Cell Reports, Volume *39*

Supplemental information

Single-cell RNA-seq-based proteogenomics identifies glioblastoma-specific transposable elements encoding HLA-I-presented peptides

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Supplementary 1

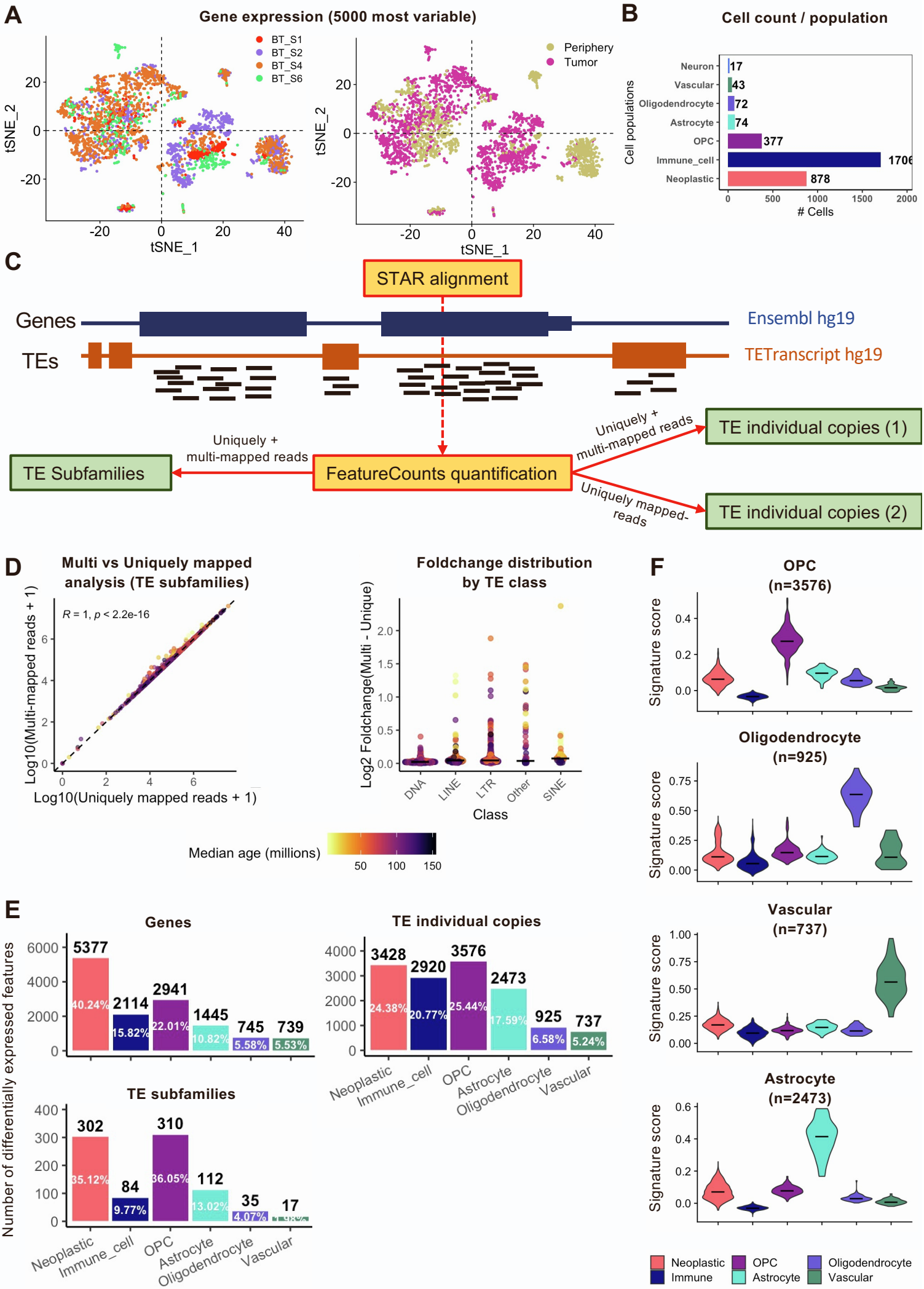


Figure S1. General description of single cel dataset, workflow, genes and TE signatures, Related to Figure 1. (A) t-Distributed Stochastic Neighbor Embedding (tSNE) visualizing all single cells after filtering (n = 3,167), colored by patient ID (left) or location (tumor core and surrounding tissue, right). (B) Barplot showing the number of cells in each cell population. (C) Workflow showing the strategy of alignment and TE quantification using uniquely or multiple mapped reads. (D) On the left, plots displaying the correlation of expression in each subfamily between the quantification with uniquely mapped reads (x-axis) and multiple mapped reads (y-axis). On the right, the log2foldchange between multiple mapped reads vs uniquely mapped read quantification. Each dot represents a TE subfamily and a scale color is used to show the median of age of TEs within each subfamily. (E) Barplots showing the number of differentially expressed genes (top left), TE subfamilies (bottom left) and TE individual copies (right) in each cell population. (F) Violin plots representing the signature score for OPC, Astrocyte, Oligodentrocyte, and Vascular cell populations based on their differentially expressed TEs. The number of differentially expressed TEs included in each signature is indicated.

Supplementary 2

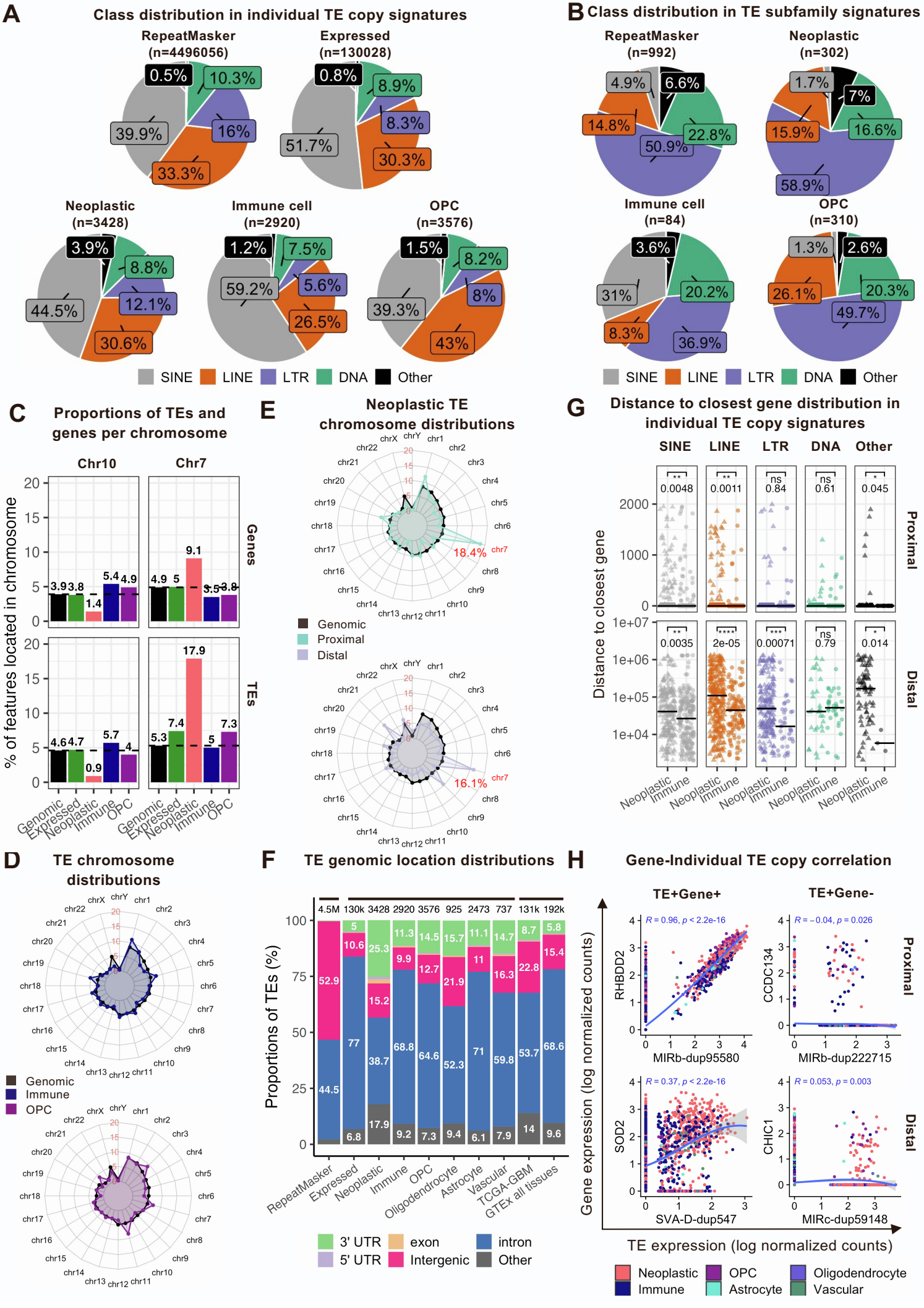
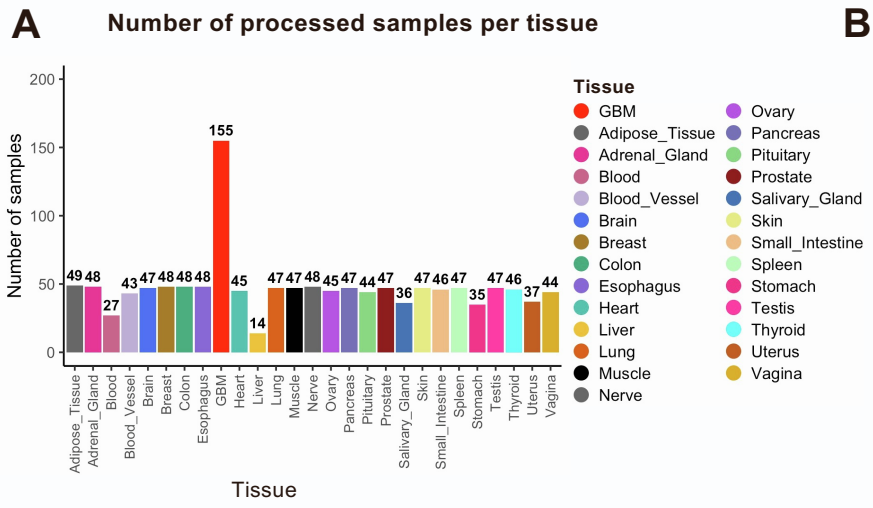
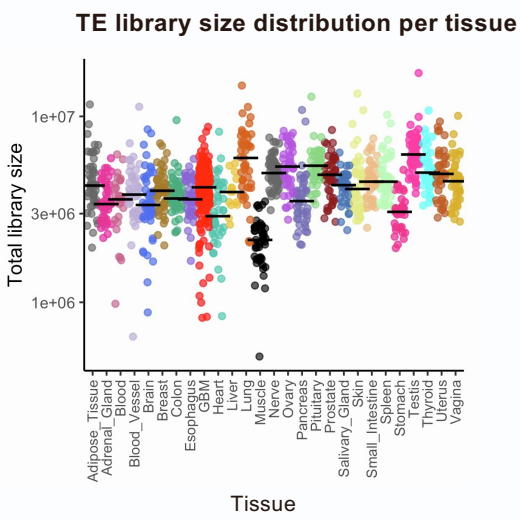
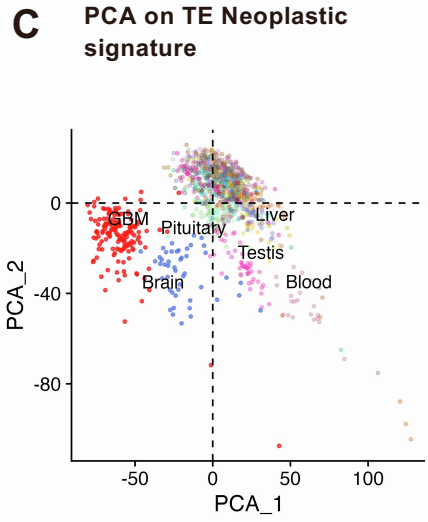


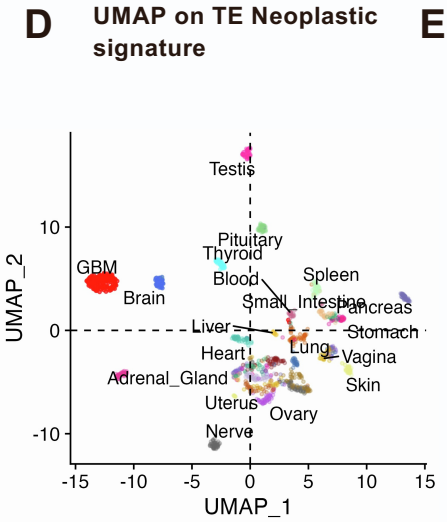
Figure S2. TE class, chromosome and genomic location distributions observed among single cell signatures, Related to Figure 1. (A-B) Pie charts showing TE class distributions within individual TE copy (A) or TE subfamily signatures (B) as compared to class distribution observed in the genome (RepeatMasker) or in all filtered expressed TEs (Expressed). (C) Barplots showing the rate of genes (first line) or TEs (second line) located in chromosome 10 (left) or 7 (right) on different subsets of features: All annotated features in the genome (Genomic), all expressed features in the data set after filtering (Expressed), all differentially expressed features from neoplastic, immune and OPC cell populations. (D) Radar plots displaying the rate of TEs along all chromosomes for immune cells (top) and OPC cells (bottom). Genomic distribution from RepeatMasker is plotted in black. (E) Radar plots displaying the rate of proximal (top) or distal (bottom) neoplastic TEs along all chromosomes. Genomic distribution from RepeatMasker is plotted in black. (F) Barplot showing the distribution of different types of RefSeq genomic locations for individual TE copies within RepeatMasker, expressed TEs in all cell populations, TE signatures for each cell population and TEs expressed in bulk RNA-seq TCGA-GBM and GTEx data sets. (G) Plot showing the distance to closest protein-coding gene per class of TEs for proximal (first line) and distal (second line) TEs comparing neoplastic and immune TE-signatures. (H) Scatter plots illustrating the correlation between TE expression and their nearest genes. The $TE^{+}gene^{+}$ category (left) represents a positive correlation when the TE and gene are both differentially expressed. The $TE^{+}gene^{-}$ category (right) represents a negative correlation when the TE is differentially expressed and not the gene. The categories are also separated according to proximal (top) and distal status (bottom).

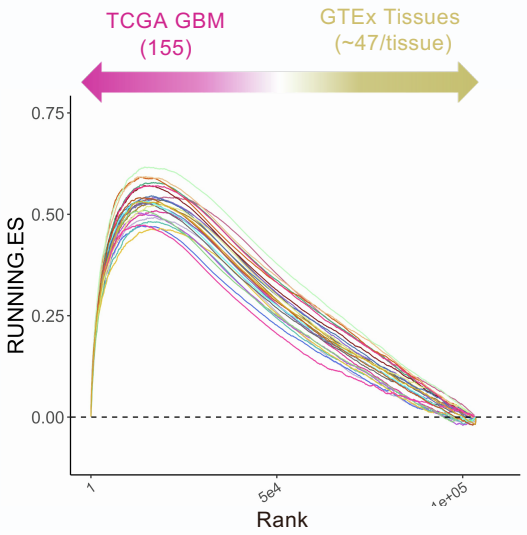
Supplementary 3











F

Tissues	FDR	NES
Adipose_Tissue	0.012	1.76
Adrenal_Gland	0.018	1.71
Blood	0.0039	1.87
Blood_Vessel	0.028	1.71
Brain	0.027	1.67
Breast	0.0068	1.77
Colon	0.0033	1.93
Esophagus	0.0082	1.82
Heart	0.025	1.69
Liver	0.029	1.66
Lung	0	1.93
Nerve	0.0097	1.7
Ovary	0.036	1.66
Pancreas	0.02	1.75
Pituitary	0.034	1.64
Prostate	0.0025	1.87
Salivary_Gland	0.0051	1.75
Skin	0.0036	1.73
Small_Intestine	0.0013	1.86
Spleen	0	1.85
Stomach	0.0047	1.8
Testis	0.039	1.6
Thyroid	0.023	1.68
Uterus	0.0075	1.77
Vagina	0.0074	1.76

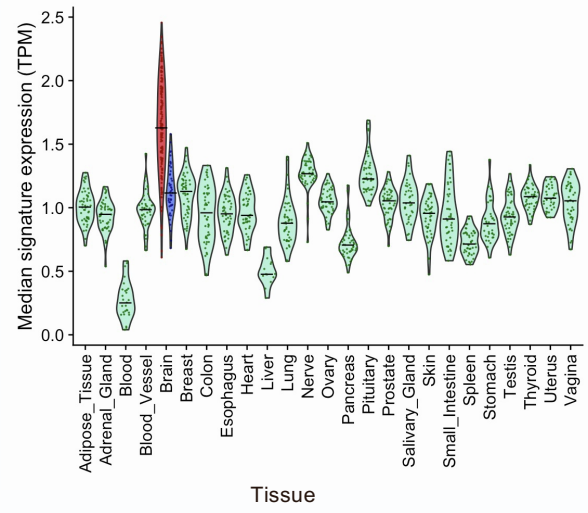
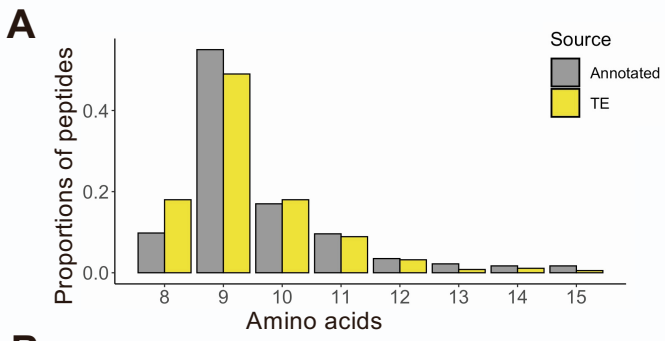
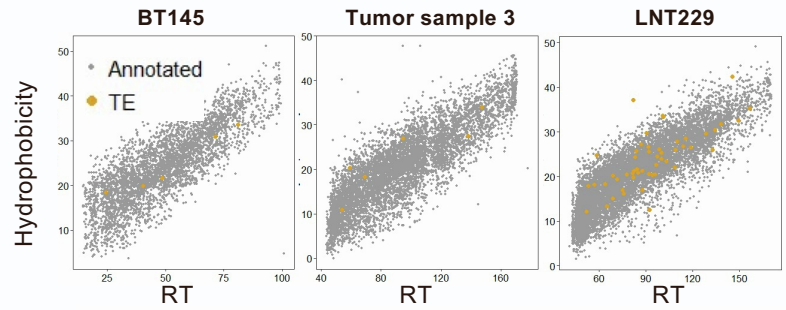
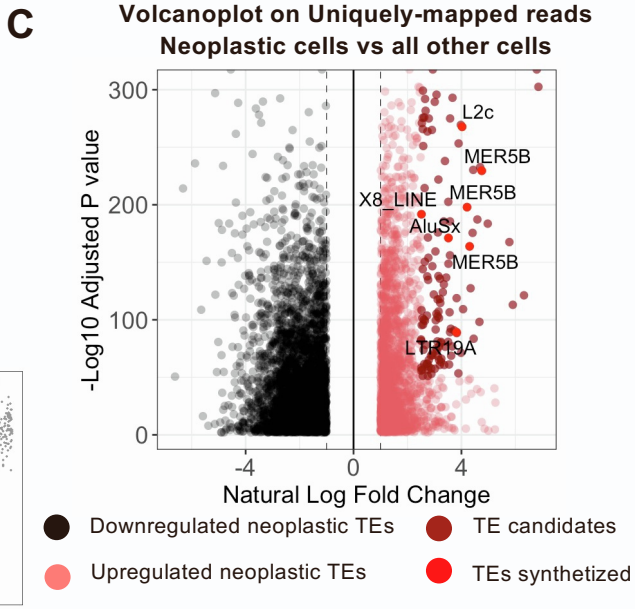


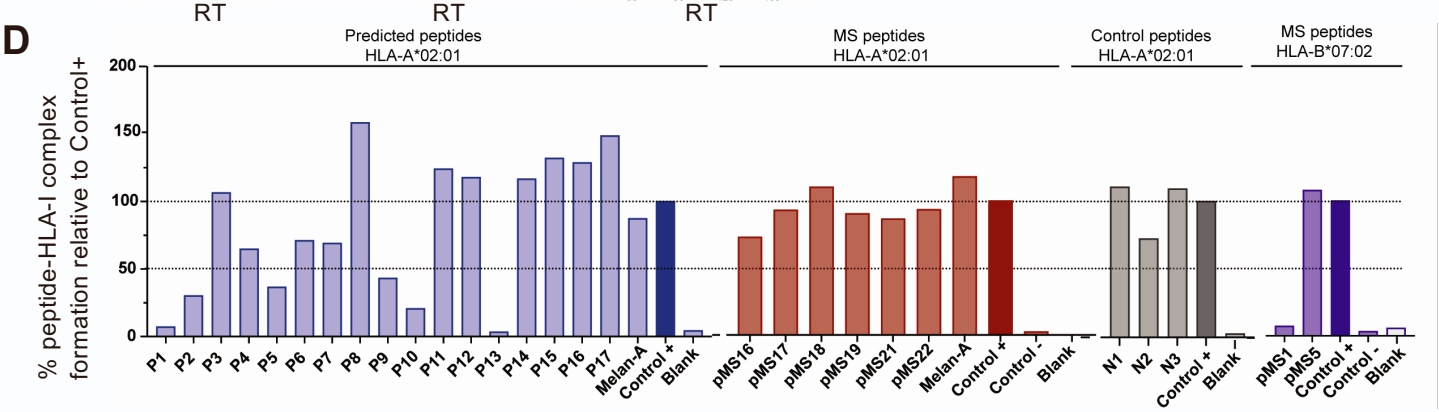
Figure S3. Validation of neoplastic TE-signature using GTEx and TCGA cohorts, Related to Figure 2. (A) Barplot showing the number of samples integrated in each condition (B) Plot representing the TE library size per data set types and tissues. (C-D) PCA and UMAP projection of TCGA. GBM tumor samples and healthy tissue samples from GTEx based on single cell neoplastic TE signature. Each point corresponds to a sample. Samples are color-coded by their tissue of origin. (E) Gene Set Enrichment Analysis (GSEA) was performed to assess the specific enrichment of the neoplastic TE-signature in TCGA GBM tumor samples compared to samples from 25 GTEx normal tissues. (F) Table showing the Normalized Enrichment Score (NES) and FDR for each tissue compared to GBM tumor. (G) Violin plots showing the median expression of single cell neoplastic signature in TCGA-GBM tumor samples and GTEx normal samples.

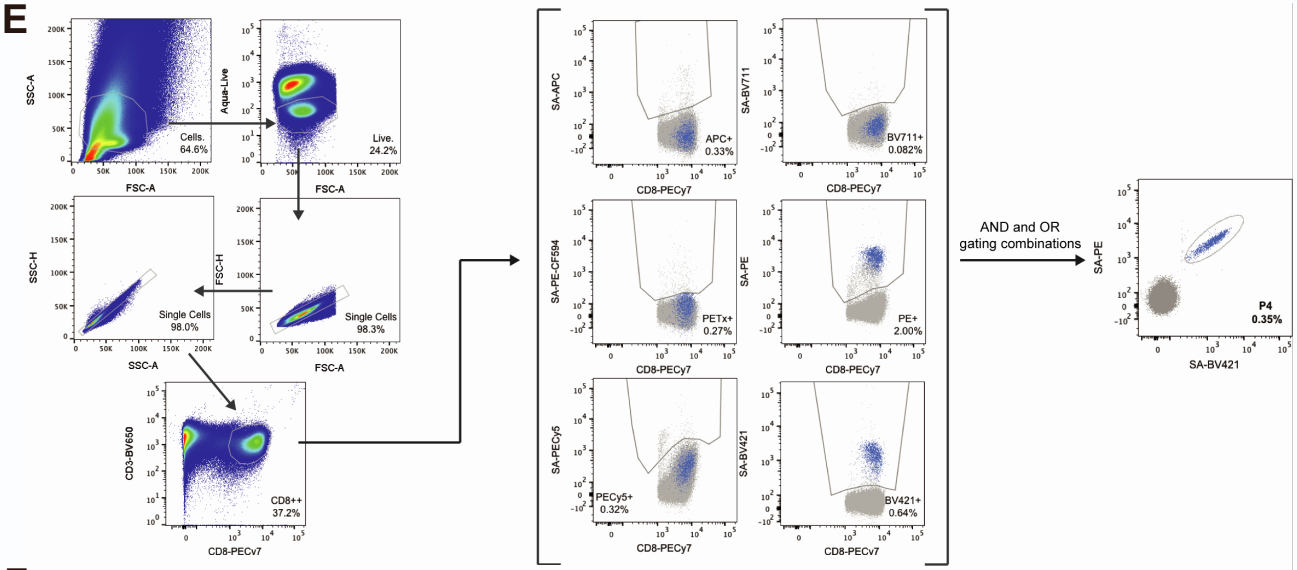
Supplementary 4











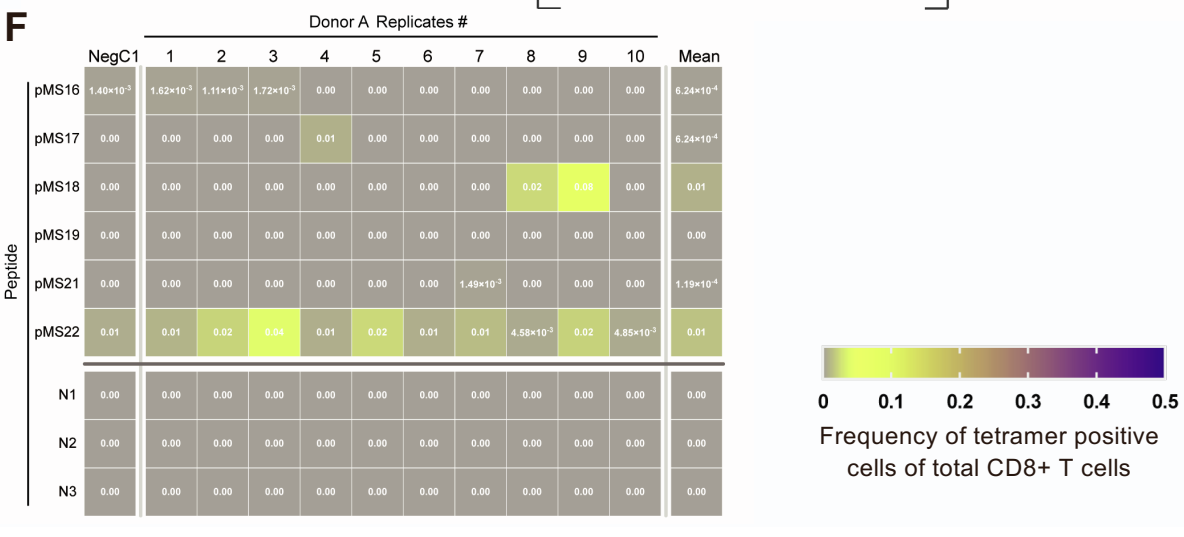


Figure S4. Quality control, strategy and validation of TE-derived peptides, Related to Figure 3. (A) Peptide length distribution (in amino acids) from annotated and TE-derived peptidomes. (B) Scatter plots comparing retention time (in minutes) and hydrophobicity index from annotated and TE-derived identifications. (C) Volcano plot displaying differential TE expression between neoplastic cells vs other cells. Up-regulated (pink) and down-regulated TEs (black) are indicated. TE candidates with low p-value (less than 1e-50) and high natural log fold change (more than 2) are indicated in dark red. TE synthetized into peptide for immunogenicity study are colored in red. (D) Binding to HLA-A02*01 and HLA-B*07:02 measured as percentage of peptide-HLA-I-complex formation compared to positive control. (E) CD8-tetramer + cells gating strategy. (F) Example of tetramer frequency analysis after *in-vitro* immunogenicity assays. Donor A and HLA-A*02:01 peptides are shown as representative example. Tetramer positive frequencies per replicate (columns) for each evaluated peptide (rows) are indicated. NegC1: negative control replicate with no peptide pulsed on the mo-DCs. Mean: total frequencies of tetramer positive populations for each peptide considering all CD8+ T cells evaluated in all replicates.

Supplementary 5

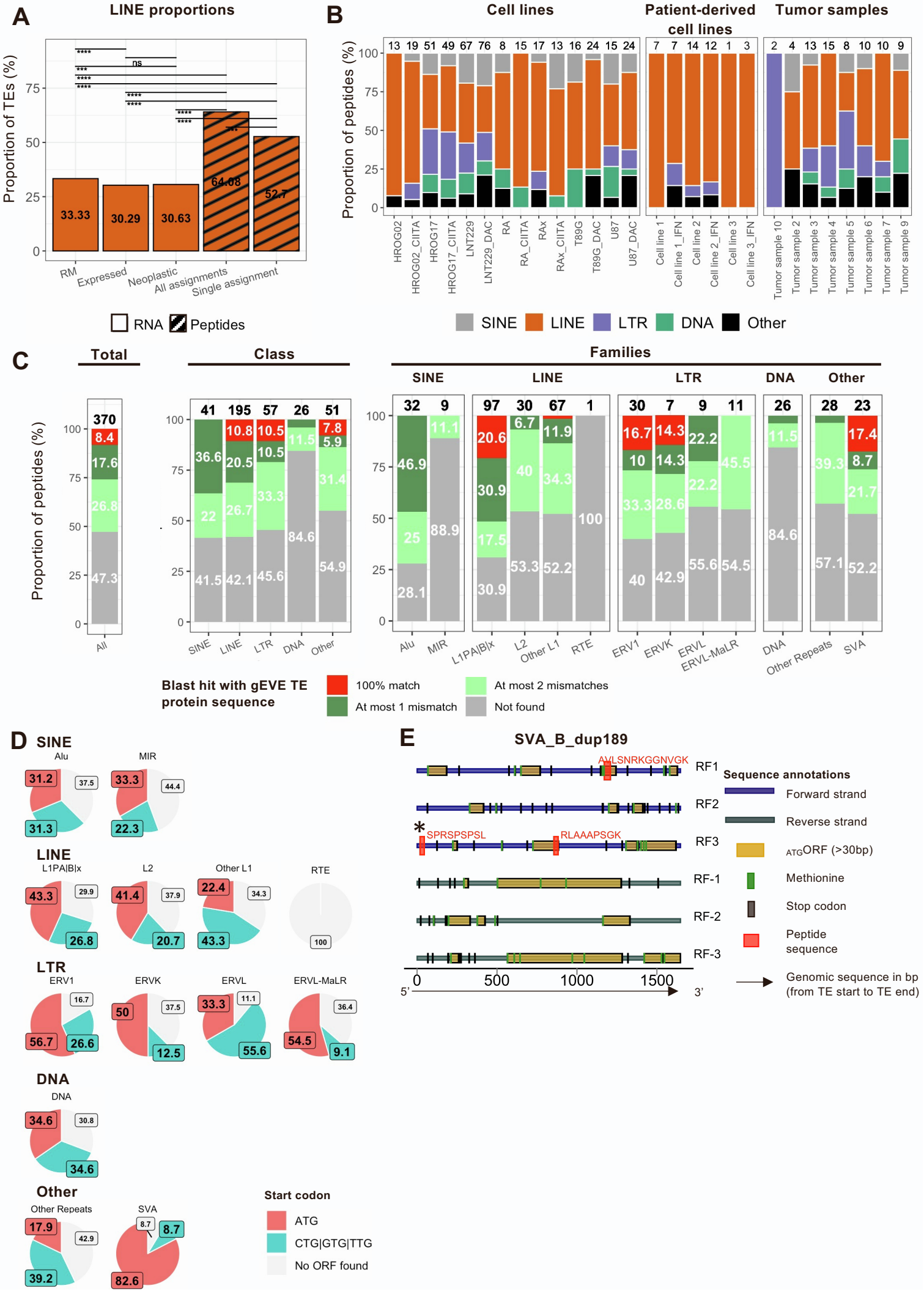


Figure S5. Characterization of TE class and genomic origin of TE-derived peptides, Related to Figure 4. (A) Barplot showing the LINE proportions at RNA level in RepeatMasker (RM), expressed TEs, TEs from neoplastic TE-signature and at peptide level using single or all assignments. (B) Barplots representing among TE-derived peptides the proportions of TE classes per sample used in immunopeptidomics. The samples were classified according to their origin (cell lines, patient-derived cell lines and tumor samples). (C) Barplots showing the percentage of peptides bearing an Endogenous Viral Element ORF documented in the gEVE database using 0 (100% match), 1 or 2 mismatches in all peptides (left panel), peptides grouped by class (middle panel) or peptides classified by TE family (right panel). (D) Pie charts showing the percentage of TE-derived peptides with canonical (red) and non-canonical codon start (blue) per class and TE family (E) Example of one peptide-coding TE: SVA_B_dup189. All reading frames (RFs) (forward strand RF1,2,3; reverse strand RF-1,-2,-3) are represented. Start codons (ATG), stop codons, identified peptides are indicated in green, black and red rectangles, respectively. Orange rectangles (ORF30) schematize ORF sequences starting with a methionine (green rectangle) and whose length is at least 30bp. The star indicates one peptide found by immunopeptidomics without detecting an ORF.

Supplementary 6

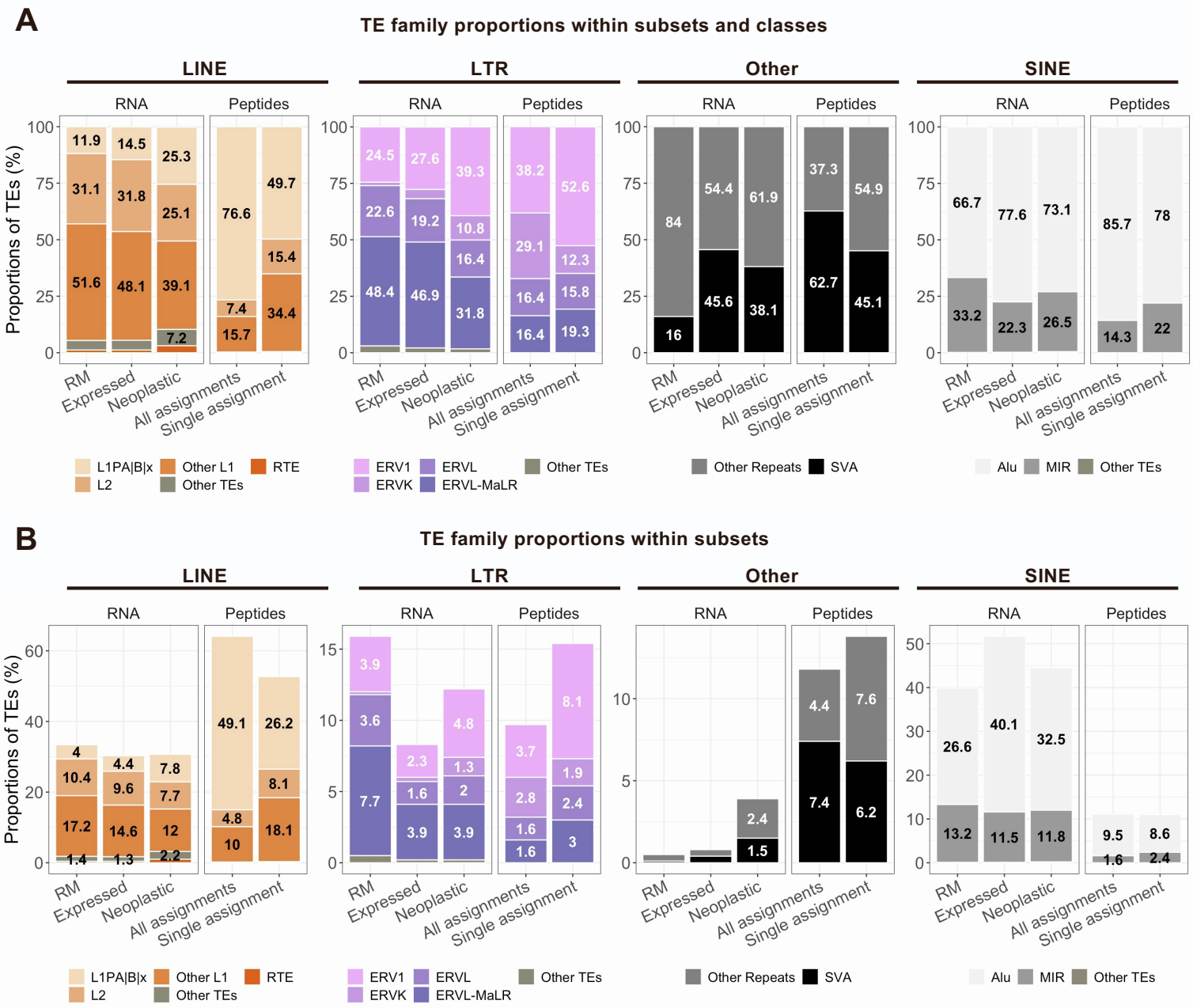


Figure S6. TE family proportions analysis at RNA and peptides levels, Related to Figure 5. (A-B) Barplots representing proportions of TE families by class (A) or globally (B) of all peptide-coding TEs identified with all or single assignment as compared to family proportions of annotated TEs in RepeatMasker, expressed TEs and TEs differentially expressed in neoplastic cells.

Supplementary 7

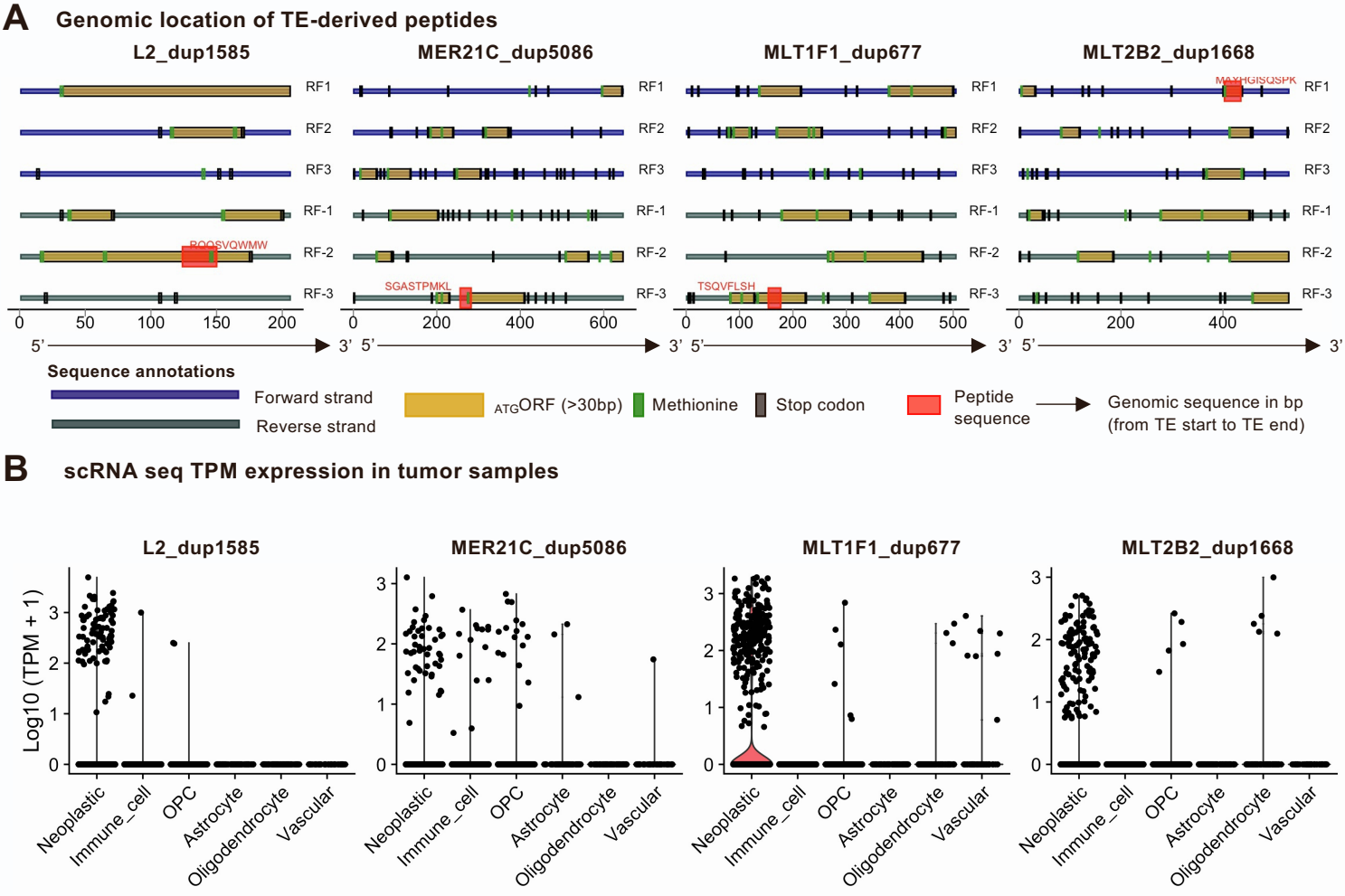


Figure S7. Examples of four TE as tumor-enriched antigens, Related to Figure 6. (A) Graphical representation showing genomic location for four examples marked with a star in (A). All RFs (sense RF1,2,3) and antisense RF-1,-2,-3) are represented. Start codons, stop codons and identified peptides are indicated in green, black and red rectangles, respectively. Orange rectangles (ORF30) schematize ORF sequences starting with a methionine (green rectangle) and whose length is at least 30bp. (B) Violin plots showing the expression in log10(TPM+1) for four peptide-coding TEs at the single cell level.

Supplementary Data S2

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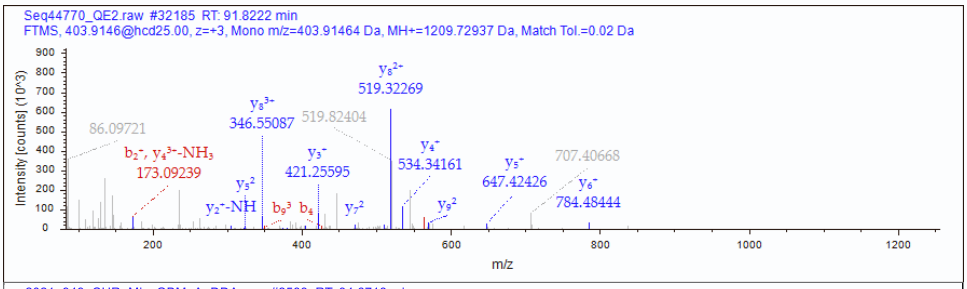

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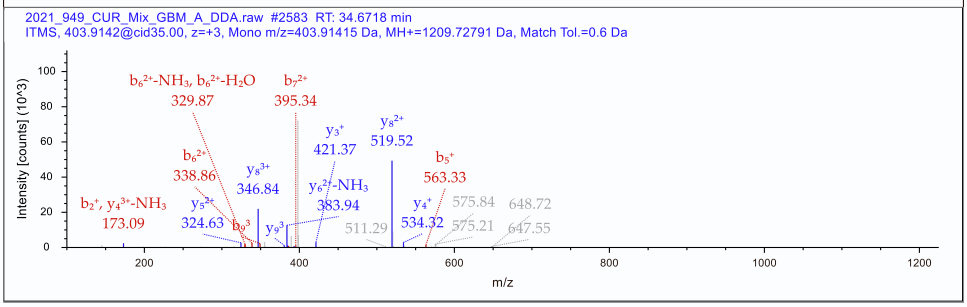

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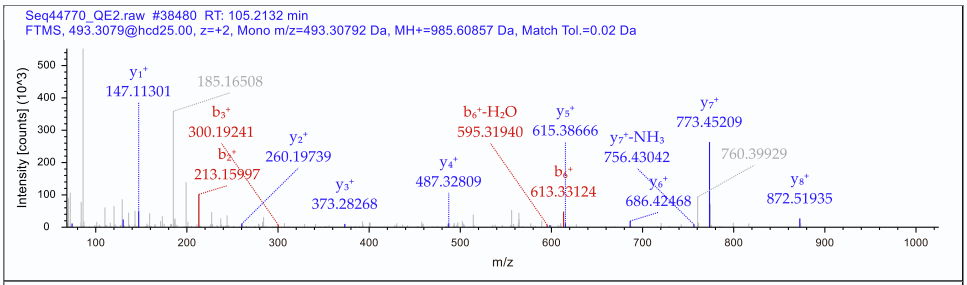

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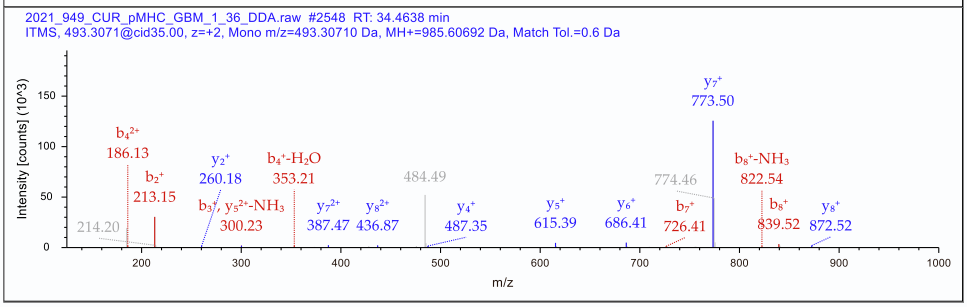

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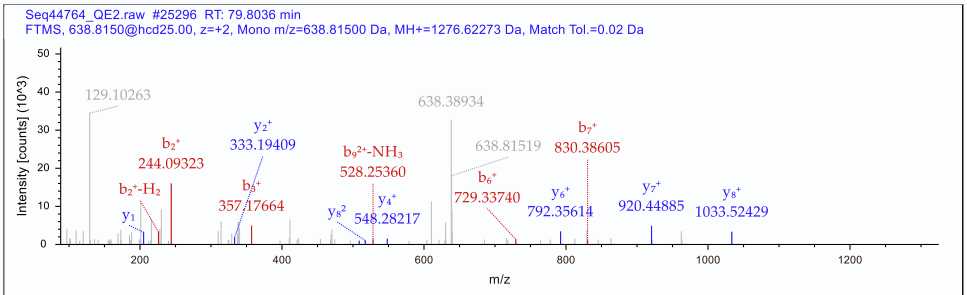

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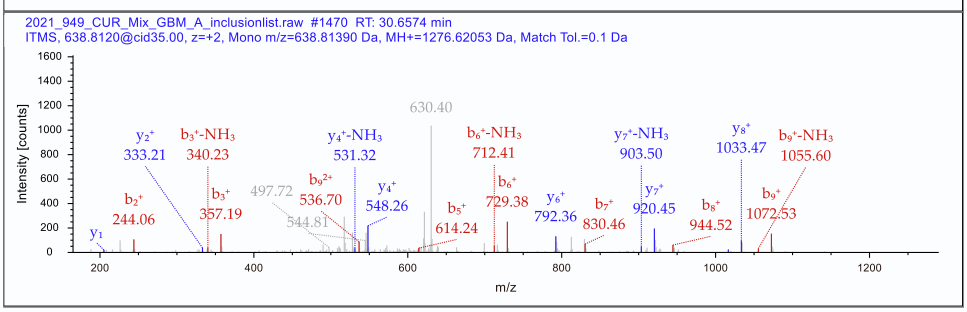

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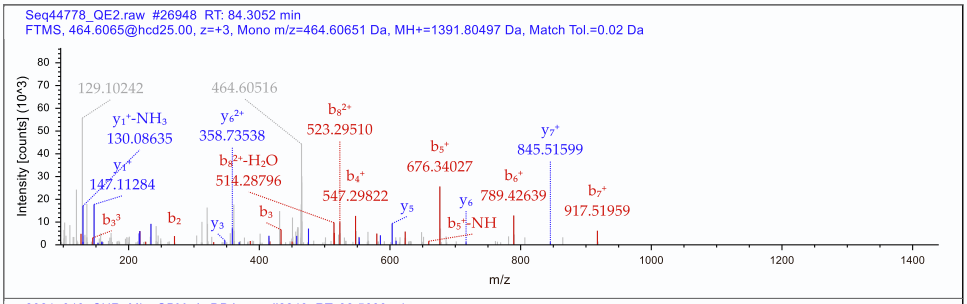

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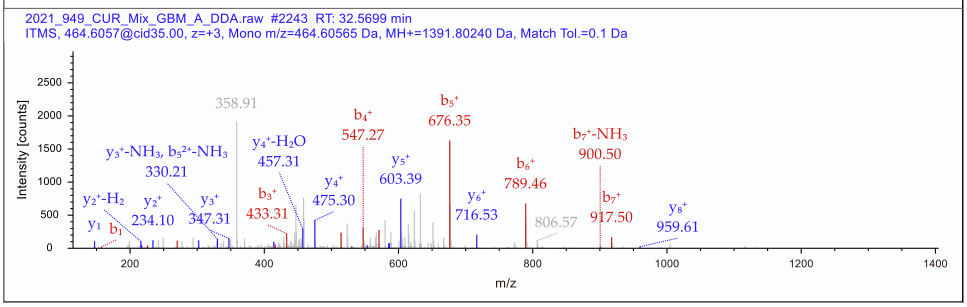

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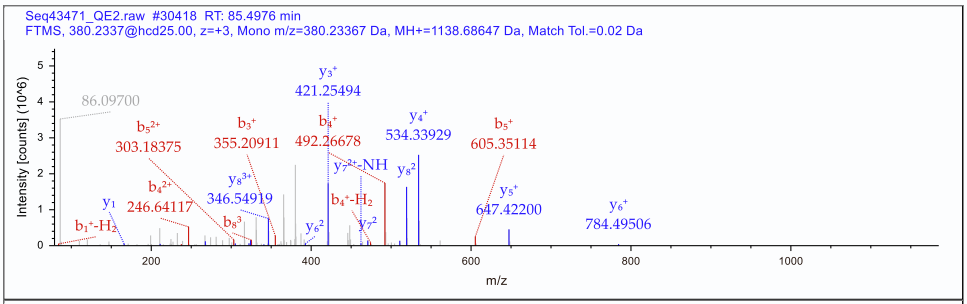

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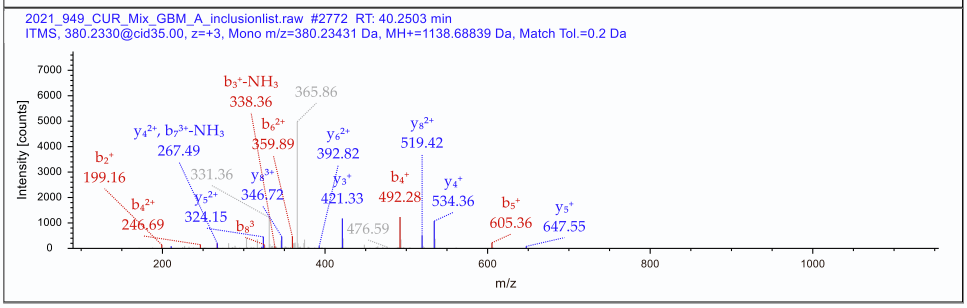

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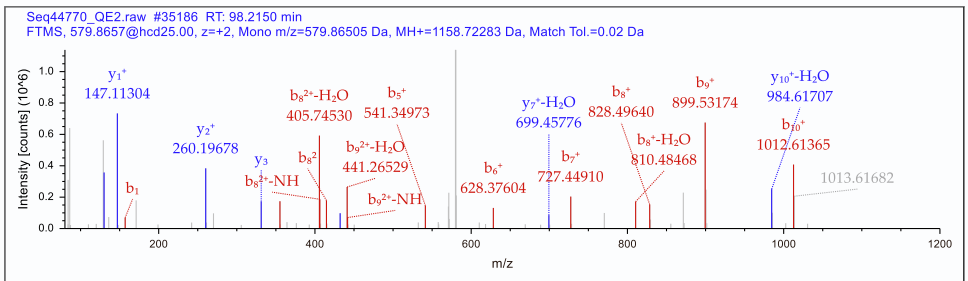

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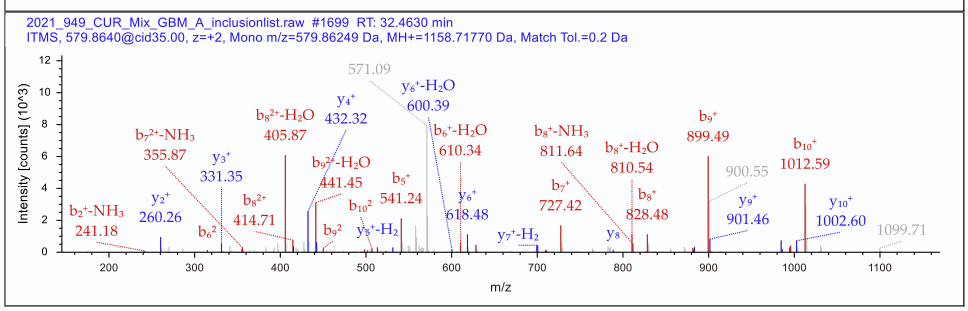

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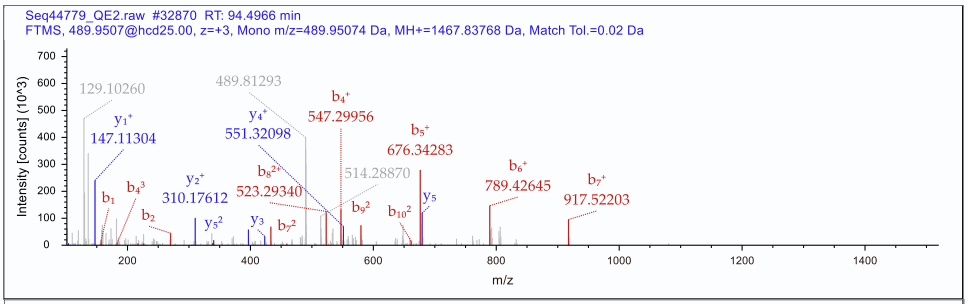

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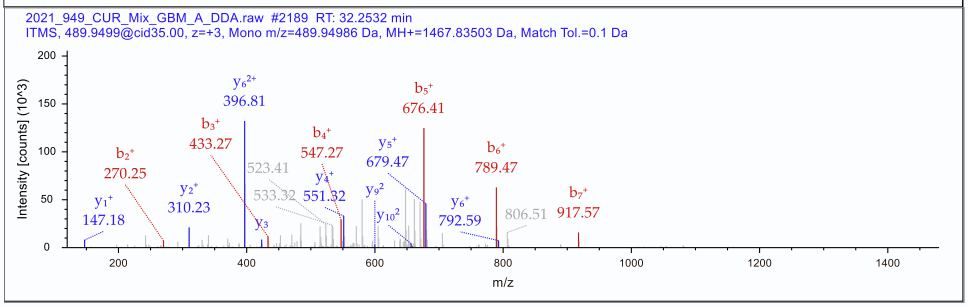

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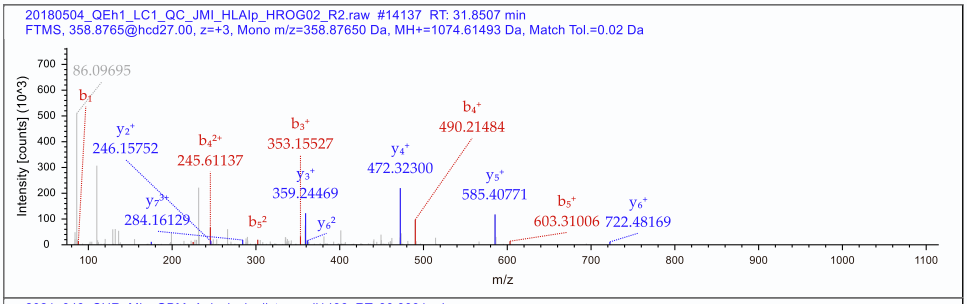

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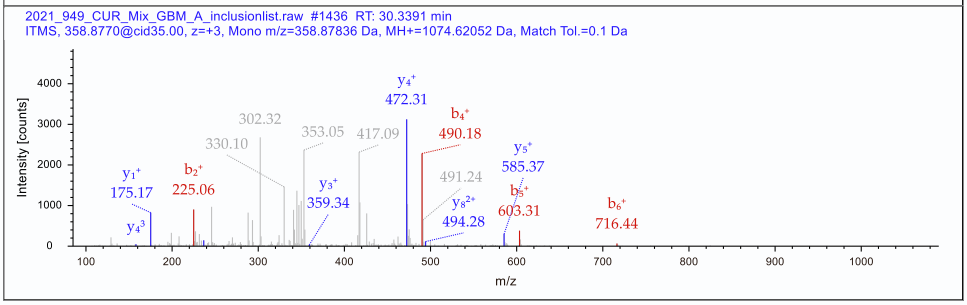

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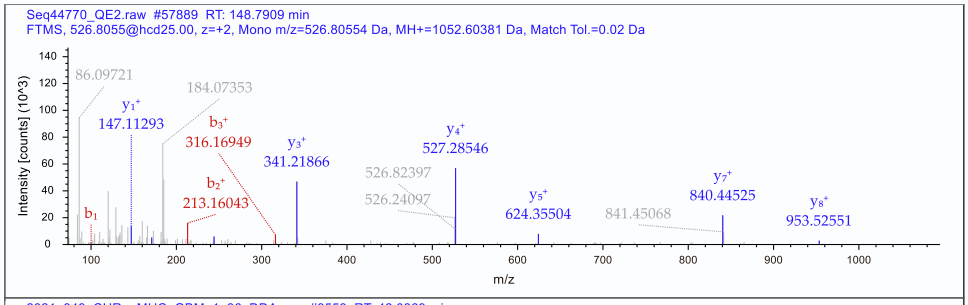

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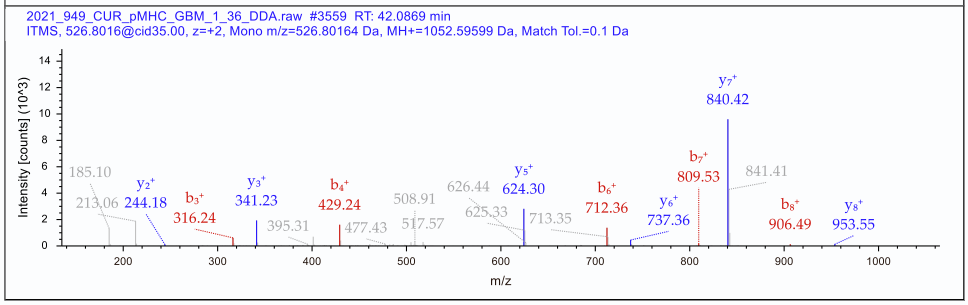

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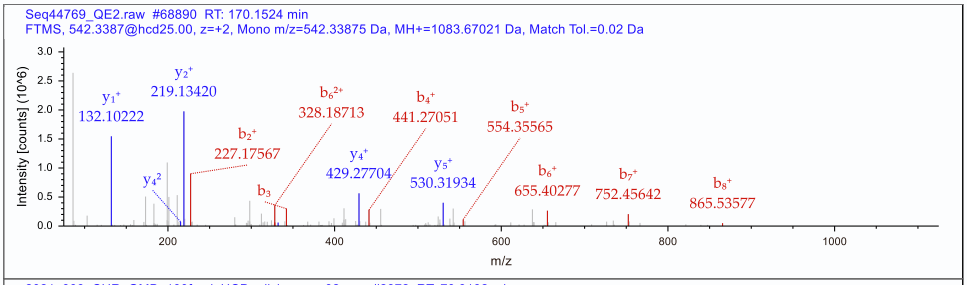

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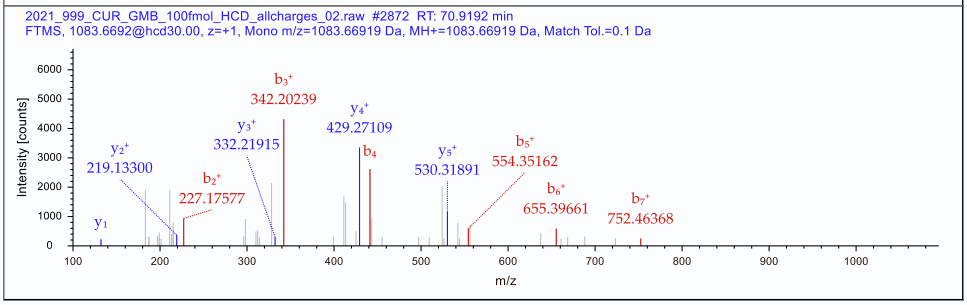

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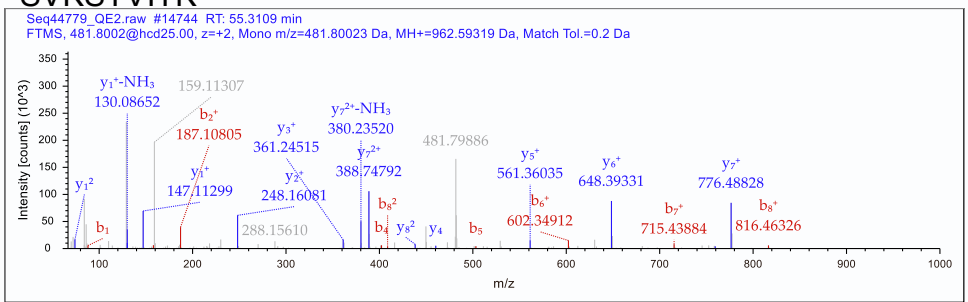

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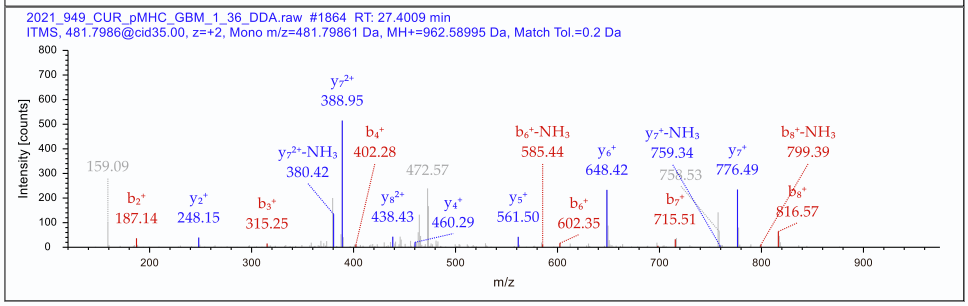

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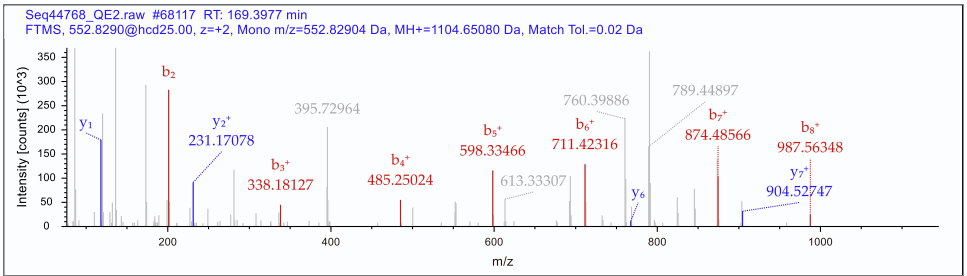

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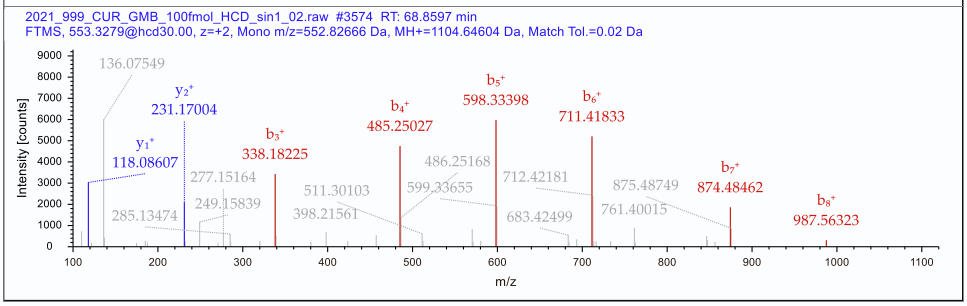

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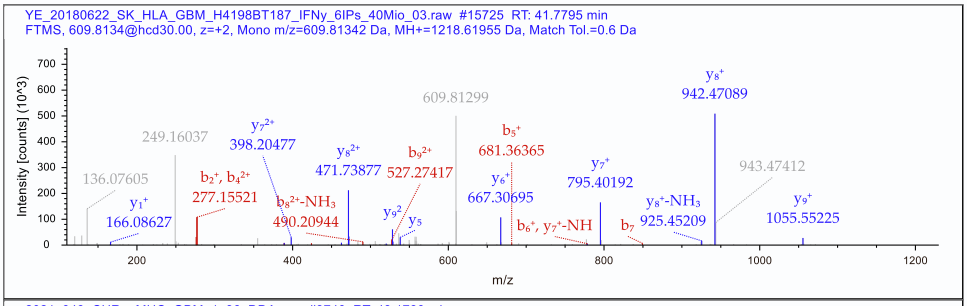

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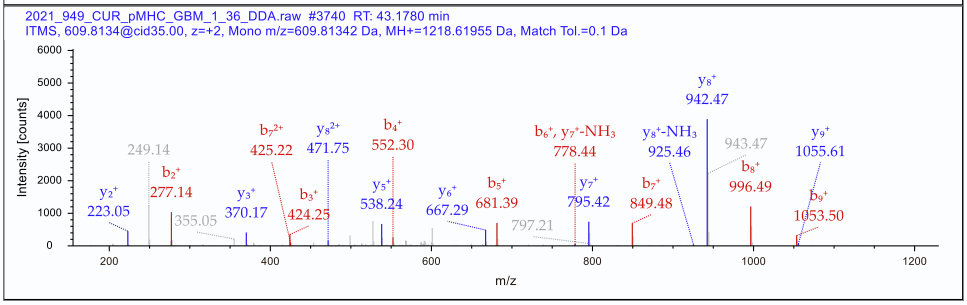

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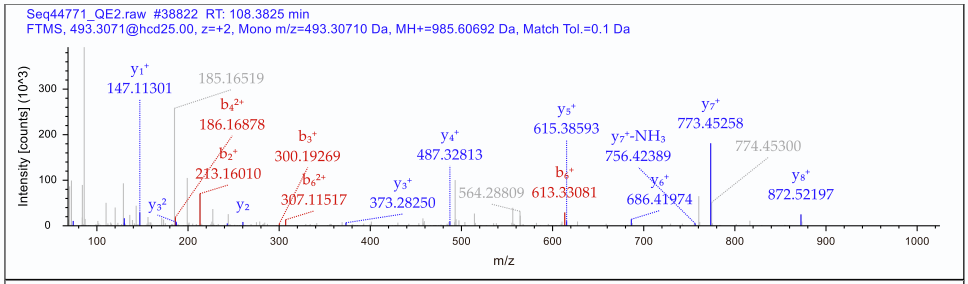
Endogenous



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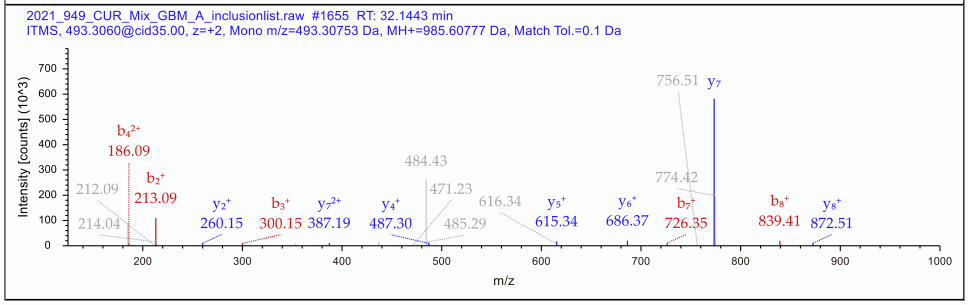

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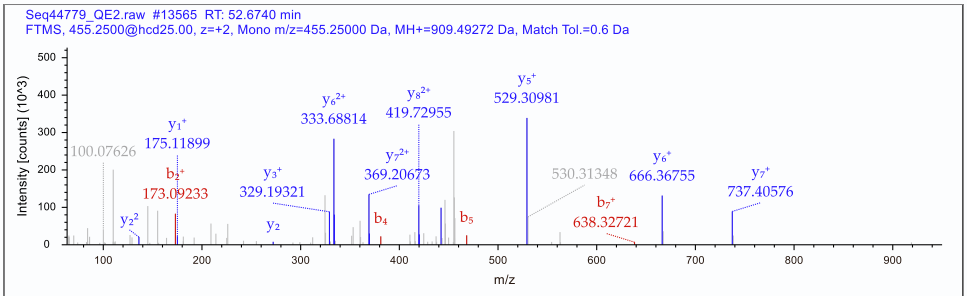

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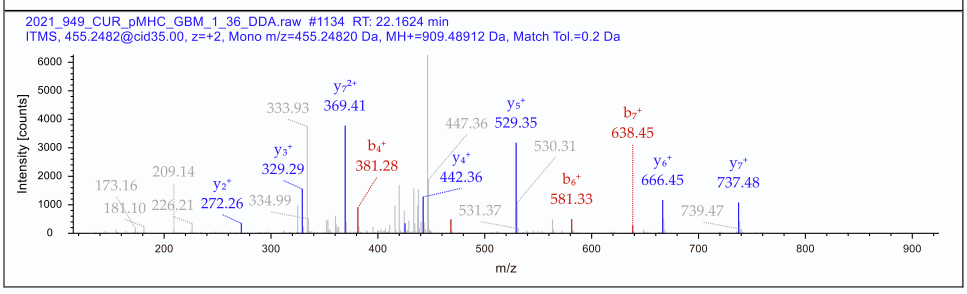

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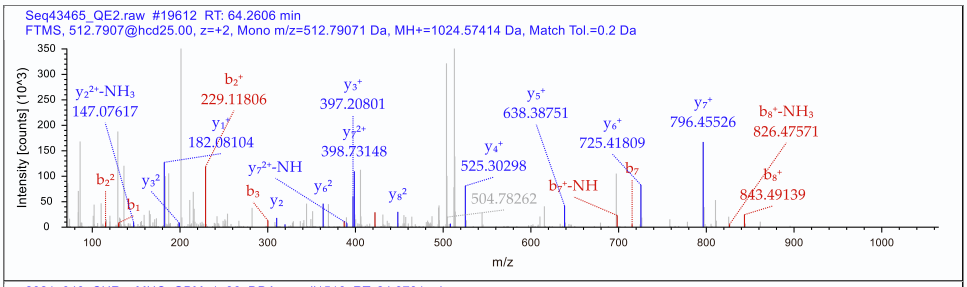

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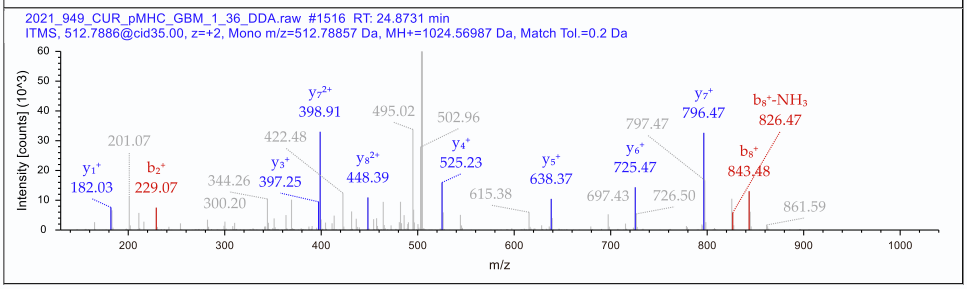

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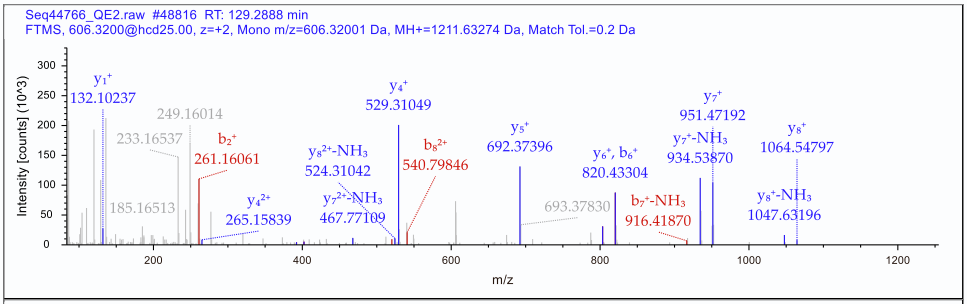

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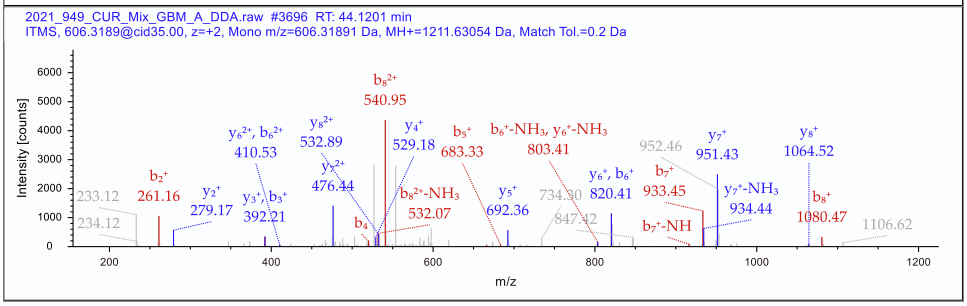

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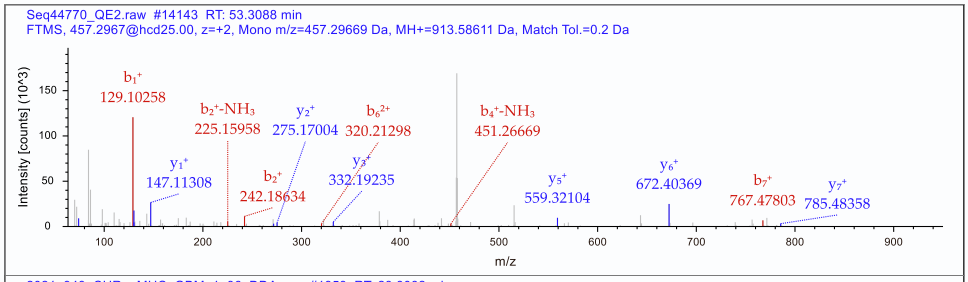

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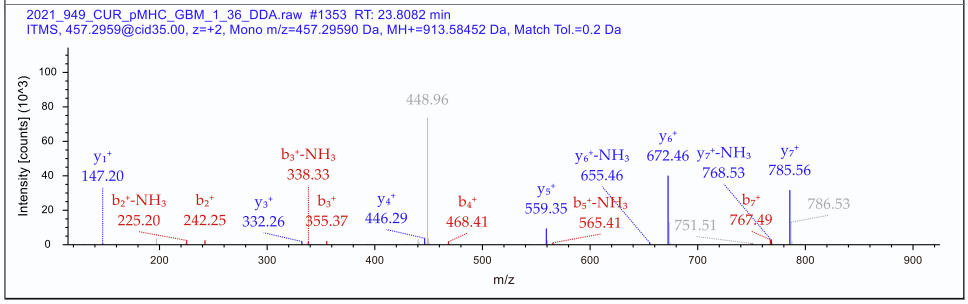

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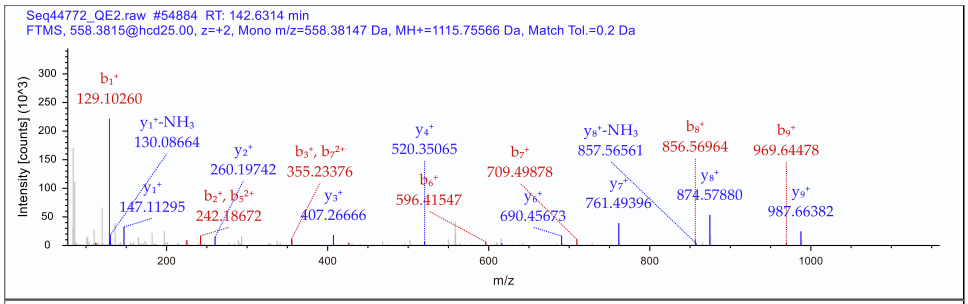

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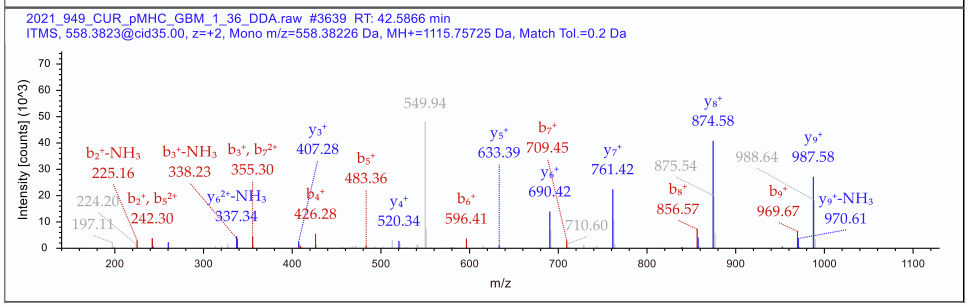

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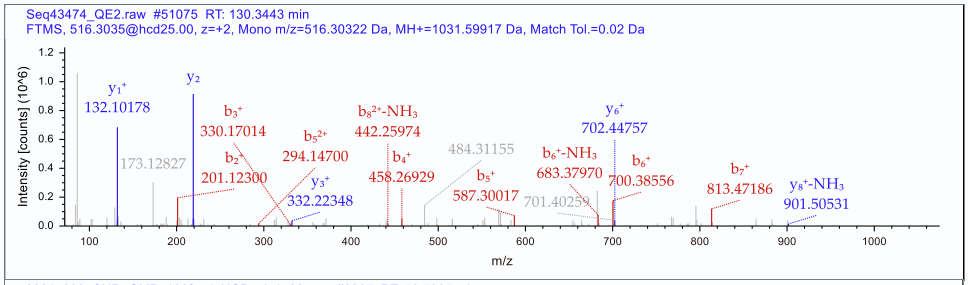

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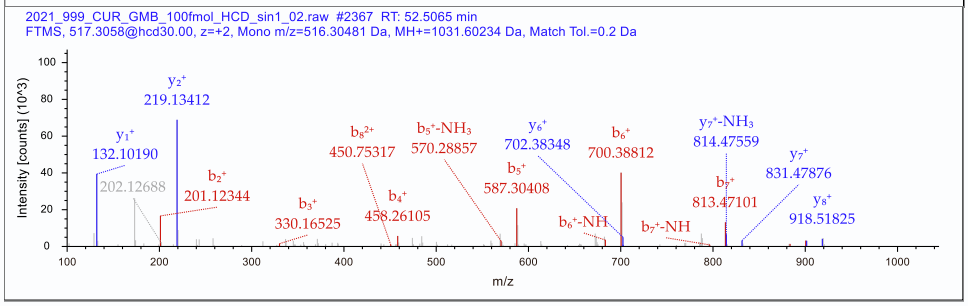

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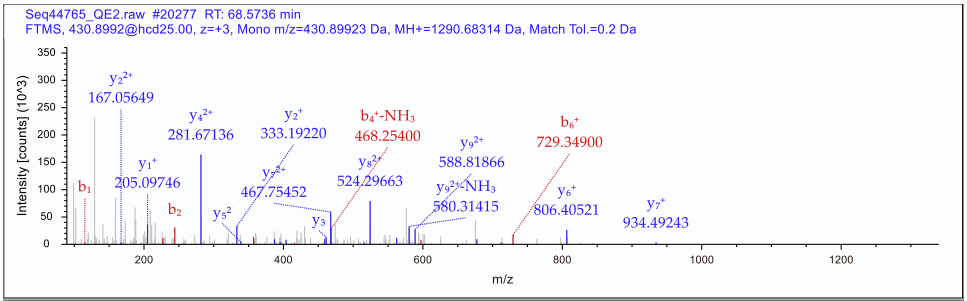

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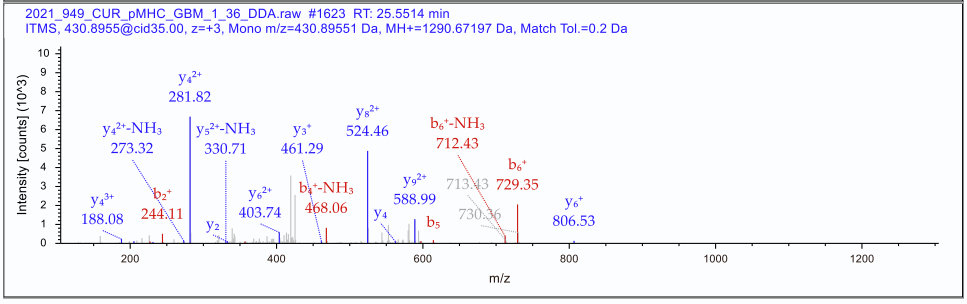

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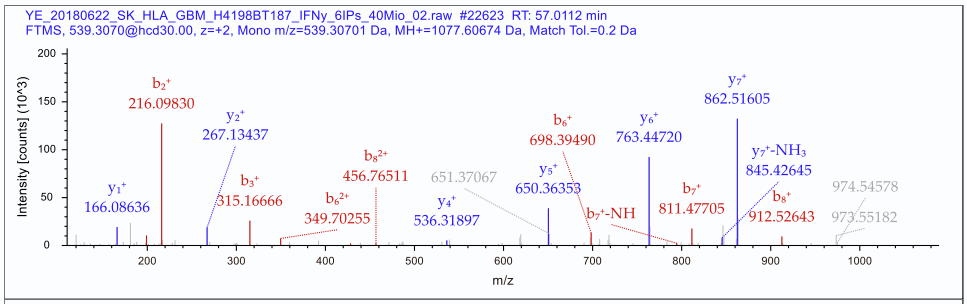

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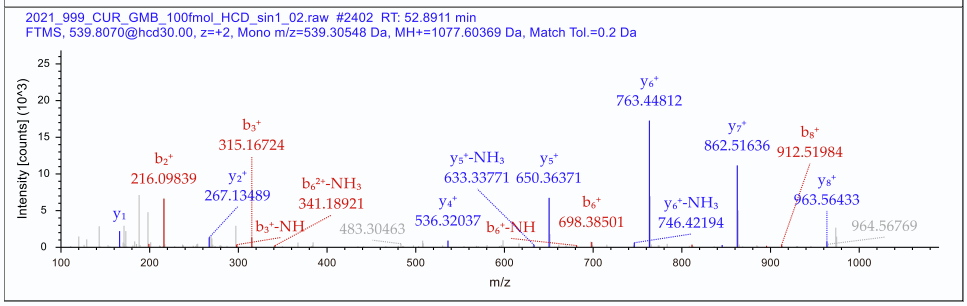
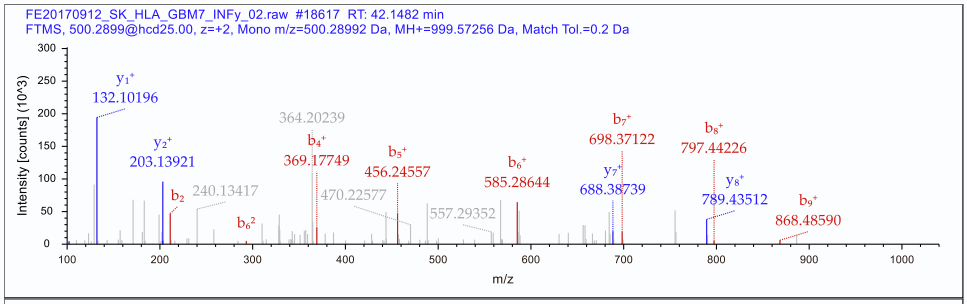


PITGSEIVAI

Endogenous



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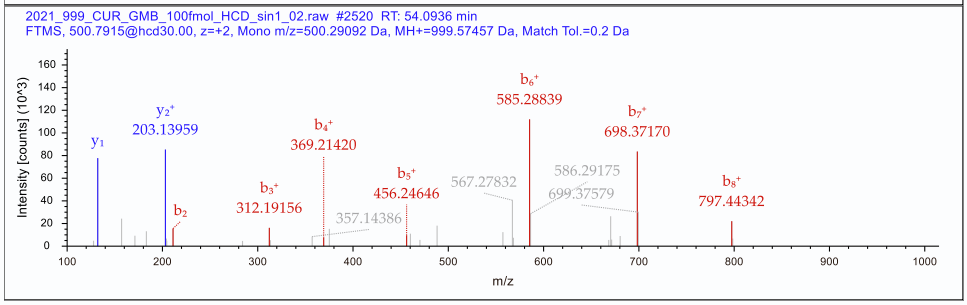

Data S2 : Spectrums from TE-derived peptides identified in glioblastoma samples, Related to Figure 3. Tumor samples (top) and their comparison with synthetic peptides (bottom) are represented. Spectrums have been extracted from Proteome Discoverer. A total of 23 TE-derived peptides have been validated after comparing the fragmentation patterns between endogenous and synthetic peptides.