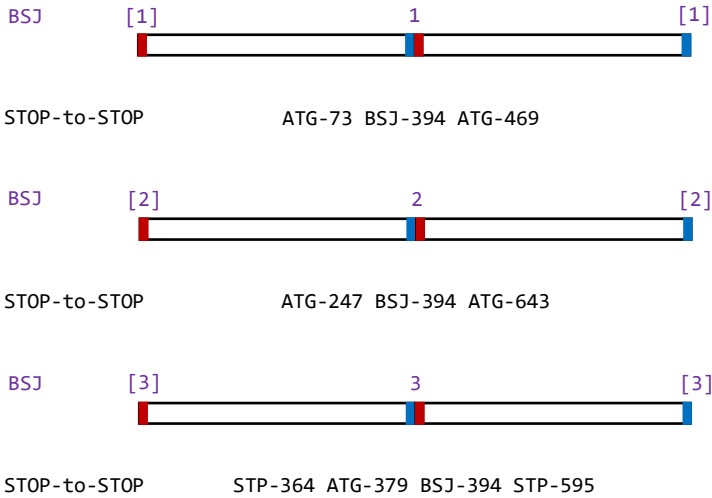
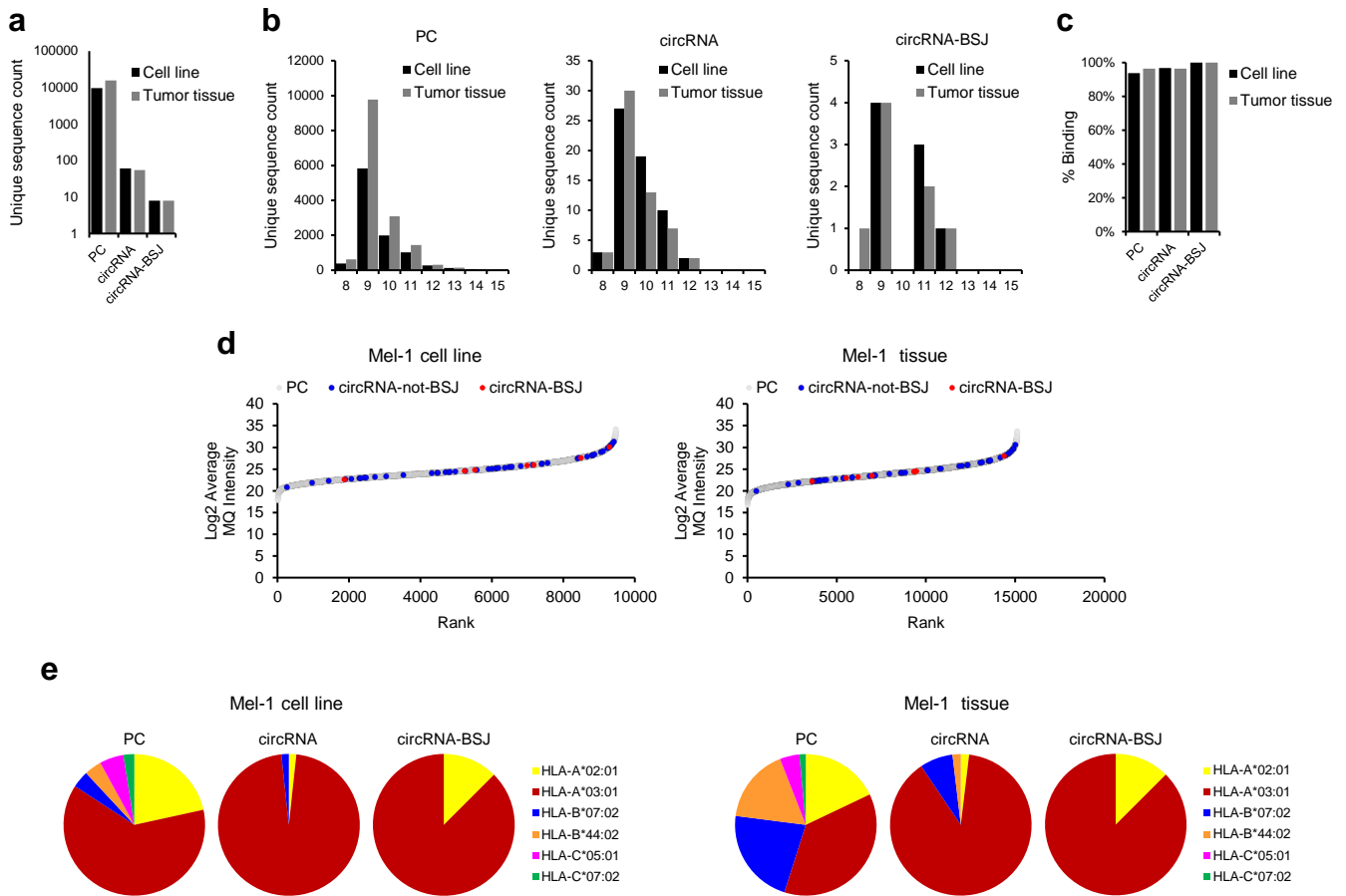


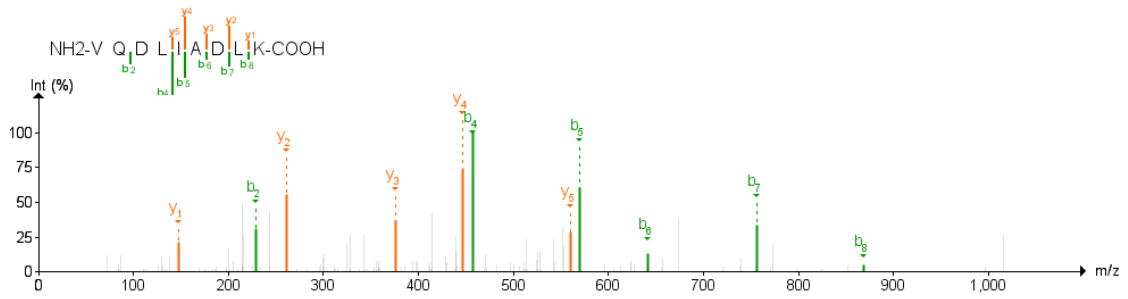
**a****b**

**Supplementary Figure 1. Method for the in silico generation and translation of BSJ-containing fragments in circRNAs.** (a) Four copies of a given circRNA sequence were concatenated into a single sequence (“4x circRNA”) and BSJs located within flanking STOP codons. In this example, three stop-to-stop fragments contain a single BSJ translated in one of the three reading frames. (b) If the circRNA sequence contains an integral number of codons, only two concatenated circRNA sequences are required since translation through the BSJ does not produce a translation frame change. Open boxes represent the circRNA sequence; blue and red boxes represent the 3'- and 5'-nucleotides contributing to the BSJ; numbers above the sequence boxes indicate sequential BSJs through the concatenated circRNA construct (partial BSJs are in square brackets).

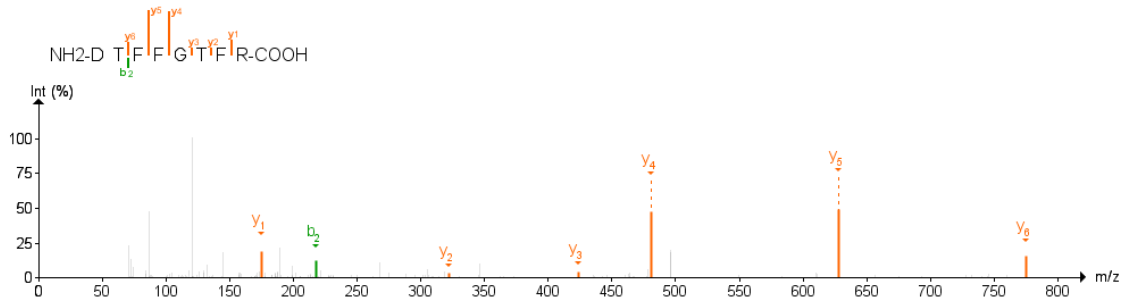


**Supplementary Figure 2. Overview of the immunopeptidomics analysis in Mel-1 patient.** (a) Total number of peptide sequences, (b) peptide length distribution and (c) percentage of peptides predicted as HLA binders (% rank < 2), in Mel-1 cell line and tumor tissues, in the PC, circRNA, and circRNA-BSJ groups. (d) Rank plots of PC, circRNA-not-BSJ and circRNA-BSJ derived peptide intensities in Mel-1 cell line and tumor tissues. (e) Predicted HLA restriction of PC, circRNA-not-BSJ and circRNA-BSJ derived peptides in cell line and tumor tissues from Mel-1 patient. Source data are provided as a Source Data file.

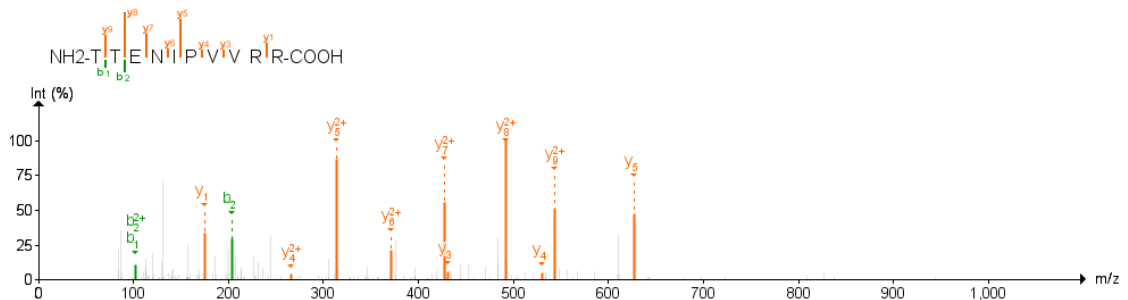
Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
ANXA6	hsa_circ_0074614_0	TRUE	VQDLIADLK	9	no_binder	VVLQGTREEDDVSEDLVQQD->v[QD]LIADLK<-YELTKKFERLIVGLMRP	T1185B	Cell line



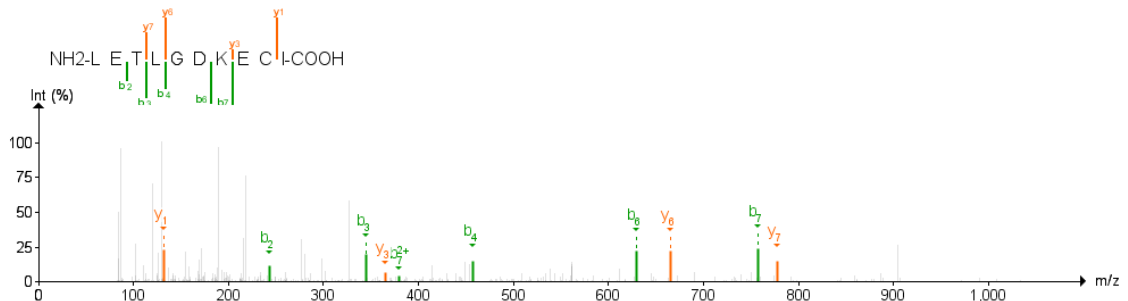
Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
CBL1	hsa_circ_0081903_0	FALSE	DTFFGTFR	8	HLA-A*68:01	GMTVKGVSCLQISEDFL->DTFFGTFR<-RI	T1185B	Cell line



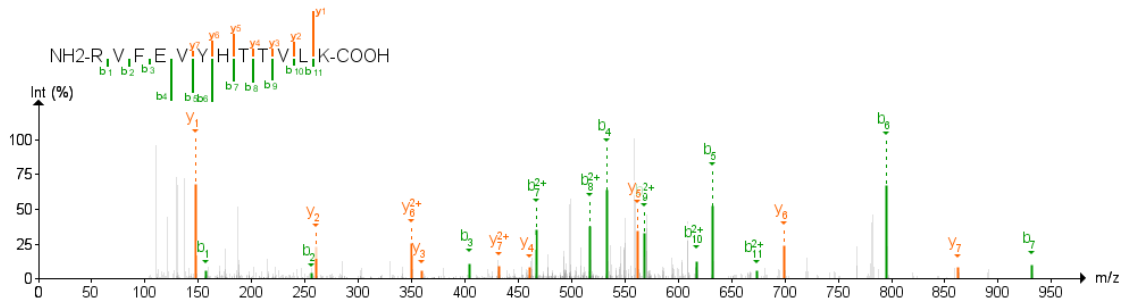
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CDC73	hsa_circ_0111569_0	TRUE	TTENIPVRR	10	HLA-A*68:01	KEETEGFKIDTMGTYHGMLKSV->[TT]ENIPVRR<-PDRKDLGLYNGEAS	T1185B	Cell line



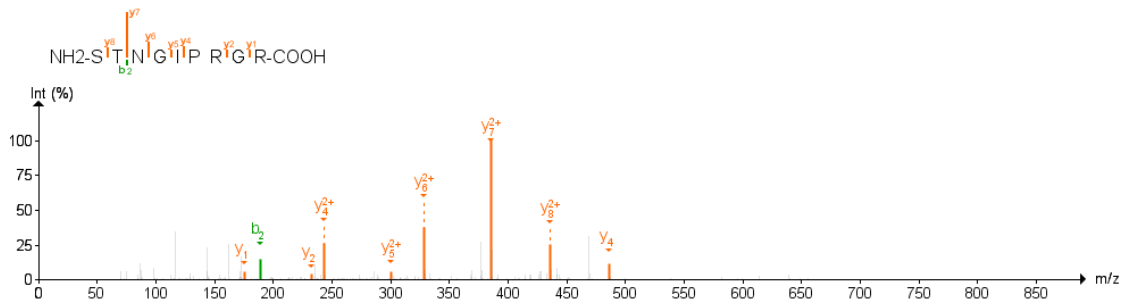
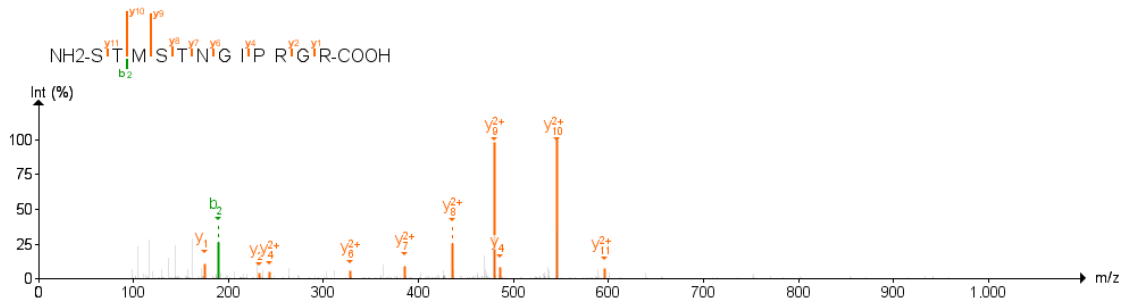
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CLASP2	hsa_circ_0123550_2	FALSE	LETLGDKECI	10	no_binder	SFSVWDEHFKTILLLL->LETLGDK[EC]I<-	T1185B	Cell line



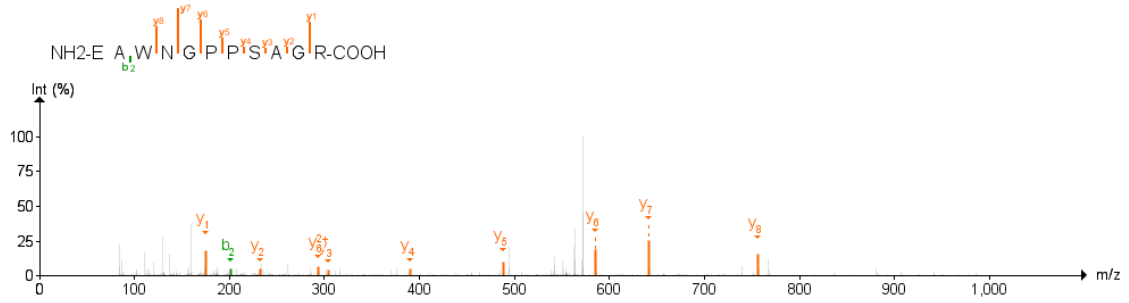
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CTNNB1	hsa_circ_0003137_1	FALSE	RVFEVYHTTVLK	12	HLA-A*68:01	MATKKA->[R]VFEVYHTTVLK<-IQRGQWLLKLI	T1185B	Cell line
	hsa_circ_0004030_1	FALSE				SWMGCLOVTAISWPGLLTCKSSF->[R]VFEVYHTTVLK<-IQRGQWLLKLI		



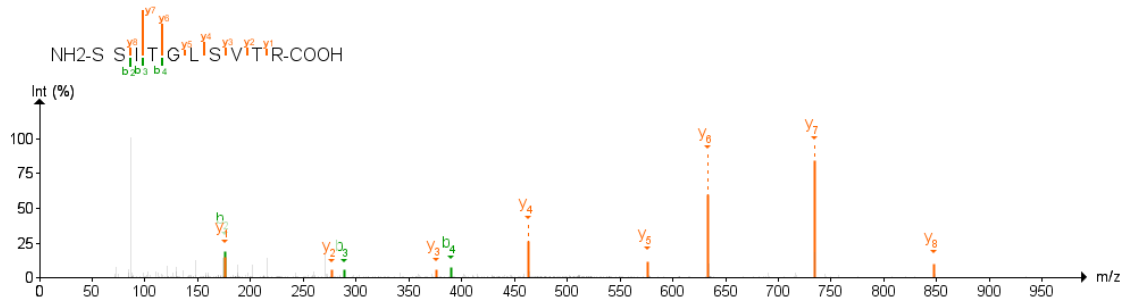
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CTSB	hsa_circ_0083357_1	FALSE	STMSTNGIPRGR	9	HLA-A*68:01	GAGPLSIPCRMSW->STMSTNGIPRGR<-GRGREGT	T1185B	Cell line
			STNGIPRGR			GAGPLSIPCRMSWSTM->STNGIPRGR<-GRGREGT		



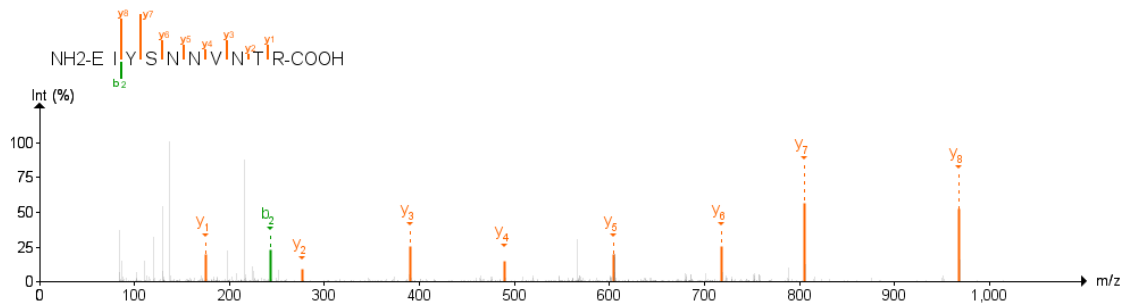
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GAS7	hsa_circ_0042080_0	FALSE	EAWNGPPSAGR	11	HLA-A*68:01	WERPSSSPGIPASPGSHRSSLPPT->[E]AWNGPPSAGR<-RKPDPGHPSTWLAEL	T1185B	Cell line



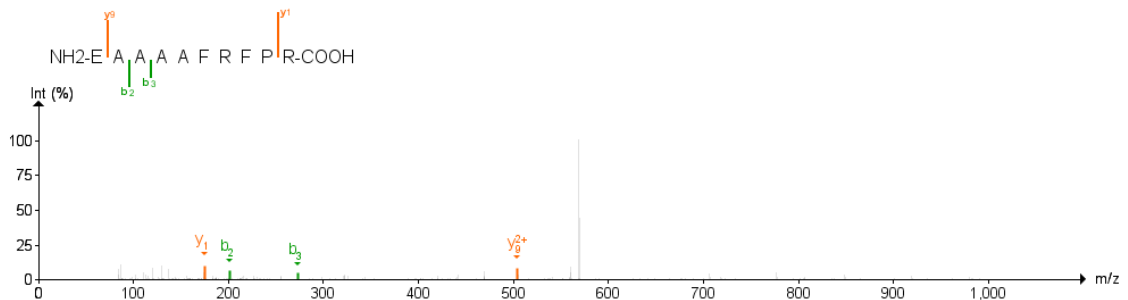
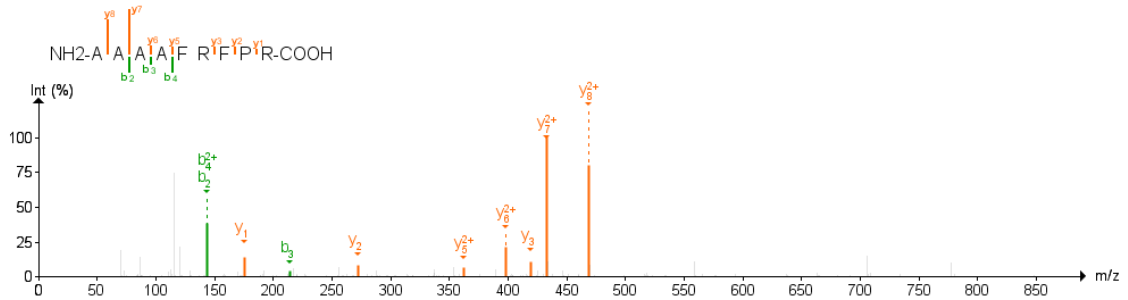
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KIAA0226	hsa_circ_0068719_1	FALSE	SSITGLSVTR	10	HLA-A*68:01	MVAWSGFAGTCRA->SSITGLSVT[R]<-REHWQLLGNLKTVEGLVSTNSPN	T1185B	Cell line



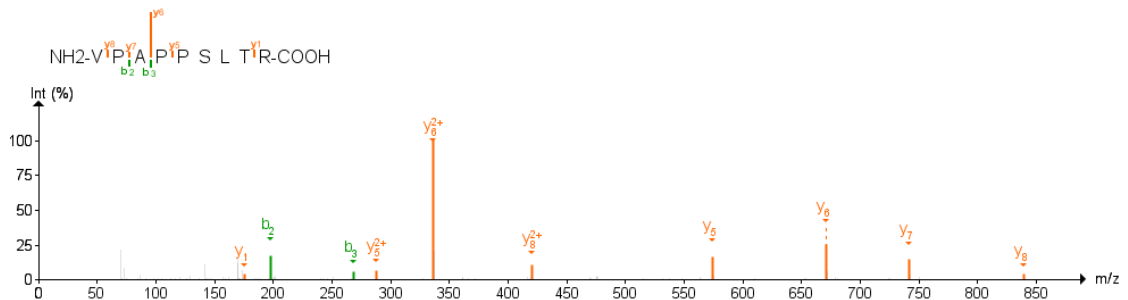
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LIN9	hsa_circ_0016709_0	FALSE	EIYSNNVNTR	10	HLA-A*68:01	ADV SQFKDLPDEIPLVLVIGTKVT->[E]IYSNNVNTR<-	T1185B	Cell line



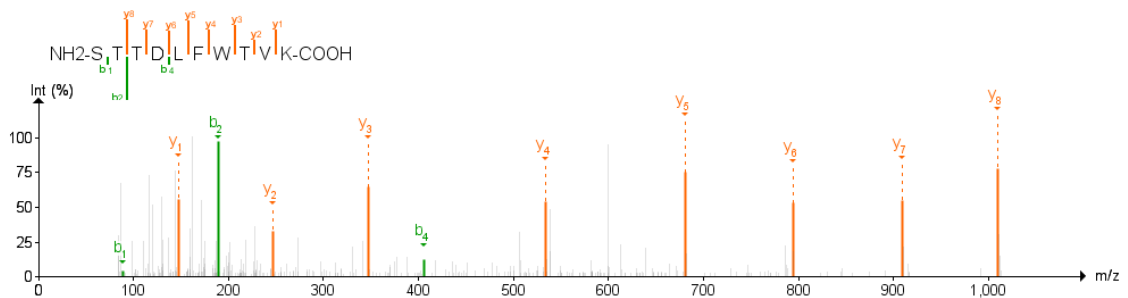
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LINC00221	hsa_circ_0101415_1	FALSE	AAAAFRFP	9	HLA-A*68:01	MPARSWLGE->AAAAFRFP[R]<-DFCVCAHIDVPTLCPSQ	T1185B	Cell line
			EAAAAFRFP	10	HLA-A*68:01	MPARSWLG->EAAAAFRFP[R]<-DFCVCAHIDVPTLCPSQ		
	hsa_circ_0101416_0	FALSE	AAAAFRFP	9	HLA-A*68:01	MPARSWLGE->AAAAFRFP[R]<-M		
			EAAAAFRFP	10	HLA-A*68:01	MPARSWLG->EAAAAFRFP[R]<-M		



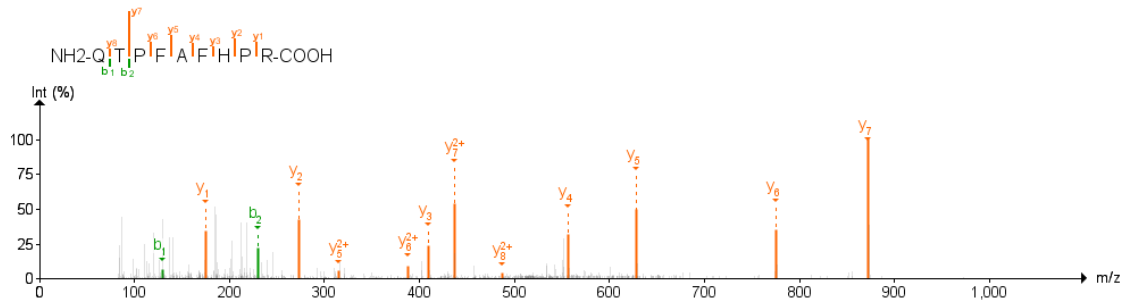
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NOD1	hsa_circ_0079703_1	FALSE	VPAPPSLTR	9	HLA-A*68:01	MTTSRPKMRRLC->VPAPPSLT[R]<-GCAE	T1185B	Cell line



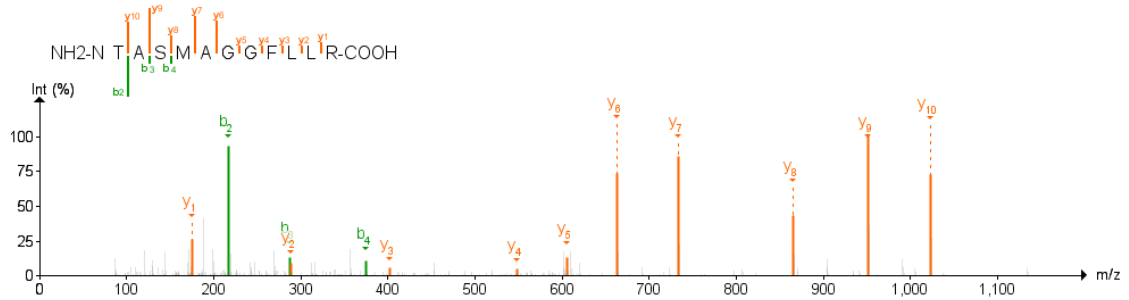
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PAFAH1B2	hsa_circ_0095083_1	FALSE	STTDLFWTVK	10	HLA-A*68:01	MENWRILSL->[S]TTLDFWTVK<-TKSLMYCSWETPWCS	T1185B	Cell line



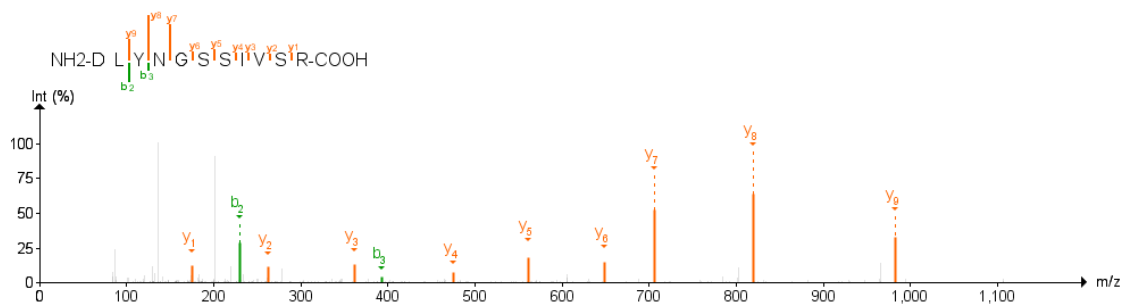
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PKNOX1	hsa_circ_0061852_2	FALSE	QTPFAFHPR	9	HLA-A*68:01	VVT <b>PQ</b> QVVTQTLS <b>P</b> GTIRIQNS-> <b>[QT]</b> PF <b>A</b> FHPR<-	T1185B	Cell line
	hsa_circ_0116109_2	FALSE				KTKM <b>N</b> SETLLSGEPGSPYSPVQS-> <b>[QT]</b> PF <b>A</b> FHPR<-		
	hsa_circ_0116112_2	FALSE				TPV <b>M</b> NVDSLQSLSSDGATLAVQ-> <b>[QT]</b> PF <b>A</b> FHPR<-		



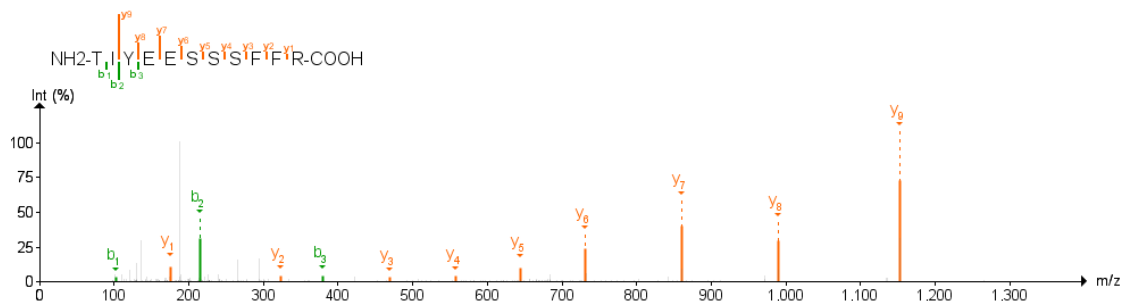
Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
PTPRG	hsa_circ_0066402_0	FALSE	NTASMAGGFLR	12	HLA-A*68:01	MAQRAL->NTASMAGGFL <b>[R]</b> <-HVTSC <b>M</b> EAWPVSSPALFVPSQ <b>P</b> A <b>P</b>	T1185B	Cell line
	hsa_circ_0066406_0	FALSE				MAQRAL->NTASMAGGFL <b>[R]</b> <-CL <b>W</b> S		
	hsa_circ_0124401_0	FALSE				MAQRAL->NTASMAGGFL <b>[R]</b> <-Y <b>R</b> TCSD		



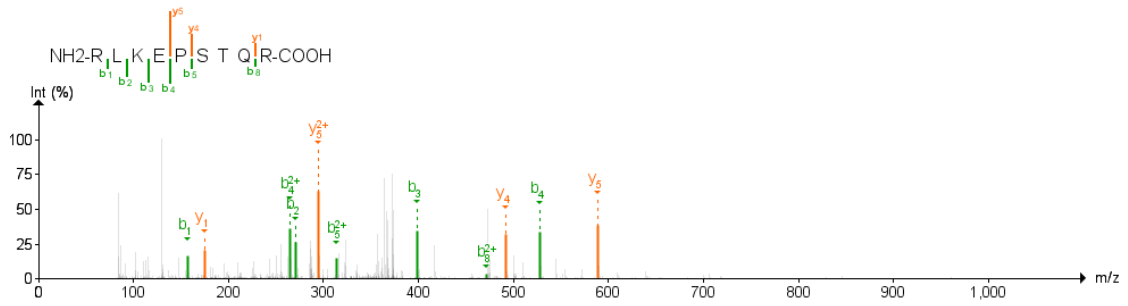
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RFWD2/ COP1	hsa_circ_0015364_0	FALSE	DLYNGSSIVSR	11	HLA-A*68:01	YNSVRPLATLSYAS->DLYNGSSIV <b>[R]</b> <-QR <b>N</b> SLGI <b>A</b> R	T1185B	Cell line
	hsa_circ_0015366_0	FALSE				YNSVRPLATLSYAS->DLYNGSSIV <b>[R]</b> <-NH <b>M</b> QPNYRFLWNSSRLQ <b>E</b> IRE <b>S</b> N		
	hsa_circ_0111261_0	FALSE				YNSVRPLATLSYAS->DLYNGSSIV <b>[R]</b> <-K		
	hsa_circ_0111262_1	FALSE				YNSVRPLATLSYAS->DLYNGSSIV <b>[R]</b> <-T <b>S</b> G <b>T</b> MMN <b>R</b> SGD <b>S</b> G <b>A</b> S		



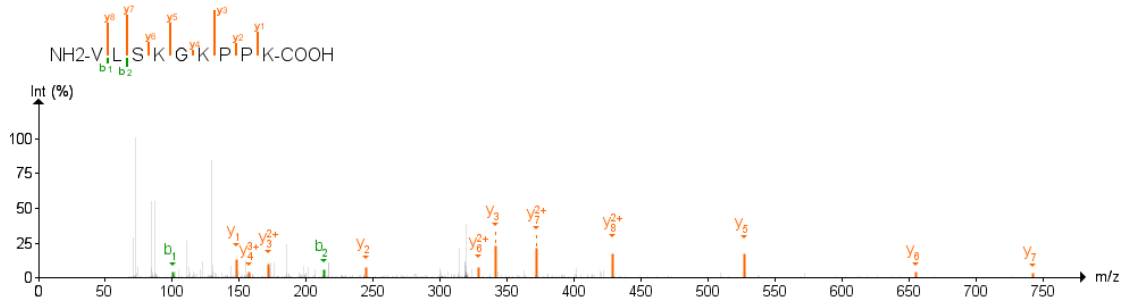
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STAT3	hsa_circ_0043818_1	TRUE	TIYE <b>S</b> SSFFR	11	HLA-A*68:01	SIA <b>A</b> CKSR <b>M</b> FSIS->TIYE <b>S</b> SSFF <b>[R]</b> <-V <b>S</b> G <b>A</b> AA <b>A</b> QTGRG <b>S</b> R <b>G</b> FR <b>R</b> RS <b>R</b> G <b>N</b> K	T1185B	Cell line
	hsa_circ_0106859_1	FALSE				SIA <b>A</b> CKSR <b>M</b> FSIS->TIYE <b>S</b> SSFF <b>[R]</b> <-M <b>A</b> Q <b>W</b> N <b>L</b> Q <b>L</b> D <b>R</b> Y <b>L</b> E <b>Q</b> L <b>H</b> Y <b>S</b> D		



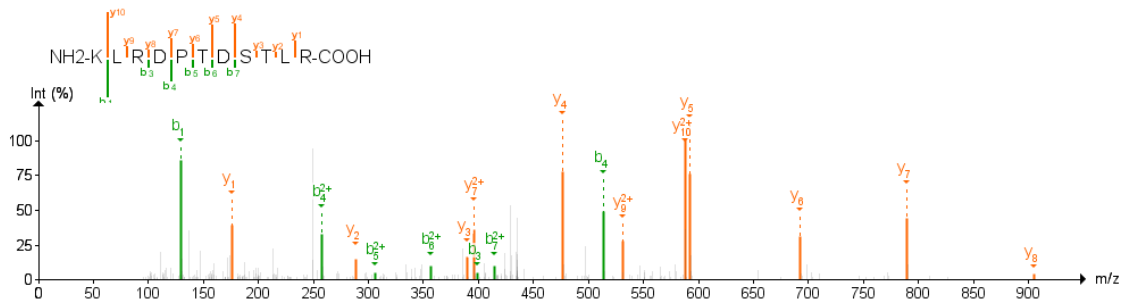
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ABCA2	hsa_circ_0089621_2	FALSE	RLKEPSTQR	9	HLA-A*03:01	ISWYVYVAMTIQHIVAKEKH->RLK[EP]STQR<-RP	Mel-1	Tumor



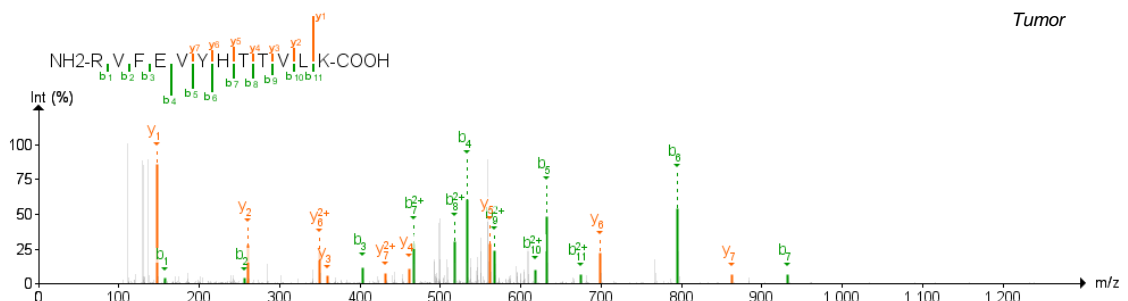
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CCAR1	hsa_circ_0018553_1	FALSE	VLSKGGPKK	9	HLA-A*03:01	ISAASITPLLOTQPGPLLQQPQK->[V]LSKGGPKK<-	Mel-1	Cell line



Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
CEP57L1	hsa_circ_0130265_1	FALSE	KLRDPTDSTLR	11	HLA-A*03:01	MGLSSCKN->KLRDPTDSTL[R]<-AWYLVQV	Mel-1	Cell line



Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
CTNNB1	hsa_circ_0003137_1	FALSE	RVFEVYHTTVLK	12	HLA-A*03:01	MATKKA->[R]VFEVYHTTVLK<-IQRGWLLKLI	Mel-1	Cell line + Tumor
	hsa_circ_0004030_1	FALSE				SWMGCLOVTAISWPGILTKSSF->[R]VFEVYHTTVLK<-IQRGWLLKLI		

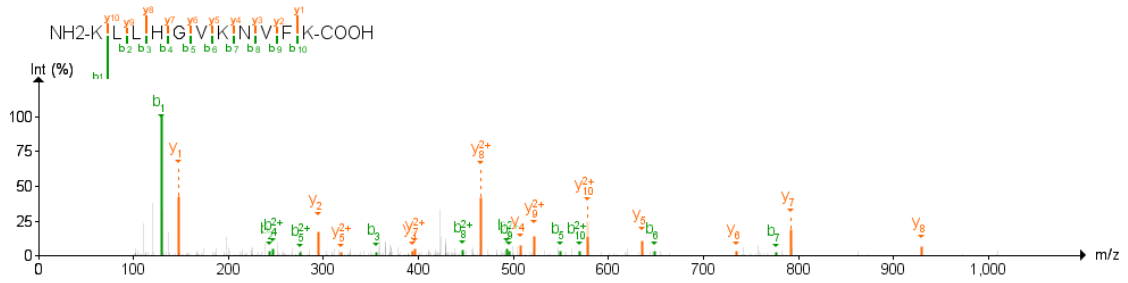


Tumor

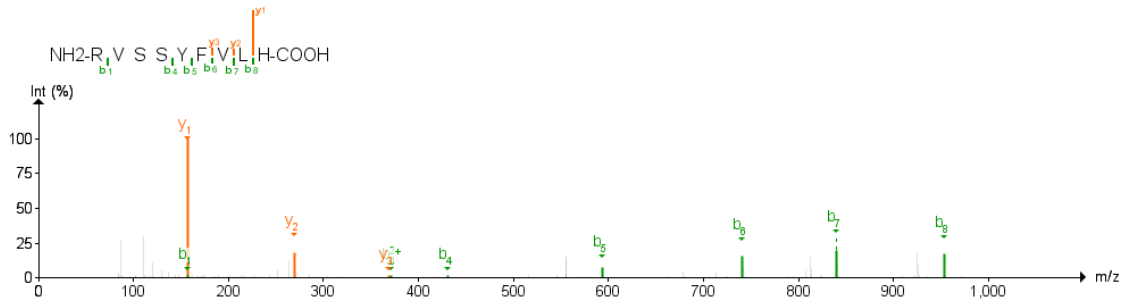


Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
MALAT1	hsa_circ_0096124_2	FALSE	KLLHGKKNVFK	11	HLA-A*03:01	M->IKLJLHGKKNVFK<-RKLRERTTEPRINT	Mel-1	Cell line + Tumor

Cell line

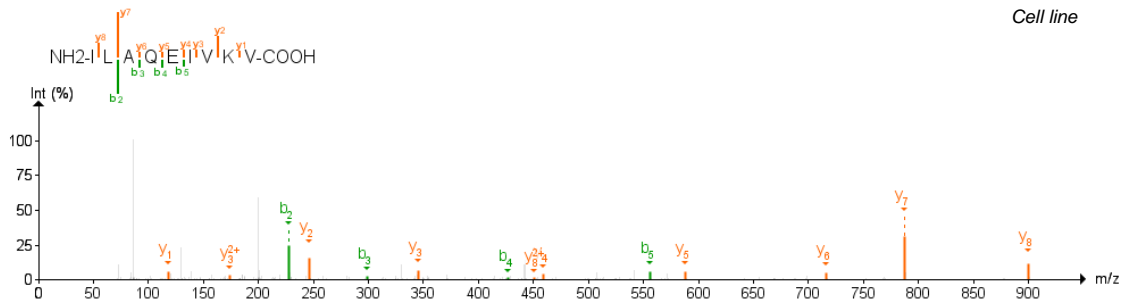


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PDIA3	hsa_circ_0035034_2	FALSE	RVSSYFVLH	9	HLA-A*03:01	LLERLLLLSELLKERSLSCRSS->[RVSSYFVLH<-]SLTSLRTRLWHIQSK	Mel-1	Cell line



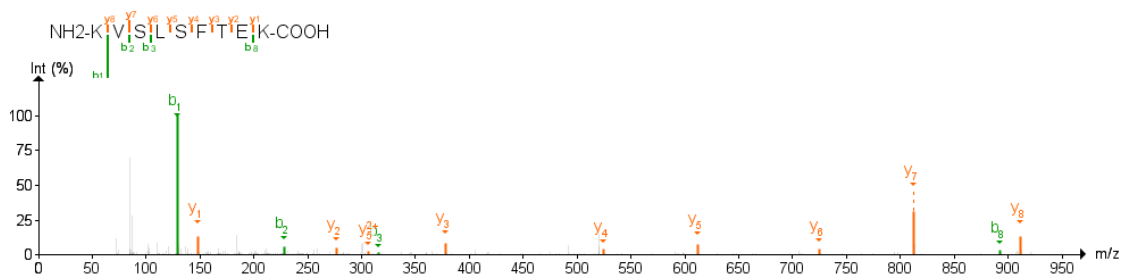
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SAE1	hsa_circ_0051662_2	FALSE	ILAQEIVKV	9	HLA-A*02:01	MAPVCAVVG->ILAQEIV[KVI<-]LLLRDGPVSCGGWRDFGTGNCEG	Mel-1	Cell line + Tumor

Cell line



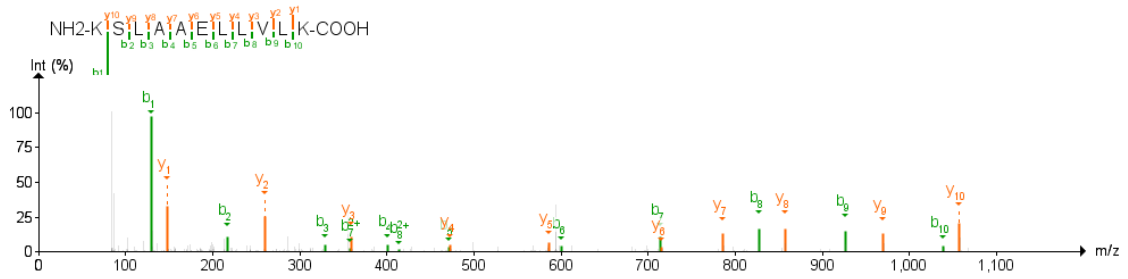
Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
PPP2R3A	hsa_circ_0122039_2	FALSE	KVLSFTEK	9	HLA-A*03:01	SPVGDKAKDTTSAVLIQQTPEVI->[KV]LSFTEK<-	Mel-1	Cell line + Tumor

Tumor

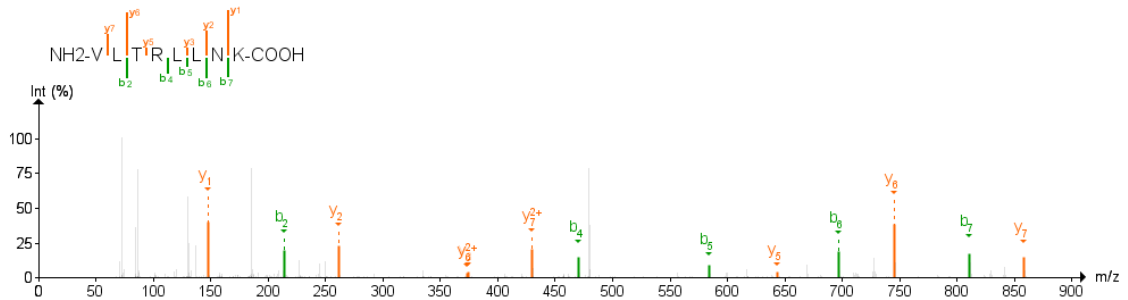


Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
TOPBP1	hsa_circ_0121989_1	FALSE	KSLAAELLVK	11	HLA-A*03:01	MYTPHCART->K[ <b>S</b> ]LAAELLVK<-	Mel-1	Cell line + Tumor

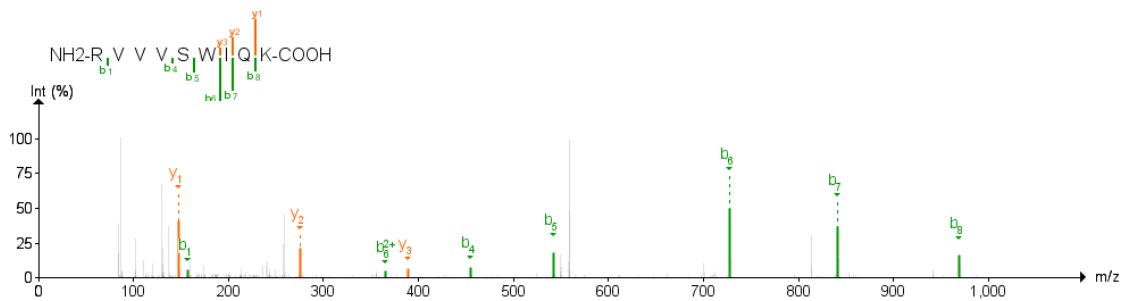
Tumor



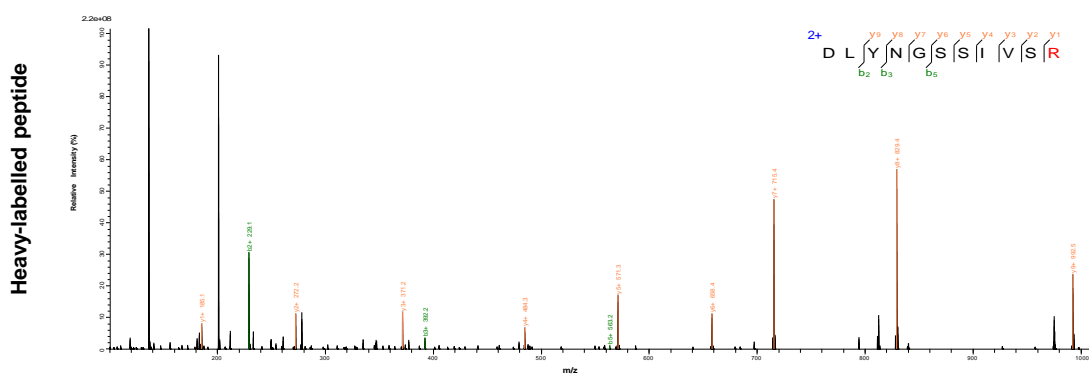
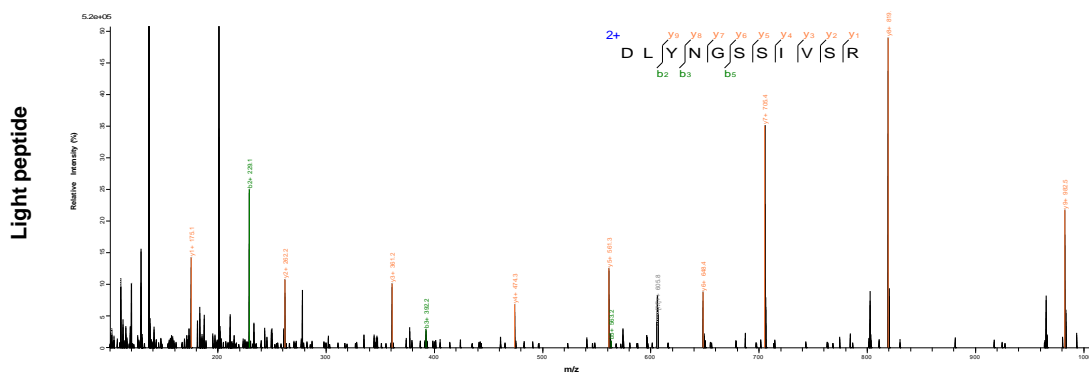
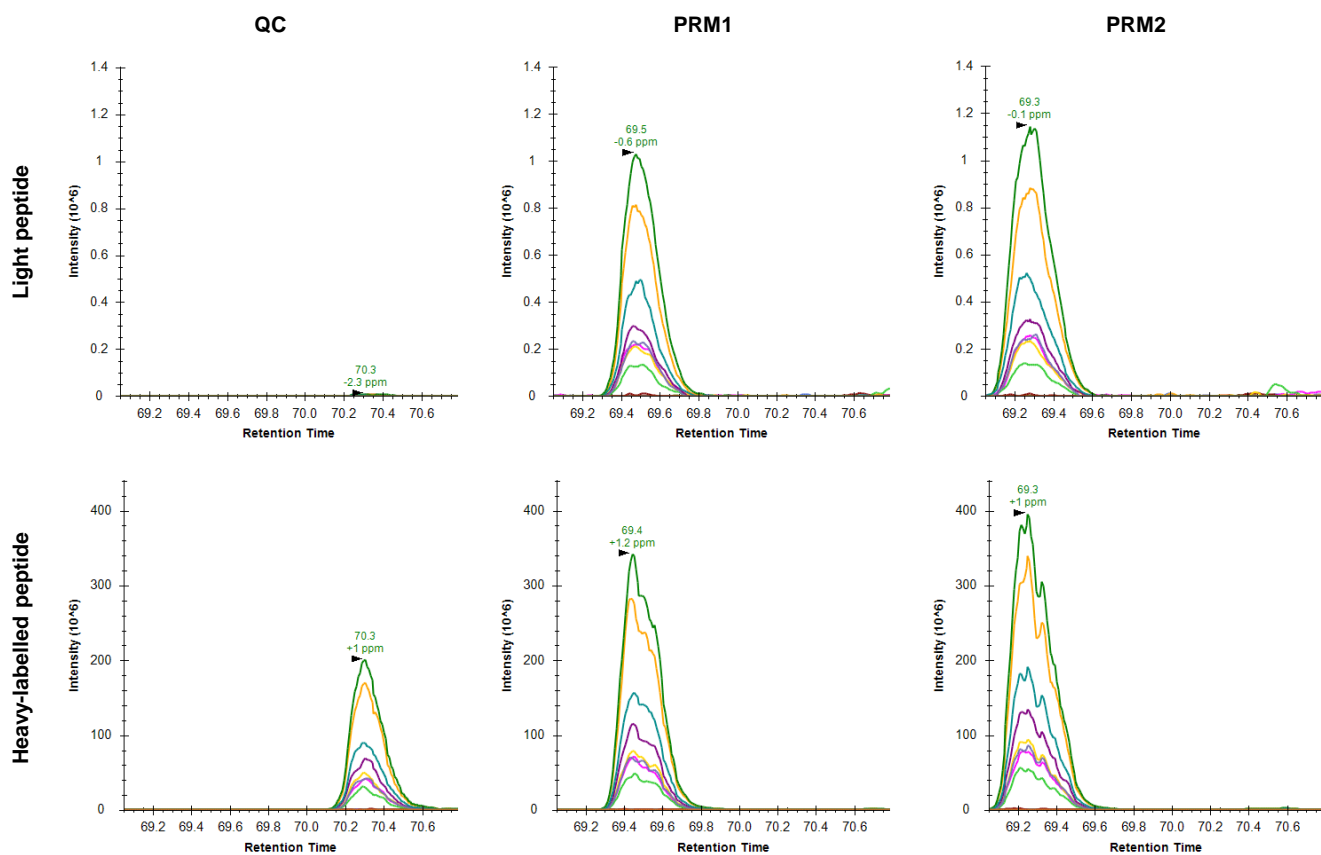
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WARS	hsa_circ_0033185_2	FALSE	VLTRLLNK	8	HLA-A*03:01	MPRTSSPVALTSTRLSYSLTWTW->[ <b>V</b> ]LTRLLNK<-SSDLRPAVNVVPER	Mel-1	Tumor
	hsa_circ_0033189_1	FALSE				SDGESAALESARPAPLLLLPVCM->[ <b>V</b> ]LTRLLNK<-SSDLRPAVNVVPER		



Gene ID	circRNAs_frame	Infinite translation candidate	Peptide Sequence	Length (AA)	NetMHCpan - 4.1 Binder/Best Allele	BSJ context (3'Exon -> Peptide [BSJ] <- 5'Exon)	Patient ID	Detection
ZMYM4	hsa_circ_0113154_2	FALSE	RVVSWIQK	9	HLA-A*03:01	DKAANQVEETLHTLHPQTPETNF->[ <b>R</b> ]V[V]VSWIQK<-CLKI	Mel-1	Tumor



**Supplementary Figure 3. Spectrum visualization of the MS identified circRNA-BSJ derived peptides.** MS/MS spectra of peptides identified in T1185B and Mel-1, encoded in circRNAs from different host genes. Sample source is indicated for spectrums from peptides detected in both cell line and tissue from Mel-1 patient.

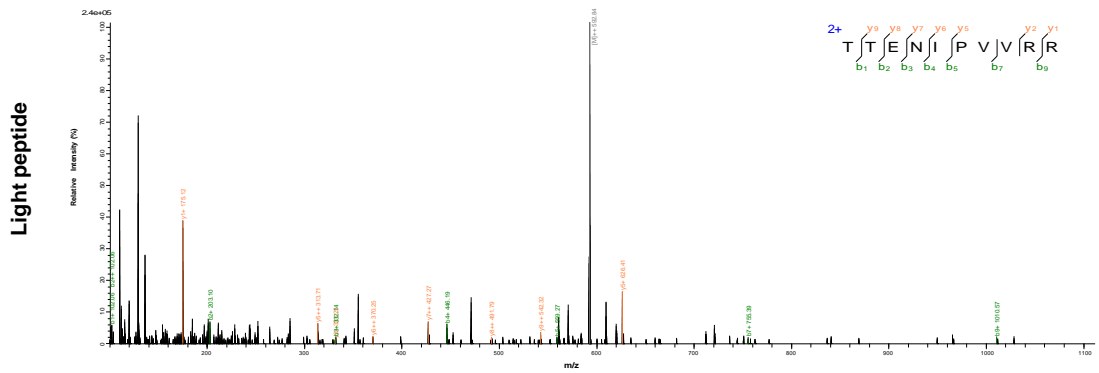
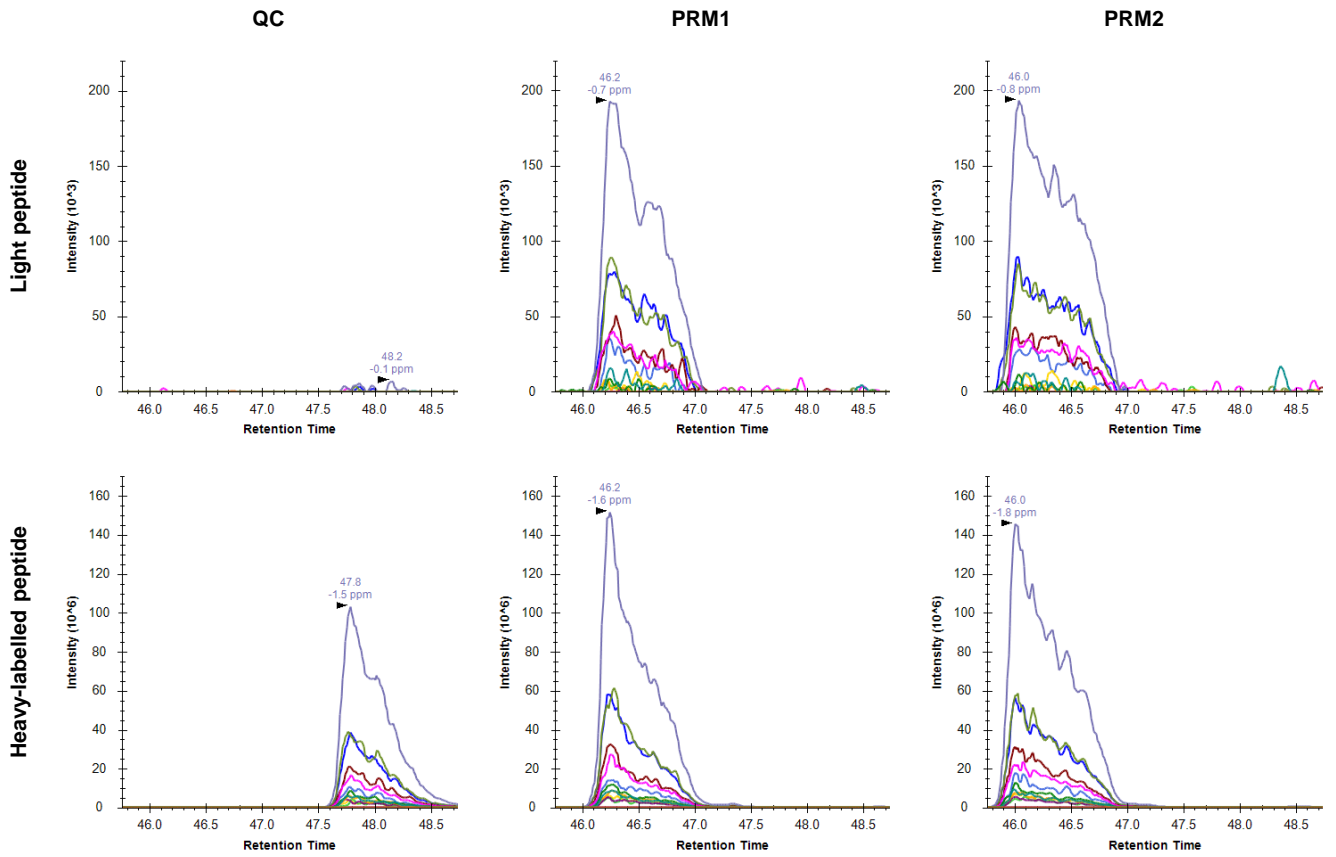
**a****COP1 (RFWD2)/T1185B****Peptide: DLYNGSSIVSR****Precursor charge: ++**

**b**

CDC73/T1185B

Peptide: TTENIPVRR

Precursor charge: ++

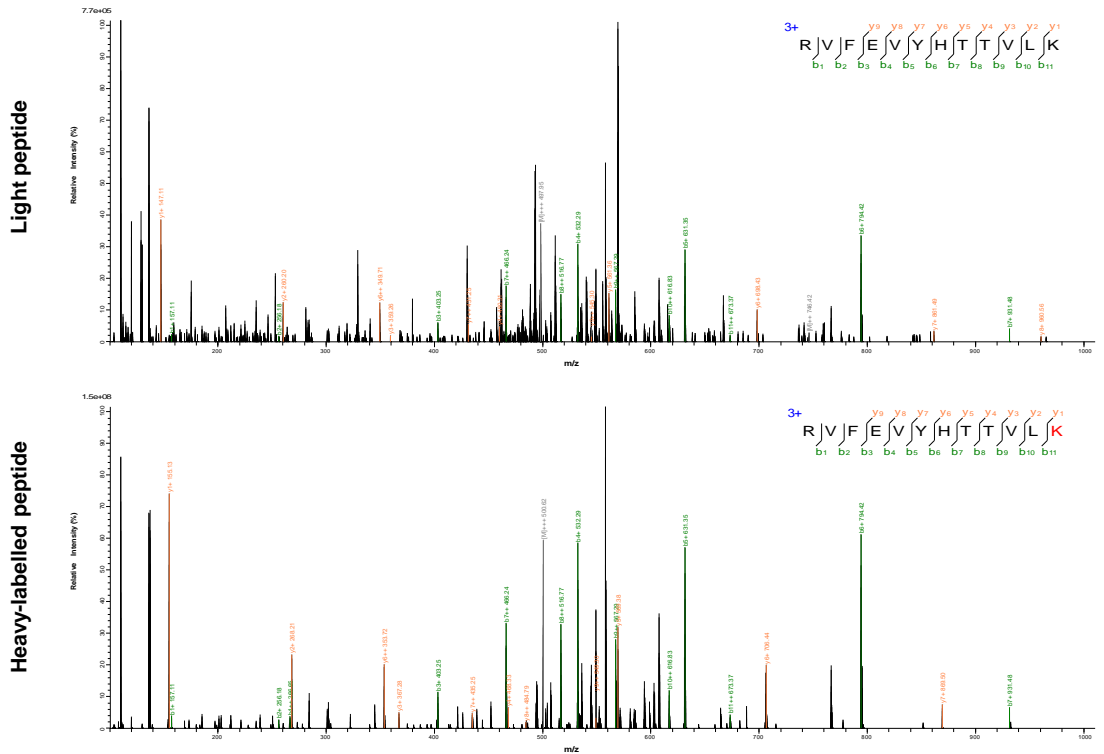
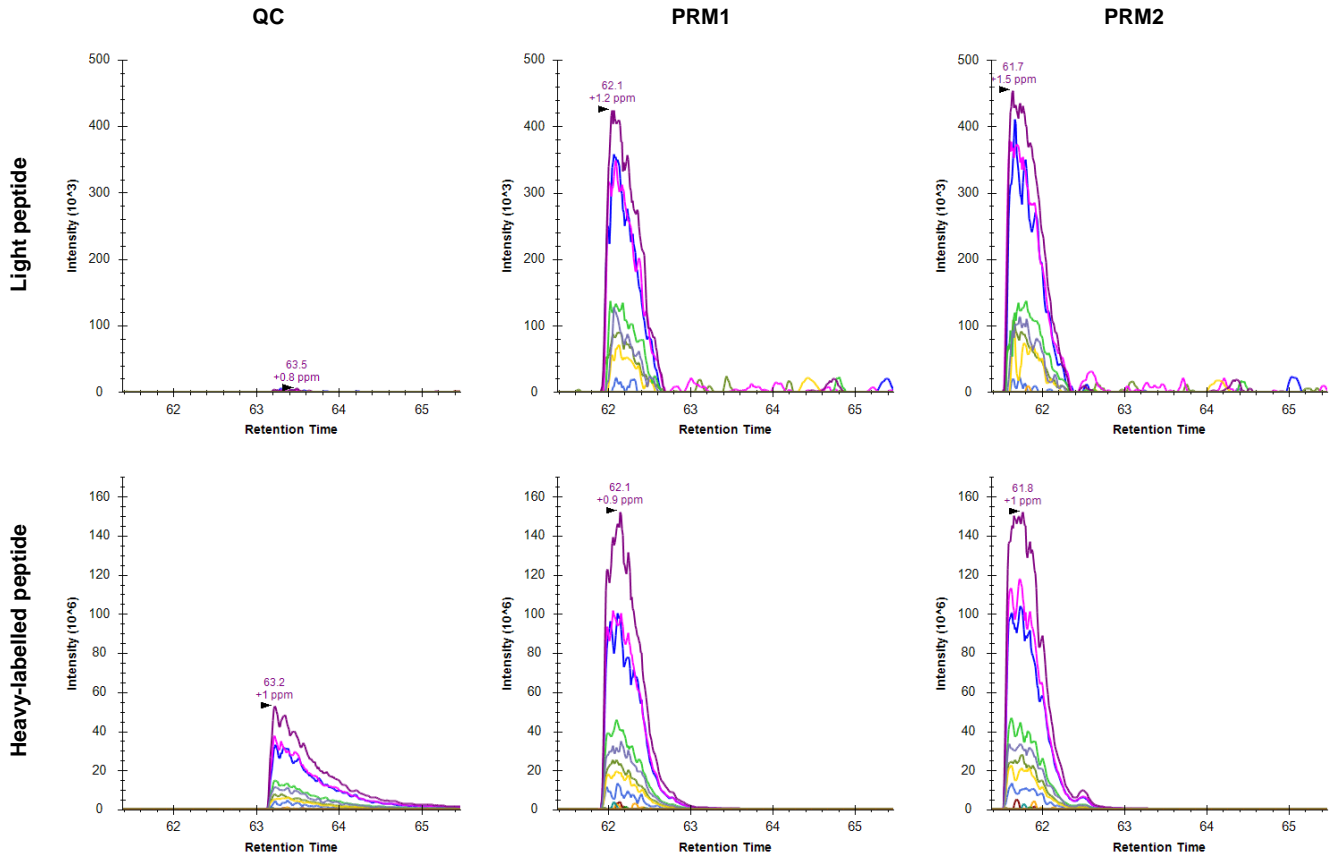


C

CTNNB1/T1185B

Peptide: RVFEVYHTTVLK

Precursor charge: +++



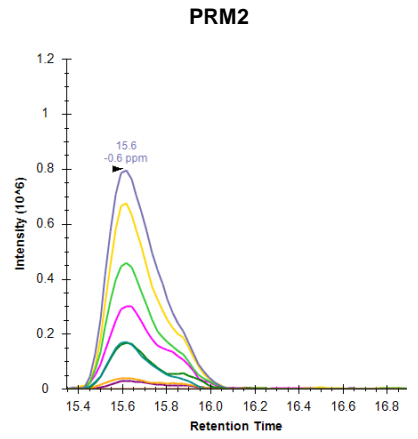
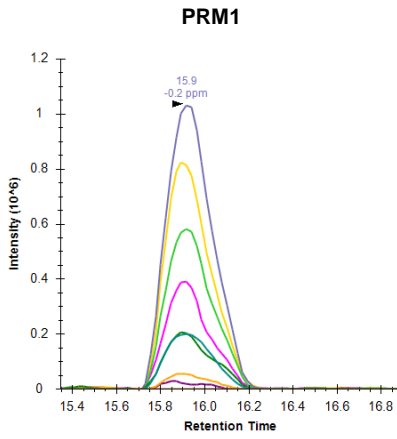
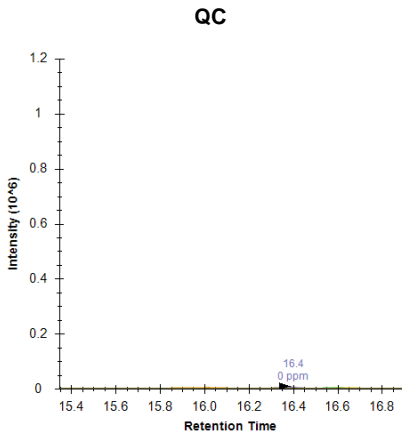
d

CTSB/T1185B

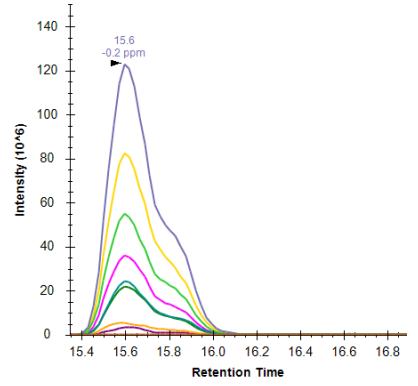
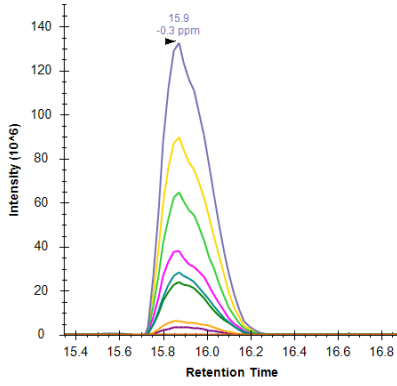
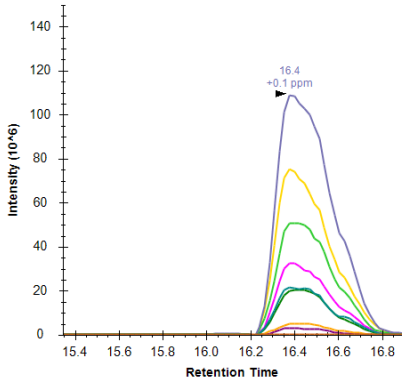
Peptide: STNGIPRGR

Precursor charge: ++

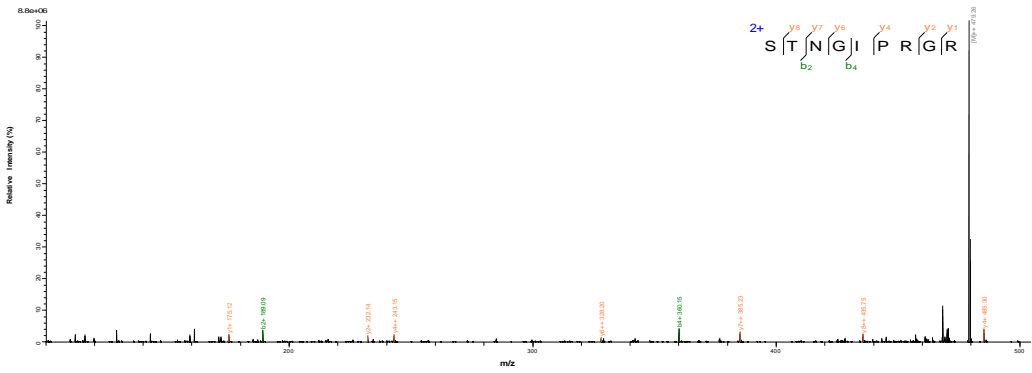
Light peptide



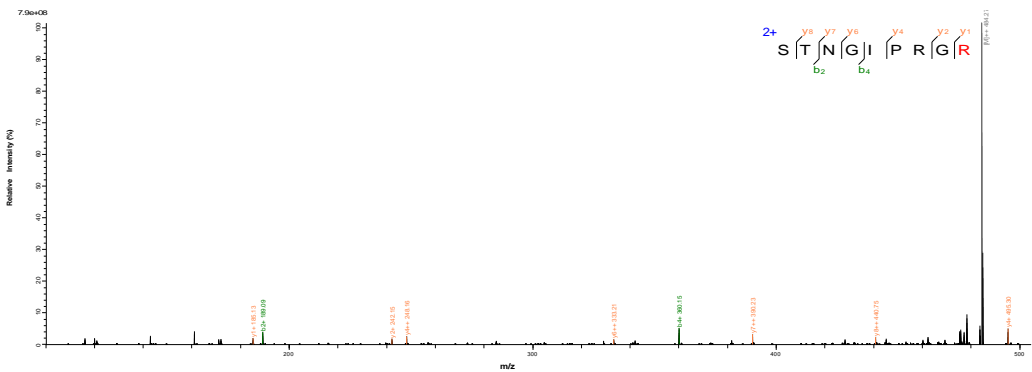
Heavy-labelled peptide



Light peptide



Heavy-labelled peptide



e

GAS7/T1185B

Peptide: EAWNGPPSAGR

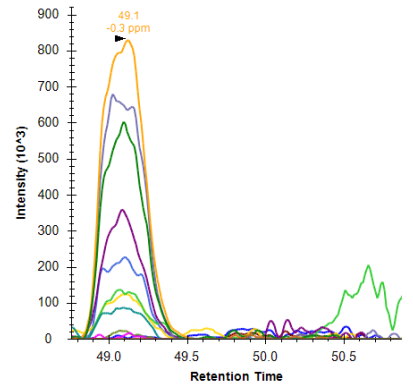
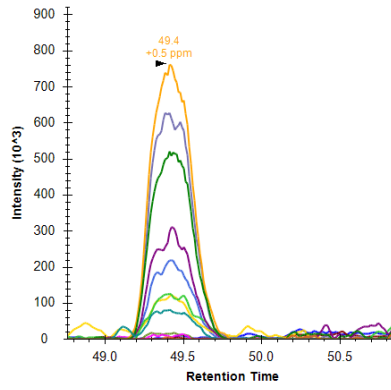
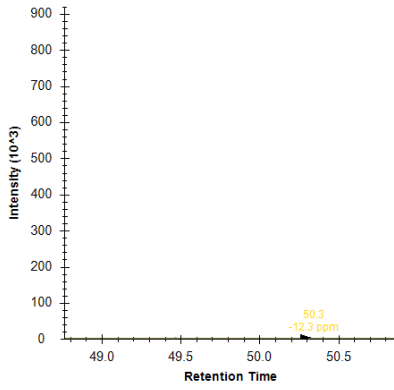
Precursor charge: ++

QC

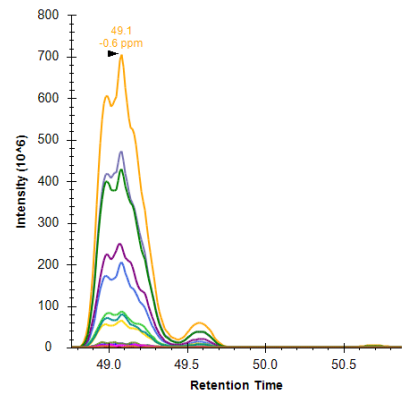
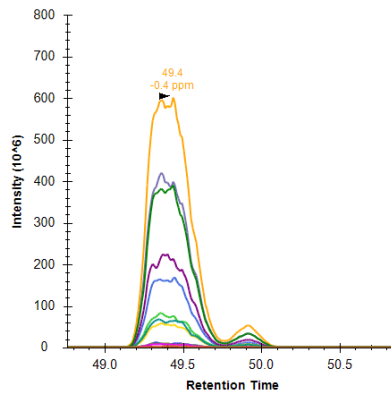
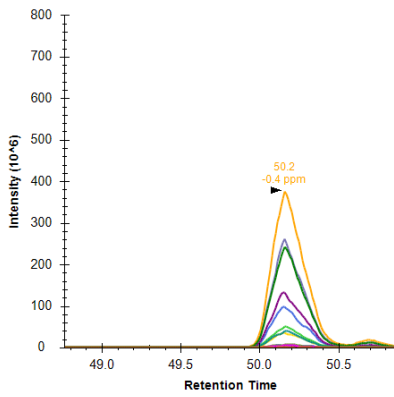
PRM1

PRM2

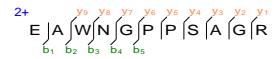
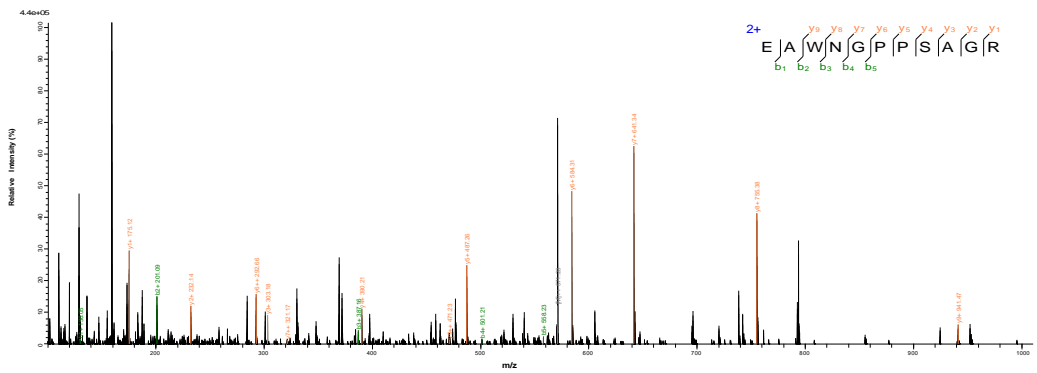
Light peptide



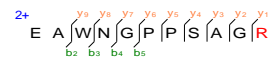
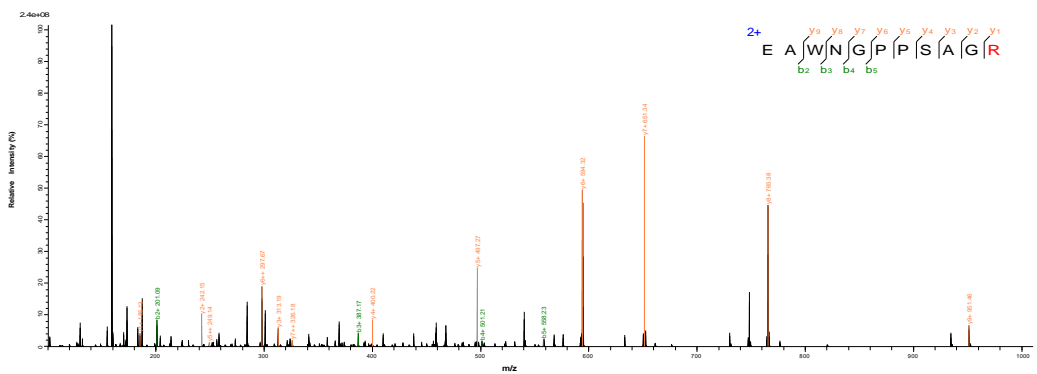
Heavy-labelled peptide



Light peptide



Heavy-labelled peptide

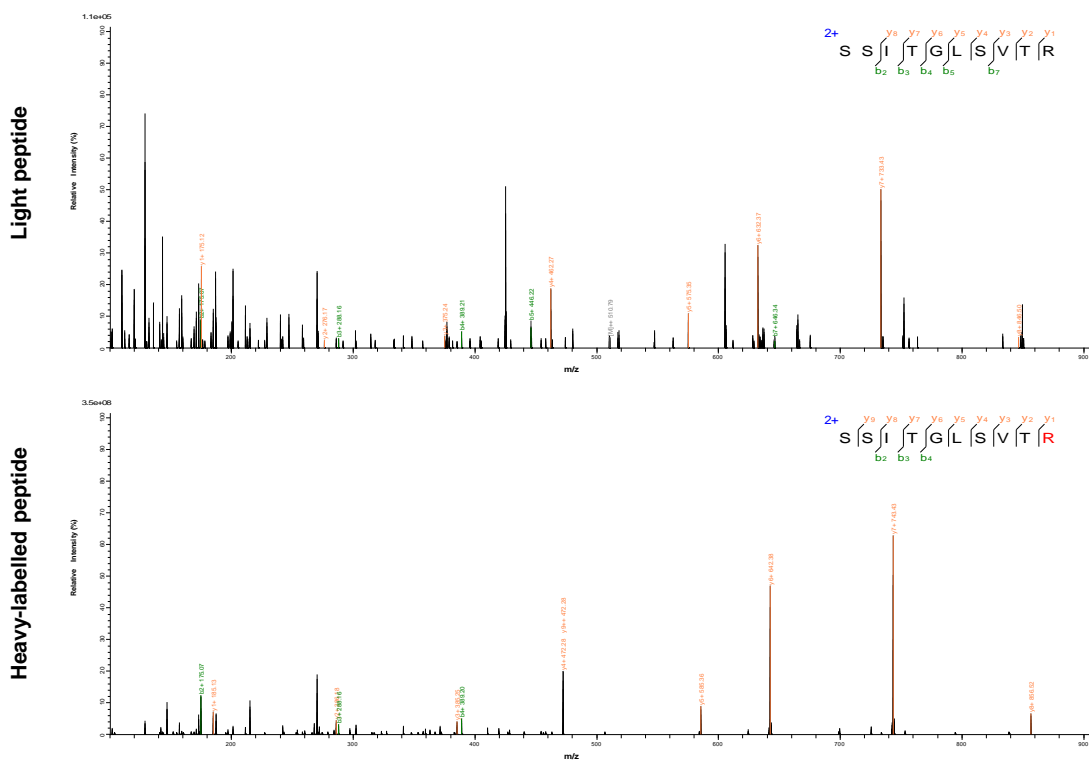
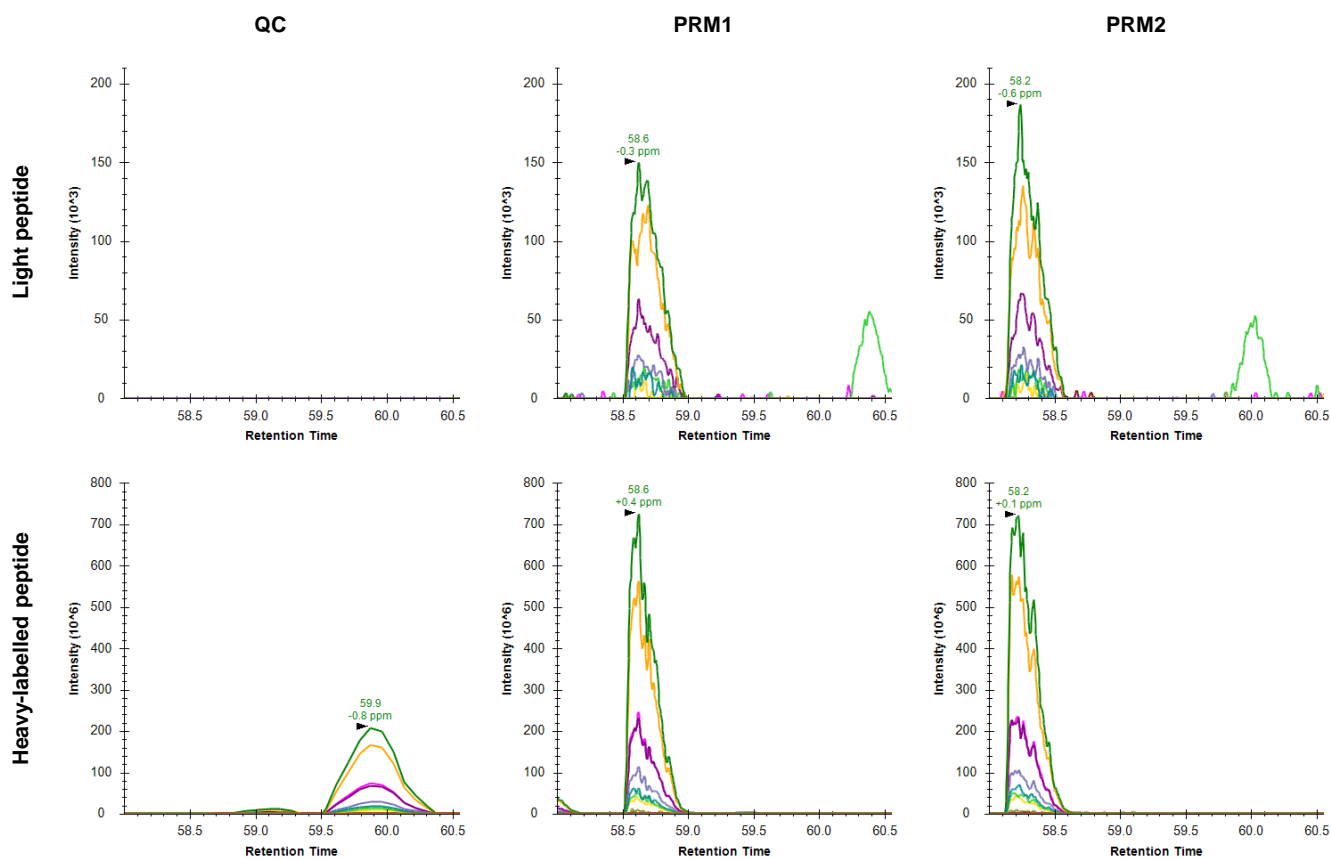


f

KIAA0226/T1185B

Peptide: SSITGLSVTR

Precursor charge: ++

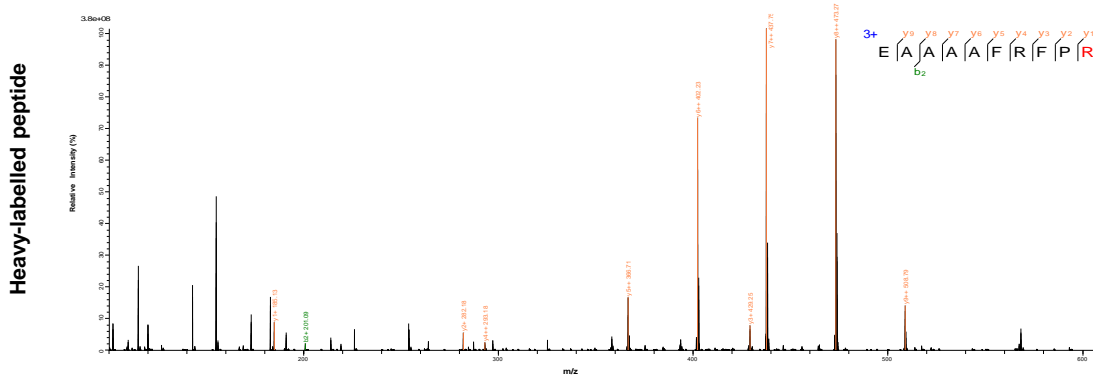
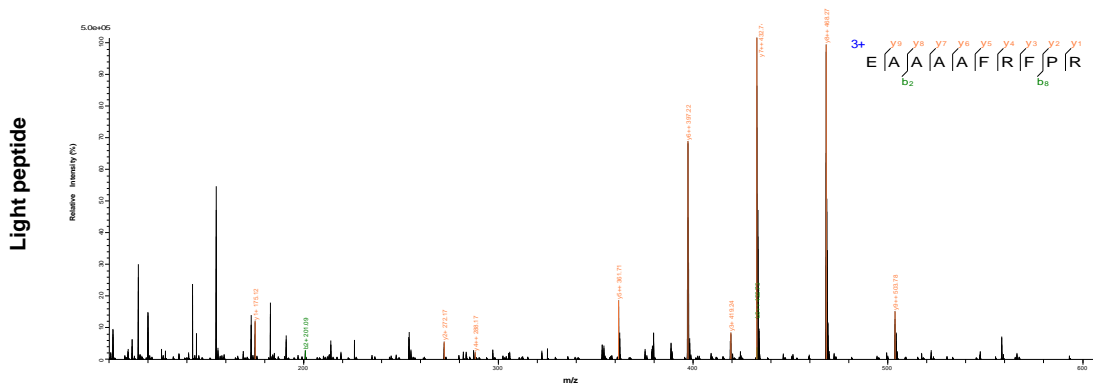
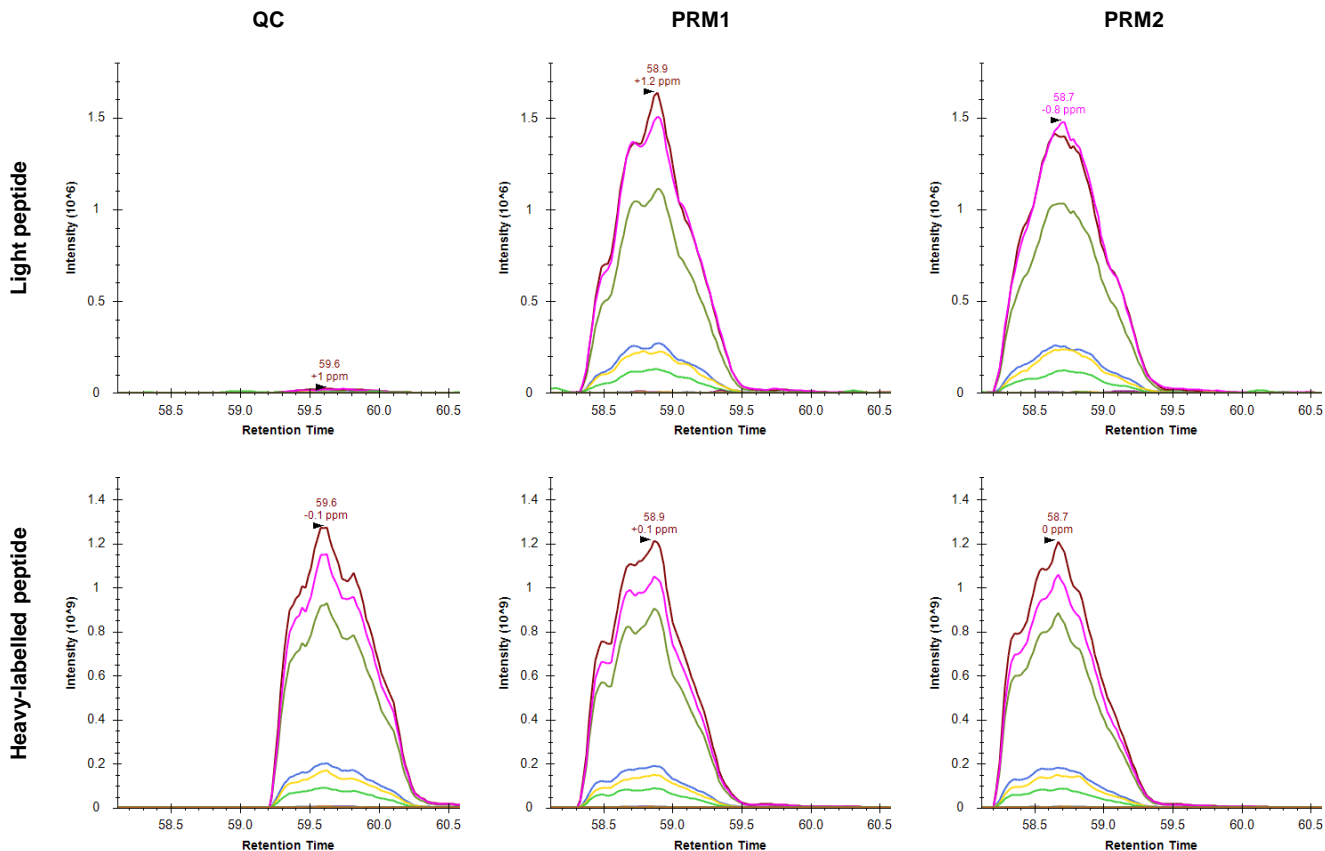




LINC00221/T1185B

Peptide: EAAAAFRFPR

Precursor charge: +++

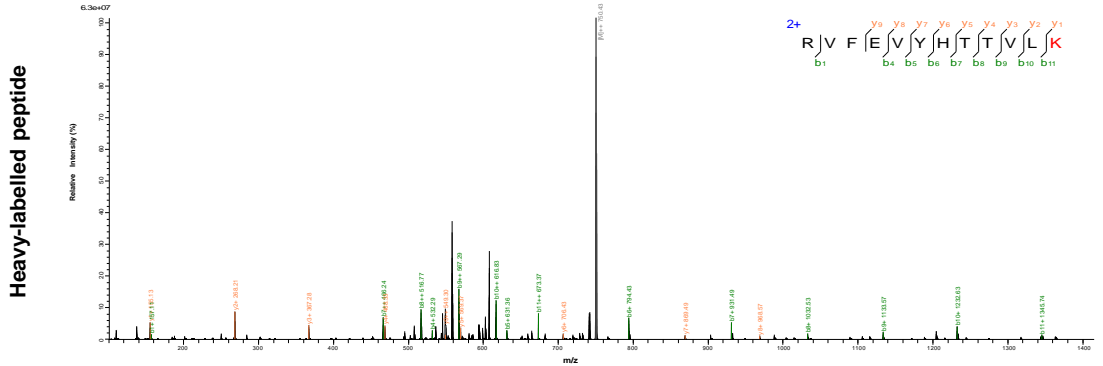
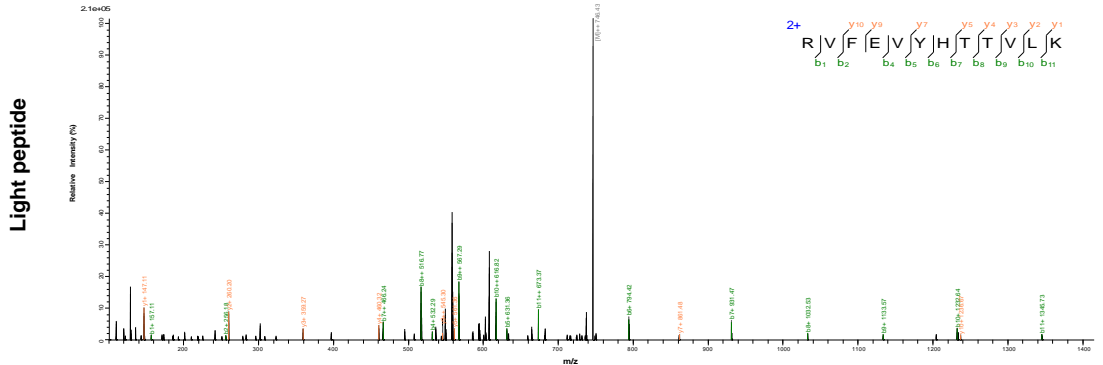
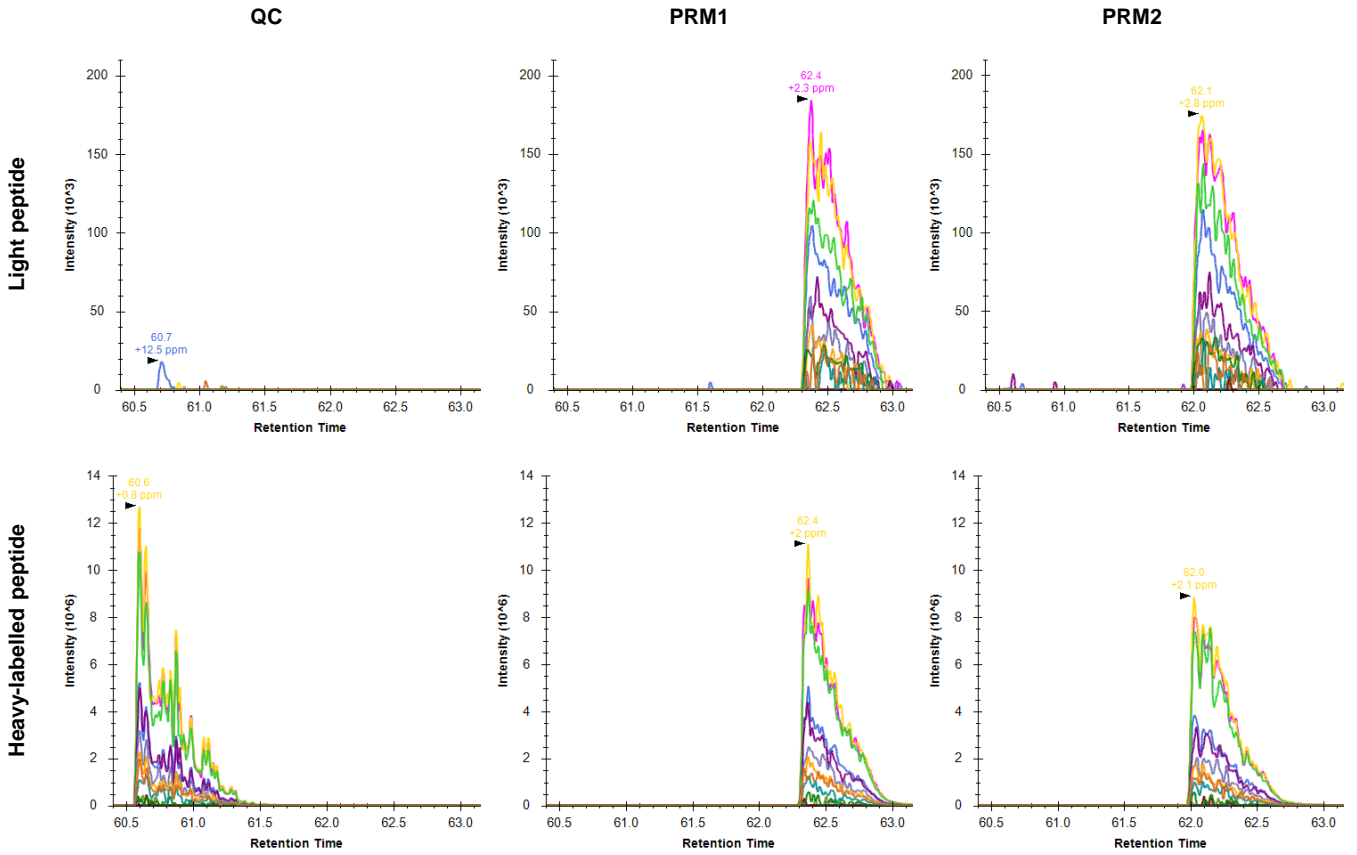


h

CTNNB1/Mel-1

Peptide: RVFEVYHTTVLK

Precursor charge: ++





j

TOPBP1/Mel-1

Peptide: KSLAAELLVLK

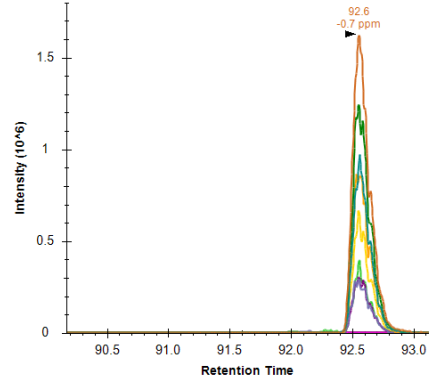
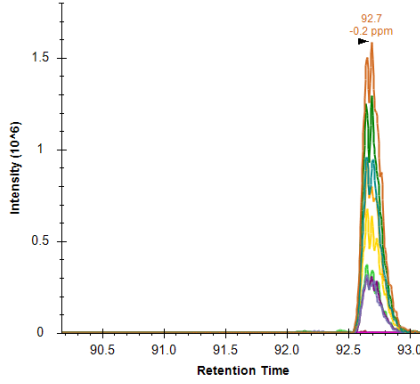
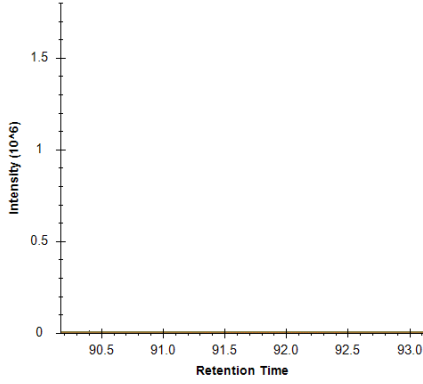
Precursor charge: ++

QC

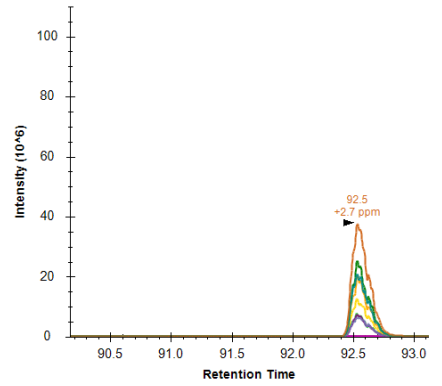
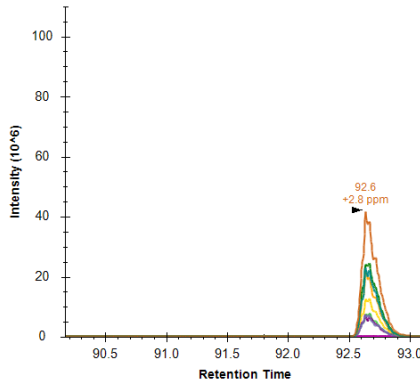
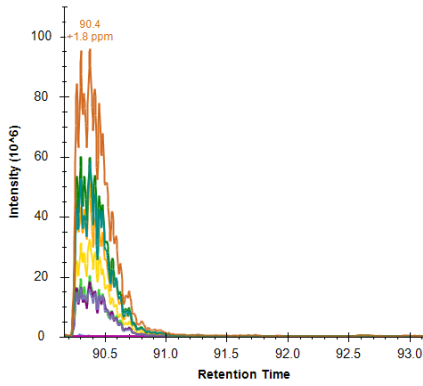
PRM1

PRM2

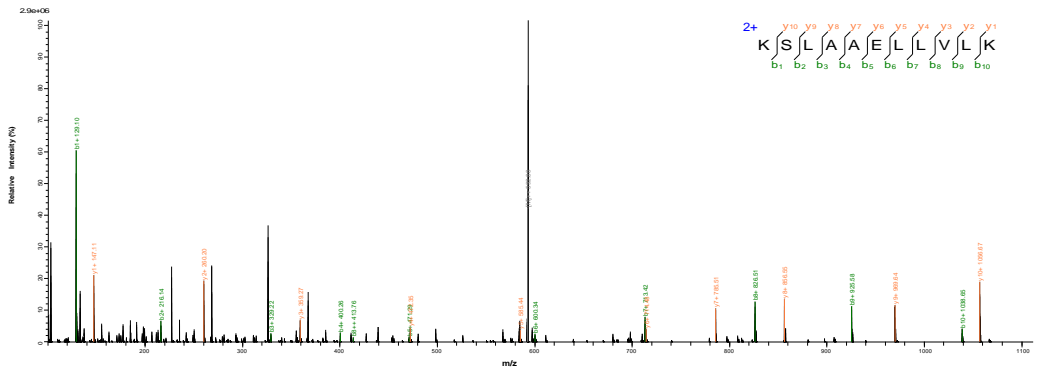
Light peptide



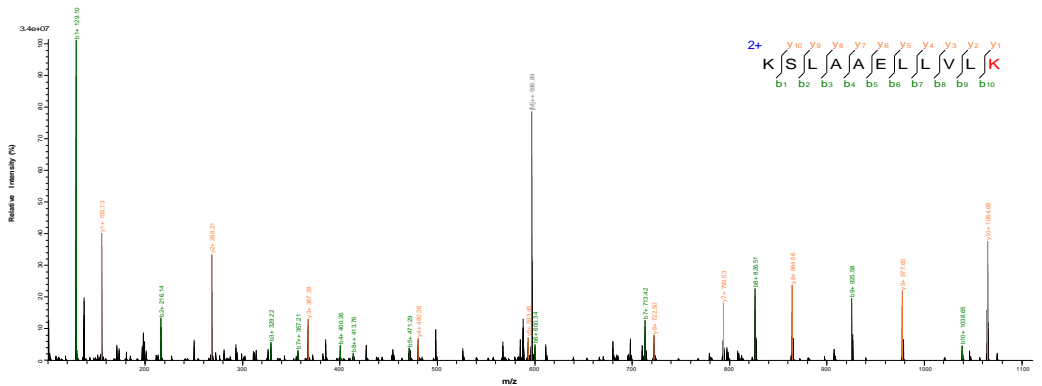
Heavy-labelled peptide

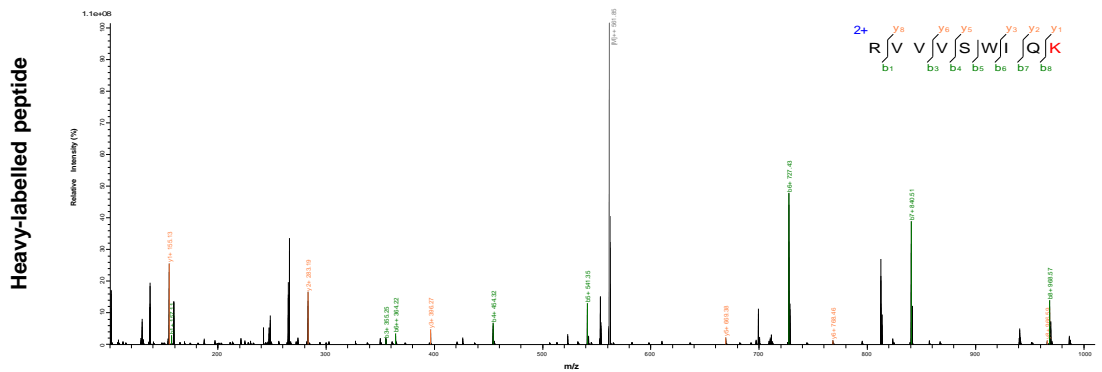
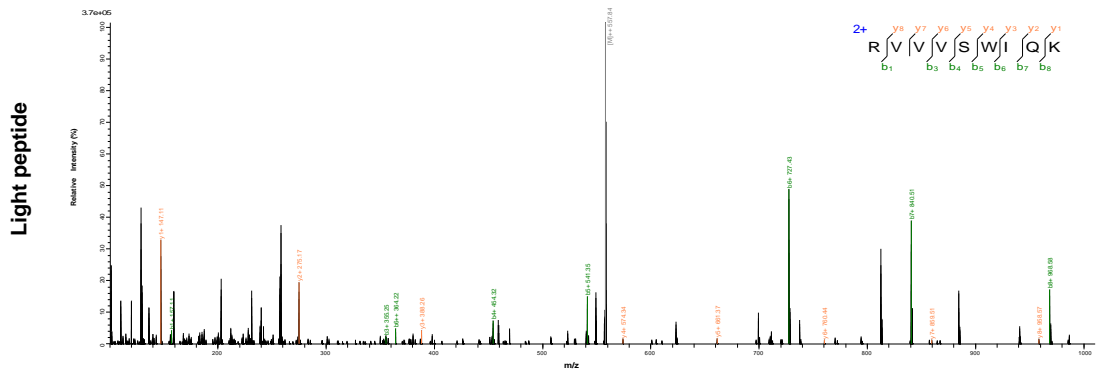
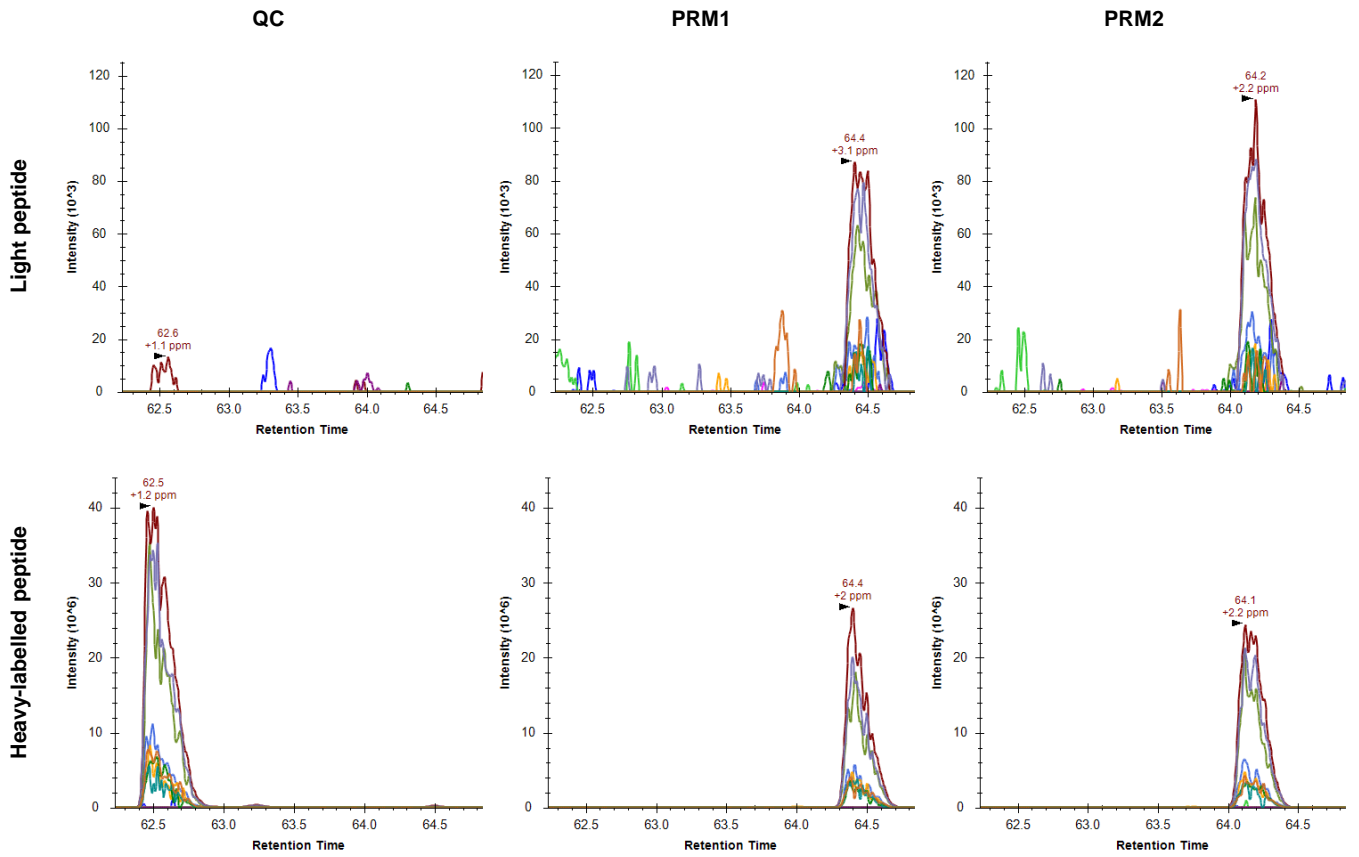


Light peptide



Heavy-labelled peptide



**k****ZMYM4/Mel-1****Peptide: RVVVSWIQK****Precursor charge: ++**

CDC73 (canonical)/T1185B

Peptide: ATENIPVRR

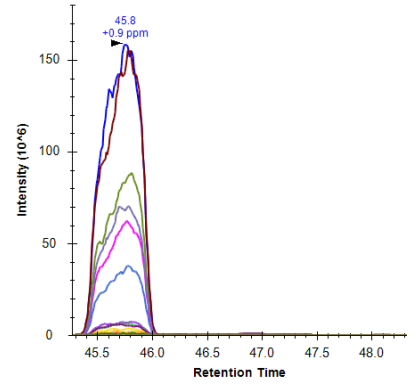
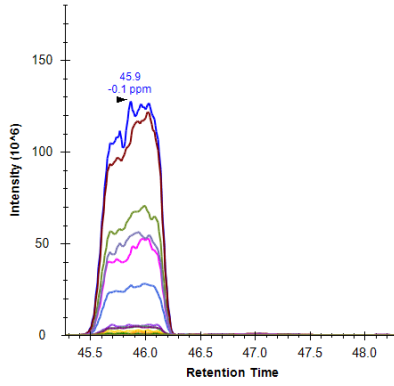
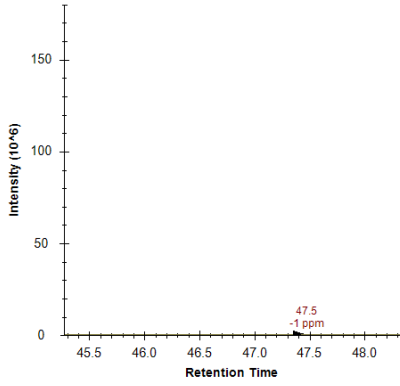
Precursor charge: +++

QC

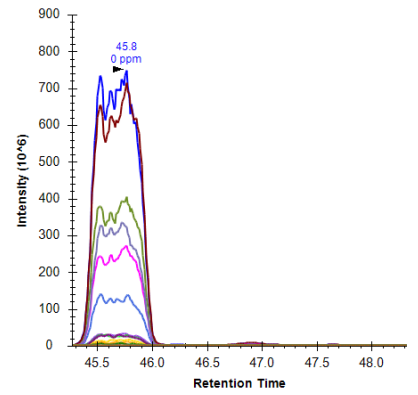
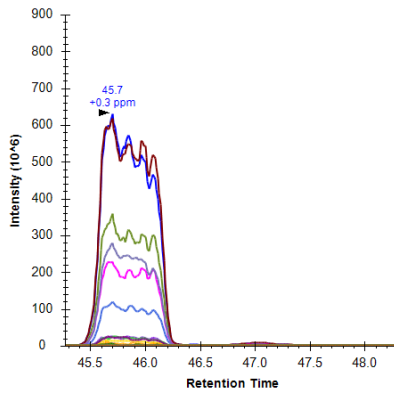
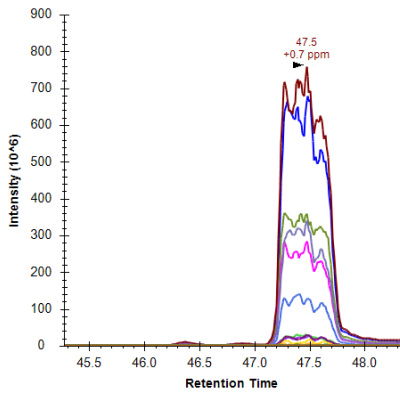
PRM1

PRM2

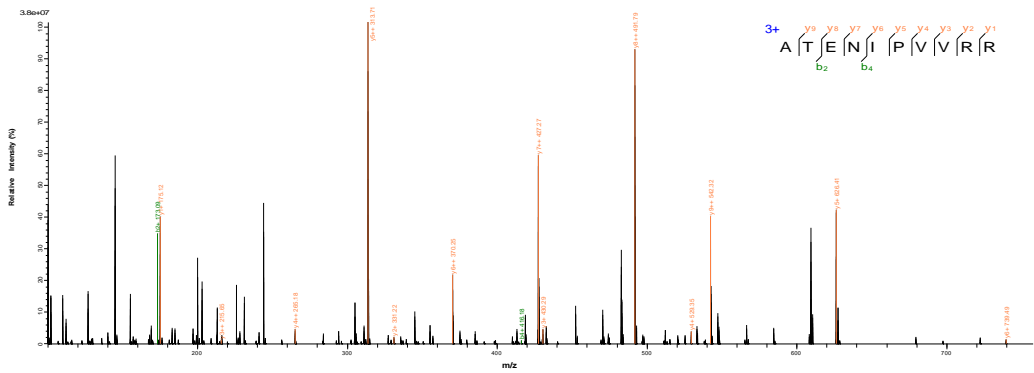
Light peptide



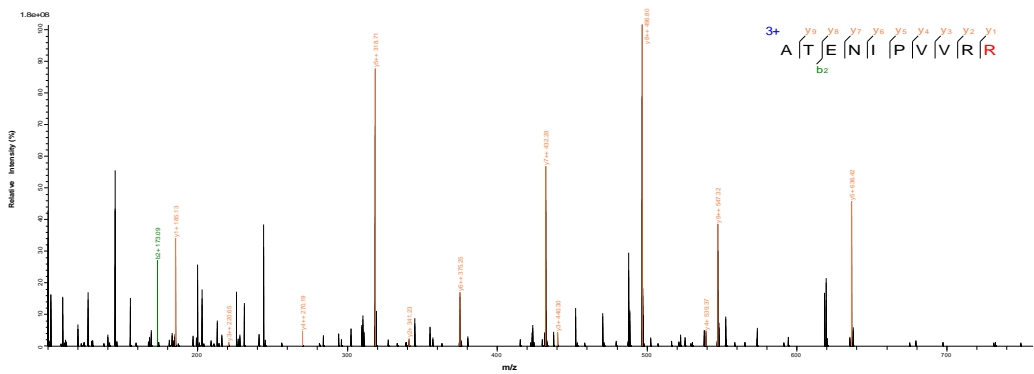
Heavy-labelled peptide



Light peptide



Heavy-labelled peptide

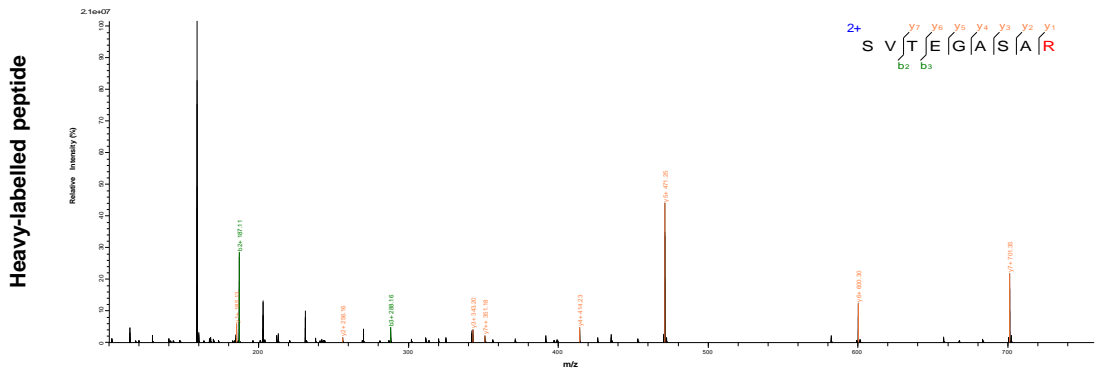
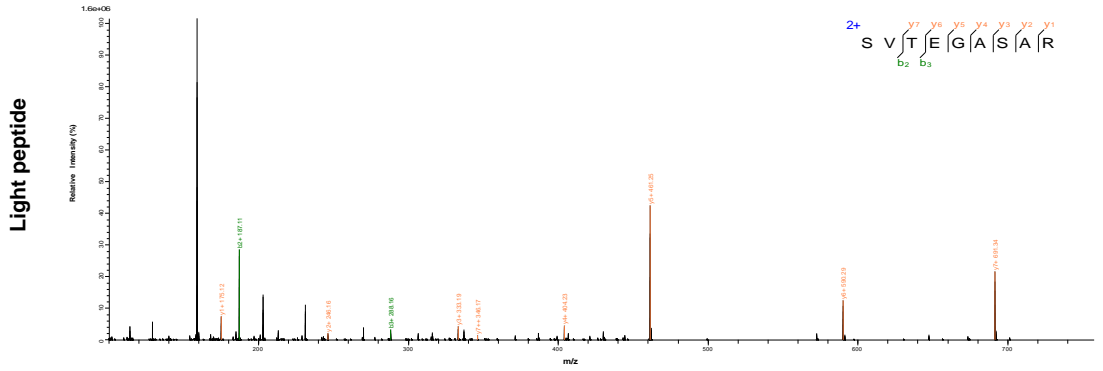
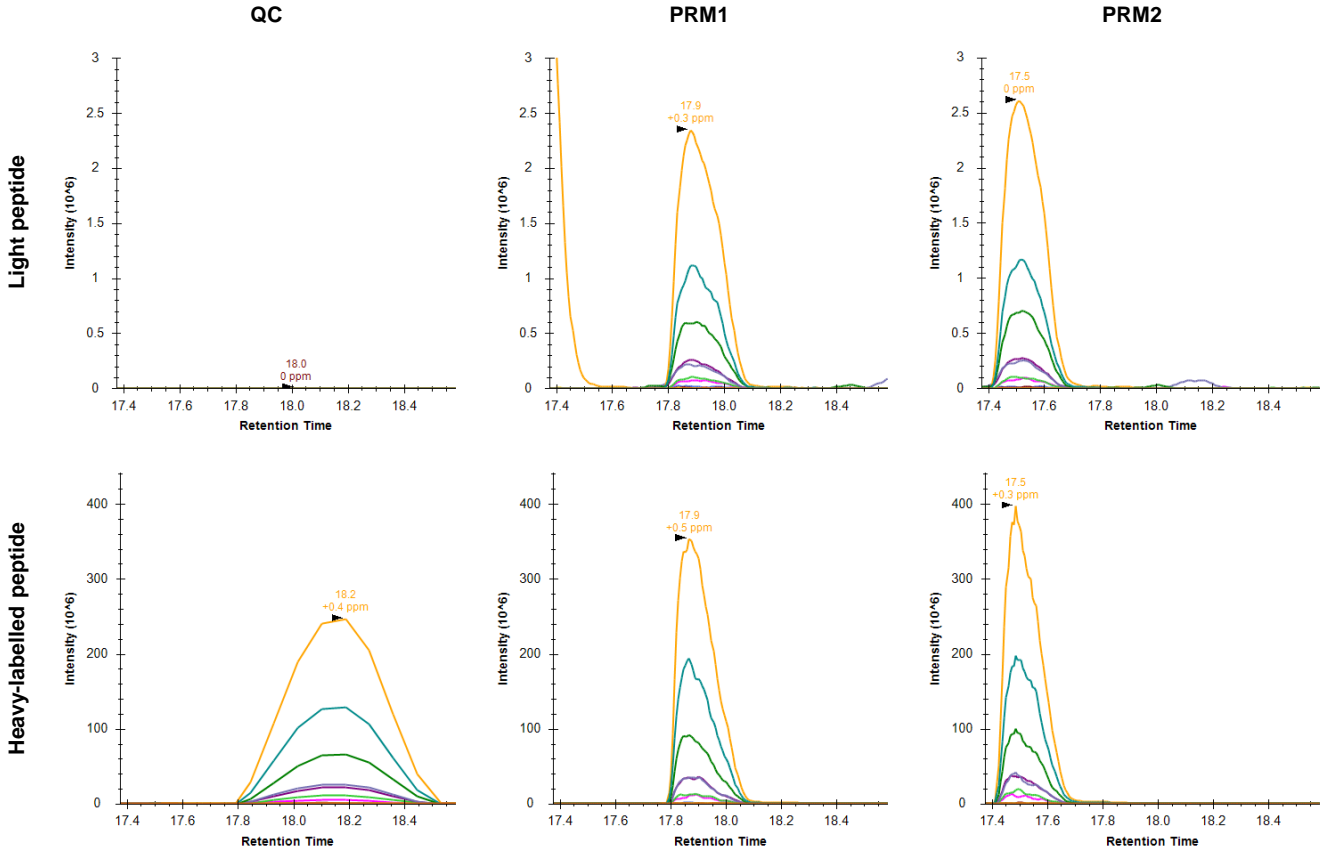


m

CDC73 (canonical)/T1185B

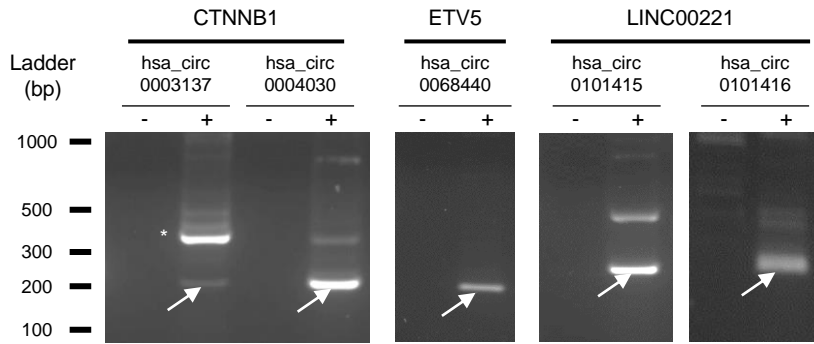
Peptide: SVTEGASAR

Precursor charge: ++

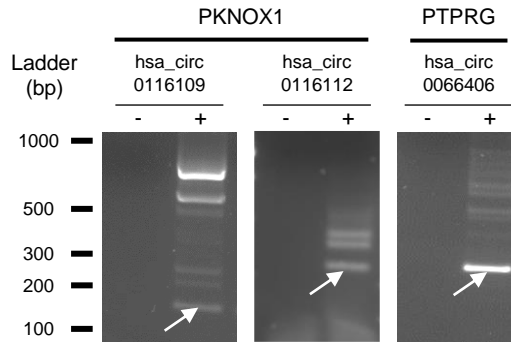
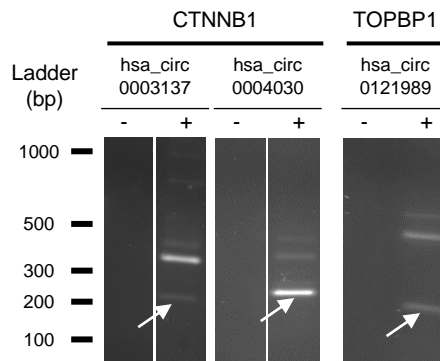


**Supplementary Figure 4. PRM validation of selected circRNA-derived peptides identified in T1185B and Mel-1 samples.** Skyline and pLabel showing the co-elution and MS/MS fragmentation patterns of light (above) and heavy-labelled (below) peptides, respectively. Visualization of MS-detected circRNA-derived peptides from host genes (a) *COP1* (*RFWD2*), (b) *CDC73*, (c) *CTNNB1* (T1185B), (d) *CTSB*, (e) *GAS7*, (f) *KIAA0226*, (g) *LINC00221*, (h) *CTNNB1* (Mel-1), (i) *PPP2R3A*, (j) *TOPBP1* and (k) *ZMYM4*. (l, m) Visualization of two MS-detected canonical peptides from *CDC73* in T1185B. Corresponding cell line and precursor charge are shown per each peptide. PRM1 and PRM2 represent technical replicates. Heavy labelled amino acid in each synthetic peptide is shown in bold.



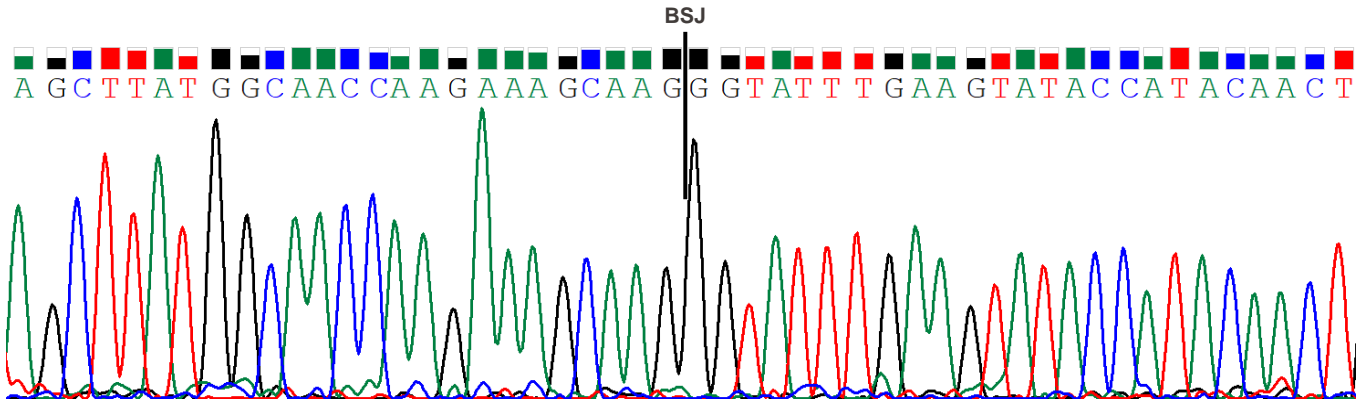
**a**

\*Note: Intense band 300-500bp was sequenced.

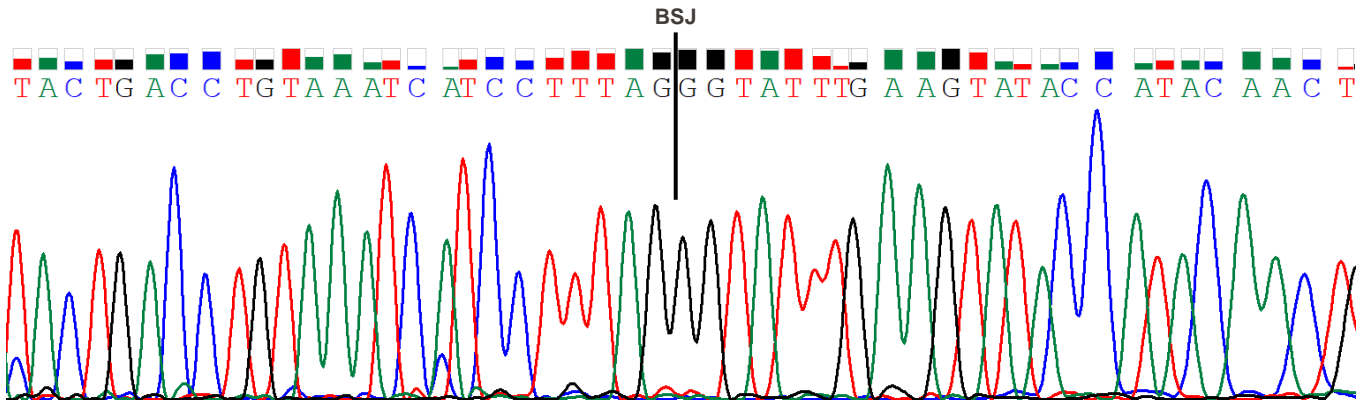
**b**

**Supplementary Figure 5. Divergent RT-PCR for circRNA validation.** (a) T1185B cell line. Agarose gel electrophoresis showing the expected size amplicons (arrows). Oligos used for RT-PCR amplification were designed to not overlap the BSJ (region aimed to be sequenced). Expectedly, several unspecificities were observed. RT-PCR amplification of the sequence containing the BSJ of *hsa\_circ0003137* showed an intense band (300-500bp). Sanger sequencing showed that the amplification corresponded to *hsa\_circ\_0004194*. CircRNAs hosted by *CTNNB1*, *ETV5*, *LINC00221*, *PKNOX1* and *PTPRG* genes were validated by divergent RT-PCR followed by Sanger Sequencing. “-”: water; “+”: T1185B-derived cDNA. (b) Mel-1 cell line. Agarose gel electrophoresis showing the expected size amplicons (arrows) for circRNAs hosted by *CTNNB1* and *TOPBP1* genes. Non-adjacent lanes corresponding to the negative controls (water) of the two circRNA amplicons from *CTNNB1* host gene, in Mel-1 cell line, derived from the same gel and they are shown separately. Remaining electrophoresis from T1185B cell line are shown in Fig. 2i (*COP1/RFWD2* host gene) and Fig. 3d (*CDC73* host gene). Images were adjusted for better visibility. This experiment was performed once. “-”: water; “+”: Mel-1-derived cDNA. Source data are provided as a Source Data file.

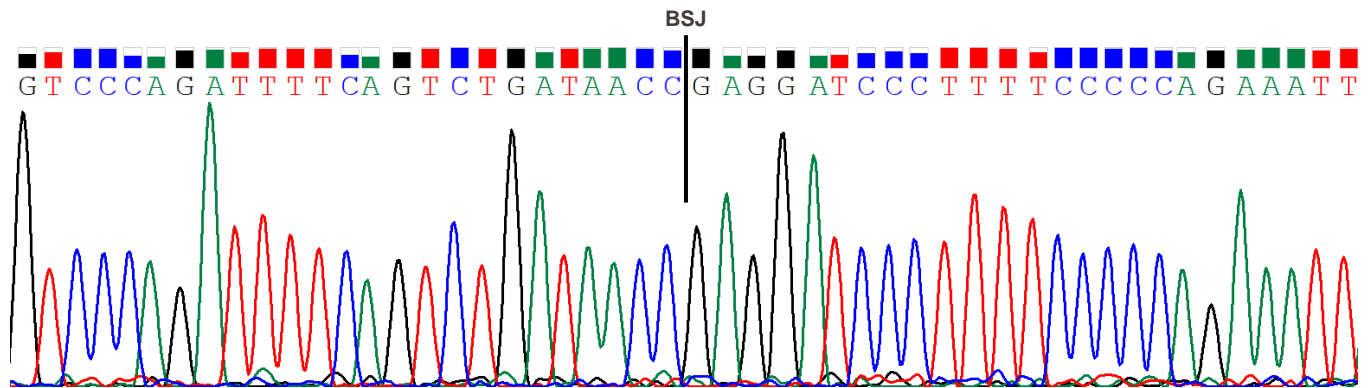
**a** Peptide: RVFEVYHTTVLK  
 Host gene: CTNNB1  
 circRNA: hsa\_circ\_0003137  
 Expected sequence around the [BSJ]: AGCTTATGGCAACCAAGAAAGCAA[GG]GTATTTGAAGTATACCATACT



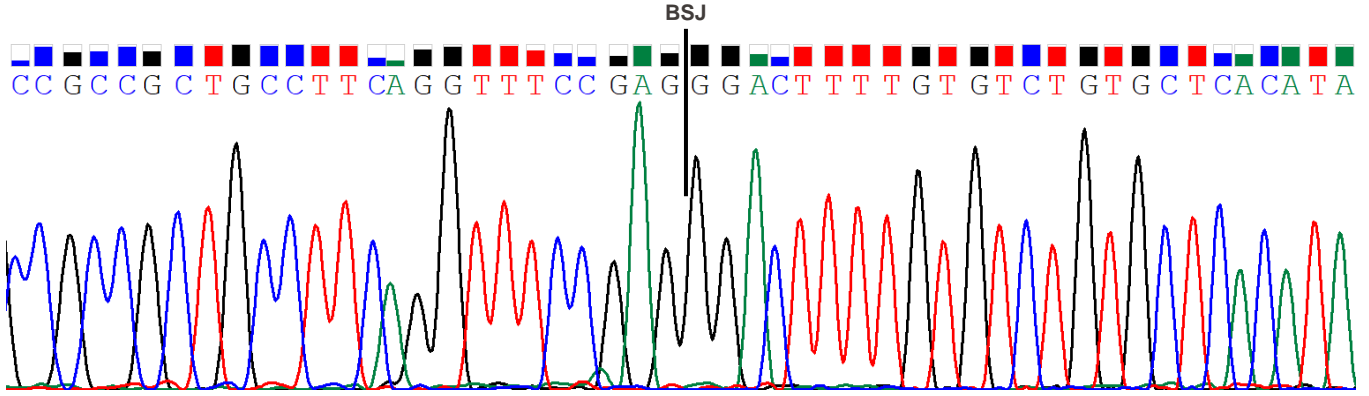
**b** Peptide: RVFEVYHTTVLK  
 Host gene: CTNNB1  
 circRNA: hsa\_circ\_0004030  
 Expected sequence around the [BSJ]: TACTGACCTGTAATCATCCTTTA[GG]GTATTTGAAGTATACCATACT



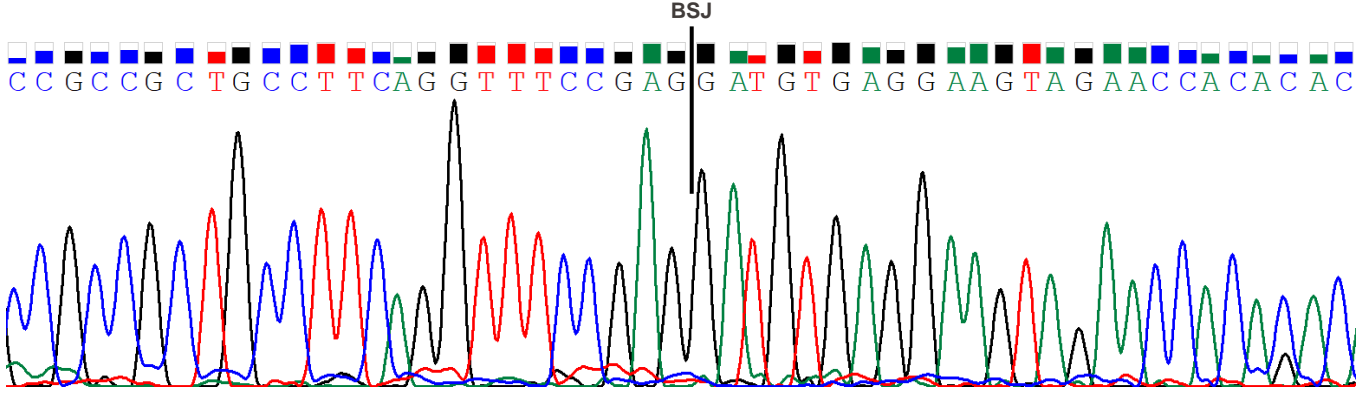
**c** Peptide: FVPDFQSDNR  
 Host gene: ETV5  
 circRNA: hsa\_circ\_0068440  
 Expected sequence around the [BSJ]: GTCCAGATTTTCA GTCTGATAAC[CG]AGGATCCCTTTTCCCCAGAAATT



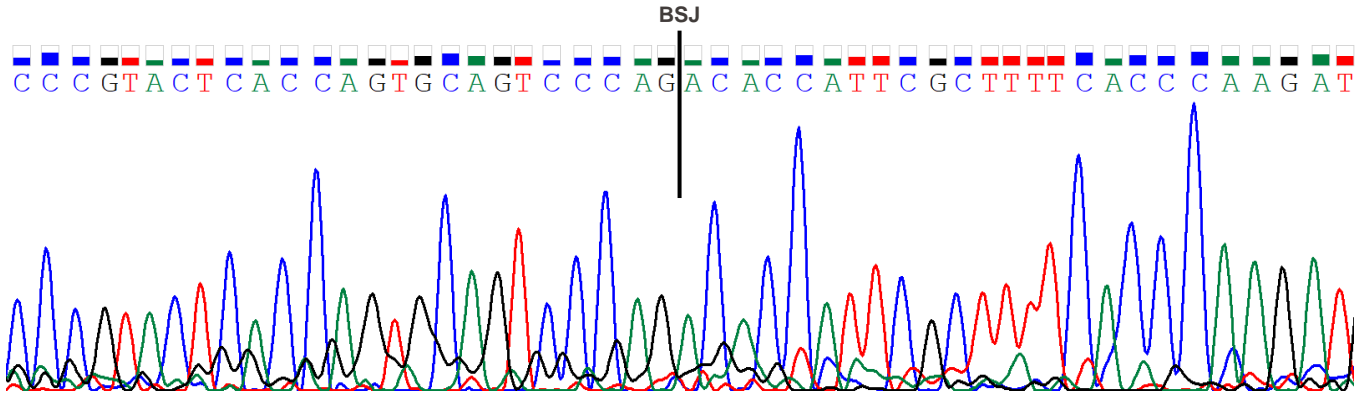
**d** Peptide: EAAAAFRFPR (not validated by PRM: AAAA FRFPR)  
 Host gene: LINC00221  
 circRNA: hsa\_circ\_0101415  
 Expected sequence around the [BSJ]: CCGCCGCTGCCTTCAGGTTTCCGA[GG]GACTTTTGTGCTGTGCTCACATA



**e** Peptide: EAAAAFRFPR (not validated by PRM: AAAA FRFPR)  
 Host gene: LINC00221  
 circRNA: hsa\_circ\_0101416  
 Expected sequence around the [BSJ]: CCGCCGCTGCCTTCAGGTTTCCGA[GG]ATGTGAGGAAGTAGAACACACAC



**f** Peptide: QTPFAFHPR  
 Host gene: PKNOX1  
 circRNA: hsa\_circ\_0116109  
 Expected sequence around the [BSJ]: CCCGTA CTCA C CAGTGCAGTCCCAGACACCATTCGC TTTT CACC CAAGAT



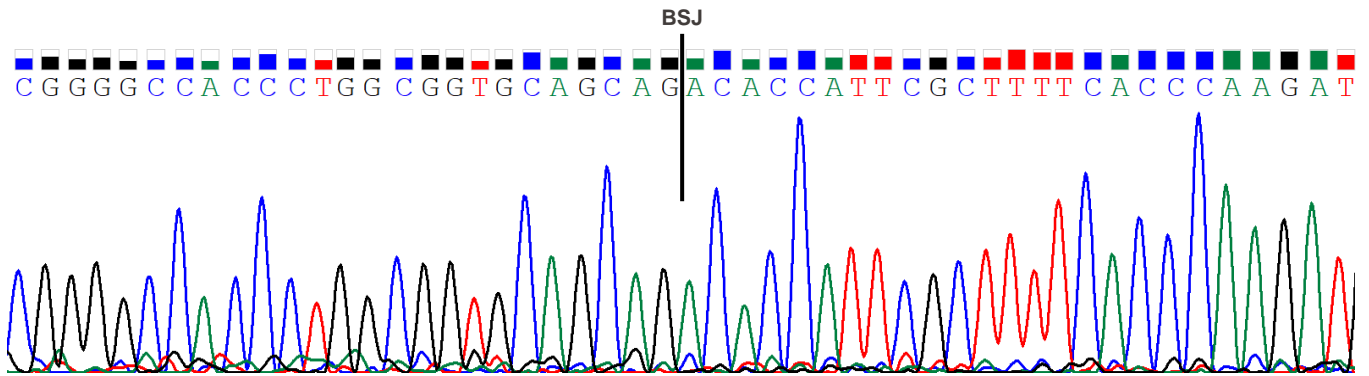
**g**

Peptide: QTPFAFHPR

Host gene: PKNOX1

circRNA: hsa\_circ\_0116112

Expected sequence around the [BSJ]: CGGGGCCACCCTGGCGGTGCAGCA[GA]CACCATTGCTTTTCACCCAAGAT

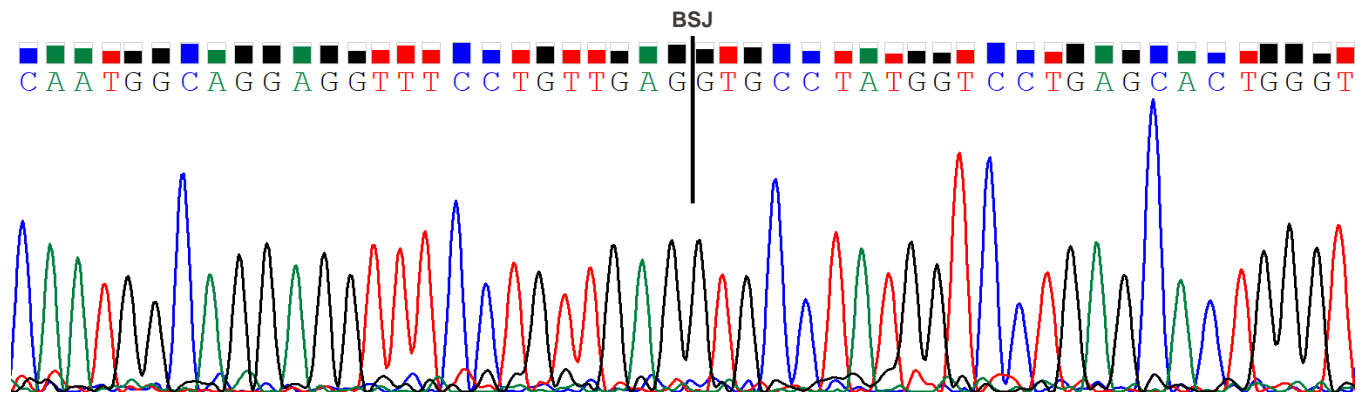
**h**

Peptide: NTASMAGGFLLR

Host gene: PTPRG

circRNA: hsa\_circ\_0066406

Expected sequence around the [BSJ]: CAATGGCAGGAGGTTTCTGTTGA[GG]TGCCTATGGTCCTGAGCACTGGGT

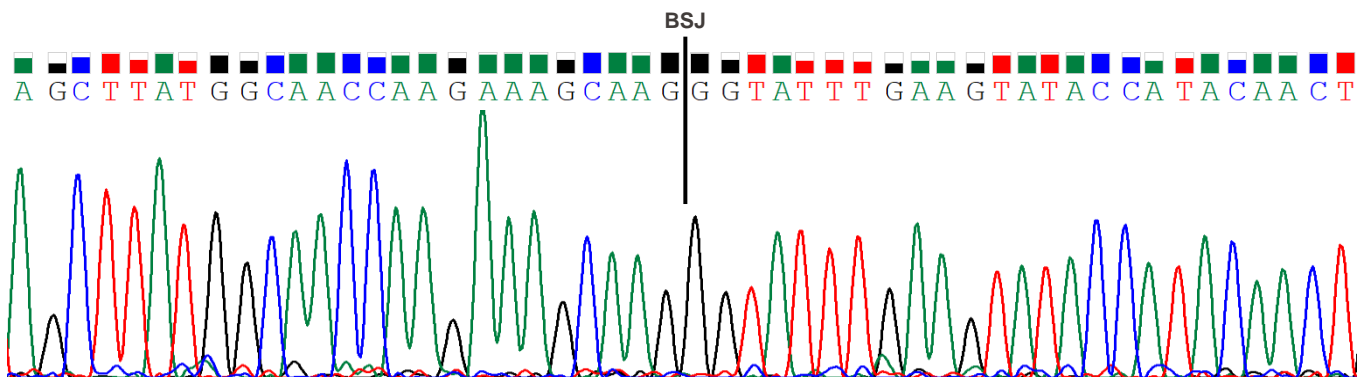
**i**

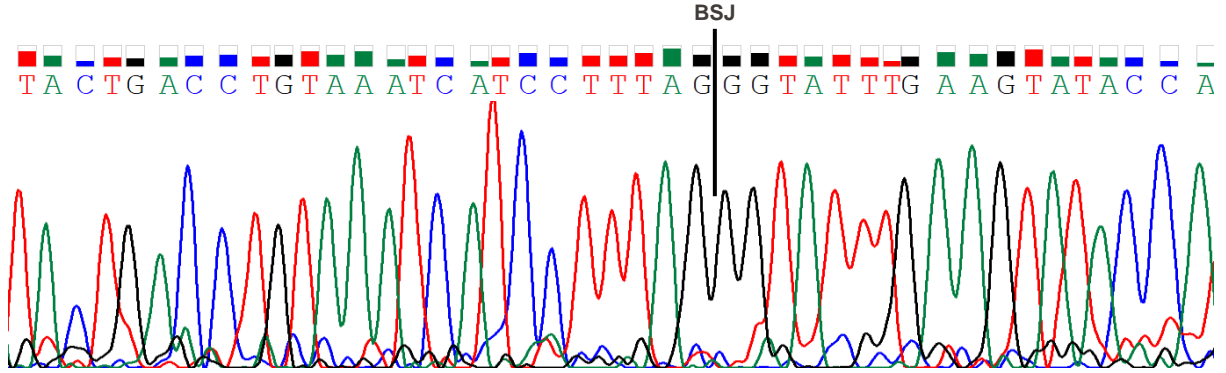
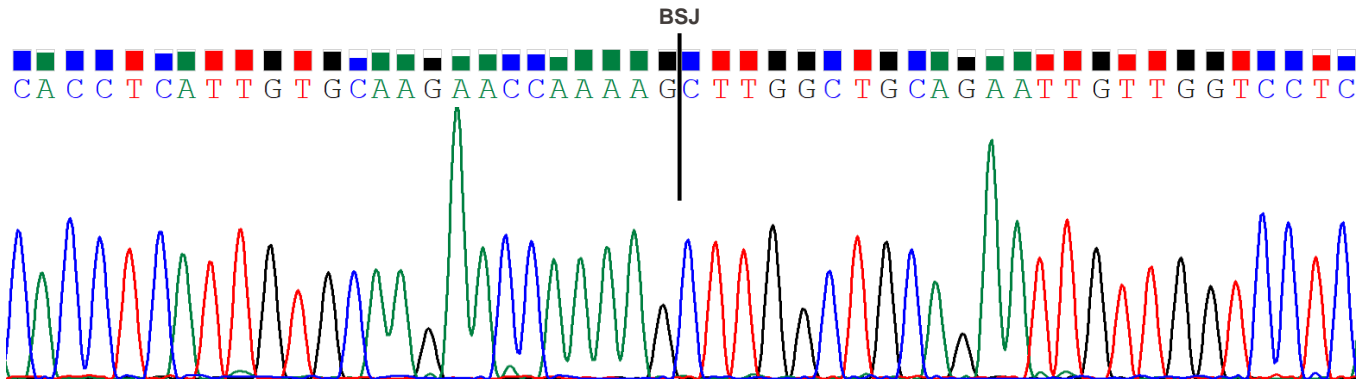
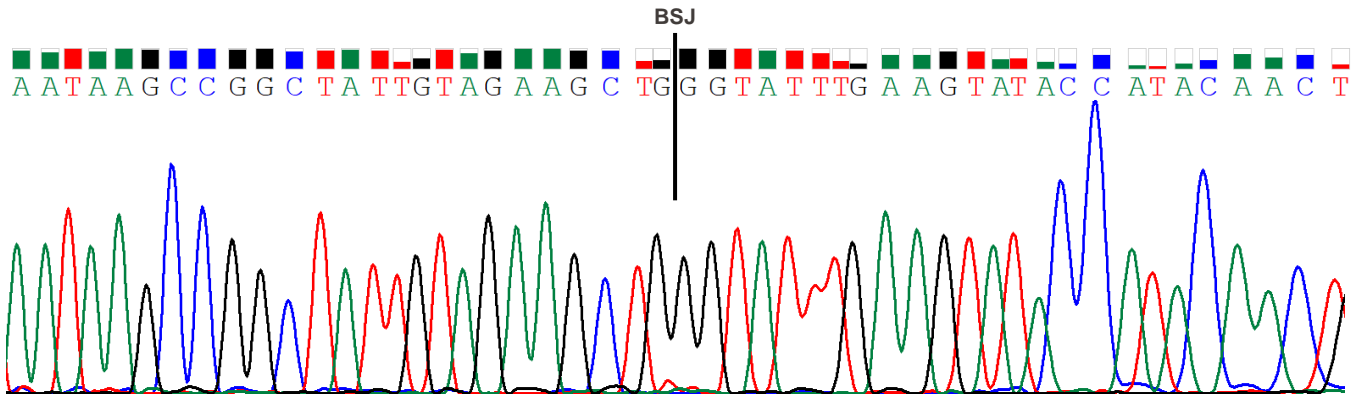
Peptide: RVFEVYHTTVLK

Host gene: CTNNB1

circRNA: hsa\_circ\_0003137

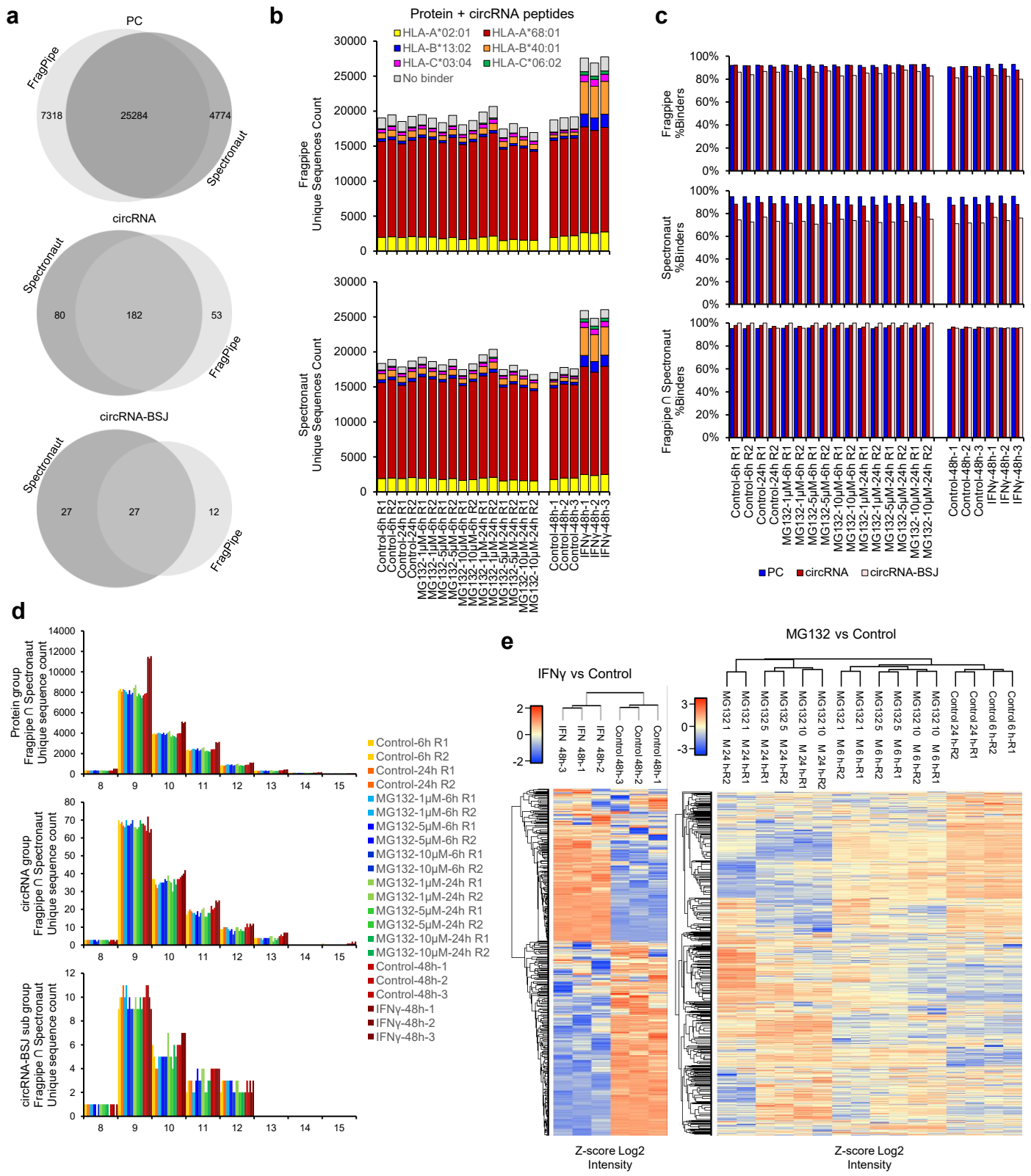
Expected sequence around the [BSJ]: AGCTTATGGCAACCAAGAAAGCAA[GG]GTATTTGAAGTATACCAATACAACCT



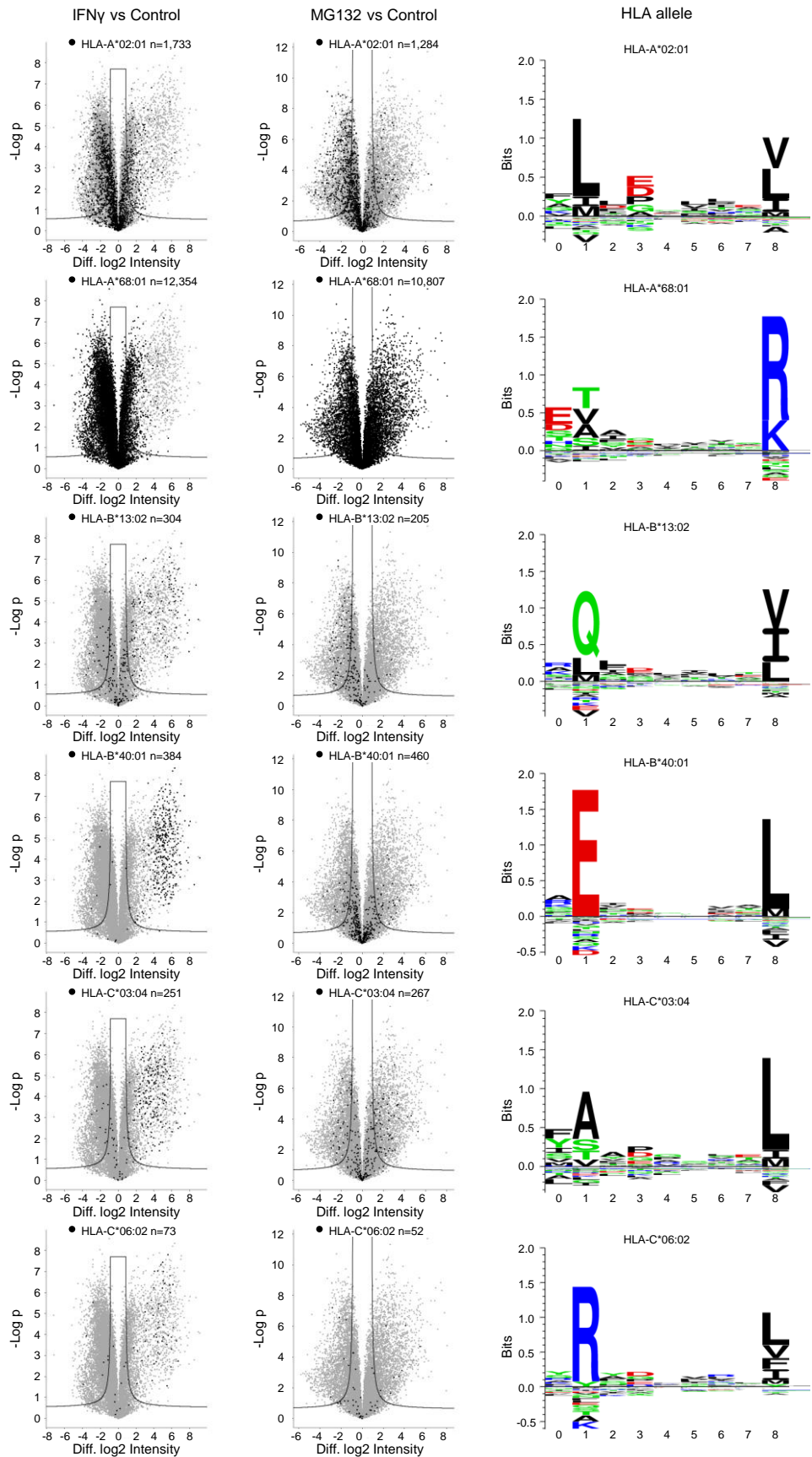
**j****Peptide:** RVFEVYHTTVLK**Host gene:** CTNNB1**circRNA:** hsa\_circ\_0004030**Expected sequence around the [BSJ]:** TACTGACCTGTAAATCATCCTTTA[GG]GTATTTGAAGTATACCATAACAAC**k****Peptide:** KSLAELLVLK**Host gene:** TOPBP1**circRNA:** hsa\_circ\_0121989**Expected sequence around the [BSJ]:** CACCTCATTGTGCAAGAACCAAAA[GC]TTGGCTGCAGAATTGTTGGTCCTC**l****Extra band obtained during amplification of hsa\_circ0003137:****Host gene:** CTNNB1**circRNA:** hsa\_circ\_0004194 (known circRNA)**Expected sequence around the [BSJ]:** AATAAGCCGGCTATTGTAGAAGCT[GG]GTATTTGAAGTATACCATAACAAC

**Supplementary Figure 6. Sanger Sequencing validation in T1185B and Mel-1 cell lines.**

Following electrophoresis, bands with the expected size were purified and DNA was analyzed by direct Sanger Sequencing, validating the sequence overlapping the BSJ of (a) *hsa\_circ\_0003137* (*CTNNB1* in T1185B), (b) *hsa\_circ\_0004030* (*CTNNB1* in T1185B), (c) *hsa\_circ\_0068440* (*ETV5*), (d) *hsa\_circ\_0101415* (*LINC00221*), (e) *hsa\_circ\_0101416* (*LINC00221*), (f) *hsa\_circ\_0116109* (*PKNOX1*), (g) *hsa\_circ\_0116112* (*PKNOX1*), (h) *hsa\_circ\_0066406* (*PTPRG*), (i) *hsa\_circ\_0003137* (*CTNNB1* in Mel-1), (j) *hsa\_circ\_0004030* (*CTNNB1* in Mel-1), (k) *hsa\_circ\_0121989* (*TOPBP1* in Mel-1) and (l) *hsa\_circ\_0004194* (*CTNNB1* extra band in T1185B). Remaining Sanger Sequencing chromatograms from T1185B cell line are shown in Fig. 2j (*hsa\_circ\_0015366* and *hsa\_circ\_0111262*, *COP1/RFWD2*) and Fig. 3e (*hsa\_circ\_0111569*, *CDC73*).



**Supplementary Figure 7. IFN $\gamma$  and MG132 treatments in T1185B cell line.** (a) Venn diagrams show the intersection between the unique peptide sequences detected in FragPipe (group-specific FDR 0.03) and Spectronaut (FDR 0.01), using a hybrid DIA approach. Illustration was done for PC, circRNA, and circRNA-BSJ groups. (b) Total number of unique peptides identified by FragPipe (top) and Spectronaut (bottom) in each sample, colored based on their predicted HLA restriction. (c) Percentage (%) of peptides predicted as HLA binders (% rank < 2) in each sample, using NetMHCpan, annotated in the PC, circRNA, and circRNA-BSJ groups, for FragPipe (top), Spectronaut (middle) and their intersection (bottom). (d) Length distribution of the common identified peptides in FragPipe and Spectronaut among PC, circRNA, and circRNA-BSJ groups. (e) Hierarchical clustering heat-maps showing the Z-score Log<sub>2</sub> intensity of the peptides following IFN $\gamma$  or MG132 treatments. Source data are provided as a Source Data file.

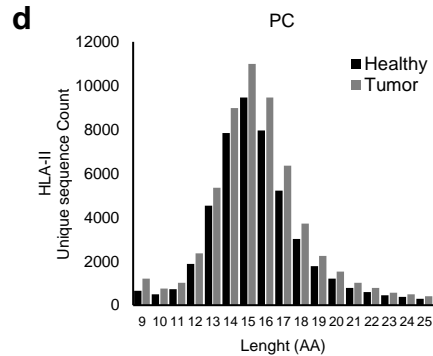
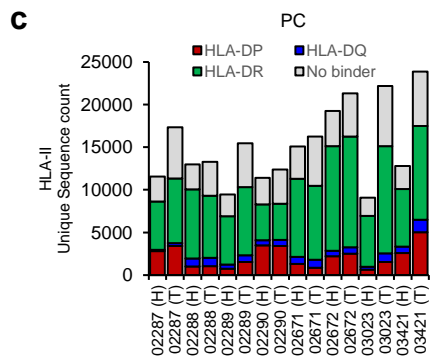
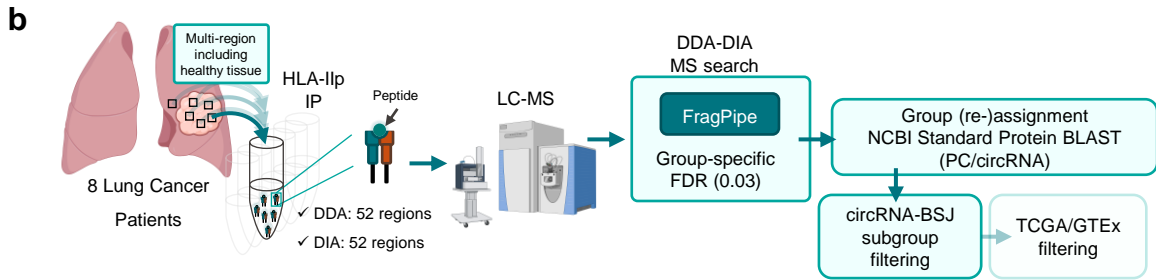


**Supplementary Figure 8. Effect of IFN $\gamma$  and MG132 in the intensity of peptides per binder allele.** Volcano plots highlighting the peptides associated to each HLA-I allele in black (best binder allele). HLA allele binding motifs based on Mass-Spectrometry Eluted Ligands (EL) are shown on the right of the volcano plots (derived from NetMHCpan - 4.1). Source data are provided as a Source Data file.



**a**

Host Gene	circRNA	Peptide	2287				2288				2289				2290				2671				2672				3023				3421						
			H	T	T	T	T	H	T	T	T	T	H	T	T	T	T	H	T	T	T	T	H	T	T	T	T	H	T	T	T	T	H	T	T	T	T
ABCC1	hsa_circ_0038107	KRQPVKVVY																																			
ANKRD10	hsa_circ_0030908	VANGAHVEI																																			
BABAM1	hsa_circ_0049967	TFNYLPGPV																																			
CTNNB1	hsa_circ_0003137	RVFEVYHTTVLK																																			
	hsa_circ_0004030																																				
EIF4B	hsa_circ_0026498	DRAKKNNKKGKTISL																																			
HSP90AB1	hsa_circ_0076651	ILDKKVEKL																																			
LIAS	hsa_circ_0069480	ETVPELQR																																			
MALAT1	hsa_circ_0096124	KLLHGVKNVFK																																			
NFKB1	hsa_circ_0070531	TSPLSSKL																																			
NPLOC4	hsa_circ_0046209	FTEAETHSY																																			
PPP2R3A	hsa_circ_0122039	KVLSFTEK																																			
PTPRG	hsa_circ_0066402	ASM(Ox)AGGFLLR																																			
	hsa_circ_0066406																																				
	hsa_circ_0124401																																				
SAE1	hsa_circ_0051662	ILAQEIVKV																																			
SCRN1	hsa_circ_0079683	SALTFQSTK																																			
SNX6	hsa_circ_0008747	SELFDKTRLK																																			
TOPBP1	hsa_circ_0121989	KSLAELLVLK																																			
YAP1	hsa_circ_0004881	KTSSGQRYFLK																																			
ZMYM4	hsa_circ_0113154	RVVVSWIQK																																			
(Intergenic)	hsa_circ_0140588	DIRKKFLR																																			



**e**

Host gene	circRNA	Peptide	2287				2288				2289				2290				2671				2672				3023				3421						
			H	T	T	T	T	H	T	T	T	T	H	T	T	T	T	H	T	T	T	T	H	T	T	T	T	H	T	T	T	T	H	T	T	T	T
LDLR	hsa_circ_0049380	KPRAIVDPVHGFG																																			
RBM10	hsa_circ_0090380	TADAGYAILEKKASQG																																			

**Supplementary Figure 9. Detection of circRNA-BSJ derived peptides across multi-region lung cancer and healthy tissues in eight patients.** (a) Identification of HLA-I peptides across the samples. (b) Workflow used to explore HLA-II immunopeptidomics DDA and DIA MS data of 8 lung cancer patients, including 52 tumoral and healthy matched tissues. MS raw files were analyzed by a DDA-DIA approach with FragPipe, applying a group-specific FDR threshold of 0.03 for protein-derived and circRNA-derived peptides groups. circRNA-derived peptides were blasted against a larger Reference Proteins database to re-assign matching peptides to the protein (PC) group. Schema was partially created with BioRender.com. (c) Unique peptide count and (d) length distribution of the protein-derived HLA-II immunopeptidome in healthy (H) and tumor (T) tissues. (e) Identification of circRNA-BSJ derived HLA-II peptides across samples. Black square: detected peptide; white square: not detected peptide; grey square: no data. Source data are provided as a Source Data file.

**Supplementary Table 1.** List of the synthetic heavy-labelled peptides correspondent to the validated targets.

Host gene	Peptide Sequence	Nterm	Cterm	MW	Cell line	#Cells	Spike-in (pmol/uL)
<i>CDC73</i>	TTENIPV <b>V</b> RR	[H]	[OH]	1194.284	T1185B	2x10 <sup>8</sup> cells	1
<i>CTNNB1</i>	RVFEVYHTT <b>V</b> LK	[H]	[OH]	1499.6896	T1185B	1x10 <sup>8</sup> cells	1
					Mel-1	1x10 <sup>8</sup> cells	1
<i>CTSB</i>	STNGIPRGR	[H]	[OH]	967.06	T1185B	1x10 <sup>8</sup> cells	0.5
<i>GAS7</i>	EAWNGPPS <b>A</b> GR	[H]	[OH]	1151.1312	T1185B	1x10 <sup>8</sup> cells	0.5
<i>KIAA0226</i>	SSITGLSV <b>T</b> R	[H]	[OH]	1030.0738	T1185B	1x10 <sup>8</sup> cells	0.5
<i>LINC00221</i>	EAAAAFR <b>F</b> PR	[H]	[OH]	1145.3	T1185B	1x10 <sup>8</sup> cells	0.5
<i>RFWD2</i>	DLYNGSSIV <b>S</b> R	[H]	[OH]	1220.31	T1185B	2x10 <sup>8</sup> cells	1
<i>PPP2R3A</i>	KVSLSF <b>T</b> EK	[H]	[OH]	1046.1452	Mel-1	1x10 <sup>8</sup> cells	1
<i>TOPBP1</i>	KSLAAELL <b>V</b> LK	[H]	[OH]	1192.4211	Mel-1	1x10 <sup>8</sup> cells	1
<i>ZMYM4</i>	RVVVSWIQ <b>K</b>	[H]	[OH]	1122.2923	Mel-1	1x10 <sup>8</sup> cells	1
<i>CDC73</i> (linear mRNA)	ATENIPV <b>V</b> RR	[H]	[OH]	1164.2577	T1185B	2x10 <sup>8</sup> cells	1
	SVTEGAS <b>A</b> R	[H]	[OH]	886.8448	T1185B	1x10 <sup>8</sup> cells	1

Note: Position of the heavy-labelled amino acid is indicated in bold.

**Supplementary Table 2.** List of oligos used for divergent PCR/ Sanger Sequencing.

Host gene	circRNA	Forward Oligo	Reverse Oligo	PCR product (bp)	Sequencing Oligo
<i>CDC73</i>	<i>hsa_circ_0111569</i>	GCACCTGTGGATCCCACTTT	ATACTTGCCGATGTTGACGC	239	ATACTTGCCGATGTTGACGC
<i>CTNNB1</i>	<i>hsa_circ_0003137</i>	GGAGCTAAAATGGCAGTGCG	ACTCCATCAAATCAGCTTGAGTA	211	GGAGCTAAAATGGCAGTGCG
<i>CTNNB1</i>	<i>hsa_circ_0004030</i>	CCACCCTGGTGCTGACTATC	ACTCCATCAAATCAGCTTGAGTA	209	ACTCCATCAAATCAGCTTGAGTA
<i>ETV5</i>	<i>hsa_circ_0068440</i>	GGACACAGATCTGGCTCACG	ATGGTGCTTTCAGCGTCTCT	204	GGACACAGATCTGGCTCACG
<i>LINC00221</i>	<i>hsa_circ_0101415</i>	TATGTGGTACAGGGTTGGGC	CAATGGGACTCGCCTTCAGT	248	TATGTGGTACAGGGTTGGGC
<i>LINC00221</i>	<i>hsa_circ_0101416</i>	AGCGGGGACTTCCTTTATGTG	GAATACAAGGCCGGGTCAGG	239	AGCGGGGACTTCCTTTATGTG
<i>PKNOX1</i>	<i>hsa_circ_0116109</i>	TCTGCAGTCGATACATTGCTTG	TGCATCTGTTGCCCATCTTGA	210	TGCATCTGTTGCCCATCTTGA
<i>PKNOX1</i>	<i>hsa_circ_0116112</i>	CCACGCCCGTGAACATGAAC	GCATCGGGTTCAGAGCAGTT	223	GCATCGGGTTCAGAGCAGTT
<i>PTPRG</i>	<i>hsa_circ_0066406</i>	GCTGGTCTACCTGGCAGATTC	TCGAAGCCATCGAGTTGCAG	244	TCGAAGCCATCGAGTTGCAG
<i>RFWD2</i>	<i>hsa_circ_0015366</i>	CTGCAAGCCAGTTGGATGAA	TGGATCTGTTCCAGTTGCTCTC	210	CTGCAAGCCAGTTGGATGAA
<i>RFWD2</i>	<i>hsa_circ_0111262</i>	CAGATGACAGTCGAACTGCAAG	TCAAGACTAGGATGCCCCGC	194	CTGCAAGCCAGTTGGATGAA
<i>TOPBP1</i>	<i>hsa_circ_0121989</i>	GTGTGACTGGCTTATGTGGC	GATGTTCCGGCTCTGGGACA	205	GTGTGACTGGCTTATGTGGC