nature research

https://doi.org/10.1038/s41586-020-03054-1

Supplementary information

Anti-tumour immunity induces aberrant peptide presentation in melanoma

In the format provided by the authors and unedited

Supplementary Methods

Reporter constructs

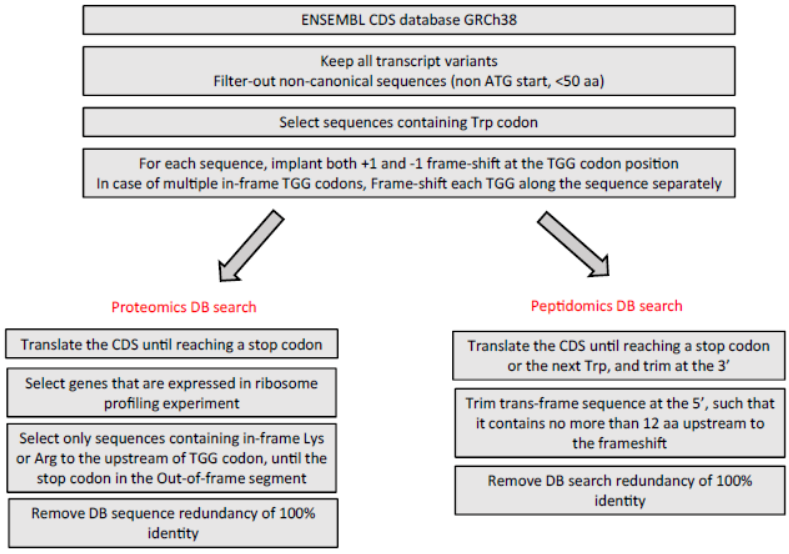
V5-ATF4-His and V5-ATF4-turboGFP reporter constructs were generated by PCR and restriction cloning into the XbaI-NotI sites of the lentiviral pCDH-Blasticidin vector (Addgene). V5-ATF4-His reporters were amplified using Phusion Polymerase (Thermo Fisher Scientific) using the primers listed below, with the pLX304-ORF clone of ATF4 (Dharmacon) as a template. V5-ATF4-tGFP reporters were amplified by a 2-step PCR, where first tGFP was amplified from a pLKO.1-tGFP vector (Addgene) with an overhang on the 5' primer that matches the previously mentioned ATF4 reporters. In the 2nd PCR the V5-ATF4 part was added to it by using the V5-ATF4-His constructs as templates. The final constructs were sequence-verified by Sanger sequencing (Eurofins).

Supplementary Table 1. Primers used for generation of reporter constructs

Name	Sequence (5'-3')
V5 ATF4 fw	ACAGCGTCTAGAGCCACCATGGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCG ATTCTACGGGCGGCGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTACG GGCGGCACCGAAATGAGCTTCCTGAG
ATF4-His Frame rev	ACAGCGGCGGCCGCTCAGTTATCTAGTGGTGATGGTGATGATGCACAGCCAGCC ATTCG
ATF4-His +1 rev	ACAGCGGCGGCCGCTCAGTTATCTAGTGGTGATGGTGATGATGACACAGCGAGC CATTCG
ATF4-His +2 rev	ACAGCGGCGGCCGCTCAGTTATCTAGTGGTGATGGTGATGATGAACACAGCCAG CCATTCG
ATF4 Frame - tGFP fw	CCGAATGGCTCGCTGTCGGAGGAATGGAGAGCGACG
ATF4 +1 - tGFP fw	CCGAATGGCTCGCTGTCGGAGGATATGGAGAGCGACG
ATF4 +2 - tGFP fw	CCGAATGGCTCGCTGTCGGAGGATAATGGAGAGCGACG
tGFP rev	ACAGCGGCGGCCGCTCAGTTATCTATTCTTCACCGGCATC
tGFP-SIINFEKL rev	ACAGCGGCGGCCGCTCAGCTATTTAGAGCTTTTCGAAGTTGATGATGGATTCCAGCT GCTCGAGTTCTTCACCGGCATCTGC

Supplementary Table 2. Aberrant peptides screened for immunogenicity

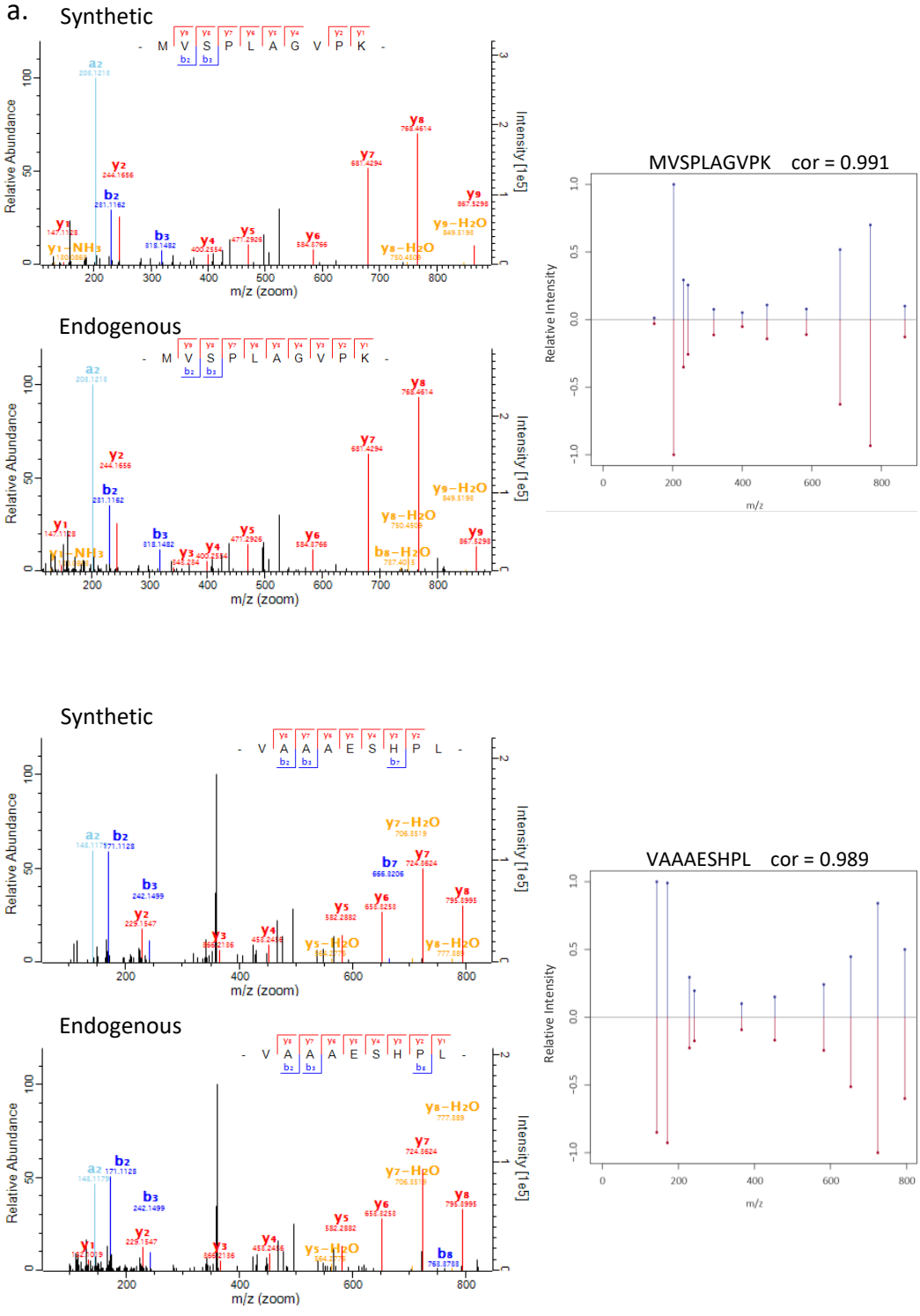
Gene	Aberrant peptide sequence	HLA	# of screened healthy donors
HSP90AB1	MVSPLAGVPK	A*03:01	5
STK25	SPALRTLTL	B*07:02, C*07:02	4, 4
MAPK12	SPAAGGQGL	B*07:02	4
RPL7A	VAAAESHPL	B*07:02	4
KCNK6	VPLSLAEHAL	B*07:02	4
GBA	APASLKASA	B*07:02	4
FCGBP	APSGVAAGL	B*07:02	4
ZNF513	VGQEGLVSL	C*07:02	4
ESPNL	LFLSHLEEI	C*07:02	4

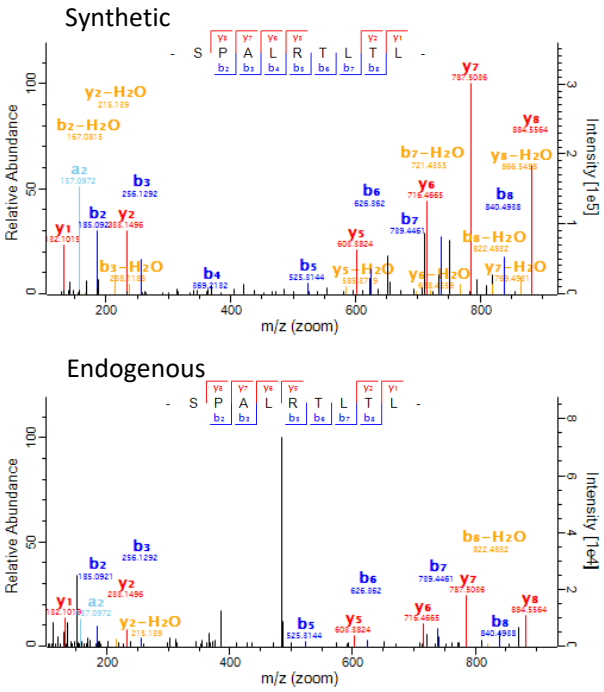


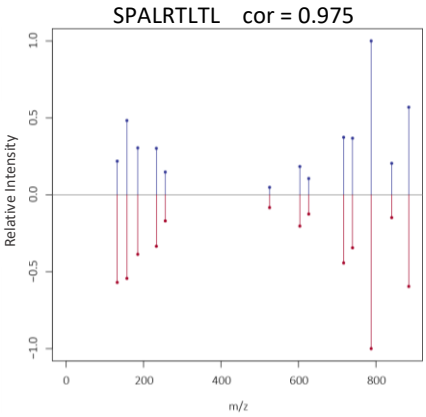
Supplementary Figure 1. Scheme for proteomics and immunopeptidomics analyses. The indicated mass spectrometry proteomics and immunopeptidomics database searches were used to identify tryptophan-associated aberrant peptides.

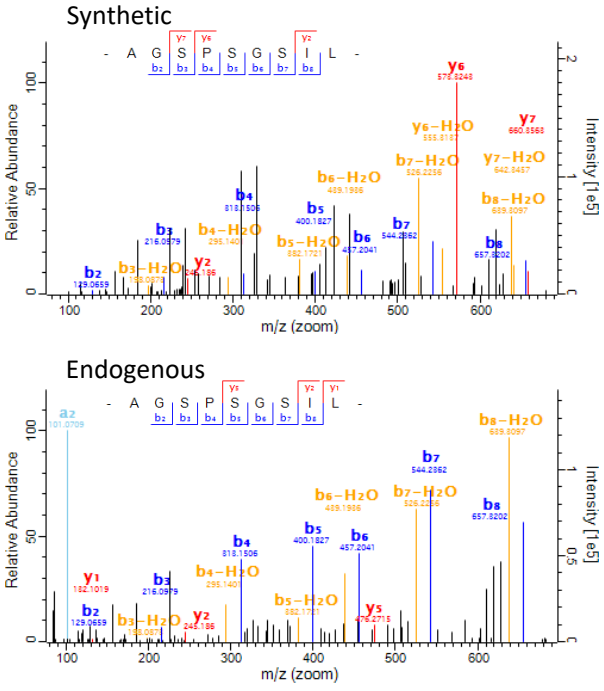
Supplementary Figure 2

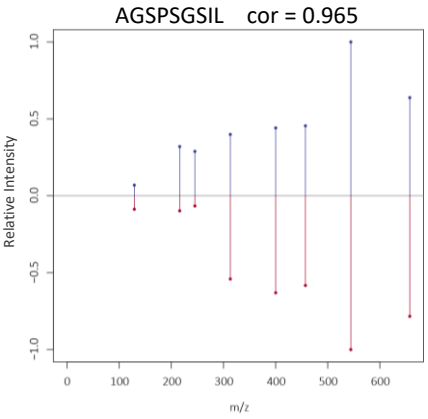
a.

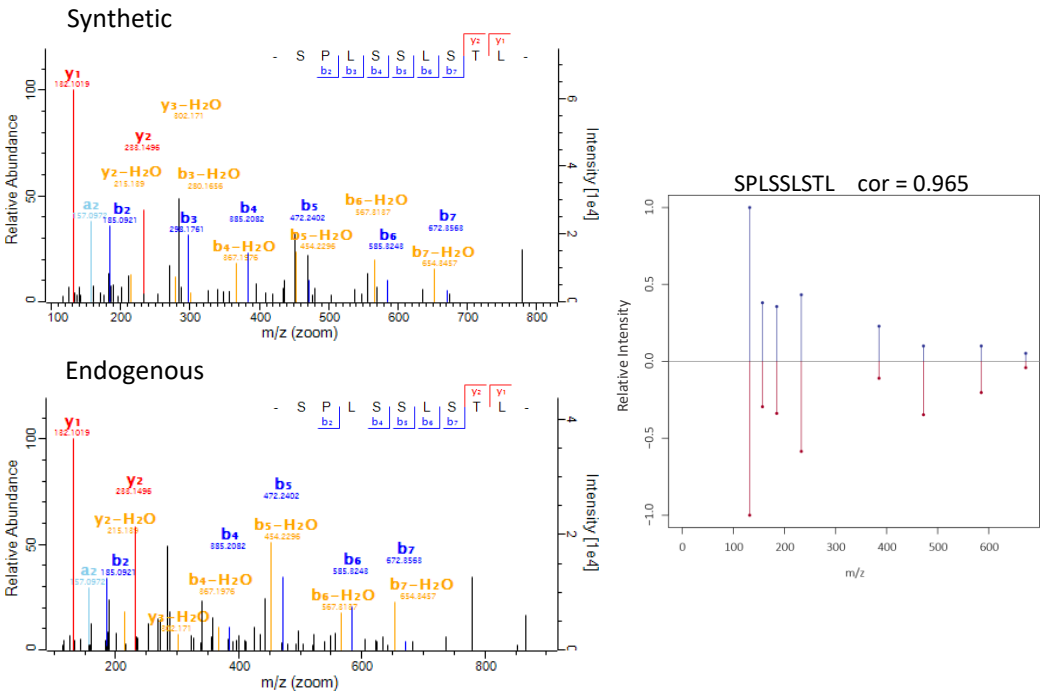


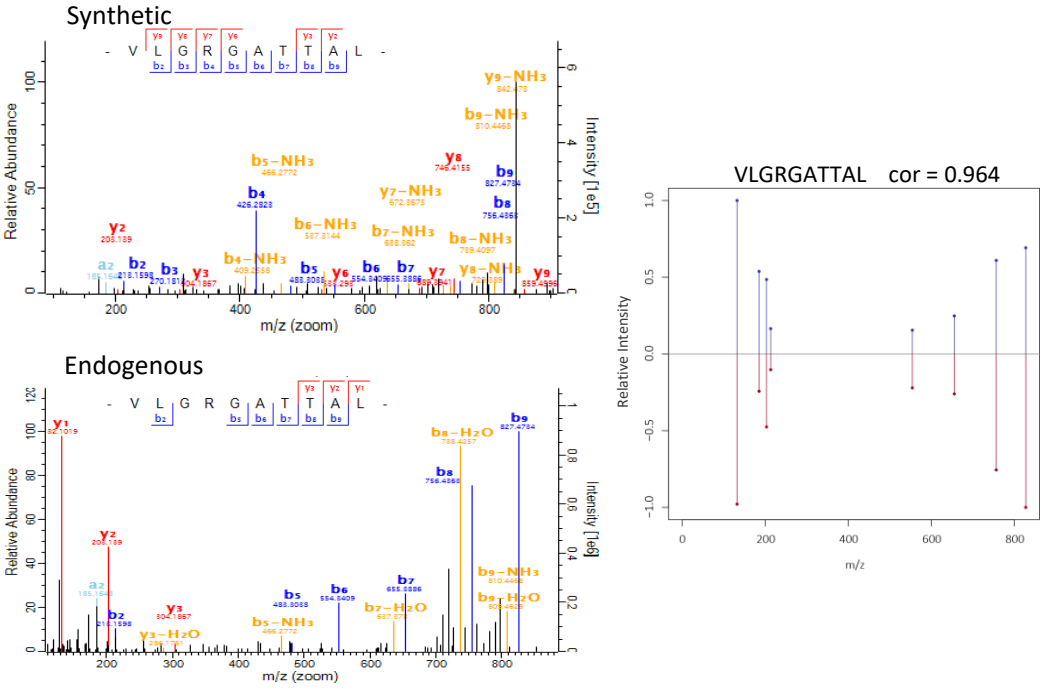

Synthetic
Endogenous
Relative Abundance
Intensity [1e5]
Intensity [1e4]
m/z (zoom)

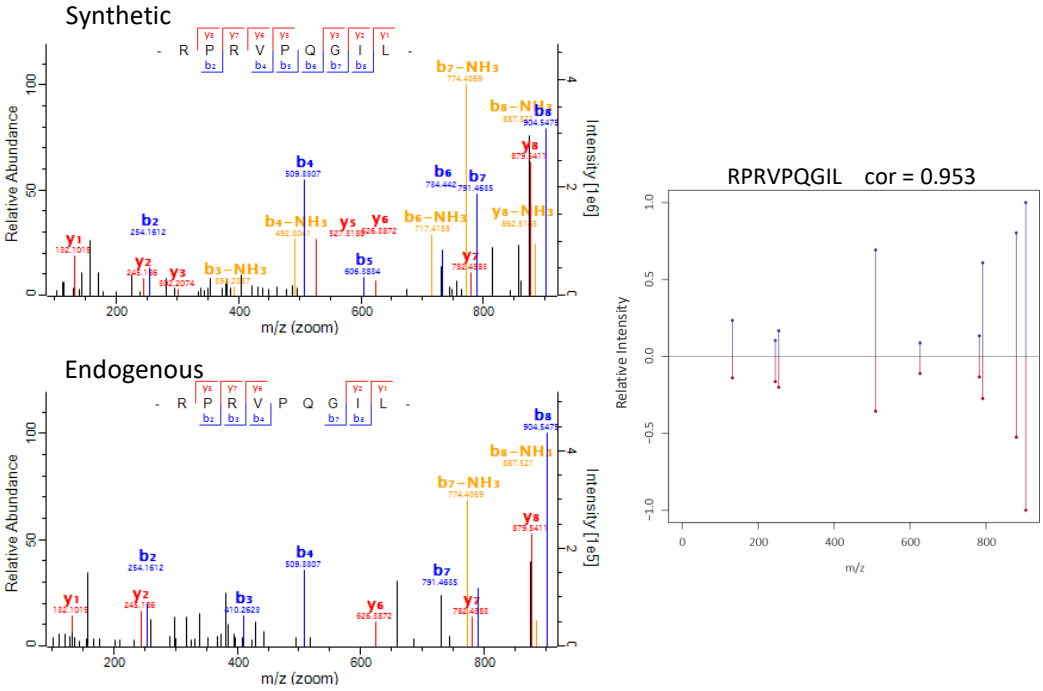

SPALRTLTL cor = 0.975
Relative Intensity
m/z

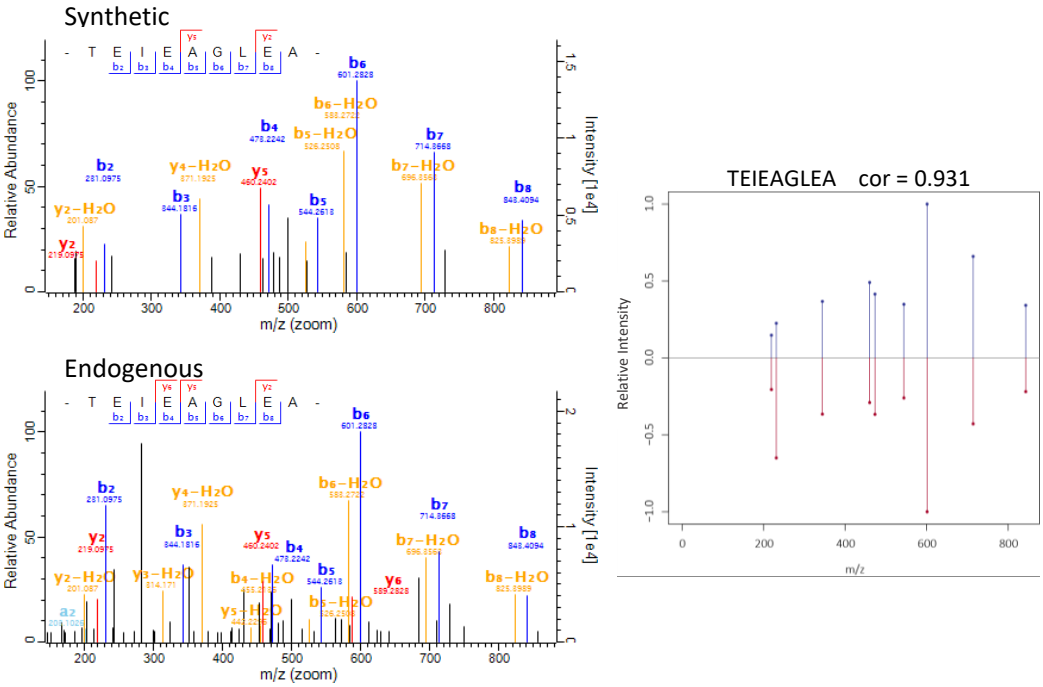

Synthetic
Endogenous
Relative Abundance
Intensity [1e5]
m/z (zoom)


AGSPSGSIL cor = 0.965
Relative Intensity
m/z

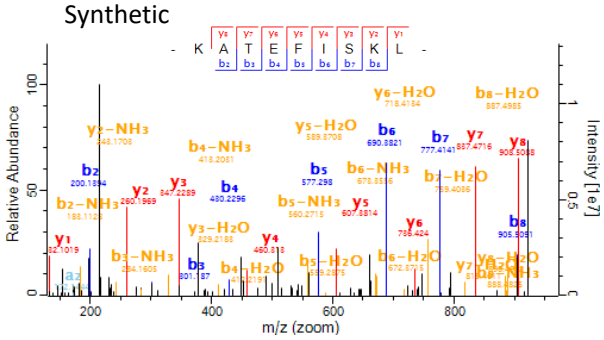

Synthetic
Endogenous
Relative Abundance
Intensity [1e4]
m/z (zoom)
SPLSSLSTL cor = 0.965
Relative Intensity
m/z


Synthetic
Endogenous
Relative Abundance
Intensity [1e5]
Intensity [1e6]
m/z (zoom)
VLGRGATTAL cor = 0.964
Relative Intensity
m/z

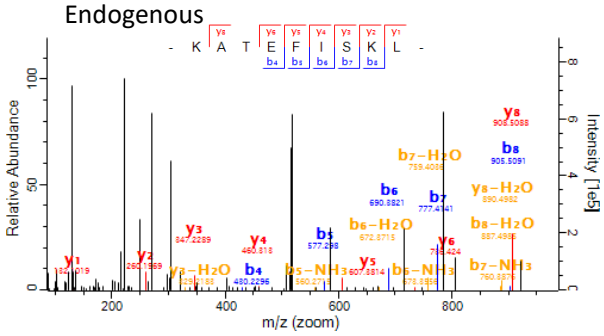

Synthetic
- R P R V P Q G I L -
Relative Abundance
Intensity [1e6]
m/z (zoom)
Endogenous
- R P R V P Q G I L -
Relative Abundance
Intensity [1e5]
m/z (zoom)
RPRVPQGIL cor = 0.953
Relative Intensity
m/z


Synthetic
- T E I E A G L E A -
Relative Abundance
Intensity [1e4]
m/z (zoom)
Endogenous
- T E I E A G L E A -
Relative Abundance
Intensity [1e4]
m/z (zoom)
TEIEAGLEA cor = 0.931
Relative Intensity
m/z

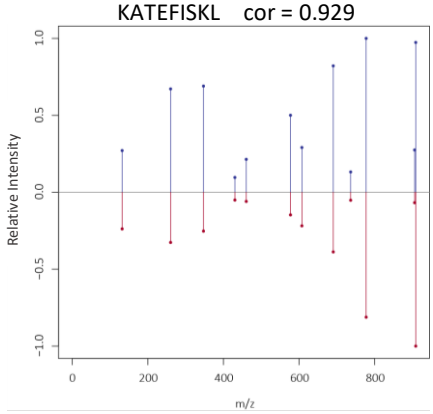
Synthetic



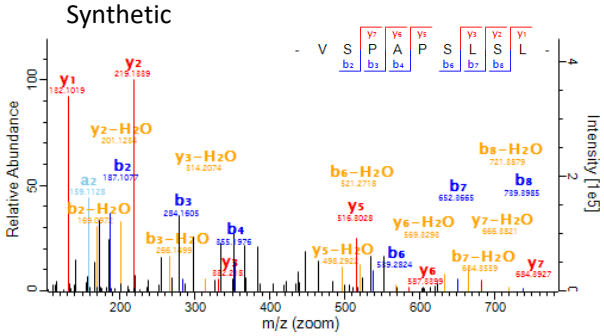
Endogenous



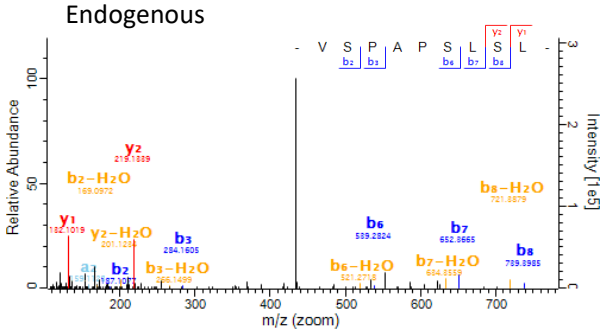
KATEFISKL cor = 0.929



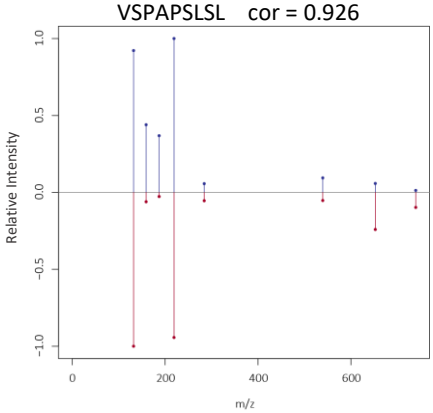
Synthetic

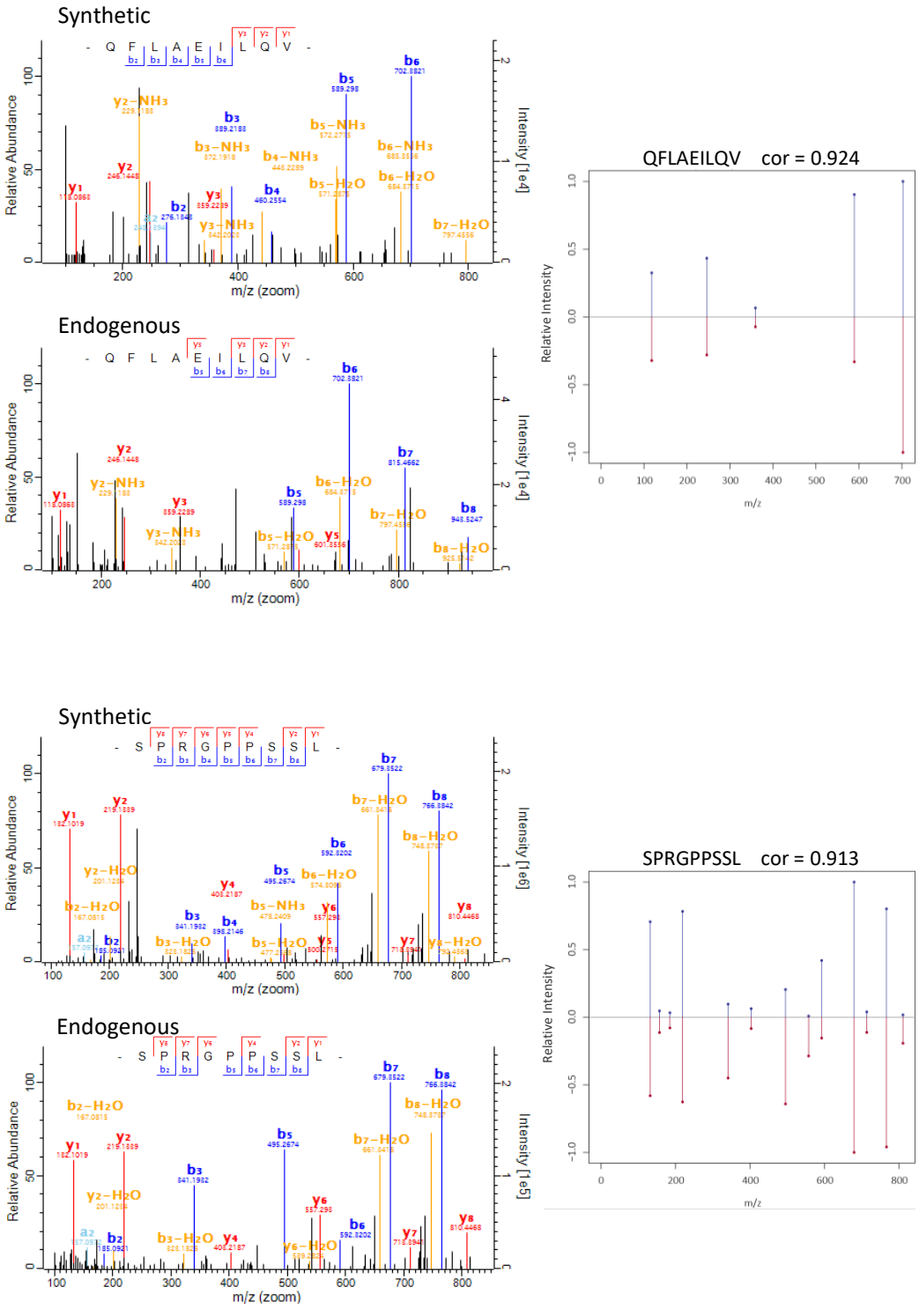


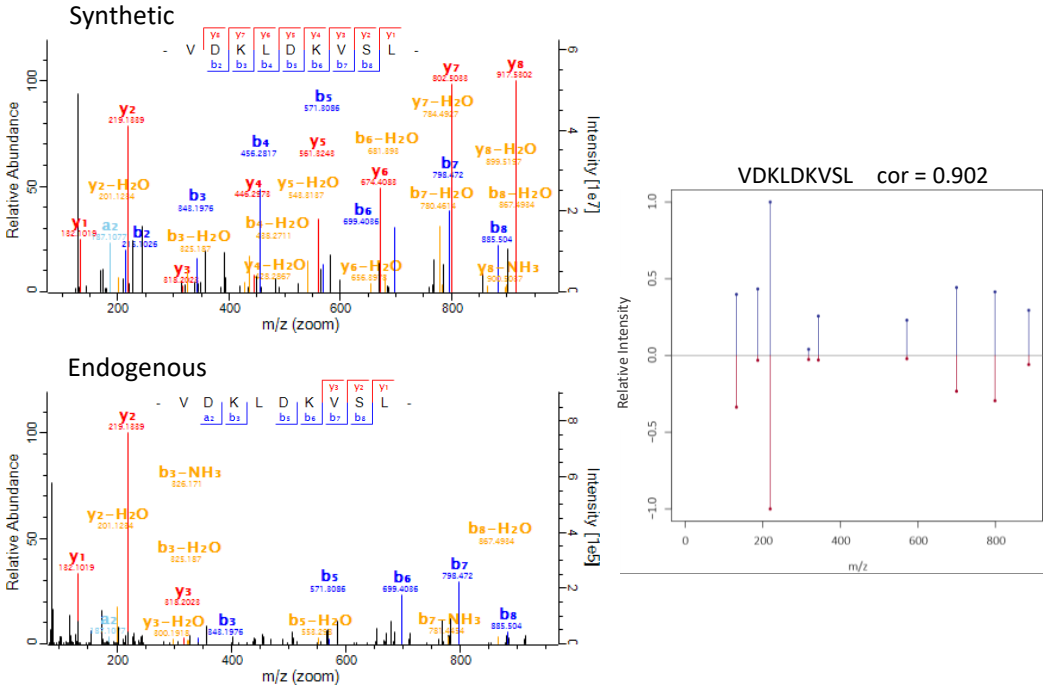
Endogenous

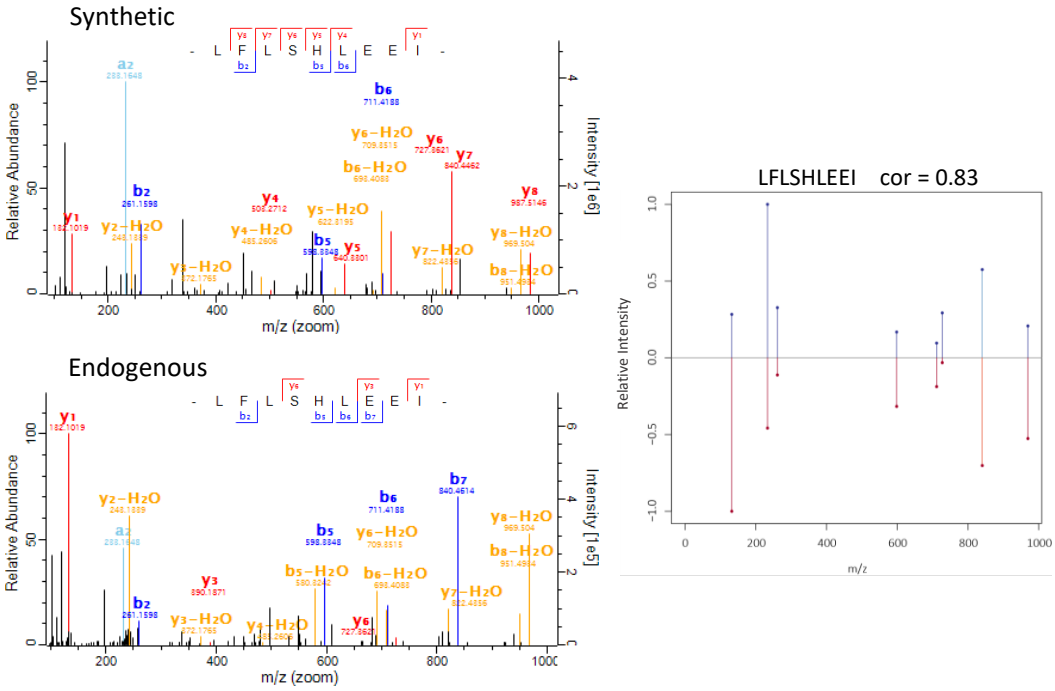


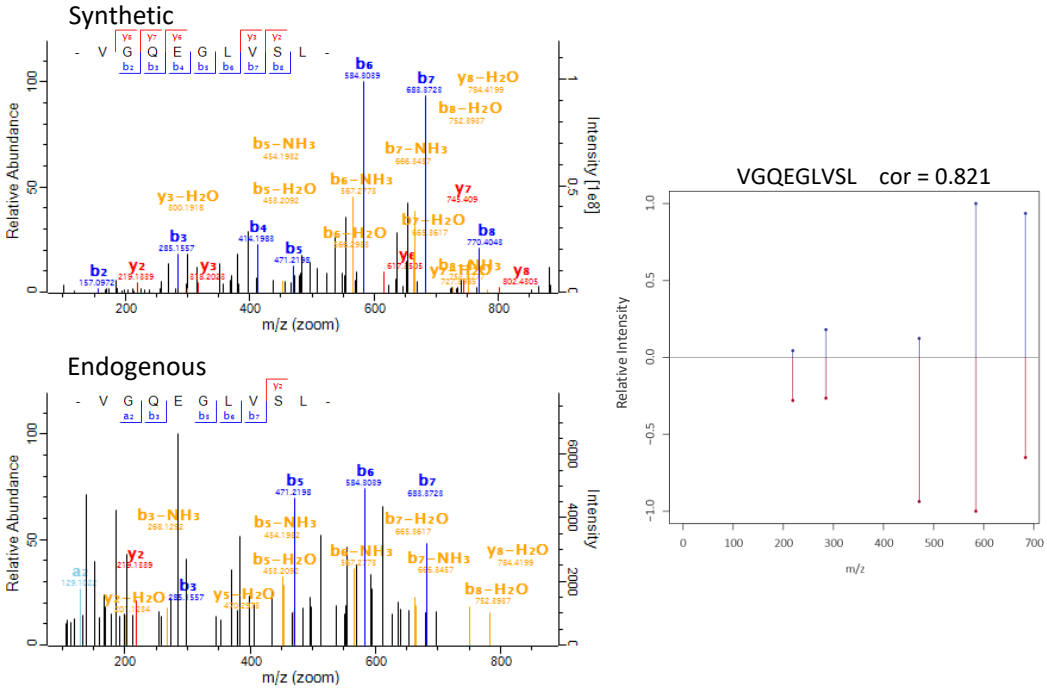
VSPAPSLSL cor = 0.926

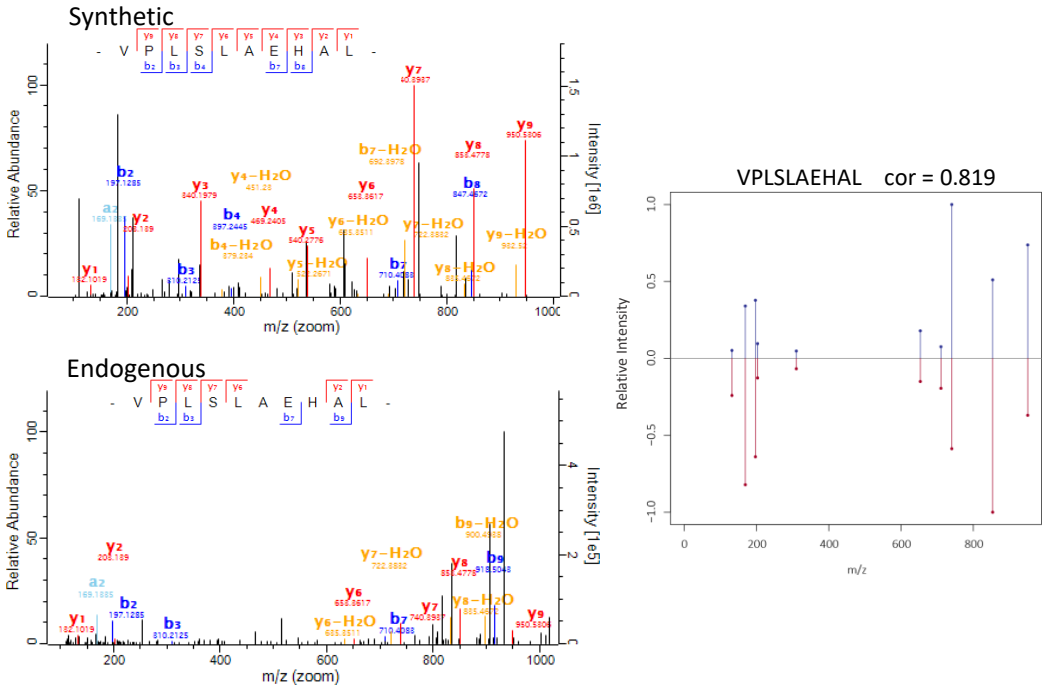


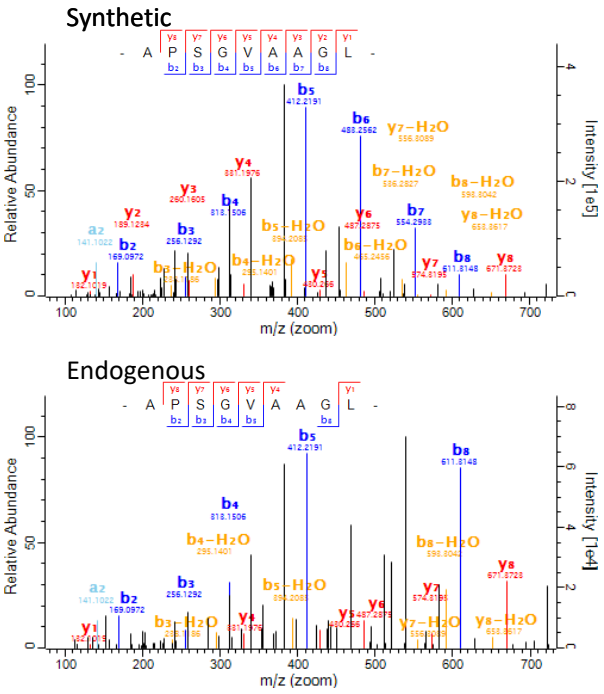

Synthetic
- Q F L A E I L Q V -
Relative Abundance
Intensity [1e4]
m/z (zoom)
Endogenous
- Q F L A E I L Q V -
Relative Abundance
Intensity [1e4]
m/z (zoom)
QFLAEILQV cor = 0.924
Relative Intensity
m/z
Synthetic
- S P R G P P S S L -
Relative Abundance
Intensity [1e5]
m/z (zoom)
Endogenous
- S P R G P P S S L -
Relative Abundance
Intensity [1e5]
m/z (zoom)
SPRGPPSSL cor = 0.913
Relative Intensity
m/z

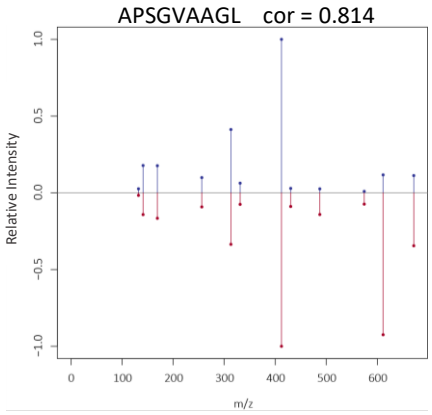

Synthetic
Endogenous
Relative Abundance
Intensity [1e7]
Intensity [1e5]
m/z (zoom)
VDKLDKVSL cor = 0.902
Relative Intensity
m/z

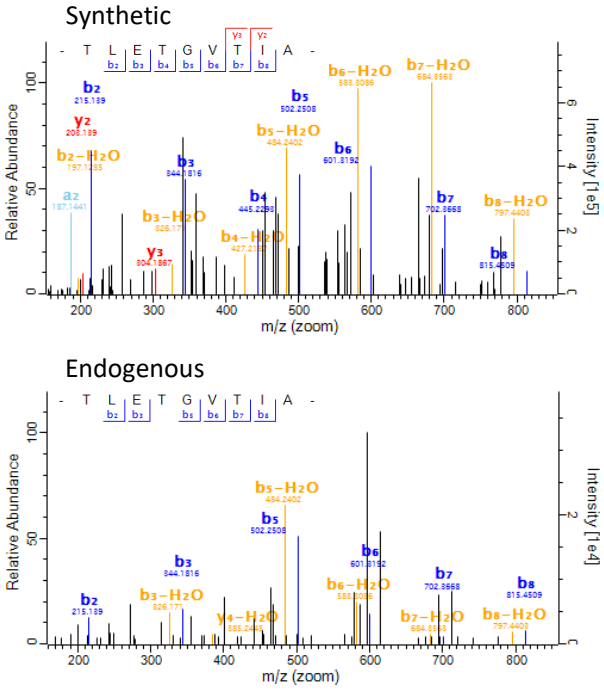

Synthetic
Endogenous
Relative Abundance
Intensity [1e6]
Intensity [1e5]
m/z (zoom)
LFLSHLEEI cor = 0.83
Relative Intensity
m/z

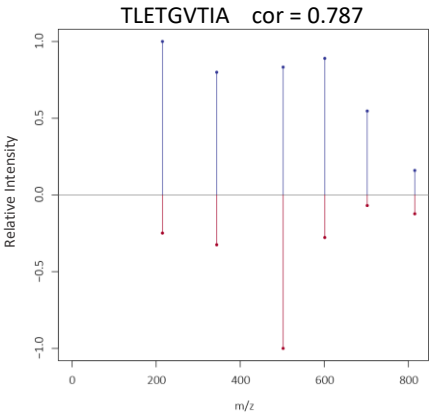

Synthetic
Endogenous
Relative Abundance
Intensity [1e8]
Intensity
m/z (zoom)
VGQEGLVSL cor = 0.821
Relative Intensity
m/z

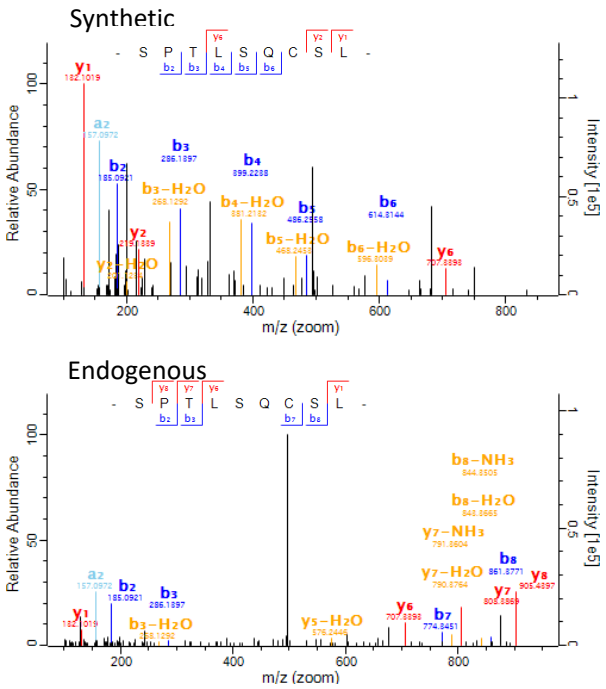

Synthetic
Endogenous
Relative Abundance
Intensity [1e6]
Intensity [1e5]
m/z (zoom)
VPLSLAEHAL cor = 0.819
Relative Intensity
m/z

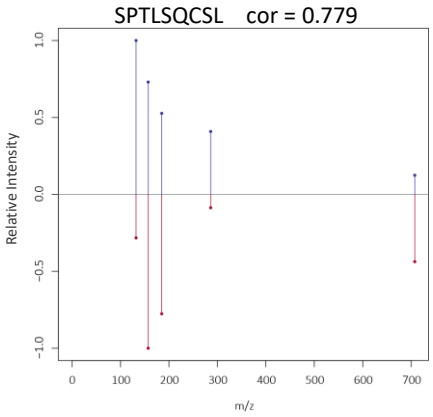

Synthetic
Endogenous
Relative Abundance
Intensity [1e5]
Intensity [1e4]
m/z (zoom)

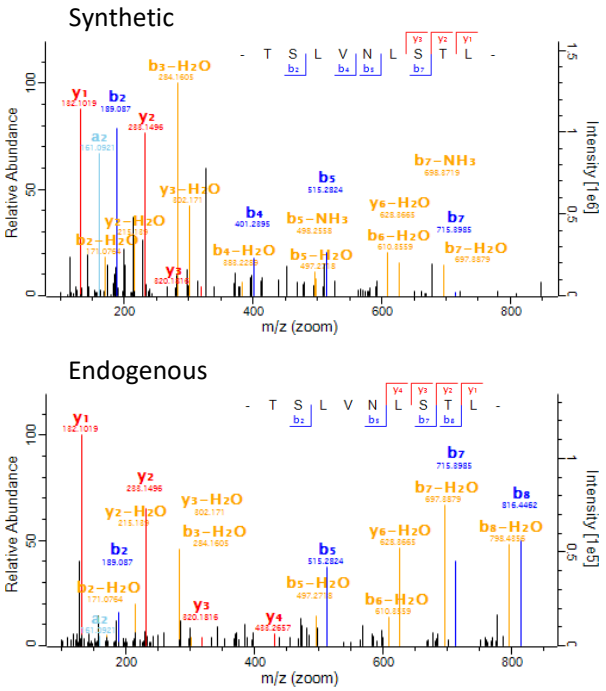

APSGVAAGL cor = 0.814
Relative Intensity
m/z

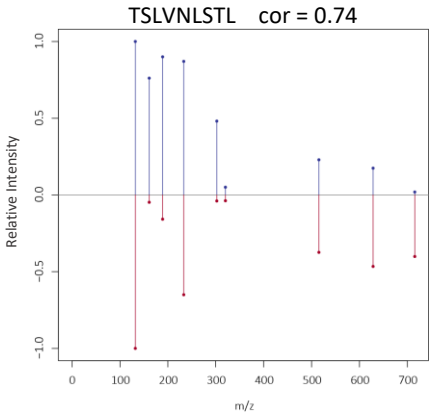

Synthetic
Endogenous
Relative Abundance
Intensity [1e5]
Intensity [1e4]
m/z (zoom)


TLETGVTIA cor = 0.787
Relative Intensity
m/z

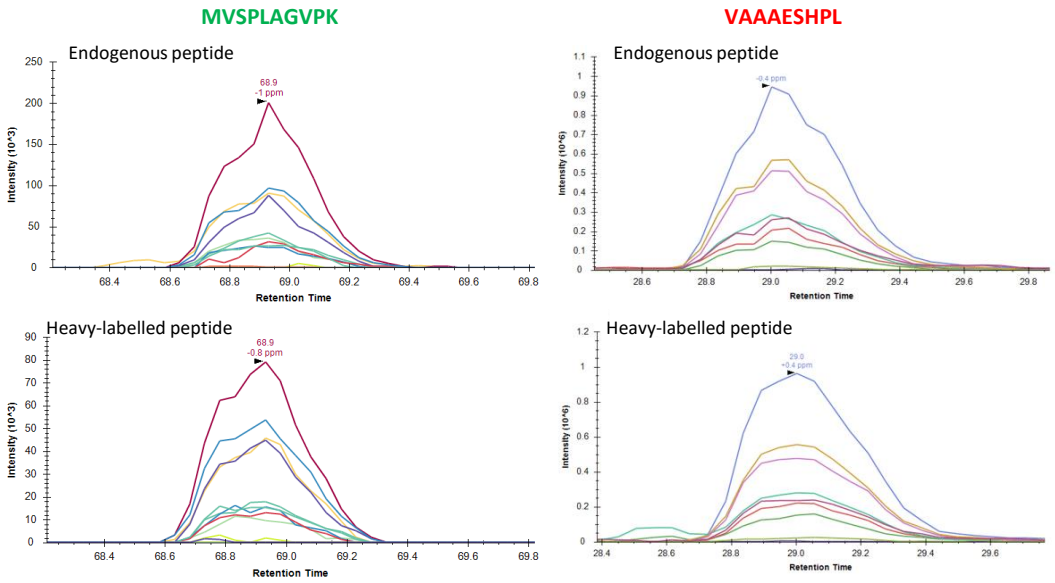

Synthetic
Endogenous
Relative Abundance
Intensity [1e5]
m/z (zoom)


SPTLSQCSL cor = 0.779
Relative Intensity
m/z


Synthetic
Endogenous
Relative Abundance
Intensity [1e5]
m/z (zoom)

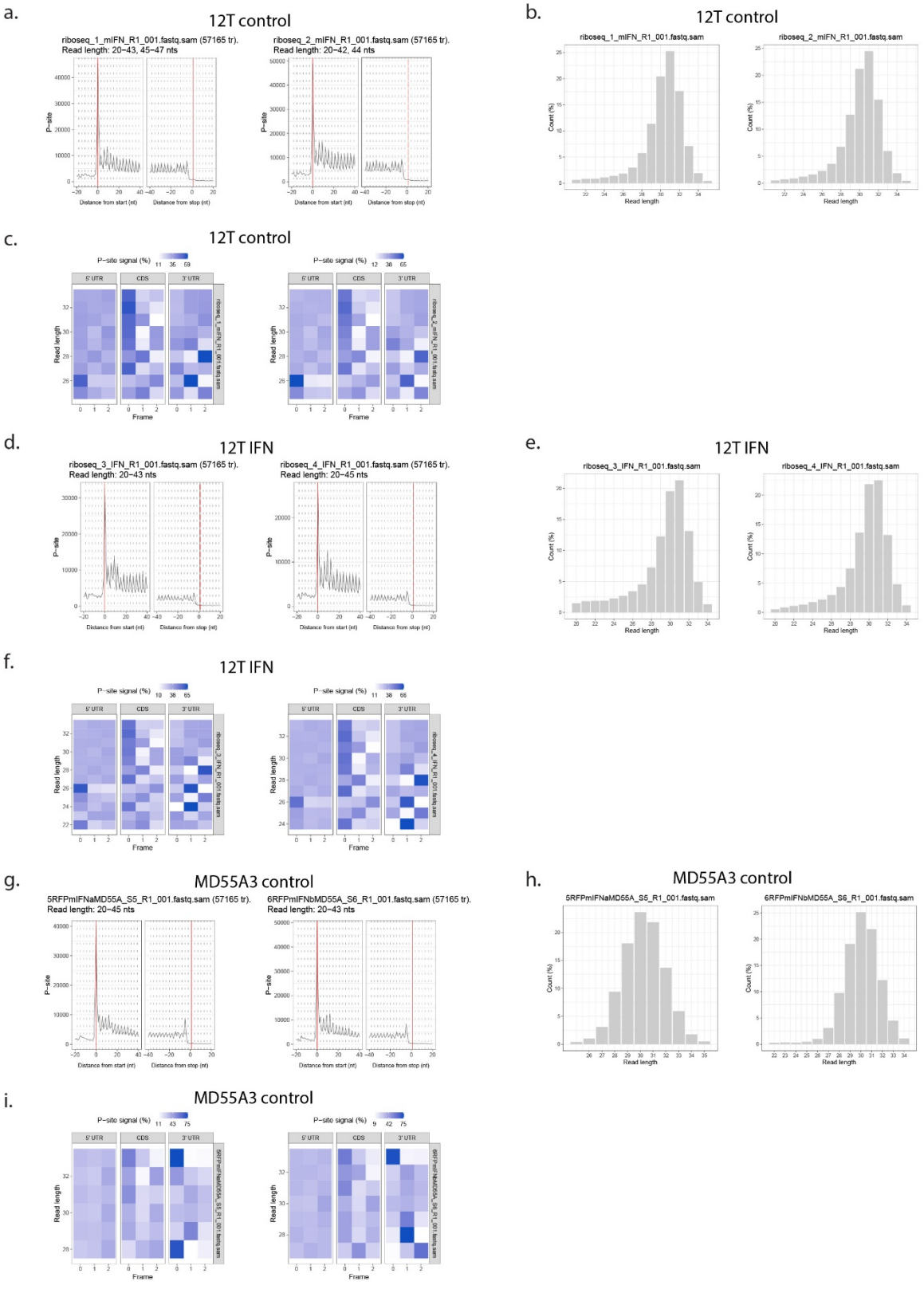

TSLVNLSTL cor = 0.74
Relative Intensity
m/z

b.

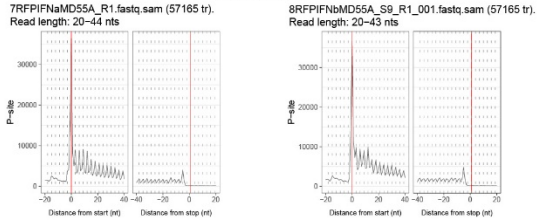


Supplementary Figure 2: MS-based validation of aberrant peptide identification: (a) Tandem mass spectra of top 20 endogenous aberrant peptides identified in MD55A3 cells, that show the highest correlation scores matched to their corresponding synthetic peptides. Upper panel – synthetic; Lower panel – Endogenous. Head-to-tail plots show similarities between overlapping relative ion intensities of the two spectra, Upper panel – Endogenous; lower panel – synthetic. (b) The indicated -1 and +1 frame-shifted antigens were synthesized in their heavy-labeled form and spiked into the original samples. Their identification confirms the presence of endogenous antigens. Chromatogram profiles of the heavy-labeled and endogenous peptides are shown.

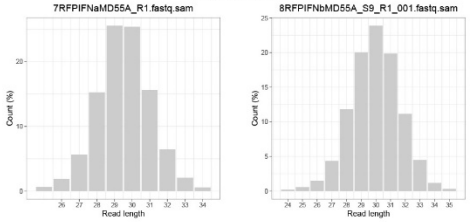
Supplementary Figure 3



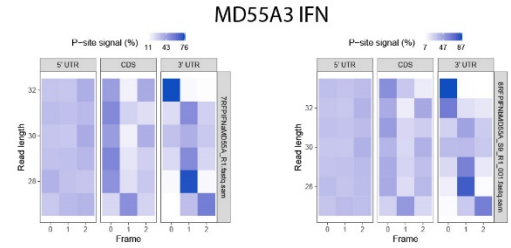
j.


MD55A3 IFN
7RFPIFNaMD55A_R1.fastq.sam (57165 tr).
Read length: 20–44 nts
8RFPIFNbMD55A_S9_R1_001.fastq.sam (57165 tr).
Read length: 20–43 nts
P–site
Distance from start (nt)
Distance from stop (nt)

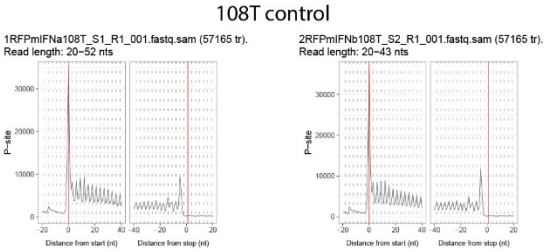
k.


MD55A3 IFN
7RFPIFNaMD55A_R1.fastq.sam
8RFPIFNbMD55A_S9_R1_001.fastq.sam
Count (%)
Read length

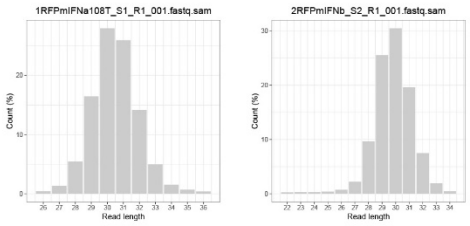
l.


MD55A3 IFN
P–site signal (%)
5' UTR
CDS
3' UTR
Read length
Frame

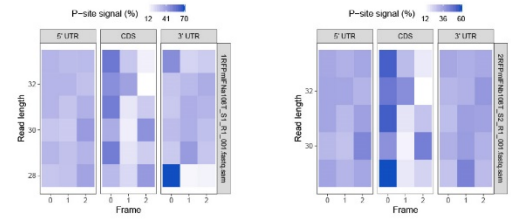
m.


108T control
1RFPmIFNa108T_S1_R1_001.fastq.sam (57165 tr).
Read length: 20–52 nts
2RFPmIFNb108T_S2_R1_001.fastq.sam (57165 tr).
Read length: 20–43 nts
P–site
Distance from start (nt)
Distance from stop (nt)

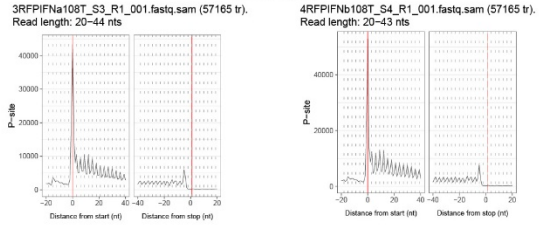
n.


108T control
1RFPmIFNa108T_S1_R1_001.fastq.sam
2RFPmIFNb_S2_R1_001.fastq.sam
Count (%)
Read length

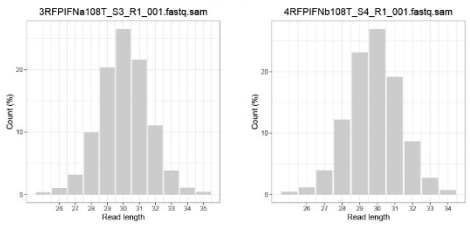
o.


108T control
P–site signal (%)
5' UTR
CDS
3' UTR
Read length
Frame

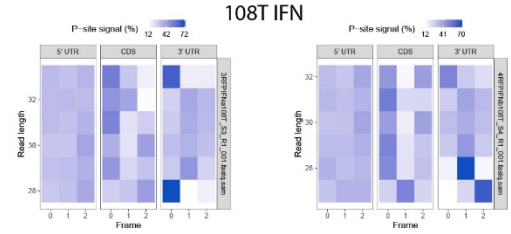
p.


108T IFN
3RFPIFNa108T_S3_R1_001.fastq.sam (57165 tr).
Read length: 20–44 nts
4RFPIFNb108T_S4_R1_001.fastq.sam (57165 tr).
Read length: 20–43 nts
P–site
Distance from start (nt)
Distance from stop (nt)

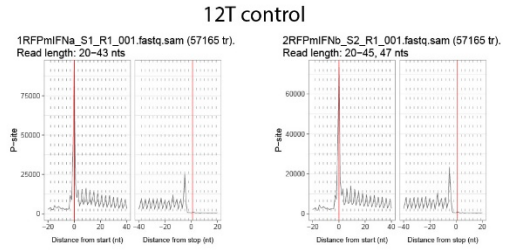
q.


108T IFN
3RFPIFNa108T_S3_R1_001.fastq.sam
4RFPIFNb108T_S4_R1_001.fastq.sam
Count (%)
Read length

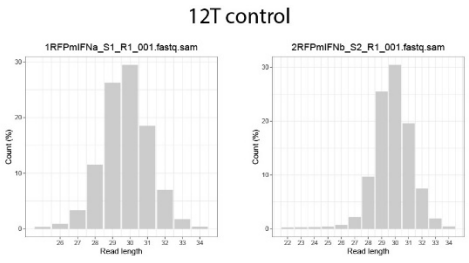
r.


108T IFN
P–site signal (%)
5' UTR
CDS
3' UTR
Read length
Frame

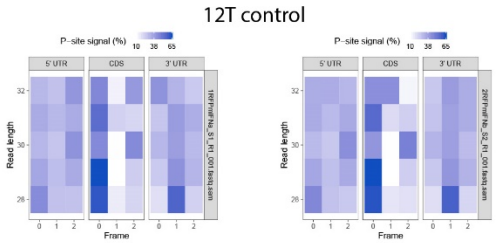
s.


12T control
1RFPmIFNa_S1_R1_001.fastq.sam (57165 tr).
Read length: 20–43 nts
2RFPmIFNb_S2_R1_001.fastq.sam (57165 tr).
Read length: 20–45, 47 nts
P–site
Distance from start (nt)
Distance from stop (nt)

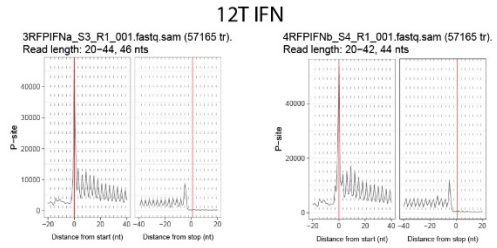
t.


12T control
1RFPmIFNa_S1_R1_001.fastq.sam
2RFPmIFNb_S2_R1_001.fastq.sam
Count (%)
Read length

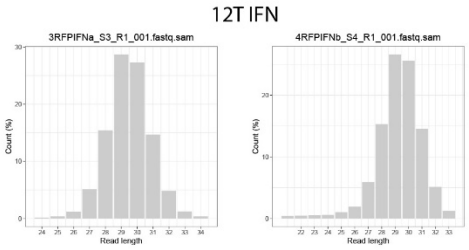
u.


12T control
P–site signal (%)
5' UTR
CDS
3' UTR
Read length
Frame

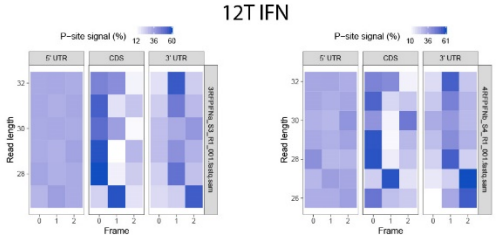
v.


12T IFN
3RFPIFNa_S3_R1_001.fastq.sam (57165 tr).
Read length: 20–44, 46 nts
4RFPIFNb_S4_R1_001.fastq.sam (57165 tr).
Read length: 20–42, 44 nts
P–site
Distance from start (nt)
Distance from stop (nt)

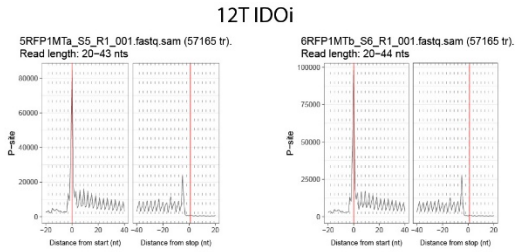
w.


12T IFN
3RFPIFNa_S3_R1_001.fastq.sam
4RFPIFNb_S4_R1_001.fastq.sam
Count (%)
Read length

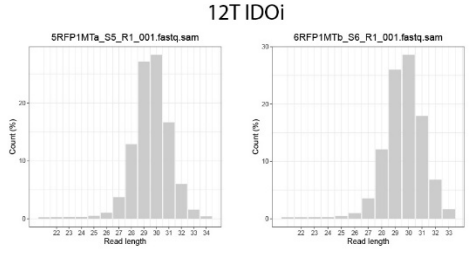
x.


12T IFN
P–site signal (%)
5' UTR
CDS
3' UTR
Read length
Frame

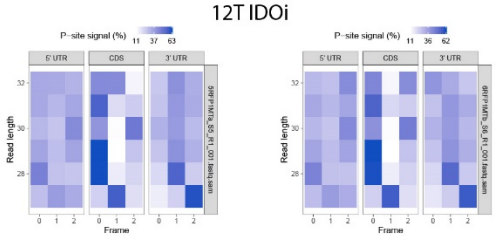
y.

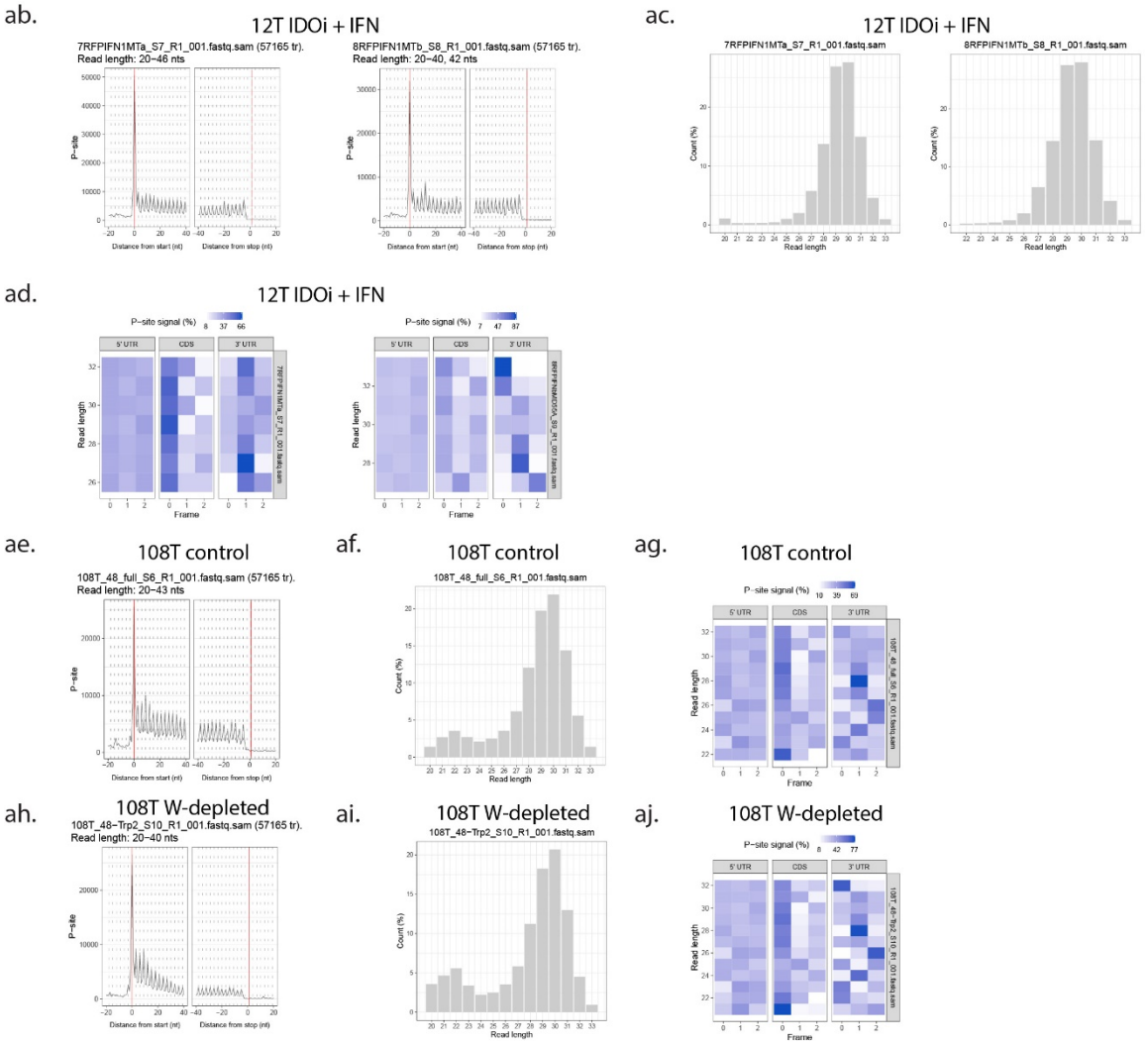

12T IDOi
5RFP1MTa_S5_R1_001.fastq.sam (57165 tr).
Read length: 20–43 nts
6RFP1MTb_S6_R1_001.fastq.sam (57165 tr).
Read length: 20–44 nts
P–site
Distance from start (nt)
Distance from stop (nt)

z.


12T IDOi
5RFP1MTa_S5_R1_001.fastq.sam
6RFP1MTb_S6_R1_001.fastq.sam
Count (%)
Read length

aa.


12T IDOi
P–site signal (%)
5' UTR
CDS
3' UTR
Read length
Frame

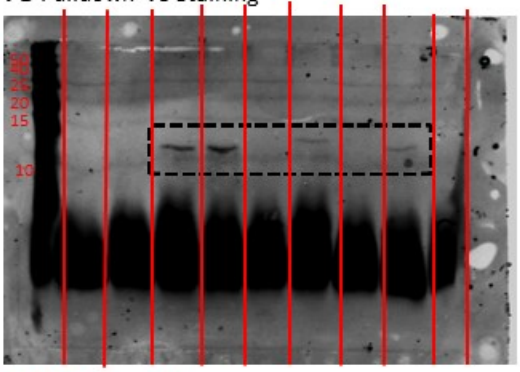


Supplementary Figure 3: Quality control plots of all RiboSeq experiments
(a) Periodicity analysis for the number of P-sites across the start and stop codons of transcripts in the ribo-seq data in two replicates of control 12T cells. (b) Barplots depicting read length distribution of the ribosome protected fragments in the ribo-seq data in control 12T cells. (c) Heatmap depicting P-site enrichment signal in three different frames with the ribosome protected fragment of various lengths as indicated on Y-axis in two replicates of control 12T cells. (d –aj) Same as (a-c) but for indicated cell lines and treatment conditions.

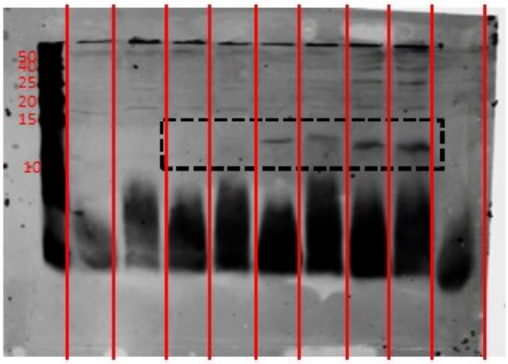
Supplementary Figure 4

Figure 3e

PD Pulldown V5 staining

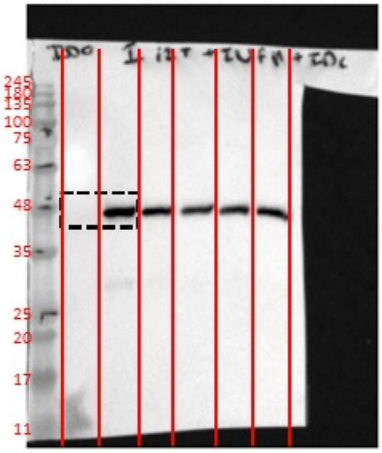


S Supernatant V5 staining

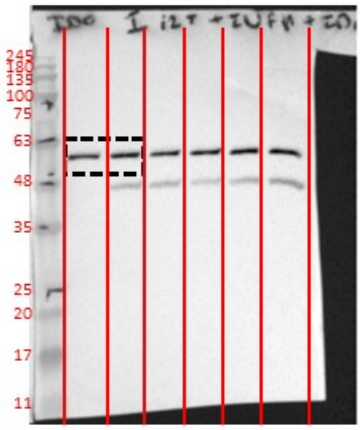


ED Figure 1a

IDO staining 12T cells (upper panel)

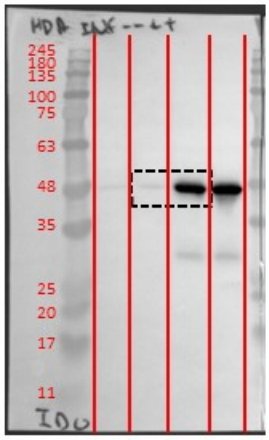


Tubulin staining 12T cells (lower panel, same blot as on the left)

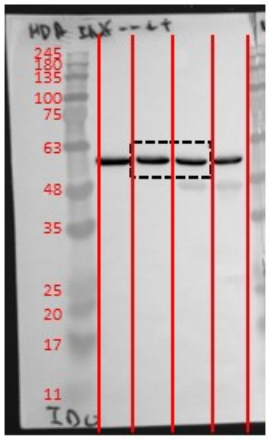


ED Figure 1a

IDO staining MD55A3 cells (upper panel)



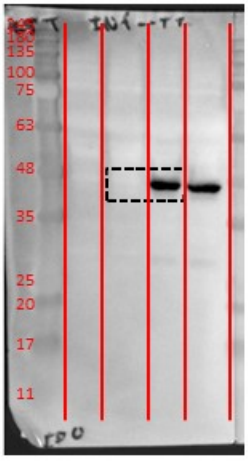
Tubulin staining MD55A3 (lower panel, same blot as on the left)



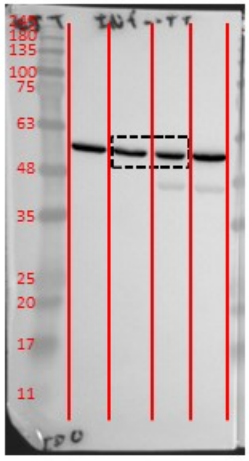
1

ED Figure 1a

IDO staining 108T cells (upper panel)

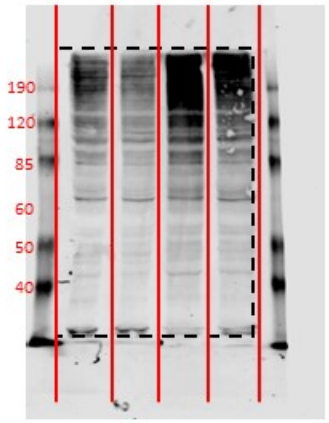


Tubulin staining 108T cells (Lower panel, same blot as on the left)

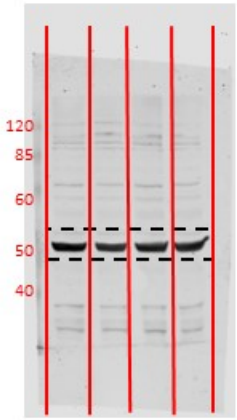


ED Figure 3c

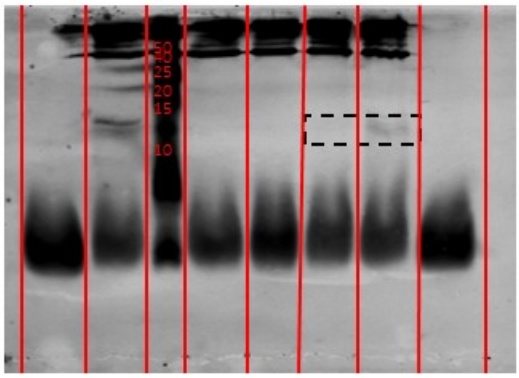
FK2 staining (upper panel)



Tubulin staining (lower panel)



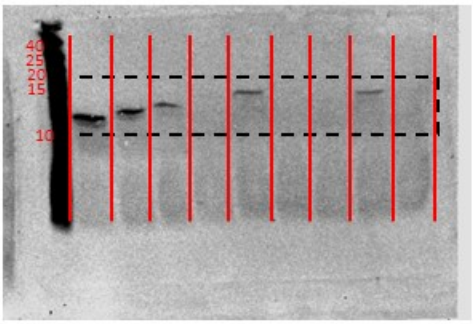
ED Figure 4d



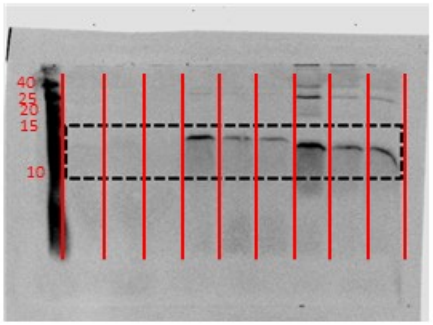
2

ED Figure 4g

PD Pulldown V5 staining (upper panel)

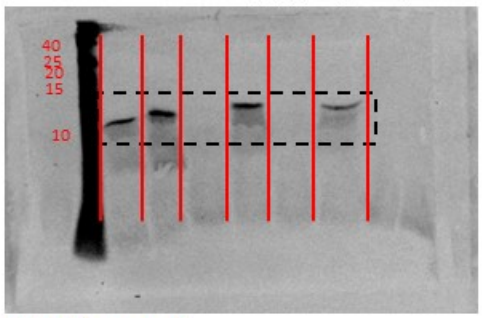


S Supernatant V5 staining (lower panel)

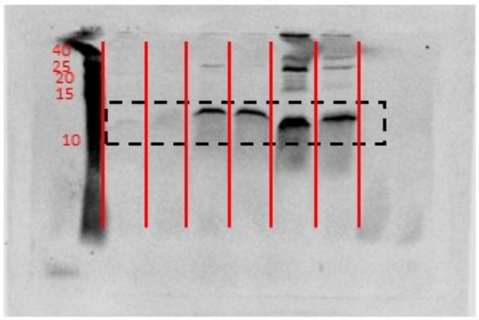


ED Figure 4h

PD Pulldown V5 staining (upper panel)

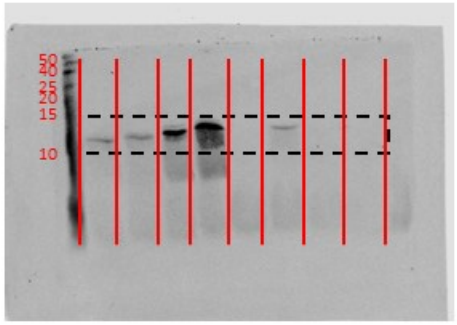


S Supernatant V5 staining (lower panel)

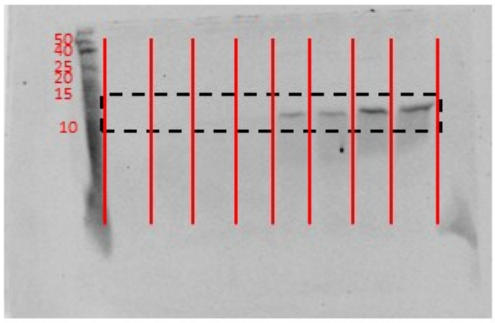


ED Figure 4i

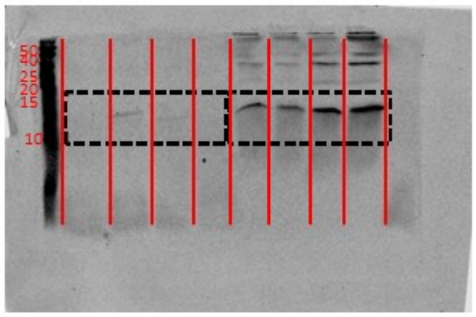
PD Pulldown V5 staining (upper left panel)



S Supernatant V5 staining (lower left panel)

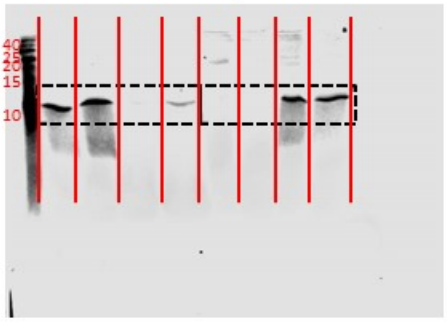


PD & S Pulldown and supernatant V5 stainings (right panels)



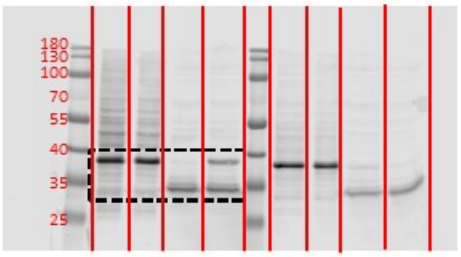
ED Figure 4k

PD & S Pulldown & Supernatant V5 staining (upper and lower panel)

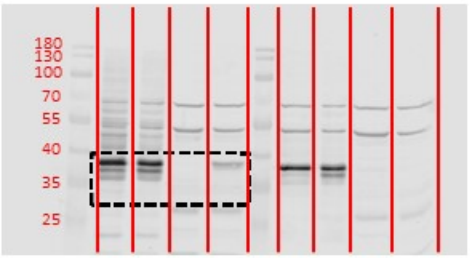


ED Figure 4n

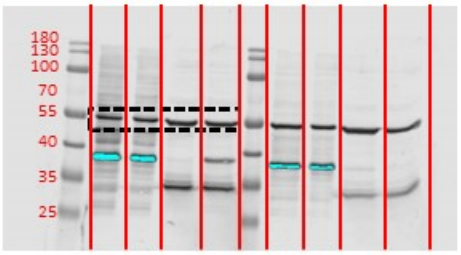
V5 staining (upper panel)



tGFP staining (middle panel, same blot as on the left)

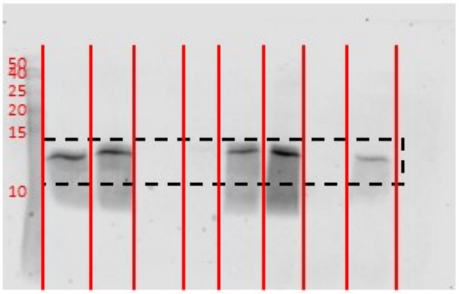


Tubulin staining (bottom panel, same blot as above)

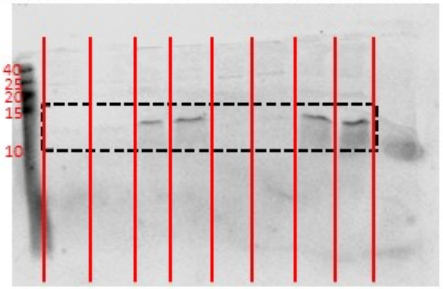


ED Figure 4o

PD Pulldown V5 staining (upper panel)

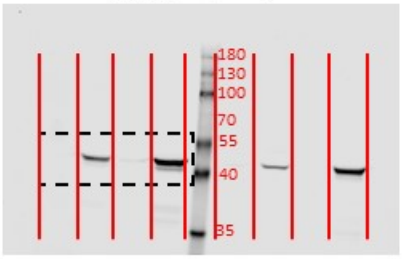


S Supernatant V5 staining (lower panel)

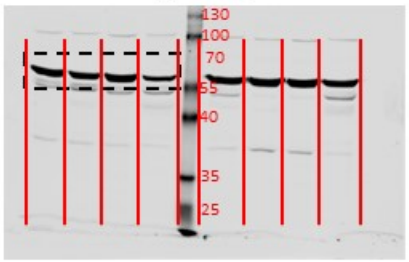


ED Figure 4p

IDO staining (upper panel)

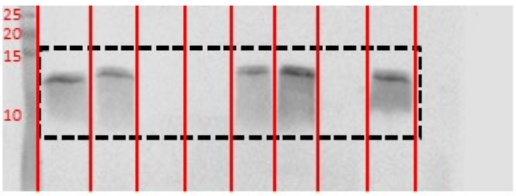


Tubulin staining (lower panel, same blot as on the left)

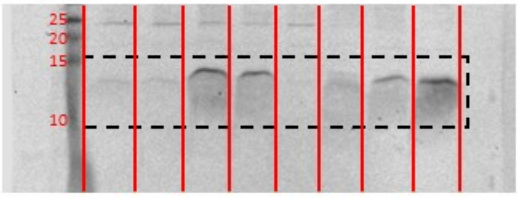


ED Figure 4q

PD Pulldown V5 staining (upper panel)

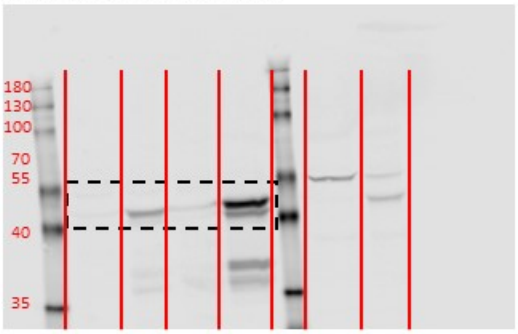


S Supernatant V5 staining (lower panel)

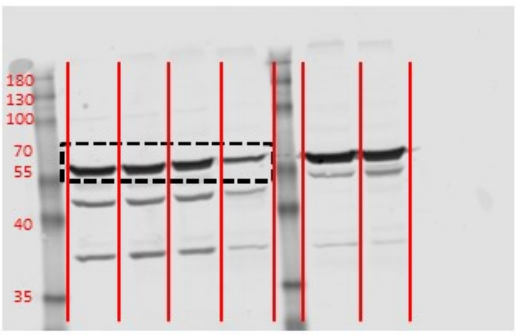


ED Figure 4r

IDO staining (upper panel)

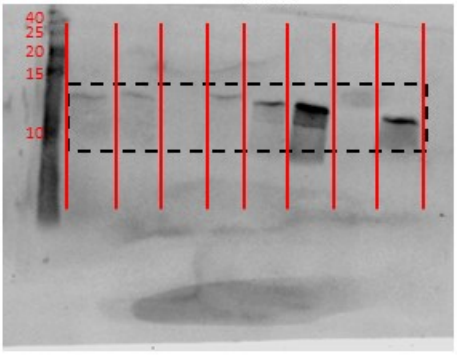


Tubulin staining (lower panel, same blot as on the left)

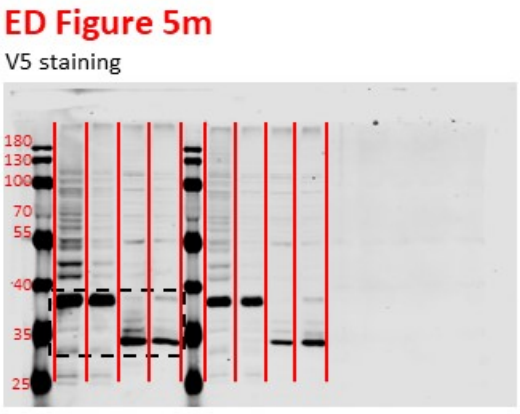


ED Figure 4t

PD Pulldown V5 staining (upper panel)



S Supernatant V5 staining (lower panel)



Supplementary Figure 4: Scans of full membranes of all western blots
With each figure it is indicated which part of the membrane was cropped to generated the indicated figures.

Supplementary Table 3

A.

Gene	Sequence	ENSG	Frame-shifted W	HLA	Strength	MQ score	PEP	Condition	Distance from tryptophan
DIP2B	QFLAEILQV	ENSG00000066084.13	13	HLA-A*02:05,HLA-C*06:02,HLA-C*07:02	WB,WB,WB	121.99	0.024982	IFN	-8
DBNDD2	SPLSSLSTL	ENSG00000244274.7	2	HLA-B*07:02,HLA-B*40:32,HLA-C*06:02,HLA-C*07:02	SB,WB,WB,WB	126.24	0.020676	IFN	8
PER1	SPTLSQCSL	ENSG00000179094.16	5	HLA-B*07:02	SB	96.492	0.063496	IFN	15
# ITGAL	TQVLSGIQV	ENSG00000005844.17	4	HLA-A*02:05,HLA-B*40:32	WB,WB	105.25	0.019415	IFN	-8
KCNK6	VPLSLAEHAL	ENSG00000099337.5	2	HLA-B*07:02	SB	110.63	0.035284	IFN	-4
FCGBP	APSGVAAGL	ENSG00000275395.6	30	HLA-B*07:02,HLA-B*40:32	SB,WB	121.14	0.025844	IFN,mets	6
# CSTF2	GVEASLAAL	ENSG00000101811.13	1	HLA-A*02:05,HLA-B*07:02	WB,WB	97.071	0.062349	IFN,mets	-1
ESPNL	LFLSHLEEI	ENSG00000144488.15	11	HLA-C*06:02,HLA-C*07:02	WB,WB	122.79	0.024172	IFN,mets	-5
* RPL7A	VAAAESHPL	ENSG00000280858.3	1	HLA-A*02:05,HLA-B*07:02,HLA-C*06:02	WB,WB,WB	114.02	0.033689	IFN,mets	7
# SGSM1	LPSLGSPVP	ENSG00000167037.18	6	HLA-B*07:02	WB	114.02	0.033689	IFN,mTRP	-4
STK25	SPALRTLTL	ENSG00000115694.15	4	HLA-B*07:02,HLA-B*40:32,HLA-C*06:02,HLA-C*07:02	SB,WB,WB,WB	96.331	0.086103	IFN,mTRP	9
GEMIN5	SPRGPPSSL	ENSG00000082516.9	1	HLA-B*07:02,HLA-B*40:32,HLA-C*06:02,HLA-C*07:02	SB,WB,WB,WB	120.15	0.026907	IFN,mTRP,mets	17
HSP90AB1	MVSPLAGVPK	ENSG00000096384.20	1	HLA-A*03:01	WB	105.57	0.044404	mTRP	15
ZNF513	VGQEGLVSL	ENSG00000163795.14	2	HLA-A*02:05,HLA-B*07:02,HLA-B*40:32,HLA-C*06:02,HLA-C*07:02	WB,WB,WB,WB,WB	85.265	0.092148	mTRP	14
TRAM1L1	TSLVNLSTL	ENSG00000174599.5	3	HLA-B*07:02,HLA-C*06:02,HLA-C*07:02	WB,WB,WB	121.62	0.025357	mTRP,mets	17
* SLC34A3	AGSPSGSIL	ENSG00000198569.9	10	HLA-B*07:02,HLA-B*40:32,HLA-C*06:02	WB,WB,WB	124.89	0.022049	mets	-1
# BCAT2	ALPEPHAAPSL	ENSG00000105552.15	2	HLA-A*02:05,HLA-B*40:32	WB,WB	85.064	0.064336	mets	8
# GBA	APASLKASA	ENSG00000262446.5	1	HLA-B*07:02	SB	104.45	0.020853	mets	6
# GALR3	APLPRALPP	ENSG00000128310.2	7	HLA-B*07:02	WB	84.359	0.094843	mets	38
PTPRM	KATEFISKL	ENSG00000173482.16	12	HLA-A*02:05,HLA-B*07:02,HLA-B*40:32,HLA-C*06:02,HLA-C*07:02	WB,WB,WB,WB,WB	113.74	0.034003	mets	9
# C1orf158	KLLGCQRSL	ENSG00000157330.10	5	HLA-A*02:05	WB	86.833	0.087487	mets	-3
* NLN	RPRVPQGIL	ENSG00000123213.23	2	HLA-B*07:02,HLA-B*40:32	SB,WB	94.262	0.06791	mets	18
# MAPK12	SPAAGGQGL	ENSG00000188130.14	4	HLA-B*07:02,HLA-B*40:32,HLA-C*07:02	SB,WB,WB	118.05	0.029234	mets	15
RBM26	TEIEAGLEA	ENSG00000139746.15	2	HLA-B*40:32	SB	134.06	0.013826	mets	10
VMO1	TLETGVTIA	ENSG00000182853.12	7	HLA-A*02:05	WB	86.866	0.087388	mets	3
HARS	VDKLDKVSL	ENSG00000170445.15	1	HLA-B*07:02,HLA-B*40:32	WB,WB	101.33	0.053923	mets	-8
PCNX2	VLGRGATTAL	ENSG00000135749.19	19	HLA-A*02:05,HLA-B*07:02	WB,WB	126.67	0.024627	mets	-4
ROBO3	VSPAPSLSL	ENSG00000154134.15	14	HLA-A*02:05,HLA-B*07:02,HLA-C*06:02,HLA-C*07:02	WB,WB,WB,WB	86.866	0.046243	mets	4

.

Table includes Ensemble transcript ID; W number found in the vicinity of the frame-shift; the peptide distance (aa) from the frameshifted W (=0); NetNHCpan allotype prediction (SB= strong binder, WB= weak binder); Maxquant (MQ) Score and Maxquant Posterior Error Probability (PEP); and the samples in which the peptides were found. * mark peptides that were detected also in the immunopeptidomics analysis of patient 42. # mark peptides whose comparison with the synthetic peptide yielded a correlation score below 0.7.

B.

Sequence	55A3.I	55A3.I.B	55A7.I	55B3.I	IFN1	IFN2	IFN3	IFN4	mTRP1	mTRP2	mTRP3	mTRP4	NT1	NT2	NT3	NT4	55A3.I	55A3.I.B	55A7.I	55B3.I	IFN1	IFN2	IFN3	IFN4	mTRP1	mTRP2	mTRP3	mTRP4	NT1	NT2	NT3	NT4
AGSPSGSIL				1																By MS/MS												
ALPEPHAAPSL			1																By MS/MS													
APASLKASA				1																By MS/MS												
APLPRALPP	1	1	1														By MS/MS	By matching	By MS/MS													
APSGVAAGL				1		1														By MS/MS		By MS/MS										
GVEASLAAL	1				1												By MS/MS				By MS/MS											
KATEFISKL	1		1														By MS/MS		By matching													
KLLGCQRSL	1	1		1													By MS/MS	By matching		By MS/MS												
LFLSHLEEI	1	1				1											By MS/MS	By matching				By matching										
LPSLGSPVP					1	1			1	1											By matching	By MS/MS			By MS/MS	By matching						
MVSPLAGVPK									1	1	1	1													By MS/MS	By MS/MS	By matching	By MS/MS				
QFLAEILQV						1																By MS/MS										
RPRVPQGIL	1	1	1														By MS/MS	By matching	By MS/MS													
SPAAGGQGL				1																By MS/MS												
SPALRTLTL							1		1	1												By MS/MS				By matching	By matching					
SPLSSLSTL								1															By MS/MS									
SPRGPPSSL	1		1	1	1	1			1	1		1					By MS/MS		By matching	By matching	By MS/MS	By matching		By MS/MS	By matching	By MS/MS		By matching				
SPTLSQCSL								1																By MS/MS								
TEIEAGLEA				1																By MS/MS												
TLETGVTIA				1																By MS/MS												
TQVLSGIQV						1																By MS/MS										
TSLVNLSTL	1									1							By MS/MS															
VAAAESHPL				1	1	1														By matching	By MS/MS	By MS/MS										
VDKLDKVSL	1																By MS/MS															
VGQEGLVSL									1																By MS/MS							
VLGRGATTAL			1	1															By MS/MS	By MS/MS												
VPLSLAEHAL						1																By MS/MS										
VSPAPSLSL				1																By MS/MS												

Table summarizing the specific samples in which each peptide was detected, and whether it was identified by MS/MS or by matching (see methods).