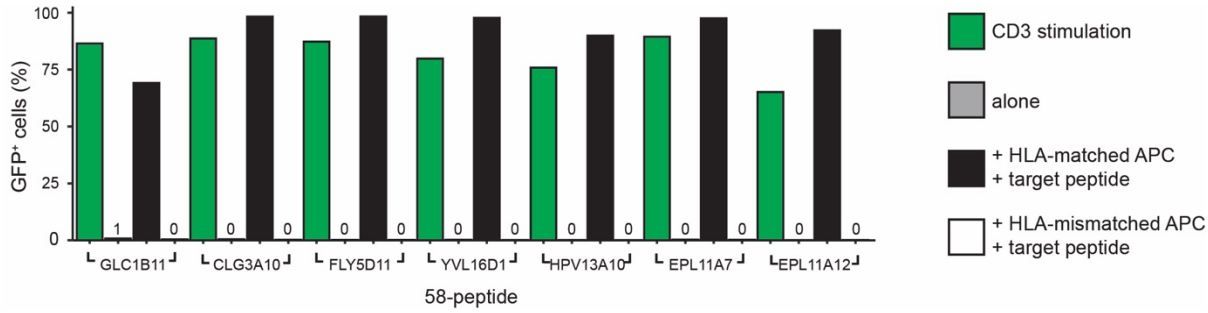
Supplementary Figure 3



Supplementary Figure 3 Gating strategy for identification of GFP expressing TCR-recombinant 58α⁻β⁻ cells

The figure shows GFP expression in 58-GLC1B11 in response to stimulation with GLC peptide-loaded antigen-presenting cells as an example. Numbers within or adjacent to gates indicate percentages.

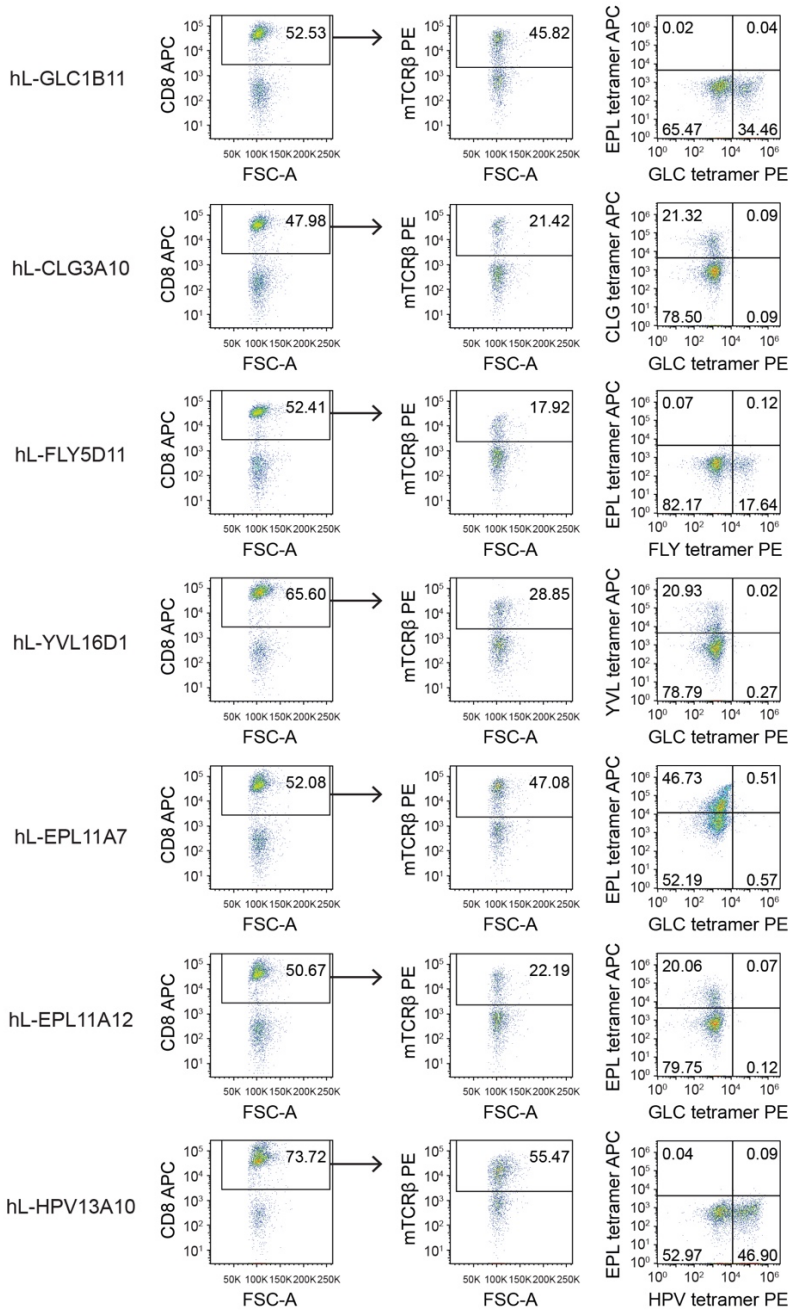
Supplementary Figure 4



Supplementary Figure 4 No cross-reactivity of four HLA-A*02:01 and three HLA-B*35:01-restricted TCRs with HLA-mismatched miniLCL

GFP expression of seven TCR-recombinant 58$\alpha^-\beta^-$ cell lines upon co-culture with HLA-matched and HLA-mismatched miniLCL loaded with the corresponding target peptides. Detailed HLA class-I data of the used miniLCL can be found in Supplementary Table 5. Numbers in places of bars indicate percentages. All bars represent mean values of two co-cultures within one experiment. APC = antigen presenting cells.

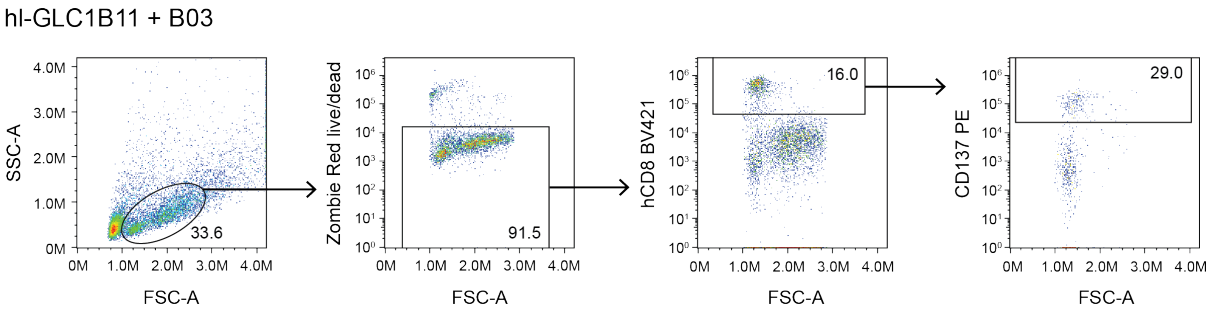
Supplementary Figure 5



Supplementary Figure 5 Recombinant TCR expression on human lymphocytes

Plots in the left column were pre-gated on lymphocytes by scatter characteristics. Plots in middle and right columns were pre-gated on $CD8^+$ lymphocytes. pMHC tetramer staining was done in a separate experiment using T cells of the same transduction and FITC-conjugated CD8 antibody. mTCRβ and pMHC tetramer gates were set based on controls with non-transduced T cells.

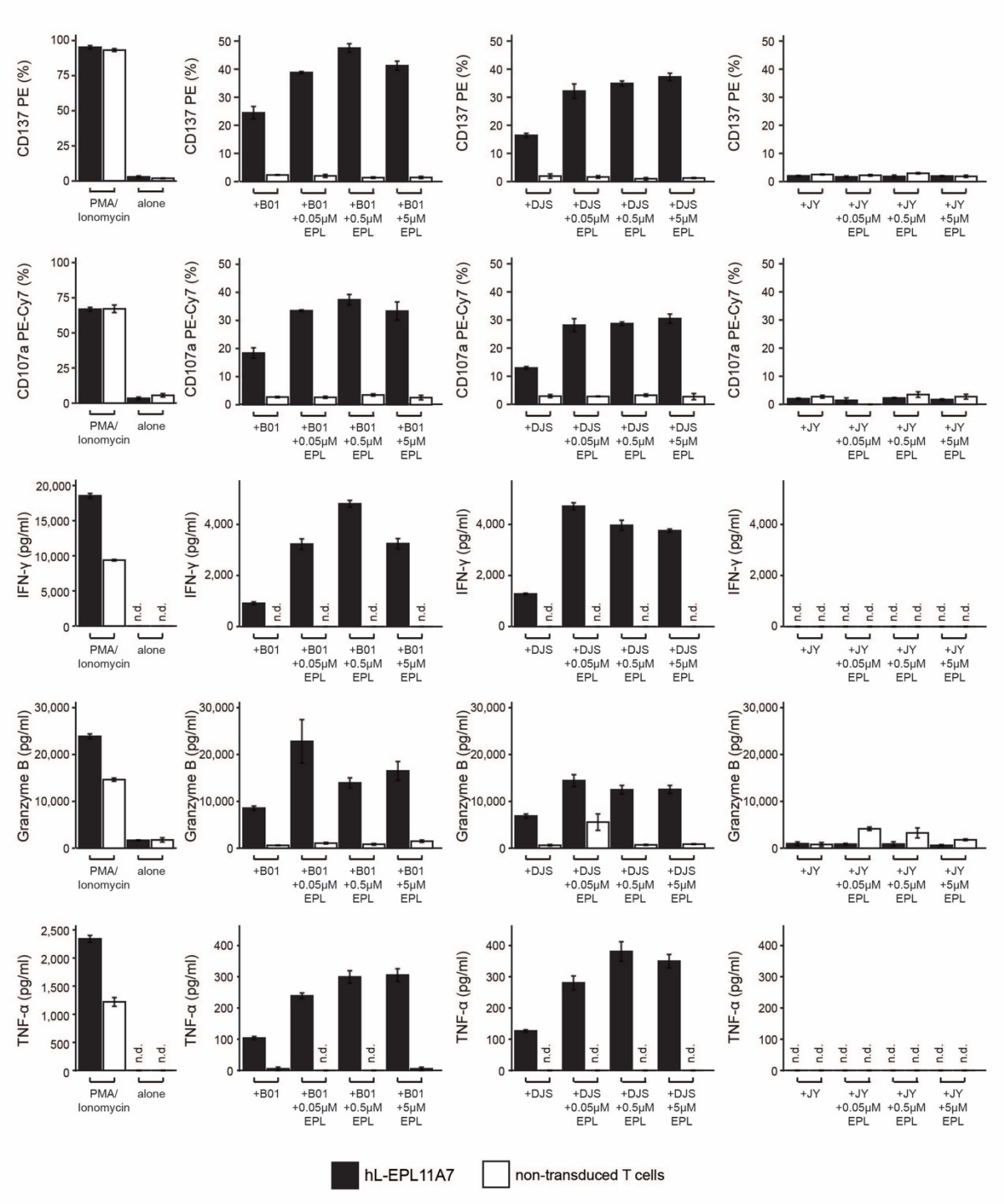
Supplementary Figure 6



Supplementary Figure 6 Gating strategy for identification of $CD8^+CD137^+$ human T cells

Data from one co-incubation of hl-GLC1B11 with LCL B03 are shown as a representative example. CD137 gates were set based on non-stimulated controls.

Supplementary Figure 7



Supplementary Figure 7 Detailed activation characteristics of hL-EPL11A7

hL-EPL11A7 were incubated with HLA-B*35:01-matched LCL (B01 and DJS) or HLA-B*35:01-mismatched LCL (JY) in presence or absence of increasing target peptide (EPL)

concentrations. CD137 and CD107a expression were determined by flow cytometry after pre-gating on live $CD8^+$ lymphocytes. IFN-γ, granzyme B, and TNF-α were measured in cell culture supernatants by ELISA. All bars represent mean values ± standard error of three co-cultures within one experiment. n.d. = not detectable.