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Supplemental Information

Healthy cells functionally present TAP-independent SSR1 peptides: implications for selection of clinically relevant antigens

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Figure S1

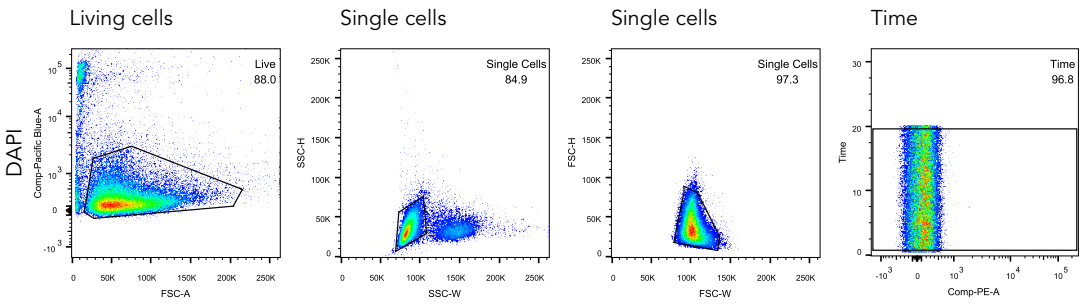

Living cells
Single cells
Single cells
Time
DAPI
Live
88.0
Single Cells
84.9
Single Cells
97.3
Time
96.8
Comp-Pacific Blue-A
FSC-A
SSC-H
SSC-W
FSC-H
FSC-W
Time
Comp-PE-A

Figure S1. Gating strategy for flow cytometry (Related to Figures 1, 3 and 4)

For all flow cytometric analyses, live cells were gated on DAPI negativity followed by two gates for single cells using SSC-H vs SSC-W and FSC-H vs FSC-W. Lastly, any potential artifacts induced by clogs were excluded from analysis by gating on time vs PE.

Figure S2

A

```
WT   /105bp/ GGG ACA GTA CTG CTA CTT CTC GCC GAC TGG GTG CTG CTC CGG ACC GCG CTG CCC CGC ATA TTC TCC
#1   /-----/ --- --- --- --- --- --- --- --- TGC TGG GTG CTG CTC CGG ACC GCG CTG CCC CGC ATA TTC TCC -129bp
#2   chr A    GGG ACA GTA CTG CTA CTT CTC GC- --C TGG GTG CTG CTC CGG ACC GCG CTG CCC CGC ATA TTC TCC -3bp
     chr B    GGG ACA GTA CTG --- --- --- -- CGA CTG GGT GCT GCT CCG GAC CGC GCT GCC CCG CAT ATT CTCC -11bp
#28           GGG ACA GTA CTG C-- --- --- --- --- --- --- --- -TC CGG ACC GCG CTG CCC CGC ATA TTC TCC -24bp
#34           GGG ACA --- --- --- --- --- --- --- --- GTG CTG CTC CGG ACC GCG CTG CCC CGC ATA TTC TCC -24bp
#37   chr A    GGG ACA GTA CTG CTA CTT CTC GCT --- --- --- --- --- --- ACC GCG CTG CCC CGC ATA TTC TCC -18bp
     chr B    GGG ACA GTA CTG CTA CTT CT- --- --- --- -T GCT GCT CCG GAC CGC GCT GCC CCG CAT ATT CTCC -11bp
```

TAP1 gRNA PAM site
– Deletions Stop codon
Substitution

B

```
          40        50        60        70        80        90        100       110
WT   RPHRGAESPRPGRDRDGVRVPMASSRCPAPRGCRCLPGASLAWLGTVLLLLADWVLLRTALPRIFSLLVPTA
#1   RPHRGAESP-------------------------------------------CWVLLRTALPRIFSLLVPTA -43aa
#2   RPHRGAESPRPGRDRDGVRVPMASSRCPAPRGCRCLPGASLAWLGTVLLLLA-WVLLRTALPRIFSLLVPTA -1aa
#28  RPHRGAESPRPGRDRDGVRVPMASSRCPAPRGCRCLPGASLAWLGTVL--------LRTALPRIFSLLVPTA -8aa
#34  RPHRGAESPRPGRDRDGVRVPMASSRCPAPRGCRCLPGASLAWLGT--------VLLRTALPRIFSLLVPTA -8aa
#37  RPHRGAESPRPGRDRDGVRVPMASSRCPAPRGCRCLPGASLAWLGTVLLLLA------TALPRIFSLLVPTA -6aa
```

– Deletions
Transmembrane domain
Substitution

C

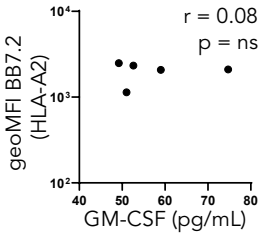
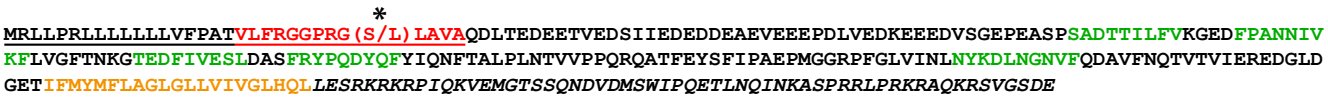


Figure S2. HAP1 TAP1 KO clone characteristics (Related to Figure 1)

(**A**) Summary of TAP1 gene editing in each TAP1 KO clone. Indicated are the gRNA targeting sequence (blue) with PAM site (green), insertions (red), deletions (dashes) and premature stop codons (orange). Clone #1 was previously described (de Waard et al., 2020). (**B**) Summary of the targeted part of the TAP1 amino acid sequence with the genome editing result depicted for each clone. Numbers above the sequence indicate the amino acid location in the wild type TAP1 protein. Transmembrane domain in wild type sequence (blue), amino acids lacking in TAP1 KO clones (dashes) and amino acid substitutions (red) are depicted. (**C**) Correlation analysis of HLA-A2 expression of HAP1 TAP1 KO clones (geometric mean fluorescent intensity, Figure 1C) with their capacity to activate SSR1-specific T cell response (Figure 1D).

Figure S3

SSR1 (284 amino acids)



Signal peptide
Recognized antigen *nonsynonymous SNP rs10004
Peptides identified by MS
Transmembrane domain
Cytosolic domain

Figure S3. Amino acid sequence and features of SSR1 (Related to Figure 2)

The location of the peptide recognized by the SSR1-specific T cell clone is depicted in red with the asterisk indicating the SNP (rs10004). The predicted signal peptide is underlined, the predicted transmembrane domain orange and the cytosolic domain in italics. Peptides eluted from various HLA-I alleles (Table S3) are depicted in green.

Figure S4

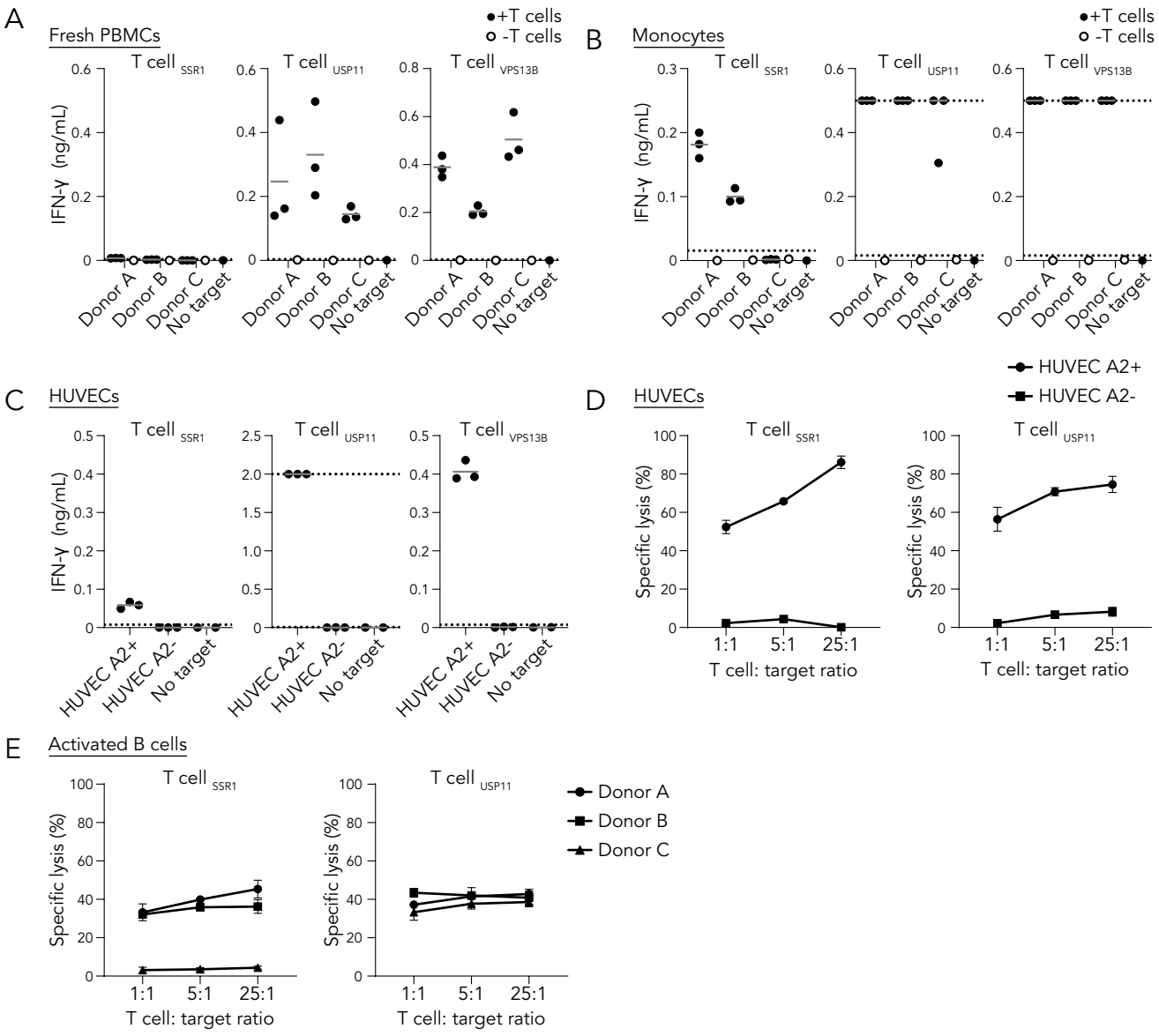

A
Fresh PBMCs
+T cells
-T cells
T cell SSR1
T cell USP11
T cell VPS13B
IFN-γ (ng/mL)
Donor A
Donor B
Donor C
No target
B
Monocytes
C
HUVECs
HUVEC A2+
HUVEC A2-
D
Specific lysis (%)
T cell: target ratio
1:1
5:1
25:1
E
Activated B cells
Donor A
Donor B
Donor C

Figure S4. Specific recognition and lysis of multiple healthy cell types (Related to Figures 3 and 5)

(**A**) T cell coculture with PBMCs from three healthy donors (see Figure 3B), culture supernatant was analyzed by IFN-γ ELISA (GM-CSF in Figure 3). Results from triplicate cultures are shown. Dotted lines represent the detection limits of the ELISA. (**B**) As in (A) but using monocytes as target cells (GM-CSF in Figure 3). (**C**) T cell coculture with HLA-A2 positive and negative HUVECs (see Figure 3F). Culture supernatant was analyzed by IFN-γ ELISA (GM-CSF in Figure 3). Each dot represents an individual measurement of triplicate cultures. (**D** and **E**) The indicated T cells were cocultured with ^{51}Cr-loaded (**D**) HLA-A2 positive or negative HUVECs (see Figure 3F) or (**E**) B cells isolated from three healthy donors (see Figure 3B) activated using CpG and B cell receptor crosslinking antibodies at indicated effector:target cell ratios. Specific lysis (%) was calculated based on the ^{51}Cr release. Each datapoint represents the mean of triplicate cultures with SD.

Table S1. All theoretical 8-14mer peptides covering the SSR1 SNP (Related to Tables 1 and 2)

Affinities for HLA-A*02:01 of all theoretical SSR1-derived peptides covering the nonsynonymous SNP rs10004 predicted using NetMHC4.0.

	Predicted affinity for HLA-A*02:01 (nM) per variant	
Peptide sequence	**L**	**S**
FRGGPRGL	36658	43601
RGGPRGLL	39127	41929
GGPRGLLA	38138	43187
GPRGLLAV	26994	33459
PRGLLAVA	35607	39522
RGLLAVAQ	34391	37964
GLLAVAQD	27129	40492
LLAVAQDL	6903	10021
LFRGGPRGL	30649	39466
FRGGPRGLL	31365	34114
RGGPRGLLA	29547	36909
GGPRGLLAV	16626	23894
GPRGLLAVA	26057	30604
PRGLLAVAQ	34960	38808
RGLLAVAQD	32556	35998
GLLAVAQDL	286	13769
LLAVAQDLT	5043	5748
VLFRGGPRGL	777	16308
LFRGGPRGLL	33415	35308
FRGGPRGLLA	26878	36619
RGGPRGLLAV	16823	23776
GGPRGLLAVA	26366	33402
GPRGLLAVAQ	32540	36539
PRGLLAVAQD	37729	40484
RGLLAVAQDL	1447	20810
GLLAVAQDLT	2684	18948
LLAVAQDLTE	21253	23246
TVLFRGGPRGL	7427	30851
VLFRGGPRGLL	4673	5524
LFRGGPRGLLA	32850	38768
FRGGPRGLLAV	18430	25942
RGGPRGLLAVA	29760	35464
GGPRGLLAVAQ	34513	38438
GPRGLLAVAQD	36423	39518
PRGLLAVAQDL	8766	30236
RGLLAVAQDLT	11515	29918

Table S1 (continued)

Peptide sequence	L	S
GLLAVAQDLTE	15158	31575
LLAVAQDLTED	32609	34026
ATVLFRGGPRGL	8580	31521
TVLFRGGPRGLL	6482	7682
VLFRGGPRGLLA	5795	10082
LFRGGPRGLLAV	20279	27386
FRGGPRGLLAVA	23552	30074
RGGPRGLLAVAQ	32152	36590
GGPRGLLAVAQD	35448	39262
GPRGLLAVAQDL	7843	30371
PRGLLAVAQDLT	13387	30950
RGLLAVAQDLTE	15461	31263
GLLAVAQDLTED	16550	34127
LLAVAQDLTEDE	33202	34697
PATVLFRGGPRGL	8936	31494
ATVLFRGGPRGLL	7517	8908
TVLFRGGPRGLLA	7199	12668
VLFRGGPRGLLAV	236	628
LFRGGPRGLLAVA	24754	31034
FRGGPRGLLAVAQ	27133	32927
RGGPRGLLAVAQD	33051	37463
GGPRGLLAVAQDL	7960	30542
GPRGLLAVAQDLT	13423	30972
PRGLLAVAQDLTE	17551	31764
RGLLAVAQDLTED	16889	33306
GLLAVAQDLTEDE	17723	35216
LLAVAQDLTEDEE	34948	36072
FPATVLFRGGPRGL	9143	27652
PATVLFRGGPRGLL	7833	9280
ATVLFRGGPRGLLA	7941	14000
TVLFRGGPRGLLAV	351	958
VLFRGGPRGLLAVA	649	1403
LFRGGPRGLLAVAQ	27460	32988
FRGGPRGLLAVAQD	28291	34050
RGGPRGLLAVAQDL	7397	29261
GGPRGLLAVAQDLT	13582	31110
GPRGLLAVAQDLTE	17371	31898
PRGLLAVAQDLTED	19135	33912
RGLLAVAQDLTEDE	17765	34570
GLLAVAQDLTEDEE	18231	35941
LLAVAQDLTEDEET	24639	25997

Table S2. SSR1-derived peptides are presented in the absence and presence of functional TAP (Related to Table 2)

SSR1 candidate peptides were eluted from wild type and TAP-deficient HAP1 cells or wild type MCF-7 cells and MCF-7 cells overexpressing the Herpes Simplex Virus-derived TAP inhibitor ICP47. Values correspond to the average log2-intensity of the LC-MS/MS data. The SADTTILF and SADTTILFV peptides are attributed to HLA-C*05:01 based on binding prediction by NetMHC4.0 (rank<=2).

		HAP1 cells			MCF-7 cells		
Peptide	HLA-I allele	WT	TAP1 KO #1	TAP1 KO #2	WT	ICP47 #C15	ICP47 #C32
SADTTILF	C*05:01	-	-	-	23.5	-	20.9
SADTTILFV	C*05:01	-	-	-	23.8	23.0	22.7
VLFRGGPRGSLAVA	A*02:01	32.5	-	33.1	-	-	-

Table S3. SSR1-derived peptides are presented on various HLA-I alleles (Related to Table 2)

Data derived from (Shraibman et al., 2019) showing SSR1-derived peptides eluted from different glioblastoma and control patients/donors and predicted to bind one or multiple of the patient-expressed HLA-I alleles by NetMHC4.0 (rank<=2).

	Peptide				
Patient	FPANNIVKF	FRYPQDYQF	NYKDLNGNVF	SADTTILFV	TEDFIVESL
11-002	B*35:03	B*35:03 C*12:03	A*24:02		
11-004			A*24:02		
11-006			A*24:02		
29/14					B*40:01
30-002	B*53:01 C*04:01				
50/14			A*24:02		
637/13			A*24:02		
BCN-004	A*26:01				
BCN-017			C*05:01		
BCN-019	C*04:01				
BCN-021			A*24:02		
CPH-07	C*04:01	B*27:05			
CPH-08					B*40:02
CPH-09		B*27:05		C*05:01	
Leiden-003	C*04:01				
Leiden-004				C*12:03	
Leiden-005	A*25:01				
AMRF*	Cw4				
CMLF*	Cw4				
DMRN*	Cw4		A*24:02		
E16*	Cw4				
E27*			A*24		
E45*	Cw4				
E48*	Cw4				
EMMS*	Cw4				
FHCR*			A*24:02		
JGMS[#]			A*24:02		
JHGP[#]			A*23:01		
MRSRCS*			A*24:02		
VSCP[#]			A*24		

*Non-cancerous arthritis control patient.

[#]Non-cancerous healthy control donor.

Transparent Methods

Cell culture

HAP1 (HLA-A*02:01, HLA-B*40:01, HLA-C*03:04), EBV-LCLs, HEK293T, U266, U2G10196, COV413b, T2, UM9, and TMD8 cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM, Gibco and Lonza) supplemented with 10% heat-inactivated fetal calf/bovine serum (Gibco, Thermo Fisher) and antibiotics (PenStrep; Invitrogen; $IMDM^{++}$) at 37°C and 5% CO_2. MCF-7 (HLA-A*02:01, HLA-B*18:01, HLA-B*44:02, HLA-C*05:01) were cultured in DMEM (Dulbecco's modified Eagle's medium), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin- streptomycin, in a humidified 5% CO2 incubator at 37°C. Primary HUVECs (kindly provided by Dr. Jaap van Buul), pooled from three different donors (Lonza), were cultured in Endothelial Cell Growth medium 2 (EGM2, Lonza) complemented with EGM2 Supplement mix (Promocel), 10% fetal calf serum and antibiotics. The HLA-A*02:01 restricted T cell clones were previously described and recognize a peptide derived from the endogenously expressed human proteins USP11, VPS13B or SSR1 (Amir et al., 2011; Van Bergen et al., 2010). The clones were expanded using irradiated feeder cells (mixed PBMCs and EBV-LCLs exposed to 30 Gy and 50 Gy, respectively) in IMDM with 5% fetal calf serum and 5% human serum (Sanquin), supplemented with 120 U/mL IL-2 (Miltenyi and Chiron), 0.8 µg/mL PHA (HA16 Remel™, ThermoFisher) and antibiotics as described (Pont et al., 2019; Spaapen et al., 2007).

PBMC isolation and activation

Blood was withdrawn from 3 HLA-A2 positive healthy volunteers (Sanquin), PBMCs were isolated by Ficoll gradient separation. Monocytes were isolated through plastic-adherence by incubating fresh PBMCs in $IMDM^{++}$ for 1 h at 37°C and 5% CO_2 on culture plastic. B cells were isolated using anti-CD19 Dynabeads and DETACHaBEAD (Invitrogen) according to manufacturer's protocol. B cell were activated by incubating them with 0.1 µM CpG (Invivogen) and 2.5 µg/mL Fab mix (anti-IGA, IgG and IgM, Jackson ImmunoResearch) in B cell medium (RPMI, Gibco; 5% FCS; 1% L-Glutamine, Life Technologies; 0.1% β-mercaptoethanol, Sigma; 20ng/mL human Transferrin, Sigma, in-house depleted for IgG) for 3 days at 37°C and 5% CO_2.

Sequencing

DNA was isolated from full PBMCs and HLA-A2 positively sorted HUVECs using the NucleoSpin Tissue kit (Machery-Nagel) according to manufacturer's protocol. DNA was amplified using Taq DNA-polymerase (VWR) with forward primer TTCTTCGGTGCATTGTAATTG and reverse primer GGGTCAATGAAACCTTTTTCC and

subsequently Sanger sequenced using the forward primer or a sequencing primer GGTGAACTGGGAGATTCTGTT (HUVECs) and BigDye v1.1 (Applied Biosystems).

Genetic engineering

Genome-editing of HAP1 cells and genomic verification was performed as previously described for TAP1 (de Waard et al., 2020). In short, cells were transduced with pL.CRISPR.efs.GFP (Addgene) containing a gRNA targeting TAP1 (ACTGCTACTTCTCGCCGACT). Transduced cells were enriched by FACS sort on GFP positivity and clones were subsequently generated by limiting dilution. The targeted region was sequenced using primer (GCTCCCCATGAGATCAGCTC) after PCR on genomic DNA of single cell-derived clonal cell lines using forward primer (CAGCCTGTTCCTGGGACTTT) and reverse primer (ACTGACAACGAAGGCGGTAG). HLA-I KO HAP1 cells were previously described (Jongsma et al., 2020). The MCF-7 cells were transfected with pcDNA3.1 containing the Herpes Simplex Virus-derived TAP inhibitor ICP47 using PEI reagent (PolyScience, Niles, Illinois) followed by G418 selection. Clones were generated of which two positive clones were selected (C#15, C#32) (Dganit Melamed Kadosh, Alan Frey, Ian Mohr, and Arie Admon, in preparation).

T cell activation assays

50,000 or 40,000 (only for activated B cells) target cells were cocultured with T cells in a 1:1 ratio in IMDM^{++} for 18 hours as previously described (Spaapen et al., 2008). IFN-γ and GM-CSF release was measured by standard sandwich ELISA (Sanquin and BioLegend, respectively) according to the manufacturer's protocol. For IFN-γ stimulation, target cells were seeded two (HAP1 cells, HUVECs) or three (PBMCs) days prior to the experiment in a culture flask in IMDM^{++} containing 20 U/mL recombinant human IFN-γ (Peprotech).

T cell-mediated killing assays

Cytotoxicity experiments were performed as previously described (Jongsma et al., 2020). In short, target cells were loaded with 100 µCi ^{51}Cr (HAP1 and HUVEC, Perkin-Elmer) or 200 µCi ^{51}Cr (B cells) for 90 min at 37°C and washed. T cells were cocultured with 5,000 HAP1 cells, 5,000 HUVECs or 10,000 B cells at indicated effector:target (E:T) ratios for 4-6 h after which 30 µL supernatant was harvested, dried and analyzed using a gamma counter (Wallac). A maximum release control was based on 0.1% triton X-100 treatment. A medium only control was used to detect spontaneous release. Specific lysis (%) was calculated using (experimental cpm – spontaneous cpm) / (maximal cpm – spontaneous cpm)] × 100%.

Flow Cytometry

Trypsinized cells were incubated with antibodies W6/32-PerCP-eFluor710 (pan-HLA; eBioscience) or BB7.2-APC (HLA-A2; eBioscience) in PBS for 30 min at 4°C. Stained cells were fixed in PBS containing 1% formaldehyde (Merck) and DAPI (1 µM, Sigma-Aldrich) for direct analysis by flow cytometry. Samples were analyzed on BD flow cytometers (LSRFortessa or LSR-II). FACS data was analyzed using FlowJo (Tree Star, Inc). Gating strategy is shown in Figure S1. The pooled primary HUVECs were incubated with BB7.2-APC antibody for 30 min at 4°C before they were sorted into HLA-A2 positive and negative cell populations in the presence of DAPI (1 µM) on a BD FACSAria II.

Sample collection and HLA-I peptide elution

Peptide elution of the cell lines shown in Table 1 and 2 was performed as outlined previously (Hassan et al., 2013). Prior to cell pellet generation, UM9, and TMD8 were retrovirally transduced with HLA alleles to express HLA-A*02:01 (UM9-A2, TMD8-A2) and magnetic-activated cell sorting (MACS) enriched for nerve growth factor receptor (NGFR) marker gene expression using anti-R-phycoerythrin (PE) microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Cell pellets were made of 5 primary acute myeloid leukemias (AML), 4 primary B cell malignancies (ALL, CLL, HCL), 2 primary ovarian carcinoma (1x ascites) and 2 multiple myeloma cell lines (U266 and UM9-A2), 1 DLBCL cell line (TMD8-A2) and the cell lines T2 and HAP1(de Waard et al., 2020). Primary ovarian carcinoma tumor material was first sliced into small pieces. Dead, clotted or non-tumor material was removed, small tumor tissue was put into a C-tube (Miltenyi Biotec) with ice cold buffer without detergent and complete protease inhibitor (Sigma-Aldrich) to prevent protein degradation and dissociated using a gentleMACS (Miltenyi Biotec) procedure until an almost homogenous cell solution. Cells were lysed in 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, and 0.5% Zwittergent 3-12 (pH 8.0) supplemented with complete protease inhibitor and Benzonase (Merck; 125 IU/mL to remove DNA/RNA complexes). After 2 hours of tumbling in lysis buffer at 4°C, cell lysates were centrifuged for 10 min at 1,000 g at 4°C. The supernatant was subsequently centrifuged for 35 min at 13,000 g at 4°C, precleared with Protein A Sepharose CL-4B beads (GE Healthcare Life Sciences), and subjected to an immunoaffinity column with dimethyl pimelimidate-immobilized W6/32 antibody (2-3 mg/ml resin) on Protein A Sepharose CL-4B beads. After flow through of the cell lysate, the column was washed with 5 to 10 column volumes of lysis buffer and 10 mM Tris-HCl (pH 8.0) buffers with 1 M, 120 mM, and no NaCl, bound HLA class I–peptide complexes were eluted from the column and dissociated with 3 to 4 column volumes of 10% acetic acid. Peptides were separated from HLA class I molecules via passage through a 10 kDa membrane (Macrosep Advance Centrifugal Devices with Supor Membrane, Pall Corp.) and freeze-dried.

Fractionation and mass spectrometry (MS) of HLA-class I peptides

Eluted peptide pools were either fractionated by strong cation exchange chromatography (SCX) or by high pH reversed phase fractionation (High pH-RP). SCX fractionation was performed with a homemade 15 cm SCX column (320 µm inner diameter; polysulfoethyl A, 3 µm, Poly LC) run at 4 µL/min. Gradients were run for 10 min at 100% solvent A (100/0.1 water/trifluoroacetic acid v/v), after which a linear gradient started to reach 100% solvent B (65/35/0.1 250 mM KCl/acetonitrile (ACN)/trifluoroacetic acid v/v/v) over 15 min, followed by 100% solvent C (65/35/0.1 500 mM KCl/ACN/trifluoroacetic acid v/v/v) over the next 15 min. The gradient remained at 100% solvent C for 5 min and then switched again to 100% solvent A. Twenty 4 µL fractions were collected in vials prefilled with 20 µL 95/3/0.1 water/ACN/formic acid v/v/v and freeze-dried. High pH-RP fractionation was performed on an Oasis HLB 1cc SPE column (Waters). The column was first equilibrated with 1 mL 10/90 water/ACN v/v, washed 2 times by passing 1 mL of 10 mM ammonium bicarbonate (ambic) buffer pH=8.4 through the column. The freeze-dried peptide mixture was diluted in 10 mM ambic pH 8.4 and gently pushed through the column and washed again with 1 mL of 10 mM ambic pH 8.4. Fractions were taken by steadily increasing the amount of ACN into the elution buffer in the range of 5% to 50% ACN and freeze-dried.

Freeze-dried peptide fractions were dissolved in 95/3/0.1 water/ACN/formic acid v/v/v and analyzed as described previously (van der Lee et al., 2019). The peptides were analyzed by data-dependent MS/MS on a Q Exactive mass spectrometer equipped with an easy-nLC 1000 (Thermo Fisher Scientific) oran LTQ FT Ultra equipped with a nanoflow liquid chromatography 1100 HPLC system (Agilent Technologies). Peptides were trapped at 6–10 µL/min on a 1.5 cm column (100 µm internal diameter; ReproSil-Pur C18-AQ, 3 µm, Dr. Maisch HPLC GmbH) followed by elution to a 20 cm column (50 µm internal diameter; ReproSil-Pur C18-AQ, 3 µm) at 150 nL/min. The column was developed with a 0-40% ACN gradient in 0.1% formic acid for 120 min. The eluent was sprayed into the mass spectrometer by drawing the end of the column to a tip (internal diameter of ~5µm). Full-scan MS spectra were acquired in the FT-ICR with a target value of 3,000,000 at a resolution of 25,000. The 2 most intense ions were isolated for accurate FT-ICR mass measurements by a selected ion monitoring scan with a target accumulation value of 50,000 at a resolution of 50,000followed by fragmentation in the linear ion trap using collision-induced dissociation at a target value of 10,000. The Q Exactive mass spectrometer was used in top10 mode with an AGC target value of 3,000,000/maximum fill time of 20 ms (full scan) at a resolution of 70,000 and an AGC target value of 100,000/maximum fill time of 60 ms for MS/MS at a resolution of 17,500 and an intensity threshold of 17,000. Apex trigger was 1-10 sec with 2-6 allowed charges. The Orbitrap Fusion LUMOS mass spectrometer was used in data-dependent MS/MS (top-N

mode), with recording of the MS2 spectrum in the orbitrap with a collision energy at 32 V. For master scan (MS1), the AGD target was 400,000/maximum fill time of 50 ms at a resolution of 60,000 and scan range 300-1,400. Charge states 1–4 were included and the dynamic exclusion was set after n=1 with a duration of 20 sec. For MS2, precursors were isolated using the quadrupole with an isolation width of 1.2 Da. HCD collision energy was at 32 V, first mass at 110 Da. The MS2 had an AGC target of 50,000/maximum fill time of 100 ms with a resolution of 30,000. To identify peptides and proteins, Proteome Discoverer version 2.1 (Thermo Fisher Scientific) was used with the mascot node for identification in mascot version 2.2.04 with the UniProt Homo Sapiens database (UP000005640; Jan 2015; 67,911 entries). Cysteine modifications were set as variably for Methionine oxidation and cysteinylation. Precursor tolerance of 10 ppm and MS/ MS fragment tolerance of 20 mmu was used for peptide assignment for the Q Exactive data and 2 ppm and 0.5 Da for LTQ FT Ultra data.

The HLA-I peptidome analyses of the HAP1 and MCF7 cells shown in Table S3 were done using exactly the HLA immunoaffinity purification and LC-MS/MS procedures as in Komov et al. 2018 (Komov et al., 2018).

Synthetic peptide stimulation

Synthetic peptides were titrated on 20,000 T2 cells prior to incubation with 5,000 T cells for 18 hours before cytokine ELISA similar as previously described (Bijen et al., 2018).

Expression analysis

Data on SSR1, CD3E, CD14 and CD19 RNA expression in blood cells and of SSR1, CD45 and PECAM1 in HUVECs was retrieved from the Human Protein Atlas (https://www.proteinatlas.org)(Uhlen et al., 2015) using the RNA HPA blood cell gene and the RNA HPA cell line gene datasets. Data for B and T cells was pooled from the different naïve and memory subsets. Microarray data of IFN-γ stimulated human cells were selected based on equal *in vitro* treatment with 200 U/mL IFN-γ for 24 hours containing expression data on SSR1 (downloaded from Interferome.org v2.01, datasets 64, 144, 311). PPIA was selected as house-hold control as it is unaffected by interferon signaling and was present in all datasets (Riemer et al., 2012). VPS13B and USP11 expression was only present in datasets 64 and 144, respectively.

Statistical Analyses

All error bars correspond to the standard deviation of the mean. Statistical evaluations were done by a student's t-test for comparison of two independent groups (T cell assay on HAP1 wild type and TAP1 KO cells), a repeated measures one-way ANOVA followed by Dunnett's

multiple comparisons test for comparison of change in gene expression derived from multiple datasets (IFN-γ regulation of mRNA), an one-way ANOVA followed by Dunnett's multiple comparisons test (IFN-γ regulation of protein) or Sidak's multiple comparisons test (T cell assay on IFN-γ stimulated HAP1 cells) for multigroup analyses with Prism software (www.graphpad.com). Statistical evaluation of primary cell cocultures was unnecessary due to the qualitative goal of the experiments.

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