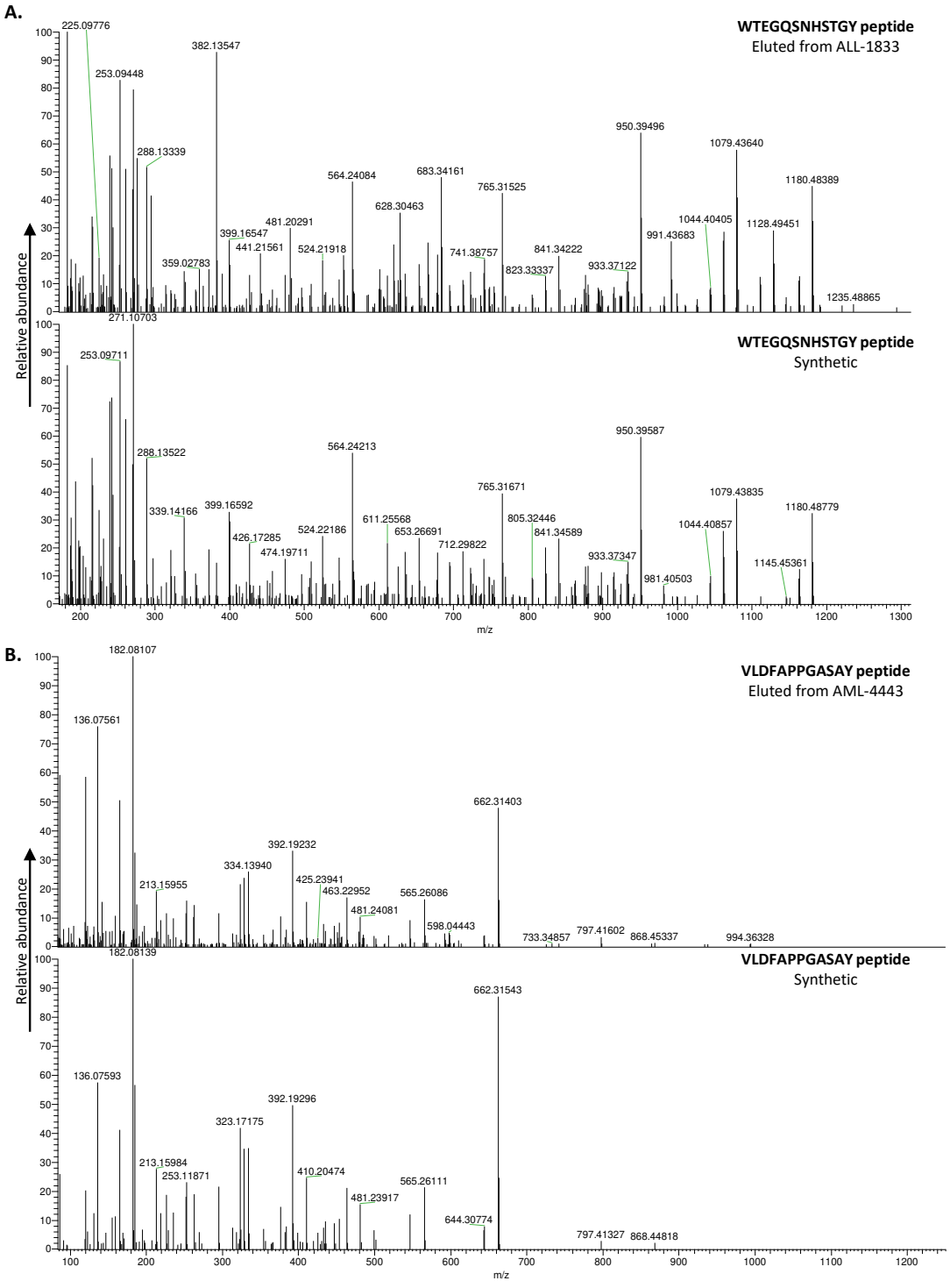
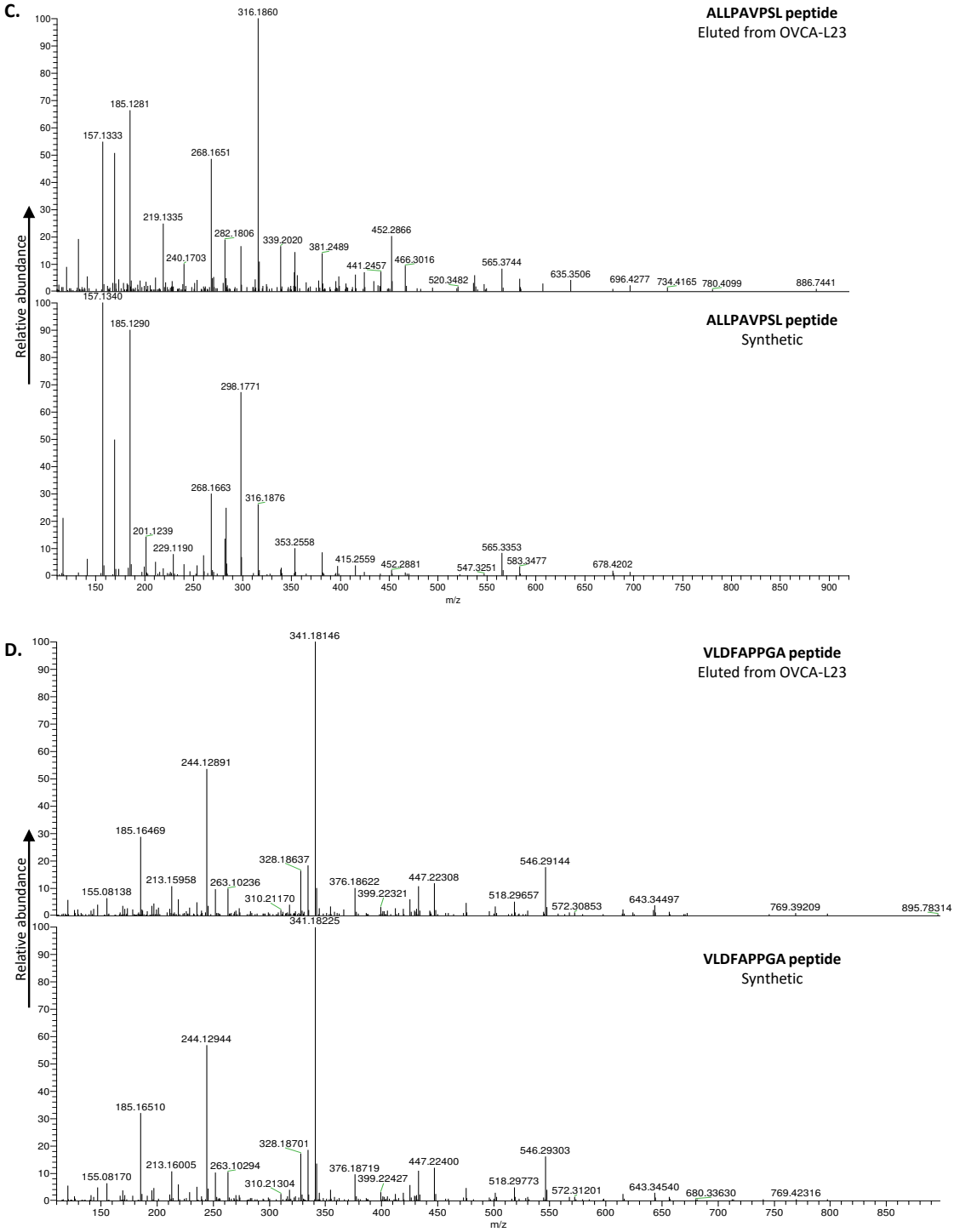
Supplemental data 1. Overview of the materials included in HLA ligandome analyses

WT1 peptides eluted				Materials included in the HLA ligandome analyses						
Nr	**Peptide**	**HLA**	**BMI score**	**Name**	**WT1 expr.**	**Material**	**Amount**	**HLA-A**	**HLA-B**	**HLA-C**
1	WTEGQSNHSTGY	A*01:01	43	OVCA-G1	162%	Solid tumor	8 gram	A*01:01 A*11:01	B*08:01 B*35:01	C*04:01 C*07:01
			33	ALL-1833	9%*	PB	62x10^9	A*01 A*03	B*18 B*35	C*04 C*07
			41	AML-4443	13%	PB	110x10^9	A*01:01	B*08:01	C*07:01
			42	AML-10197	27%	PB	550x10^9	A*01:01 A*02:01	B*08:01 B*44:03	C*07:01 C*16:01
2	VLDFAPPGASAY	A*01:01	36	AML-4443	13%	PB	110x10^9	A*01:01	B*08:01	C*07:01
3	ALLPAVPSL	A*02:01	31	OVCA-L23	57%	Ascites	7x10^9	A*02:01 A*26:01	B*38:01, B*44:02	C*05:01 C*12:03
4	VLDFAPPGA	A*02:01	26	OVCA-L23	57%	Ascites	7x10^9	A*02:01 A*26:01	B*38:01, B*44:02	C*05:01 C*12:03
5	FGPPPPSQA	A*02:01	42	OVCA-L23	57%	Ascites	7x10^9	A*02:01 A*26:01	B*38:01, B*44:02	C*05:01 C*12:03
			20	AML-10197	27%	PB	550x10^9	A*01:01 A*02:01	B*08:01 B*44:03	C*07:01 C*16:01
6	AQFPNHSFK	A*03:01	20	Cell line-COV362.4	9%	Cell line	2x10^9	A*03:01	B*40:01	C*03:04
7	HAAQFPNHSF	B*35:01	36	HCL-4512	n.d.	Spleen	100x10^9	A*02 A*29	B*35	C*04
8	TPYSSDNLY	B*35:01	41	ALL-2184	9%	PB	610x10^9	A*11:01	B*35:01 B*40:02	C*02:02 C*04:01

Supplemental data 2. MS data validation of the eight WT1-derived peptides




C.
ALLPAVPSL peptide
Eluted from OVCA-L23
316.1860
185.1281
157.1333
268.1651
219.1335
282.1806
339.2020
381.2489
452.2866
240.1703
441.2457
466.3016
565.3744
520.3482
635.3506
696.4277
734.4165
780.4099
886.7441
Relative abundance
ALLPAVPSL peptide
Synthetic
157.1340
185.1290
298.1771
268.1663
316.1876
201.1239
229.1190
353.2558
415.2559
452.2881
565.3353
547.3251
583.3477
678.4202
m/z
D.
VLDFAPPGA peptide
Eluted from OVCA-L23
341.18146
244.12891
185.16469
328.18637
546.29144
213.15958
263.10236
376.18622
447.22308
155.08138
310.21170
399.22321
518.29657
572.30853
643.34497
769.39209
895.78314
Relative abundance
VLDFAPPGA peptide
Synthetic
341.18225
244.12944
185.16510
328.18701
546.29303
213.16005
263.10294
376.18719
447.22400
155.08170
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399.22427
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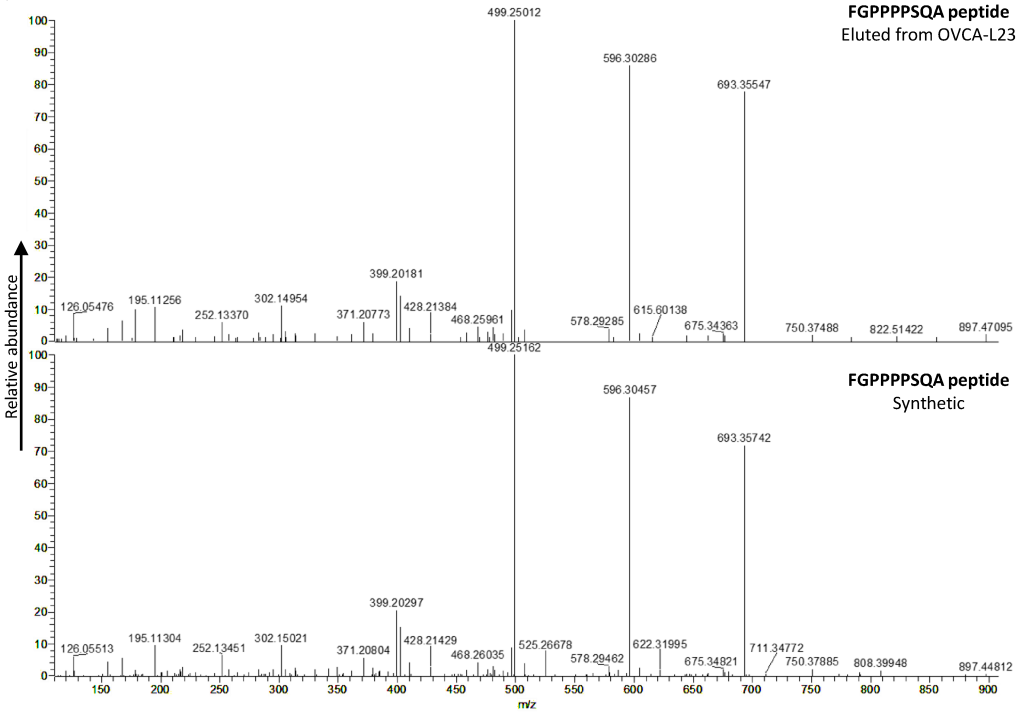
643.34540
680.33630
769.42316
m/z

E.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Eluted from OVCA-L23
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693.35547
399.20181
126.05476
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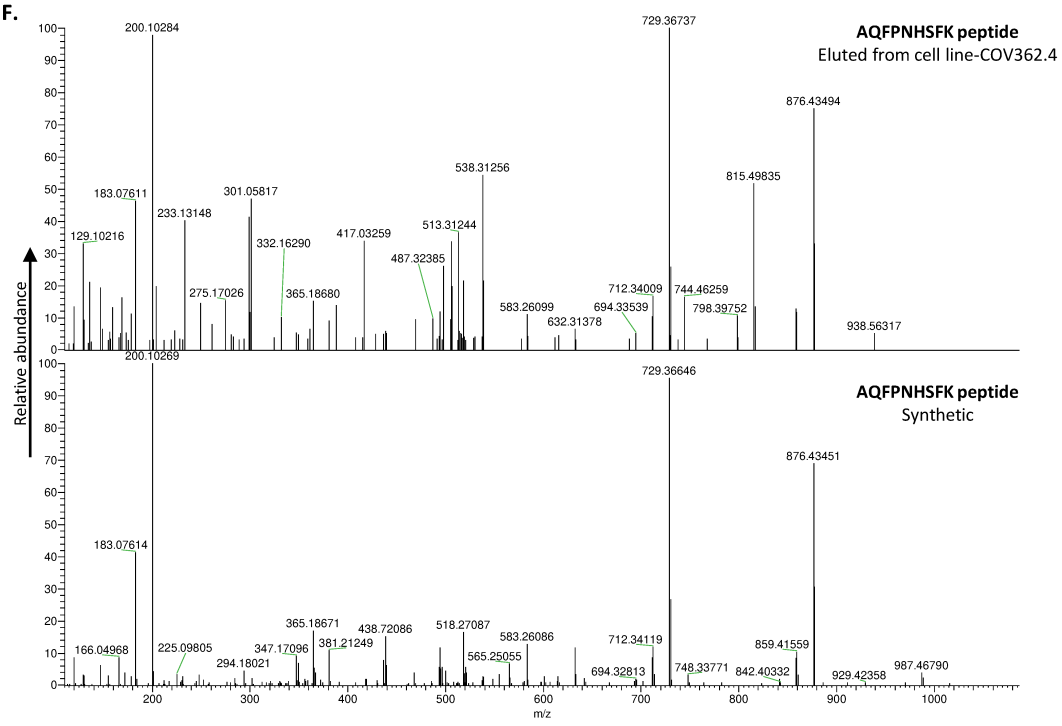
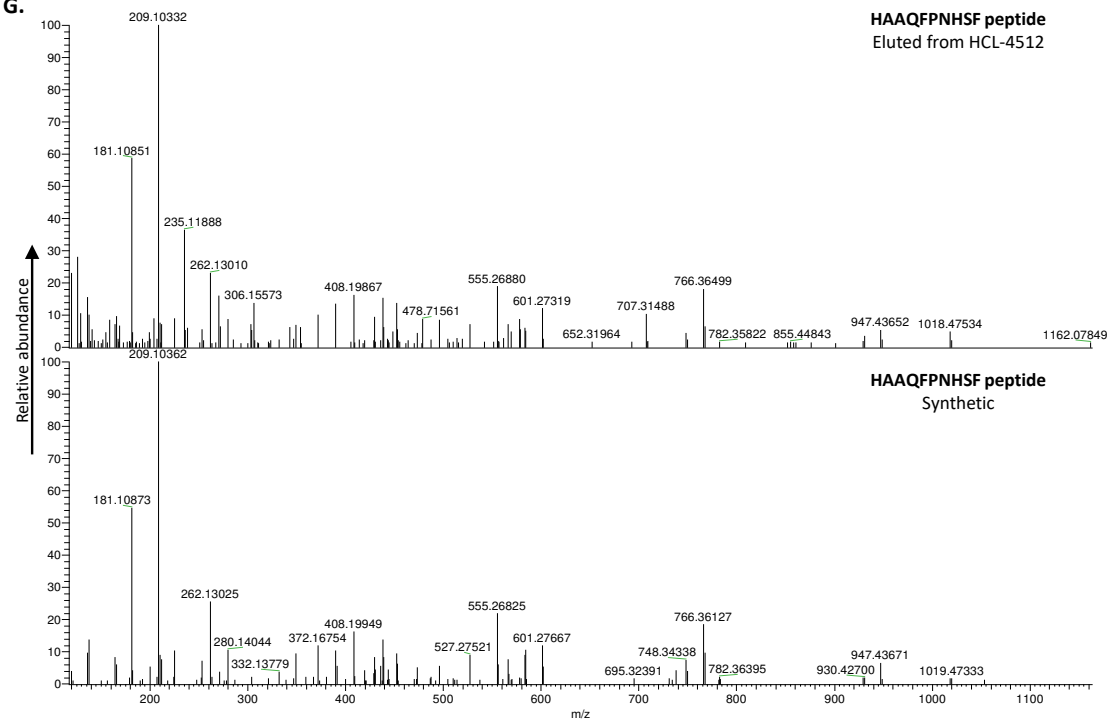
371.20773
428.21384
468.25961
578.29285
615.60138
675.34363
750.37488
822.51422
897.47095
FGPPPPSQA peptide
Synthetic
499.25162
596.30457
693.35742
399.20297
126.05513
195.11304
252.13451
302.15021
371.20804
428.21429
468.26035
525.26678
578.29462
622.31995
675.34821
711.34772
750.37885
808.39948
897.44812
Relative abundance
m/z

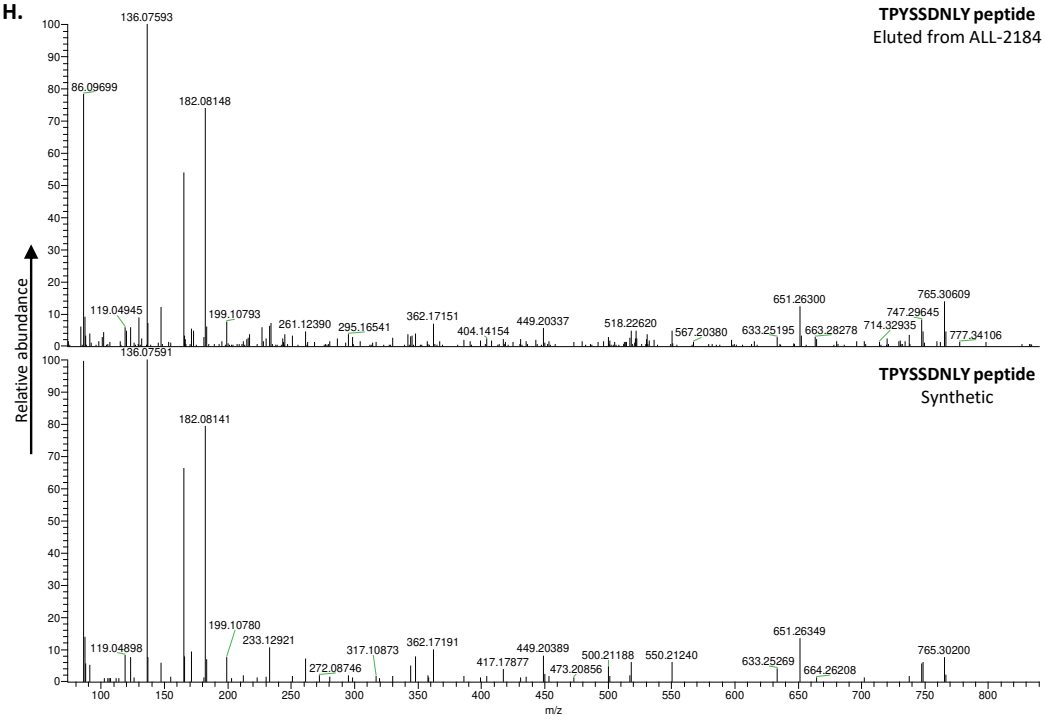
F.


AQFPNHSFK peptide
Eluted from cell line-COV362.4
200.10284
729.36737
876.43494
538.31256
815.49835
183.07611
301.05817
233.13148
129.10216
417.03259
513.31244
332.16290
487.32385
275.17026
365.18680
712.34009
744.46259
583.26099
694.33539
798.39752
632.31378
938.56317
AQFPNHSFK peptide
Synthetic
200.10269
729.36646
876.43451
183.07614
365.18671
438.72086
518.27087
583.26086
166.04968
225.09805
347.17096
381.21249
294.18021
565.25055
712.34119
859.41559
694.32813
748.33771
842.40332
929.42358
987.46790
Relative abundance
m/z

G.


HAAQFPNHSF peptide
Eluted from HCL-4512
HAAQFPNHSF peptide
Synthetic
Relative abundance
m/z

H.


TPYSSDNLY peptide
Eluted from ALL-2184
TPYSSDNLY peptide
Synthetic
Relative abundance
m/z

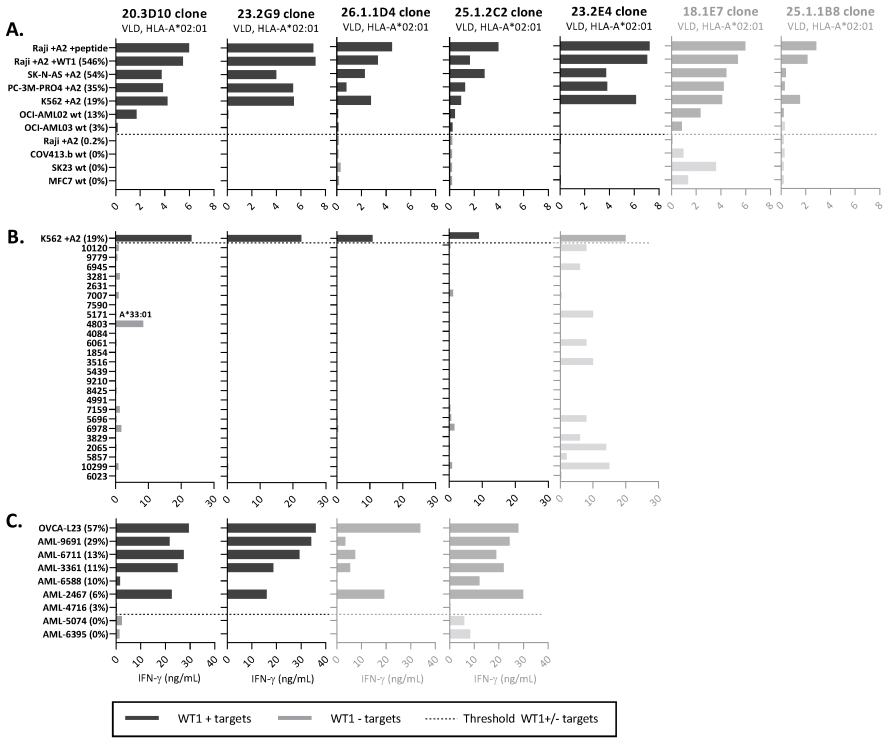
Supplemental data 3. Overview of the number of T-cell clones screened per healthy donor and T-cell clones reactive against WT1 peptide and/or transduced WT1

Name	HLA class I type	Number of PBMCs (x10^6)	Screened T-cell clones	T-cell clones reactive against WT1 peptide	T-cell clones reactive against transduced WT1
OLA.01	A03, A29, B35, C unknown	595	69	13	2
OLA.02	A01, A31, B51, C04, C07	350	48	12	1
OLA.03	A01, A03, B13, B35, C04, C06	1150	102	18	0
OLA.04	A24, A29, B15, C03, C16	565	134	9	0
OLA.05	A03, B51, C03, C07	995	137	24	1
OLA.06	A24, B07, B27, C unknown	900	576	21	6
OLA.07	A03, A31, B07, B60, C07, C10	445	238	9	1
OLA.08	A24, A29, B44, B51, C unknown	795	658	38	8
OLA.09	A03, A32, B07, B44, C05, C07	825	145	11	1
OLA.10	A09, A28, B08, B18, C05, C07	755	520	20	0
OLA.11	A23, A31, B44, B51, C04, C15	490	315	13	0
OLA.12	A31, A28, B51, B60, C03	545	493	22	4
OLA.13	A24, A32, B55, B61, C02, C03	305	199	8	0
OLA.14	A24, A26, B39, B44, C02, C12	850	456	6	2
OLA.15	A24, A33, B14, B40, C03, C08	490	20	1	0
OLA.16	A30, A31, B18, B39, C05, C12	730	384	17	7
OLA.17	A32, A33, B14, B40, C03, C08	455	331	7	3
OLA.18	A01, A03, B57, B35, C04	575	87	24	3
OLA.19	A03, A24, B44, B63, C05	585	514	4	0
OLA.20	A03, A31, B50, B60, C06, C10	544	576	19	1
OLA.21	A01, A68, B51, B08, C07	724	269	11	0
OLA.22	A03, A24, B07, B35, C04, C07	710	267	11	2
OLA.23	A03, B07, B35, C04, C07	580	179	44	11
OLA.24	A32, B44, B18, C05, C07	565	316	14	2
OLA.25	A01, A25, B37, B51, C unknown	556	308	25	7
OLA.26	A01, A03, B07, B44, C07	310	233	29	7
OLA.27	A01, B39, B44, C05, C12	940	174	11	1
OLA.28	A01, A11, B15, B35, C unknown	800	168	20	1
		18,129	**7,916**	**461**	**71**

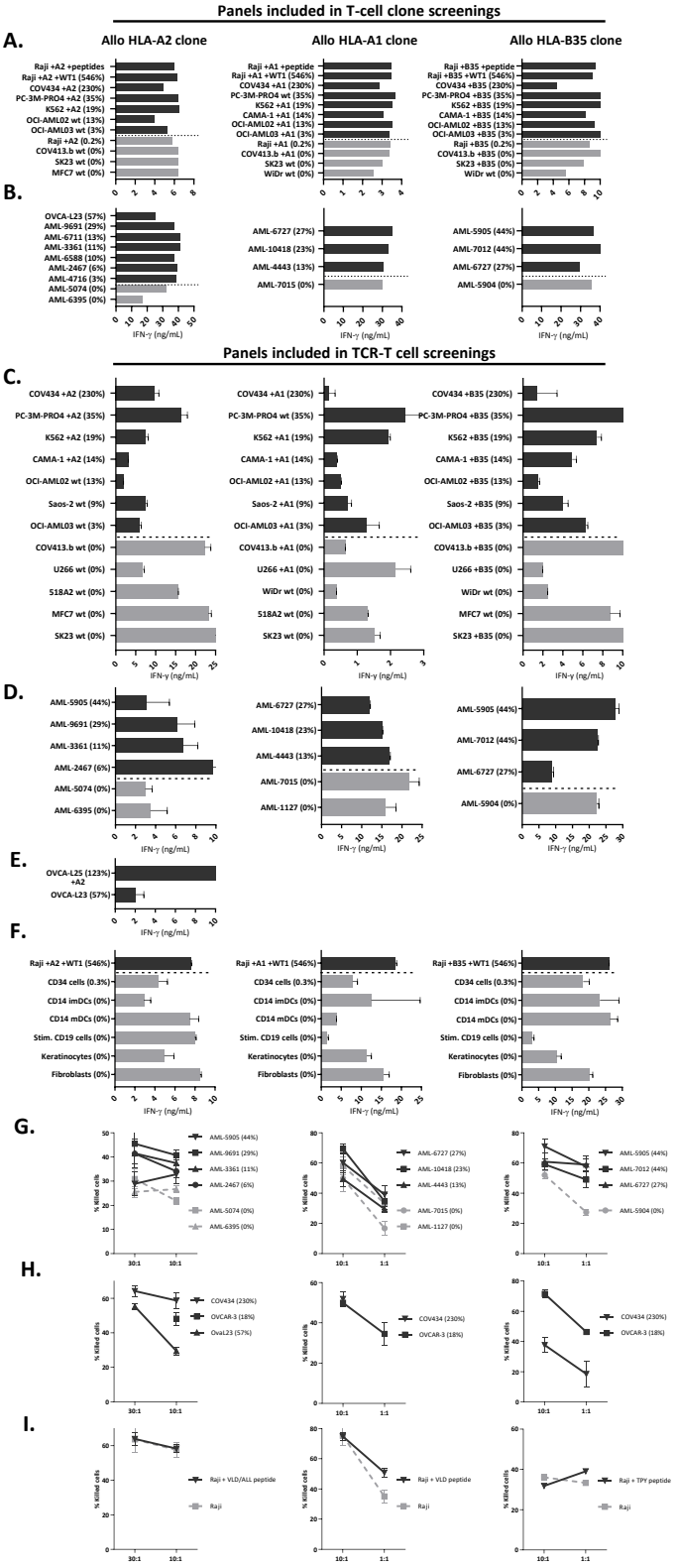
Supplemental data 4. Overview of T-cell clones reactive against transduced WT1 and those TCRs further analyzed

Nr	Peptide	HLA	Donors used for T-cell isolation	WT1-reactive T-cell clones	TCR analyzed
1	WTEGQSNHSTGY	A*01:01	23		
2	VLDFAPPGASAY	A*01:01	23	4	12.5H9
3	ALLPAVPSL	A*02:01	11	5	22.1H1
4	VLDFAPPGA	A*02:01	11	19	20.3D10
5	FGPPPPSQA	A*02:01	11	7	
6	AQFPNHSFK	A*03:01	5	1	
7	HAAQFPNHSF	B*35:01	19	18	
8	TPYSSDNLY	B*35:01	19	3	17.2G4
9	RMFPNAPYL	A*02:01	17	12	
10	CMTWNQMNL	A*02:01	17	2	
11	RWPSCQKKF	A*24:02	11		
12	CMTWNQMNL	A*24:02	11		
				71	**4**

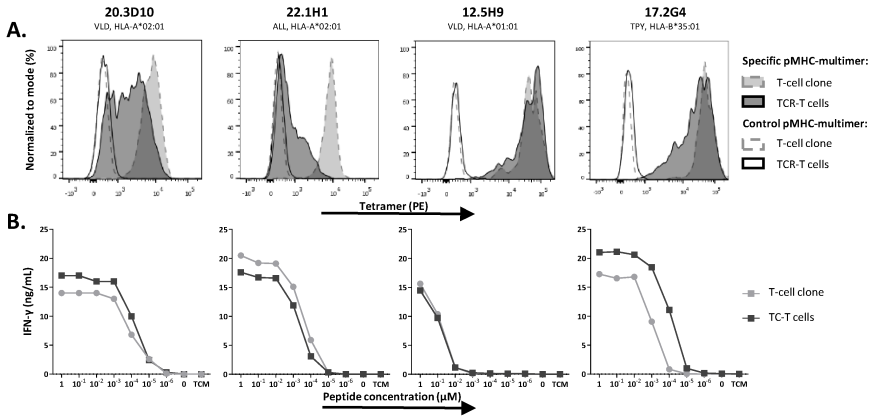
Supplemental data 5. Screening approach to select specific and potent WT1-specific T-cell clones



Supplemental data 6. Reactivities by allo-HLA reactive T-cell clones in the included screening panels



Supplemental data 7. pMHC-multimer staining and peptide sensitivity of TCR-T cells and their parental T-cell clones



LEGENDS

Supplemental data 1. Overview of the materials included in HLA ligandome analyses

Overview of the characteristics of materials included in the HLA ligandome analyses from which the WT1 peptides were identified. The materials are indicated per WT1 peptide, including their predicted HLA binding molecule based on NetMHC and best Mascot ion score (BMI). WT1 expression was determined by RT-qPCR (*ALL-1833 by Illumina HT-12.0 microarray). OVCA: primary ovarian carcinoma, ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, HCL: hairy cell leukemia, n.d. = not determined.

Supplemental data 2. MS data validation of the eight WT1-derived peptides

The eight WT1 peptides identified in our HLA ligandome analyses were validated by comparing tandem mass spectra of eluted peptides and synthetic peptides. (**A** – **H**) For each peptide the tandem mass spectra of the eluted and synthetic peptides are shown, including the source of the eluted peptide.

Supplemental data 3. Overview of the number of T-cell clones screened per healthy donor and T-cell clones reactive against WT1 peptide and/or transduced WT1

For each healthy donor, HLA class I typing, number of PBMCs, and expanded single-cell sorted T-cell clones are listed. In total 461 of 7916 screened T-cell clones were reactive against Raji pulsed with the WT1 peptide pool (1 μM), and 71 clones were also reactive against Raji transduced with WT1. The reactivity is based on IFN-γ production (ng/mL) after an overnight co-culture stimulation assay.

Supplemental data 4: Overview of T-cell clones reactive against transduced WT1 and those TCRs further analyzed

Number of healthy donors included in the T-cell search is listed. In total 71 of the isolated T-cell clones were reactive against both Raji pulsed with the WT1 peptide pool and Raji transduced with WT1. The TCRs of four T-cell clones were sequenced and further analyzed.

Supplemental data 5: Screening approach to select specific and potent WT1-specific T-cell clones

Recognition patterns by 7 of the 19 T-cell clones recognizing the VLDFAPPGA peptide presented in HLA-A*02:01. Clone 20.3D10 and 23.2G9 were selected as the most specific and potent T-cell clones. Excluded T-cell clones based on recognition in a panel are blurred. Recognition is based on IFN-γ production (ng/mL) after overnight co-culture assays. (**A**) Panel with WT1+ and WT- tumor cell lines (E:T = 1:6). (**B**) Panel with 25 EBV-LCLs, expressing all frequent HLA alleles (with an allele frequency > 1%) present in the Caucasian population (E:T = 1:6). The HLA-allele is depicted if only one HLA-allele is recognized by the T-cell clone, meeting the requirement that all EBV-LCLs with this HLA-allele are recognized. (**C**) Panel with primary AML samples (E:T = 1:16) and OVCA patient samples (E:T = 1:6). All cell lines in panel A and C were HLA-A*02:01 positive, either wildtype (wt) or the HLA-allele was introduced by transduction (+A2). Percentage relative WT1 expression is depicted, as determined by RT-qPCR. Dark grey bars depict high WT1+ targets and light grey bars the WT1- targets. Bars represent averaged duplicate values and are representative of two independent experiments.

Supplemental data 6. Reactivities by allo-HLA reactive T-cell clones in the included screening panels

Reactivities of the positive control T-cell clones that are reactive against a housekeeping gene presented in HLA-A*02:01, HLA-A*01:01 or HLA-B*35:01. (**A-B**) IFN-γ production (ng/mL) against the tumor cell line panel and primary AML panel included in T-cell clone screenings, depicted in Supplementary data 5 and Figure 2. (**C-F**) IFN-γ production against the tumor cell line panel, primary AML panel, primary OVCA panel, and healthy cell subsets panel included in TCR-T cell screenings, depicted in Figure 4. (**G-I**) Killing percentages of the primary AML panel, OVCA cell line panel, and control panel included in the TCR-T cell screenings, depicted in Figure 5. Values and error bars represent mean and SD of technical duplicates and are representative of two independent experiments.

Supplemental data 7. pMHC-multimer staining and peptide sensitivity of TCR-T cells and their parental T-cell clones

T-cell receptors (TCRs) of the four most promising WT1-specific T-cell clones were constructed and introduced in CD8+ cells via retroviral transduction. Shown are representative results of two independent experiments and two CD8+ donors, at day 10 post isolation. (**A**) Flow cytometry plots of purified TCR-T cells and their parental T-cell clones stained with the specific and a control pMHC-multimer. (**B**) IFN-γ production (ng/ml) of T cells stimulated with Raji cells (transduced with HLA-A*01:01 or -B*35:01) or T2 cells (wildtype HLA-A*02:01 positive) pulsed with titrated peptide concentrations.