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Supplemental Information

A broad and systematic approach to identify B cell malignancy-targeting TCRs for multi-antigen-based T cell therapy

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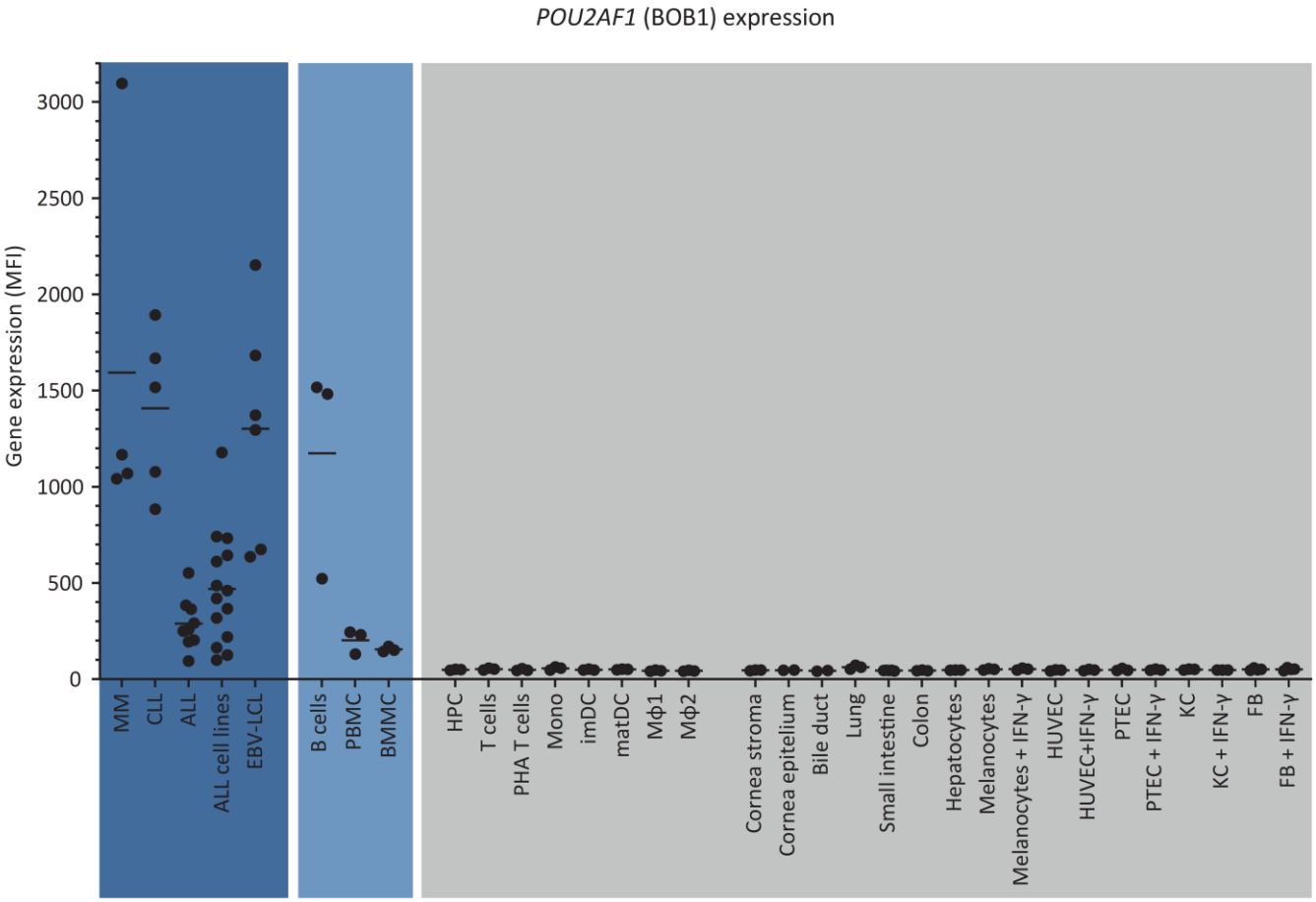

Figure S1 Example of a complete gene expression profile used to generate summary data in figure 1. Gene expression was retrieved from an Illumina HT12.0 microarray dataset.(25) *POU2AF1* (BOB1) gene expression (Mean Fluorescence Intensity; MFI) per cell type, individual samples and average (mean) gene expression is shown. Expression in patient derived B-cell malignancies or B-cell malignancy cell lines (dark blue), healthy B cells ($CD19^{pos}$) or B-cell containing PBMCs and BMMCs (light blue), healthy hematopoietic and non-hematopoietic cell types (grey). Abbreviations: MM, multiple myeloma; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; EBV-LCL, Epstein-Barr virus-transformed lymphoblastoid cell lines; PBMC, peripheral blood mononuclear cells; BMMC, bone marrow mononuclear cells; HPC, hematopoietic precursor cells; Mono, monocytes; imDC, immature dendritic cells; matDC, mature dendritic cells; MΦ1, type 1 macrophages; MΦ2, type 2 macrophages; HUVEC, human umbilical vein endothelial cells; IFN-γ, interferon-γ; PTEC, proximal tubular epithelial cells; KC, keratinocytes; FB, fibroblasts.

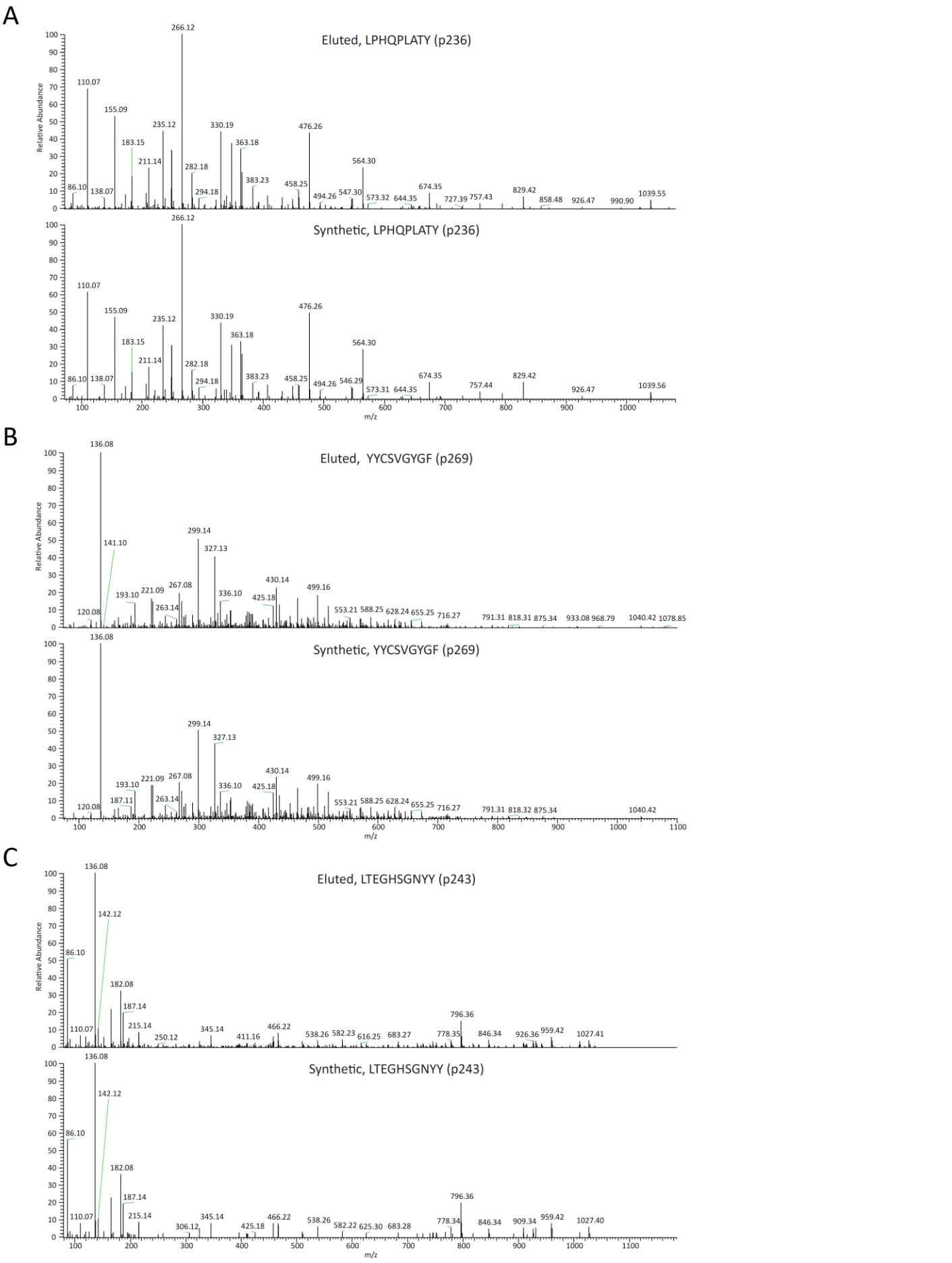

Figure S2 Examples of matching tandem mass spectra of eluted (top) and synthetic (bottom) peptides. (A) Tandem mass spectra of peptide 236 (p236, LPHQPLATY) derived from BOB1 presented in HLA-B35. (B) Tandem mass spectra of p269 (YYCSVGYGF) derived from VPREB3 presented in HLA-A24. (C) Tandem mass spectra of p243 (LTEGHSGNYY) derived from FCRL5 presented in HLA-A1.

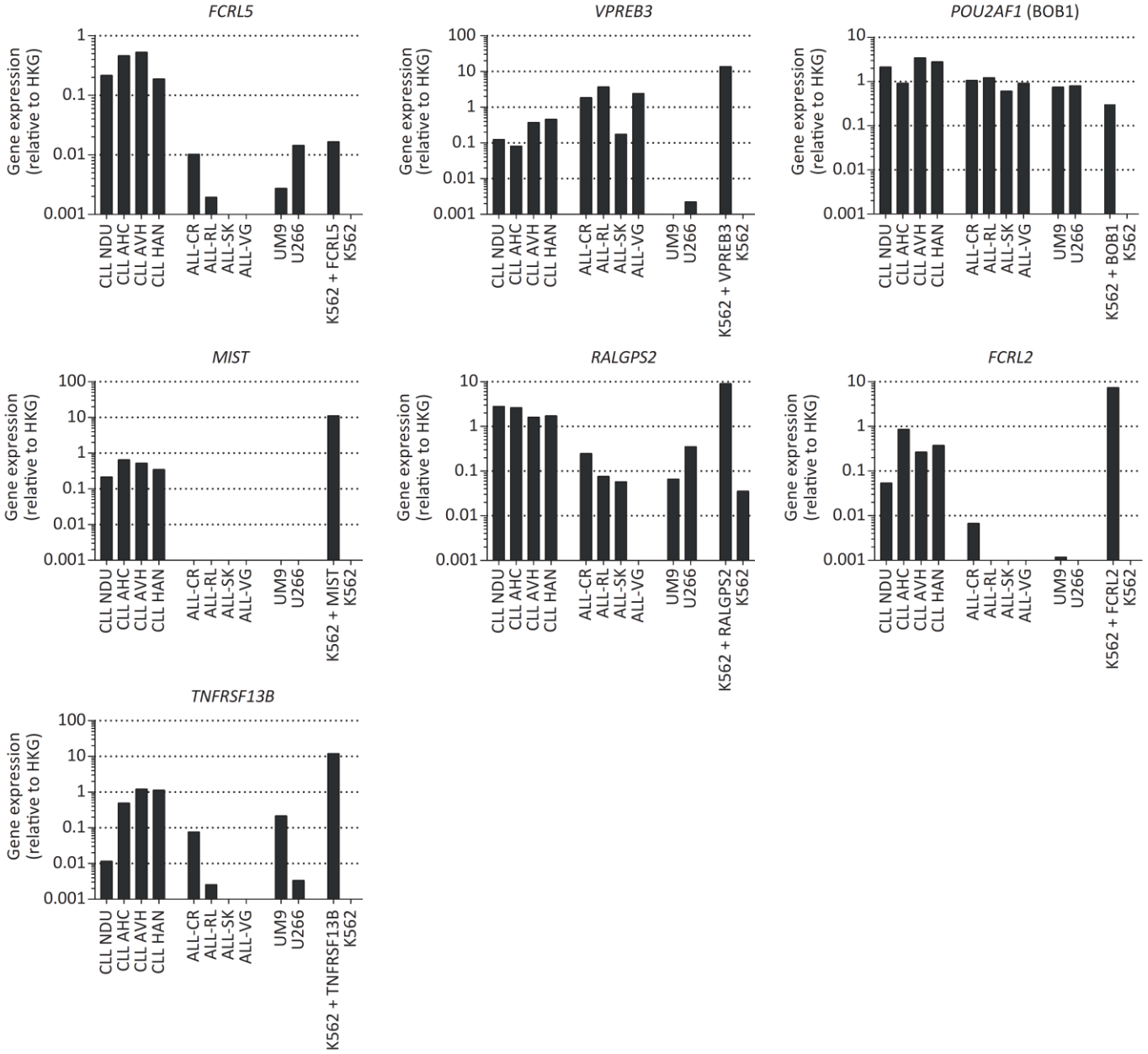

Figure S3 Target gene expression of targets used for T-cell selection. *FCRL5*, *VPREB3*, *POU2AF1*, *MIST*, *RALGPS2*, *FCRL2* and *TNFRSF13B* mRNA levels of patient derived chronic lymphocytic leukemia (CLL) samples, acute lymphoblastic leukemia (ALL) cell lines, multiple myeloma (MM) cell lines, target gene transduced K562 cells and K562 cells used in figure 4. Gene expression, measured by qPCR, depicted relative to house keeping genes (HKG). Values not depicted are <0.001 relative gene expression.

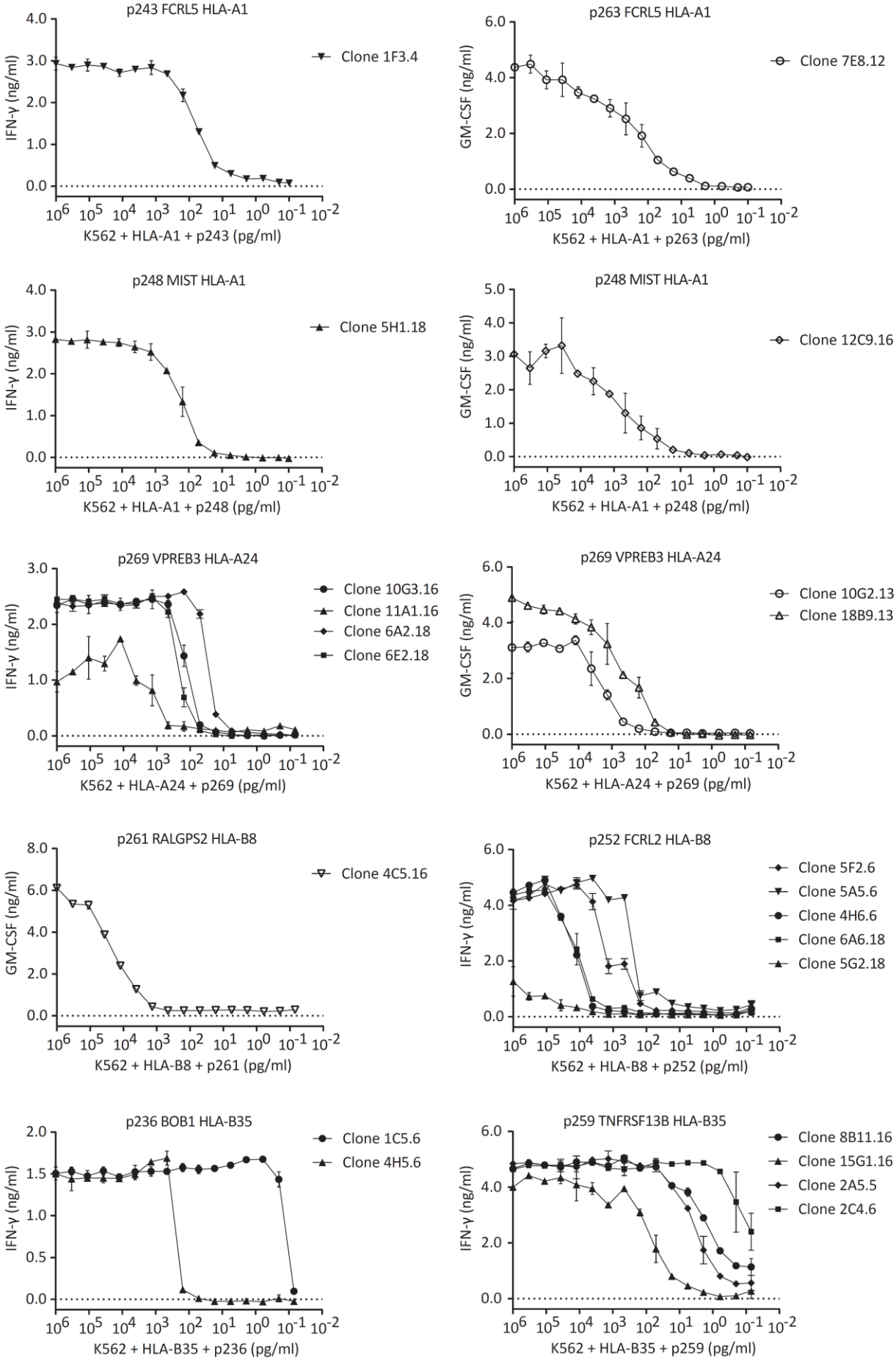

Figure S4 Peptide titrations of T-cell clones selected to recognize gene transduced (Td) K562 cells. IFN-γ (closed symbols) or GM-CSF (open symbols) production by T-cell clones from figure 3 after overnight stimulation with K562 cells Td to express target HLA, loaded with decreasing concentrations of target peptide in an effector:target ratio 1:6. Graphs are separated based on T-cell specificity and cytokine production. Values and error bars represent mean and standard deviations of technical duplicates.

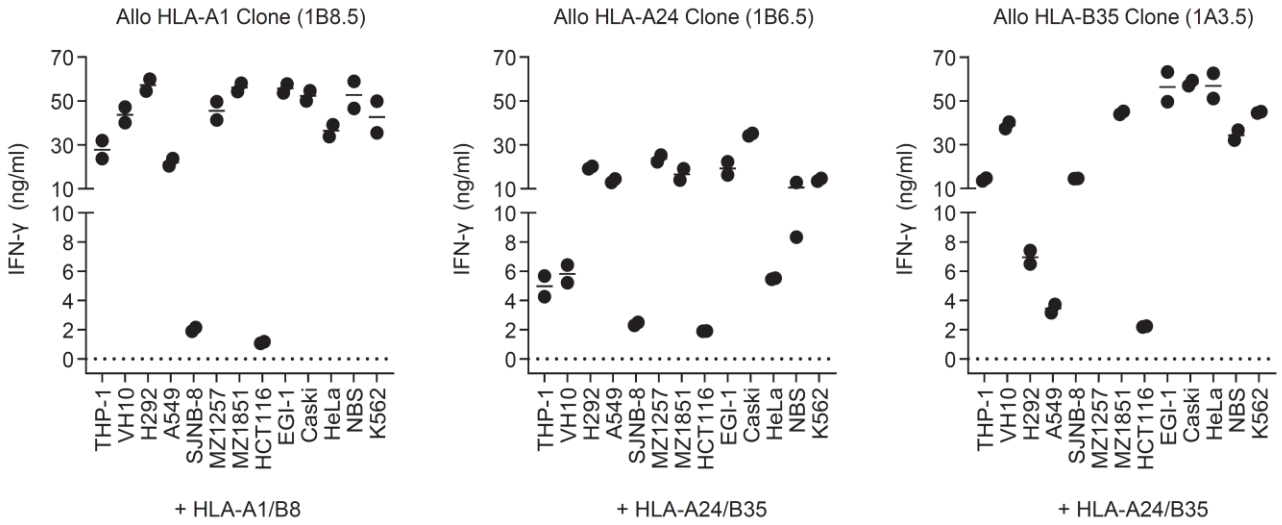

Figure S5 Recognition by allo HLA T-cell clones of cell lines used in safety screenings. IFN-γ production by allo HLA-A1 (left panel), A24 (middle panel) and B35 (right panel) T cell clones after overnight co-culture with HLA-A1 and HLA-B8 (+HLA-A1/B8) or HLA-A24 and HLA-B35 (+HLA-A24/B35) transduced cell lines. IFN-γ measured by ELISA, technical duplicates are depicted. Data obtained from the same experiment as shown in Figure 4.

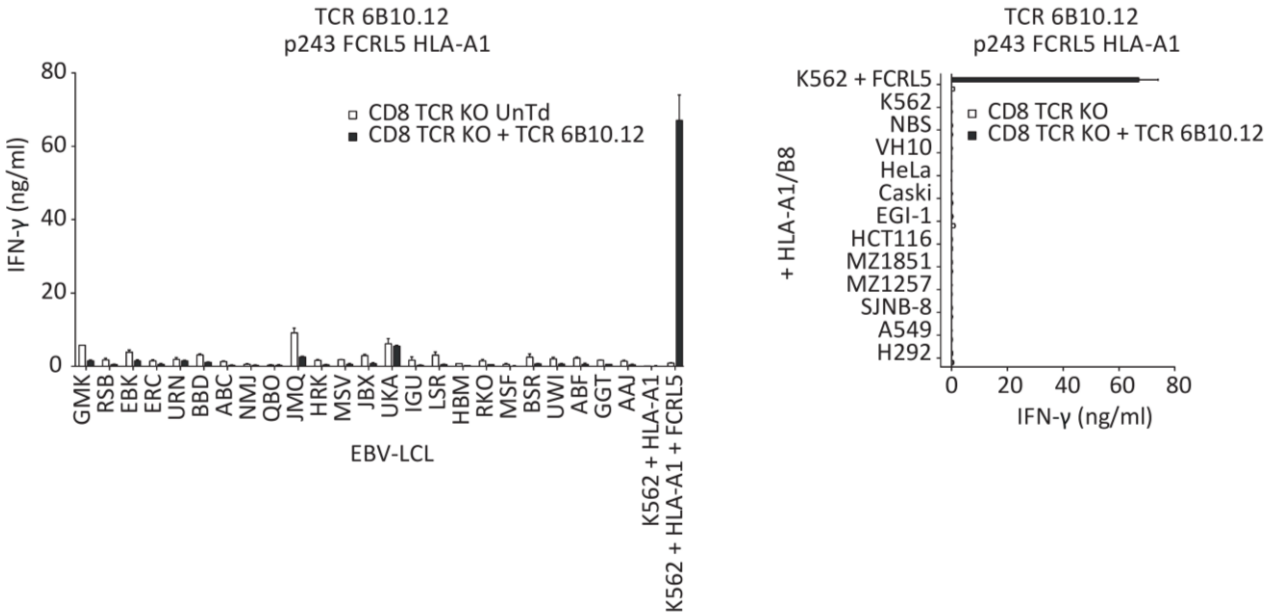

Figure S6 Safety screening of TCR 6B10.12 (FCRL5 HLA-A1). Endogenous TCR knock out CD8 T cells (5% residual huTCR^{pos} cells) untransduced or Td with TCR 6B10.12 (FCRL5 HLA-A1) were used because of lack of *in vitro* expansion of parental T-cell clone 6B10.12. Experiment performed as described in figure 5. Values represent means and standard deviations of technical duplicates.

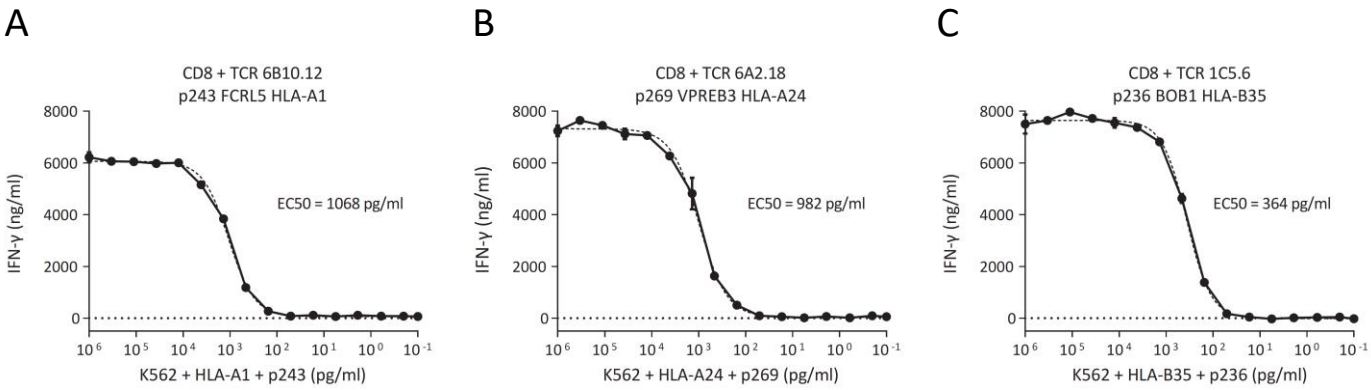

Figure S7 TCR avidity determined by peptide titrations. TCR transduced CD8 T cells were enriched for mTCR expression by MACS. IFN-γ production measured by ELISA after overnight co-culture with HLA transduced K562, loaded with decreasing concentrations of target peptide in an effector:target ratio 1:6. Sigmoidal curves (dotted lines) are plotted based on measured concentrations (solid lines). EC50 values were calculated based on sigmoidal curves and represent peptide concentrations required to induce 50% of the maximum cytokine production. Values represent means and standard deviations of technical duplicates. (A) CD8 T cells transduced with FCRL5 HLA-A1 p243 restricted TCR 6B10.12. (B) CD8 T cells transduced with VPREB3 HLA-A24 p269 restricted TCR 6A2.18. (C) CD8 T cells transduced with BOB1 HLA-B35 restricted TCR 1C5.6.

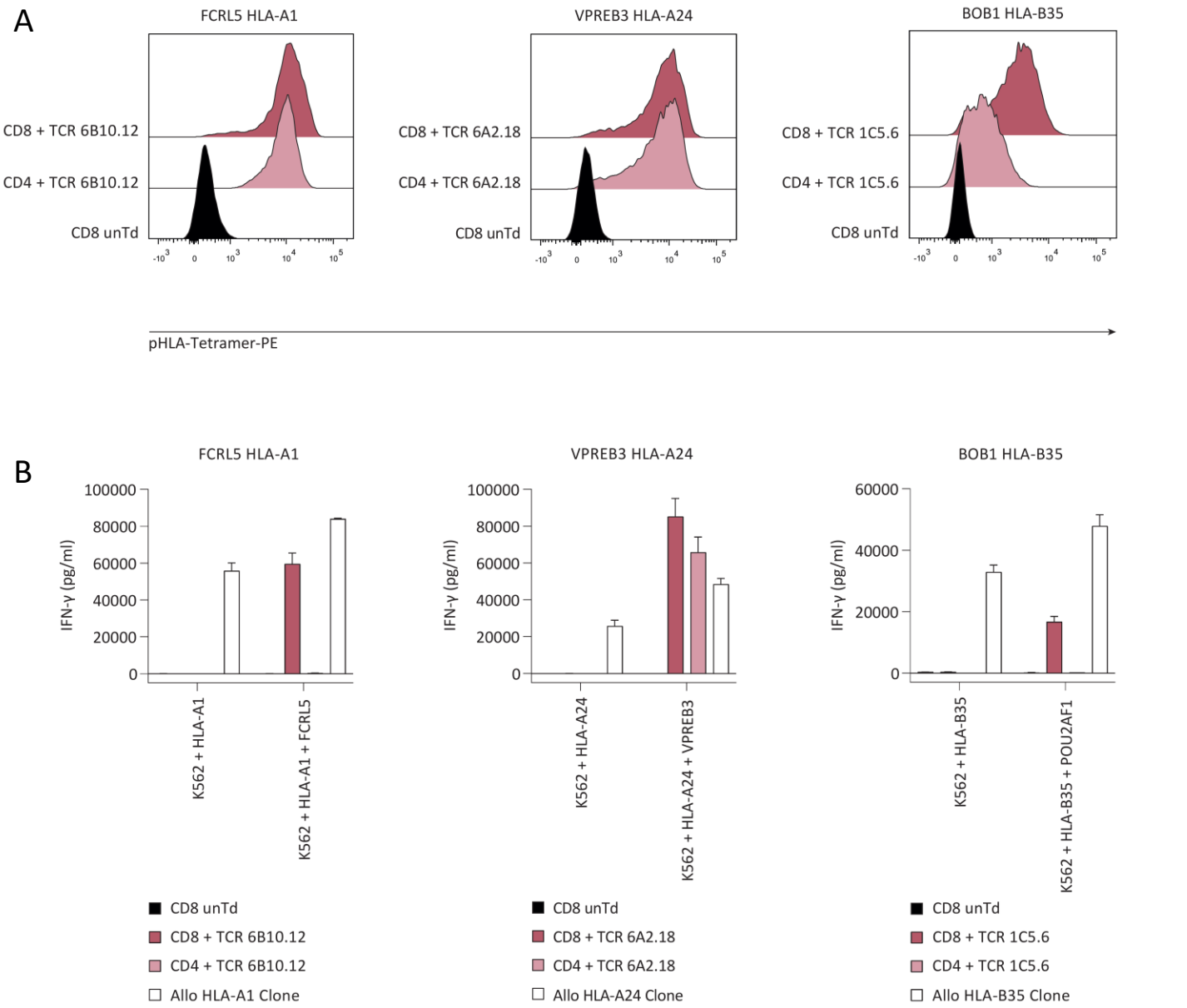

Figure S8 CD8 co-receptor dependence of identified TCRs. CD8 (dark pink) and CD4 (light pink) cells were isolated separately and transduced with identified TCRs or left untransduced (CD8 only, black). T cells were enriched for mTCR expression by MACS and used for analysis on day 10-12 after stimulation. T cells were transduced with FCRL5 HLA-A1 p243 restricted TCR 6B10.12. (left panels), VPREB3 HLA-A24 p269 restricted TCR 6A2.18. (middle panels) or BOB1 HLA-B35 restricted TCR 1C5.6 (right panels). (A) FACS analysis of T cells stained with specific PE-labeled pHLA-tetramers, gated on CD8 or CD4 positive cells. (B) IFN-γ production by T cells from experiment shown in A, after overnight co-culture with K562 cells transduced with target HLA alone or with target HLA and target gene combined in an effector:target ratio 1:6. Allo-HLA T-cell clones were included as positive control for HLA expression. Values represent means and standard deviations of technical duplicates.

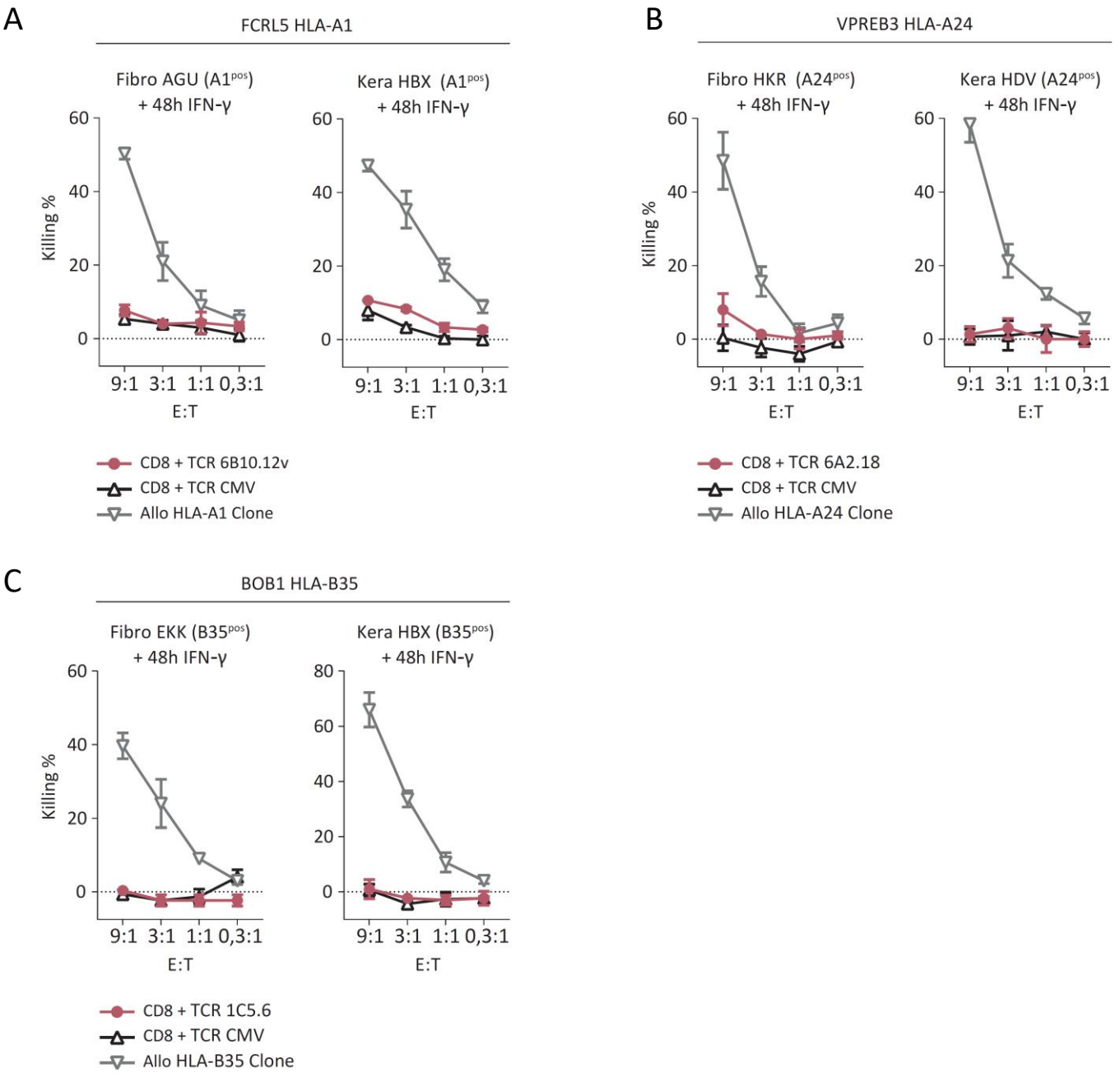

Figure S9 Cytotoxicity of antigen negative target cells by TCR transduced (Td) CD8 T cells. Killing by CD8 T cells Td with selected TCRs (pink), CMV (pp65-NLV-HLA-A2) TCR Td CD8 T cells (black) as negative control and allo-HLA-A1, A24 or B35 T-cell clones (grey) as positive controls. Target cells were fibroblasts and keratinocytes expressing target HLA alleles as indicated between brackets, pre-treated for 48 hours with 100 IU/ml IFN-γ to upregulate HLA expression. Killing was measured by 51CR release assay after 6-hour co-culture in different effector:target (E:T) ratios. Values and error bars represent mean and standard deviations of technical triplicates. Data was obtained from the same experiment as shown in figure 6. Fibroblasts and keratinocytes were negative (<0,0003 relative expression compared to house keeping genes) for *VPEB3*, *FCRL5* and *POU2AF1* expression measured by qPCR as described in figure S3. (A) Killing by FCRL5 HLA-A1 specific TCR 6B10.12 Td CD8 T cells. (B) Killing by VPREB3 HLA-A24 specific TCR 6A2.18 Td CD8 T cells. (C) Killing by BOB1 HLA-B35 specific TCR 1C5.6 Td CD8 T cells. Abbreviations: Fibro, fibroblasts; Kera, Keratinocytes.

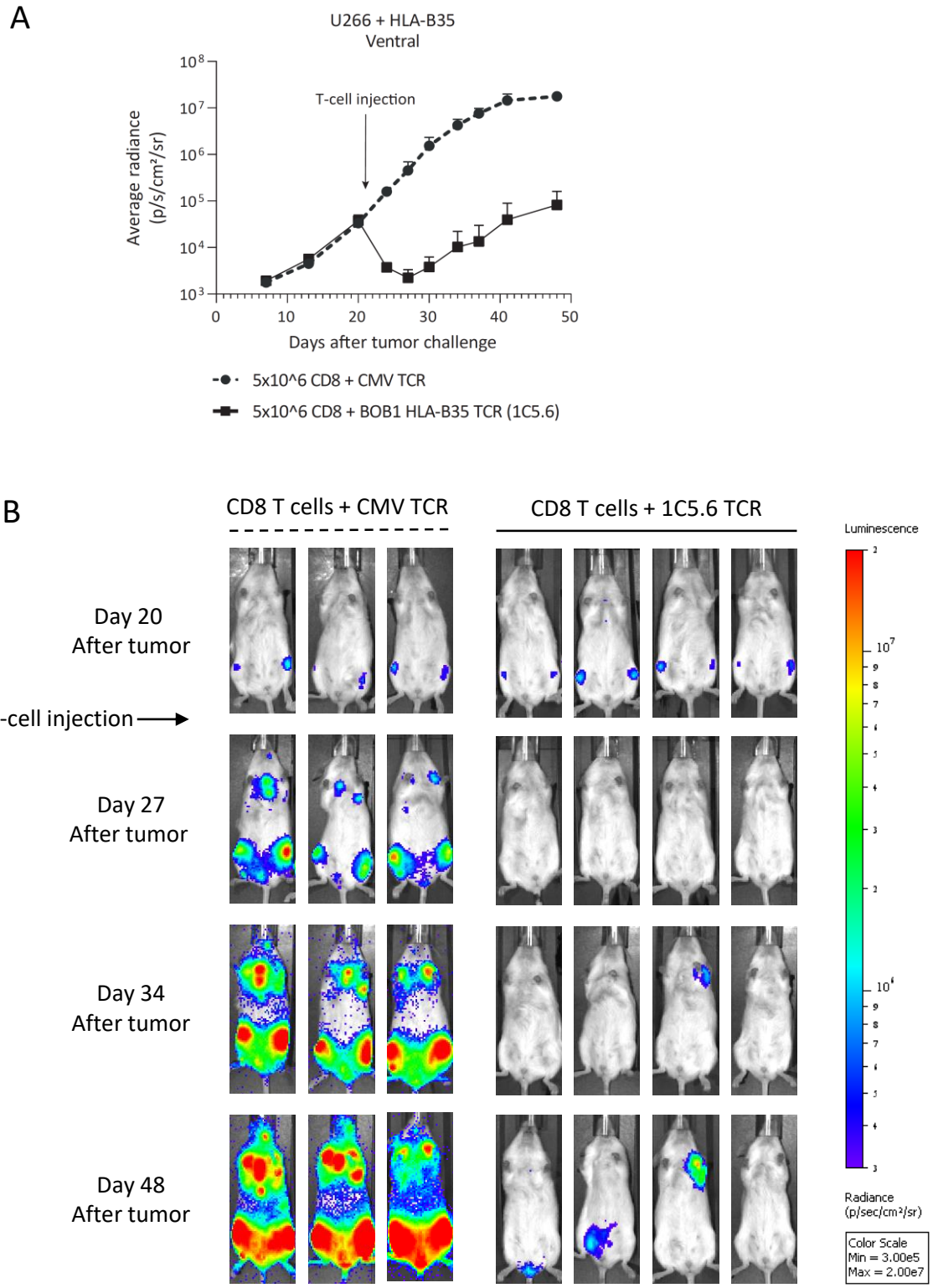

Figure S10 *In vivo* antitumor efficacy of BOB1 HLA-B35 restricted TCR transduced CD8 T cells. NSG mice engrafted with $2x10^6$ U266 multiple myeloma cells transduced with luciferase and HLA-B35, were i.v. injected with $5x10^6$ TCR transduced CD8 T cells after 21 days. T cells were transduced with BOB1 HLA-B35 restricted TCR 1C5.6 (n=4) or control CMV (pp65-NLV-HLA-A2) TCR (n=3) and enriched for mTCR expression by MACS. Tumor outgrowth was frequently tracked by bioluminescence imaging. (A) Mean and standard deviations of tumor outgrowth over time on the ventral side of CMV TCR treated control mice (dashed line) and BOB1 HLA-B35 TCR (solid line) treated mice. (B) Tumor outgrowth for individual CMV TCR (left) or BOB1 HLA-B35 TCR (right) treated mice measured on day 20, 27, 34 and 48 after tumor cell injection.

Table S1: Origin and HLA typing of HLA-A1, A24, B8 or B35^{pos} B-cell malignancies used for peptide elution and the number of unique peptides that were identified by mass spectrometry with Ion scores ≥20 or ≥35.

Patient Code	Diagnosis	Material	HLA-A	HLA-B	HLA-C	Experiment Tag	Cells used for peptide elution (x10^9)	Unique Peptides Ion Score≥20	Unique Peptides Ion Score≥35
AGP	ALL	Peripheral blood	A*01:01 - A*02:01	B*08:01 - B*38:01	C*07:01 - C*12:03	Exp1	233	36773	17449
CDT	HCL	Spleen	A*02:01 - A*29:02	B*35:01	C*04:01	Exp2	500	63562	26981
SWD	CLL	Spleen	A*01:01 - A*02:01	B*08:01 - B*40:01	C*03:04 - C*07:01	Exp3	180	68892	30168
						Exp15	0,1	1351	415
						Exp22	0,2	4981	1645
MWY	ALL	Peripheral blood	A*11:01 - A*NT	B*35:01 - B*40:02	C*02:02 - C*04:01	Exp4	610	24873	13655
						Exp14	0,1	539	322
WSG	ALL	Peripheral blood	A*01:01 - A*03:01	B*18:01 - B*35:08	C*04:01 - C*12:03	Exp5	62	44098	23211
SLE	ALL	Peripheral blood	A*01:01 - A*32:01	B*08:01 - B*45:01	C*06:02 - C*07:01	Exp6	512	32830	11242
NBA	ALL	Peripheral blood	A*24:02 - A*26:01	B*38:01	C*12:03	Exp7	210	35330	17375
HJS	FL	Peripheral blood	A*24:02 - A*32:01	B*44:02 - B*44:05	C*02:02 - C*05:01	Exp8	243	2696	1296
KYE	ALL	Peripheral blood	A*02:01 - A*24:02	B*18:01 - B*40:02	C*03:04 - C*07:01	Exp10	284	45721	13332
AHC	CLL	Peripheral blood	A*01:01 - A*03:01	B*07:02 - B*08:01	C*07:01 - C*07:02	Exp16	0,2	3476	1002
ALA	ALL	Peripheral blood	A*02 - A*11	B*07 - B*35	C*04 - C*07	Exp17	0,2	3628	1233
EMY	ALL	Peripheral blood	A*11 - A*32	B*35 - B*44	C*03 - C*04	Exp18	0,2	3800	1662
HAN	CLL	Bone marrow	A*02:01	B*08:01 - B*15:01	C*03:03 - C*07:01	Exp20	0,2	3257	895
HBP	ALL	Peripheral blood	A*02:01 - A*24:02	B*39:01 - B*57:01	C*06:02 - C*12:03	Exp21	0,2	7335	2373
UM9 + A2	MM	Celline	A*01:01 - A*11:01 (A*02:01 Td)	B*07:02 - B*55:01	C*03:03 - C*07:02	Exp107	30	82504	34115

Table S2: Peptides derived from selected genes identified by mass spectrometry from B-cell malignancy samples.

Patient Code	Diagnosis	Target HLA expressed	Experiment Tag	RALGPS2-LTDSEKGNSY-A1 p242	FCRL5-LTEGHSGNYY-A1 p243	FCRL5-TTENSGNYY-A1 p263	MIST-ESEYADTHY-A1 p248	TLR10-YLDHNSFDY-A1 p265	IGJ-YTAVVPLVY-A1 p268	BLK-AYIERMNSI-A24 p246	VPREB3-YYCSVGYGF-A24 p269	RALGPS2-QYIEELQKF-A24 p258	TLR10-ELFKRTIQL-B8 p247	TLR10-LPHLKTLIL-B8 p256	IGLL1-HGLLRPTAA-B8 p251	KLHL14-DMNTKRAIHTL-B8 p239	FCRL2-IVKIKVQEL-B8 p252	FAM129C-LPALRAQTL-B8 p255	FAM129C-YLRLLDAL-B8 p271	RALGPS2-TLKIRAEVL-B8 p261	TNFRSF13B-SADQVALVY-B35 p259	POU2AF1/BOB1-APAPTAVVL-B35 p233	POU2AF1/BOB1-LPHQPLATY-B35 p236
AGP	ALL	A*01:01, B*08:01	Exp1					34,2	44,2						35,9			23,0		36,8			
CDT	HCL	B*35:01	Exp2																			39,9	40,0
SWD	CLL	A*01:01, B*08:01	Exp3	39,2	34,9	32,3	45,2	33,5	49,3				32,3	39,1		25,7			43,3	50,4			
SWD	CLL	A*01:01, B*08:01	Exp15						41,8											26,2			
SWD	CLL	A*01:01, B*08:01	Exp22	36,2					35,6											29,3			
MWY	ALL	B*35:01	Exp4																		21,8		35,7
MWY	ALL	B*35:01	Exp14																				27,5
WSG	ALL	A*01:01	Exp5	40,0				25,2															
SLE	ALL	A*01:01, B*08:01	Exp6					35,5	43,3				25,1				32,4			30,6			
NBA	ALL	A*24:02	Exp7							32,0		46,5											
HJS	FL	A*24:02	Exp8									44,6											
KYE	ALL	A*24:02	Exp10									22,1											
AHC	CLL	A*01:01, B*08:01	Exp16	34,7	23,9	27,1											32,4	21,8		30,5			
ALA	ALL	B*35	Exp17																				32,1
EMY	ALL	B*35	Exp18																				
HAN	CLL	B*08:01	Exp20																	31,6			
HBP	ALL	A*24:02	Exp21								22,3												
UM9	MM	A*01:01	Exp107						45,6														

* For each material, the patient code, diagnosis, expressed target HLA alleles and experiment tag as used in supplementary table 1 is shown. Columns indicate different identified target peptides. Values represent the highest detected Ion score per material, when a peptide was not detected in a material, no value is shown.

Table S3: HLA typing of healthy donor PBMCs used for T-cell isolation

Donor number	HLA-A	HLA-B	HLA-C
3	A*01 - A*30	B*08 - B*39	C*07 - C*07
4	A*03 - A*03	B*07 - B*07	C*07 - C*07
5	A*02 - A*03	B*07 - B*27	C*01 - C*07
6	A*02 - A*03	B*07 - B*44	C*05 - C*07
7	A*03 - A*32	B*15 - B*44	C*03 - C*05
8	A*02 - A*02	B*44 - B*62	unknown
12	A*02 - A*02	B*07 - B*55	C*03 - C*07
13	A*02 - A*23	B*07 - B*44	C*05 - C*07
14	A*03 - A*03	B*07 - B*07	C*07 - C*07
15[a]	A*24 - A*29	B*07 - B*55	C*03 - C*03
16	A*23 - A*30	B*44 - B*49	C*04 - C*07
17	A*02 - A*03	B*07 - B*44	C*01 - C*07
18	A*03 - A*11	B*07 - B*56	C*01 - C*07

[a] When target HLA is expressed, tetramers with this HLA restriction are excluded from experiment

Table S4: HLA typing of EBV-LCLs used in EBV-LCL panels

EBV-LCL	HLA-A	HLA-B	HLA-C
GMK	23:01:01 - 02:01	41:01 - 40:01	17:01:01:01 - 03:04:01:01
RSB	02:01 - 03:01/03:03/03:04	44:02 - 57:01	06:02 - 07:04/07:12/07:11
EBK	02:05 - 02:05	58:01 - 58:01	unknown
ERC	02:01 - 02:01	13:02 - 44:02	05:01 - 06:02
BBD	02:01 - 02:05	15:01 - 45:01	01:02 - 06:02
ABC	02:01:01 - 11:01:01:01	44:05:01 - 51:01:01:01	02:02:02 - 14:02:01
NMJ	02:01 - 66:01/66:04	40:01/40:11/40:14 - 41:02	03:04/03:08/03:09 - 17
HRK	03:01 - 25:01	15:17 - 18:01/18:03/18:05	07:01/07:05/07:06 - 12:03/12:06
MSV	03:01 - 33:01	07:02 - 14:02	07:02 - 08:02
JBX	02:01 - 30:02	15:01 - 39:01	03:03 - 12:03
IGU	03:01 - 26:01	07:02:01 - 14:01	07:02 - 08:02
LSR	32:01 - 68:01	35:03 - 52:01	12:02 - 12:03
HBM	02:01:01 - 02:01:01	15:01:01:01 - 51:01:01	03:03:01 - 15:02:01
RKO	02:05 - 29:02	27:05 - 44:03	01:02 - 16:01:01
MSF	03:01/03:03/03:04 - 30:01	07:02 - 38:01	07:02/07:03/07:05 - 12:03/12:06
BSR	02:01 - 68:01	35:03 - 37:01	04:01 - 06:02
ABF	30:04 - 68:02	38:01 - 55:01	03:03 - 12:03
GGT	26:01/26:08/26:02 - 31:01/31:02/31:06	14:01 - 49:01	07:01/07:05/07:06 - 08:02/08:07
AAJ	03:01/03:03/03:04 - 11:01/11:02/11:03	40:02/40:35/40:37 - 56:01	01:02/01:06/01:07 - 02:02/02:04/02:08
QBO	24:02:01:01 - 31:01:02	07:02/07:61 - 35:08:01	04:01 - 07:02
MMG	01:01:01 - 32:01	35:08 - 35:08	04:01 - 04:01
UKA	03:01 - 25:01	18:01 - 35:01	04:01 - 12:03
JBZ	01:01 - 02:01	07:02 - 18:01	07:01 - 07:02
JMQ	02:01 - 24:02:01:01	35:02 - 44:02	04:01 - 05:01
UJE	01:01:01:01 - 33:03:01	44:03:02 - 51:01:01	07:06/07:18 - 14:02:01
UWI	02:01 - 24:02	07:02:01 - 40:02:01	02:02:02 - 07:02:01
CAA	02:01 - 02:01	40:02 - 40:02	02:02 - 02:02
URN	02:01 - 03:01	08:01:01 - 50:01:01	06:02:01 - 07:01
AKB	01:01 - 02:01	37:01 - 39:01	06:02 - 07:02
APZ	01:01 - 68:01	44:02 - 44:02	05:01 - 07:04

Table S5: Primers used to measure target gene expression by qPCR

Gene	Forward primer	Reverse primer
GUSB	ACTGAACAGTCACCGACGAG	GGAACGCTGCACTTTTTGGT
PSMB4	GTTTCCGCAACATCTCTCGC	CATCAATCACCATCTGGCCG
VPS29	TGAGAGGAGACTTCGATGAGAATC	TCTGCAACAGGGCTAAGCTG
FCRL5	TGCAAATCCTAGAGGAGAAAATGTG	TAGGGGAACCCTTGTTCCTGA
VPREB3	GGGGACCTTCCTGTCAGTTTC	CGTAGTCCCTGATGGTGACG
POU2AF1	GACATGTATGTGCAGCCCGT	GAGCTTCTTGTCGTGACATTGG
MIST	GGACTCAGAGGAGATGAGAAGTT	GTTTCCTGTGGACCCCAGTT
RALGPS2	GCTGACTGACTCTGAGAAAGGAAA	CAGGCTGCACTCAAATGCTT
FCRL2	TCTCTGGGGACTGTTTGGTGT	GAAGCCCCTCTGGGTTCATTAGT
TNFRSF13B	GTGGCTATGAGATCCTGCCC	CAGCTGAGTGACCTGCAGAA