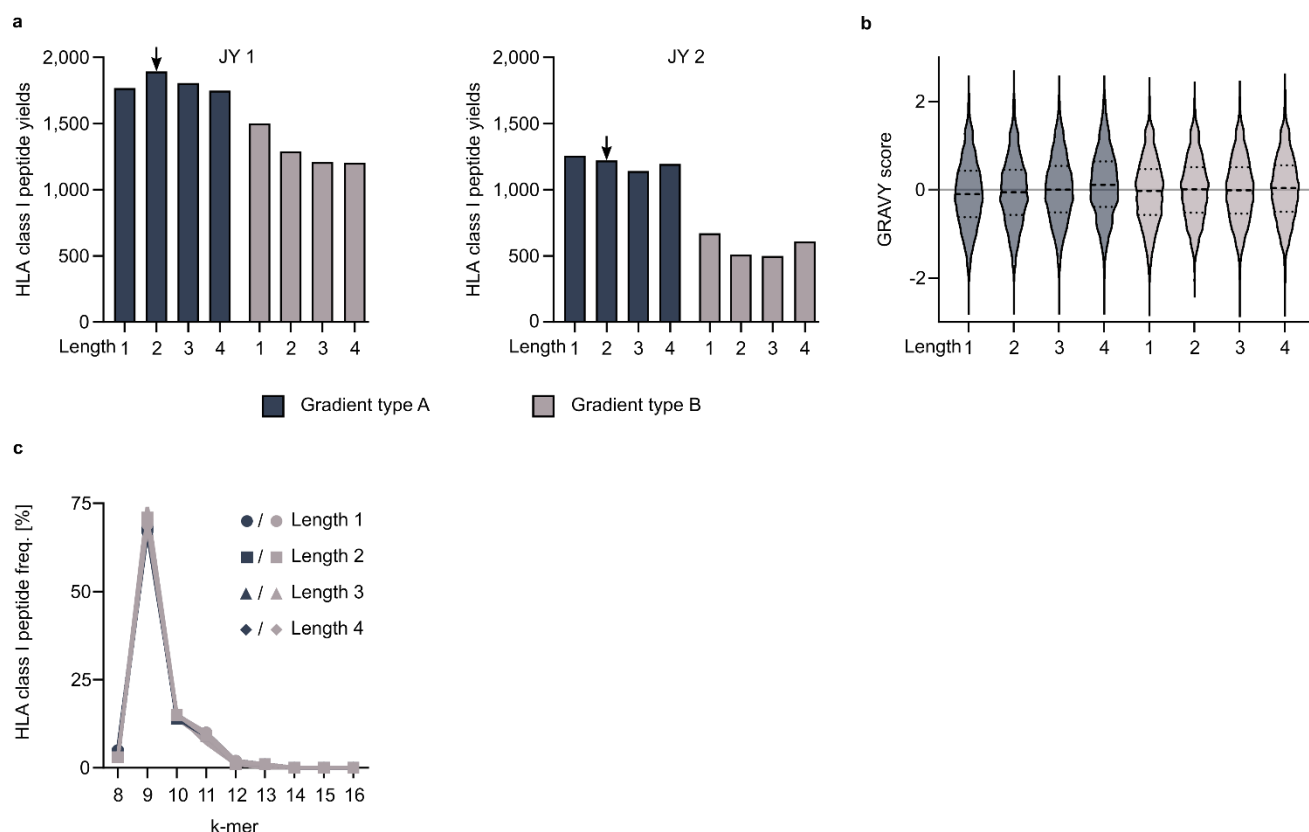
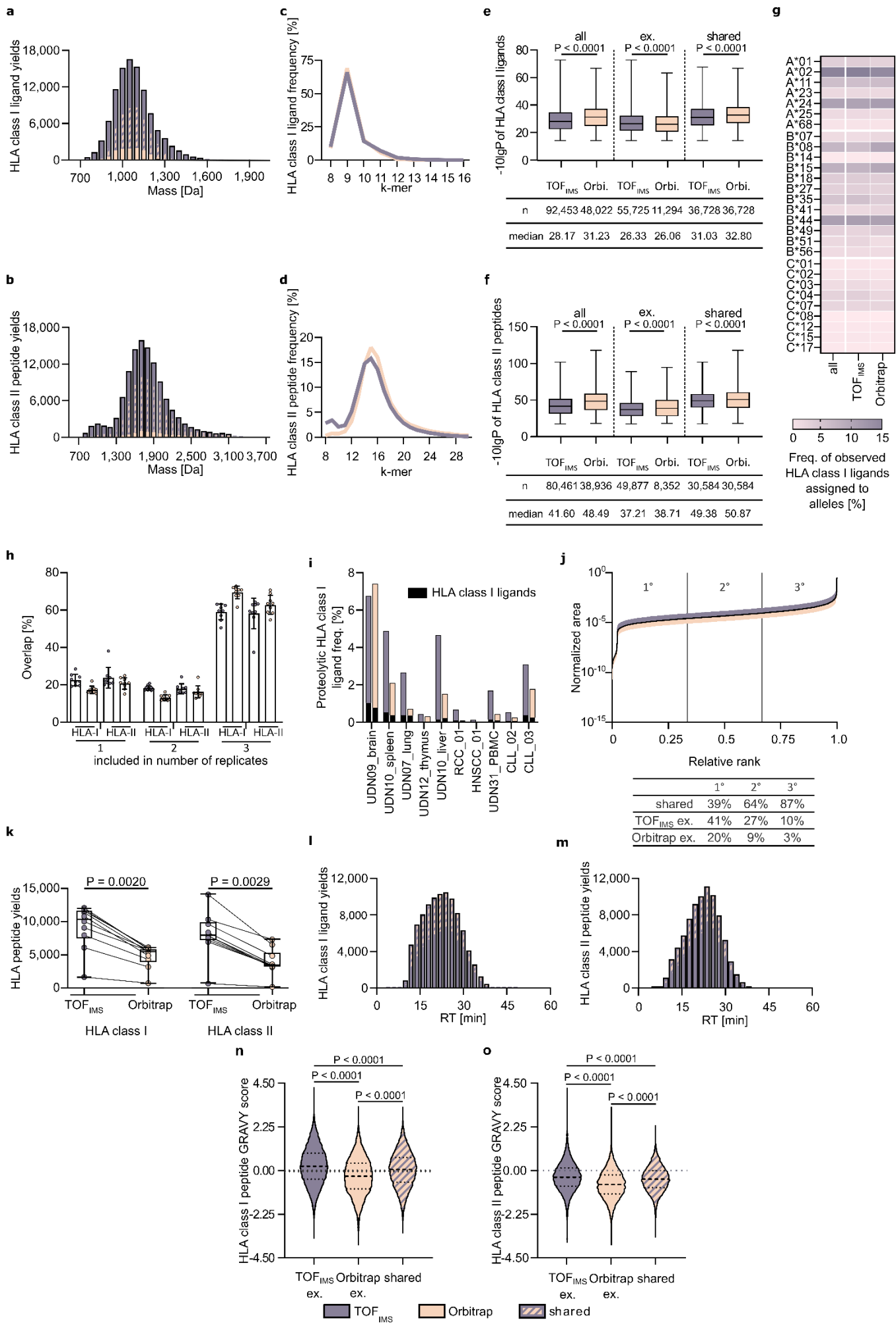
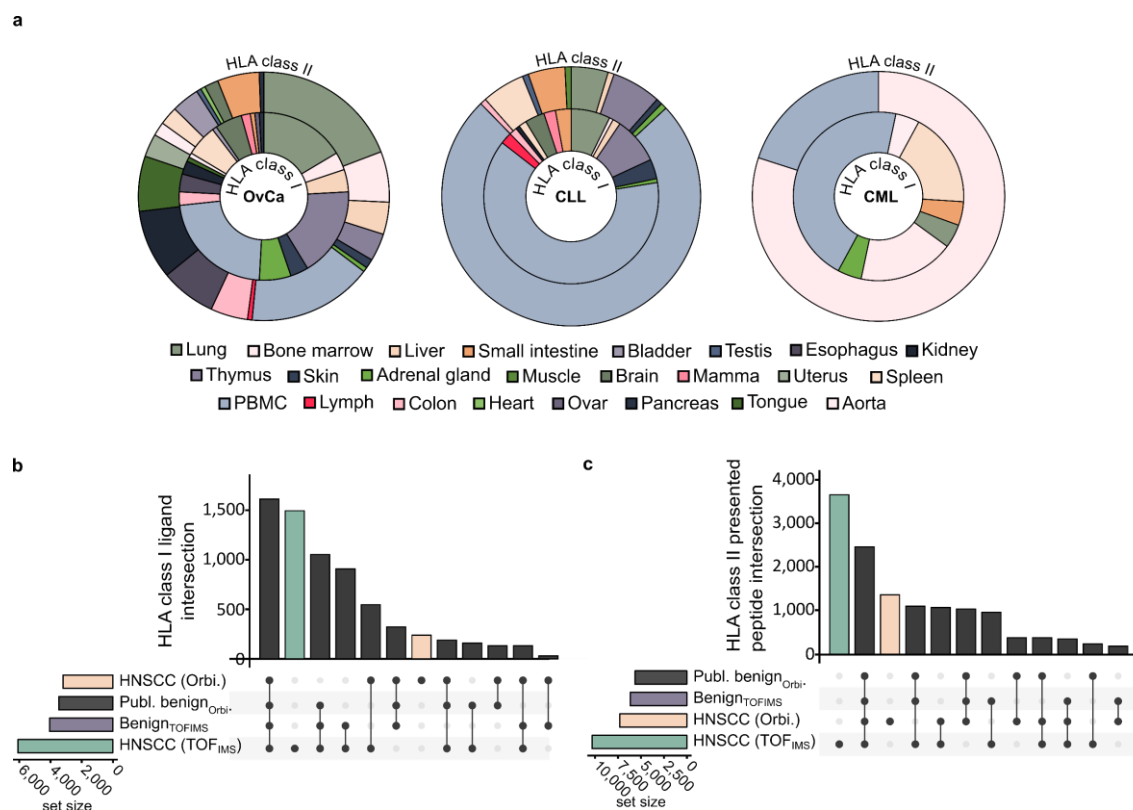




Supplementary Fig. 1 | Method development for immunopeptidomics using liquid chromatography coupled TOF_{IMS} MS. **a**, HLA class I peptide yields according to different gradient lengths (1, 2, 3, and 4) and gradient types (A, B) for different JY cell line sample concentrations, JY 1 (left) and JY 2 (right). **b**, Violin plots of grand average of hydrophobicity (GRAVY) score of HLA class I-presented peptides identified using different gradient lengths (1, 2, 3, and 4) and gradient types (A, B) from JY 2 sample. Median and 25th to 75th percentiles are indicated by dashed and dotted lines, respectively. **c**, Length distribution of identified HLA class I-presented peptides using the different gradient lengths (1, 2, 3 and 4) and types (A and B). Abbreviations: HLA, Human leukocyte antigen; GRAVY, grand average of hydropathy; freq., frequency. Source data are provided as a Source Data file.

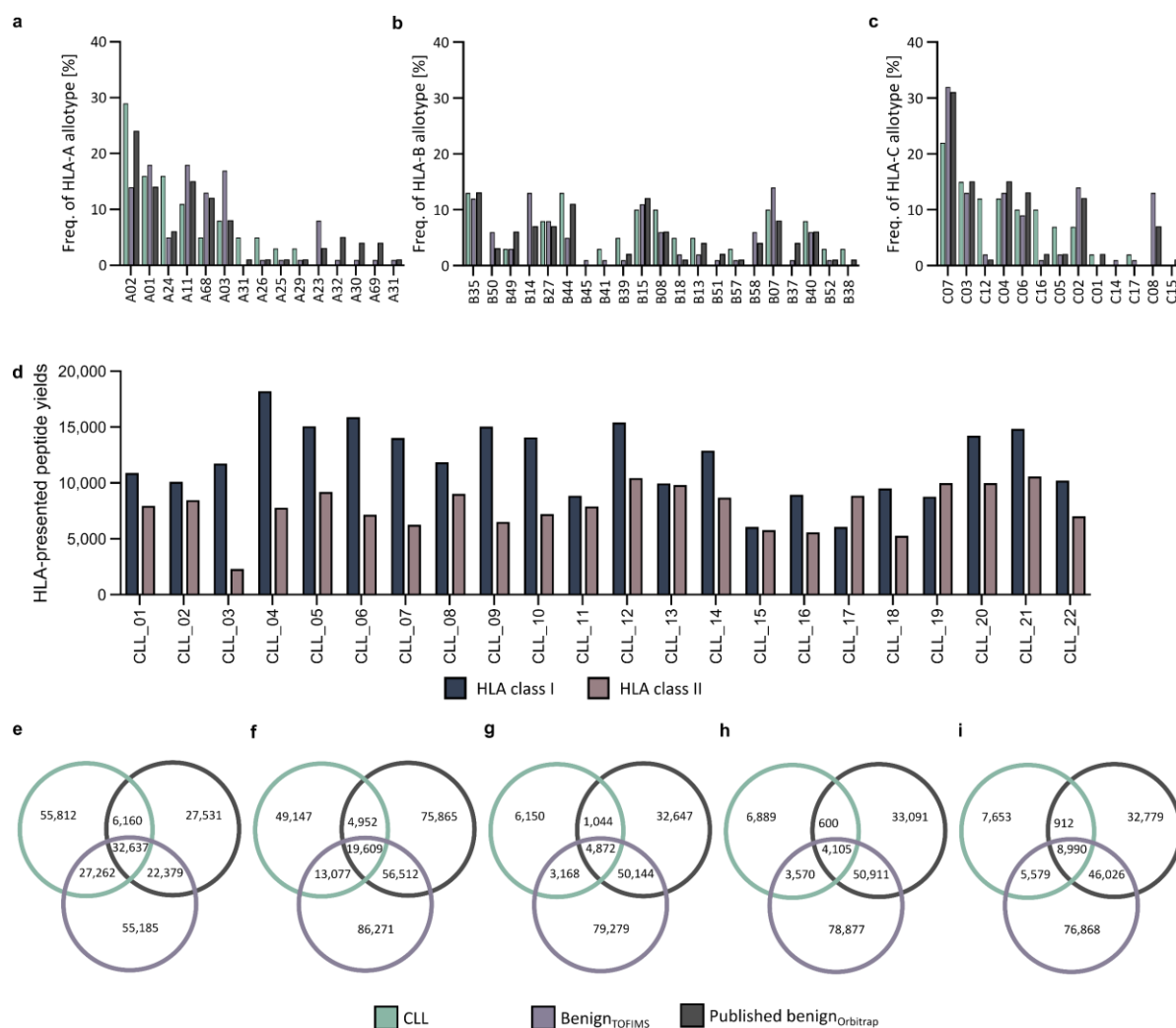


Supplementary Fig. 2 | Characterization of peptides identified by TOF_{IMS}. **a, b**, Mass distribution of exclusive TOF_{IMS}, Orbitrap or shared HLA class I (e) and II peptides (f) across all samples (n=10). **c, d**, Length distribution of TOF_{IMS}- or Orbitrap-identified HLA class I (c) and II peptides (d) of all analyzed samples (n=10). **e, f**, -10lgP of TOF_{IMS} and Orbitrap HLA class I (c) and II peptides (d) for total (all), exclusive (ex.) and shared peptides, respectively across samples (n=10). Boxes represent median and 25th to 75th percentiles, whiskers are minimum to maximum, unpaired Kruskal-Wallis t-test. **g**, Heatmap of HLA allotype distribution across HLA class I ligands from all samples (n=10) identified by TOF_{IMS} and Orbitrap. **h**, Median overlap percentage ($\pm$ standard deviation) between technical triplicates per sample of HLA class I and II peptides acquired using TOF_{IMS} and Orbitrap. **i**, Frequency of proteolytic HLA class I peptides per sample identified by TOF_{IMS} and Orbitrap. Black indicates the percentage of HLA class I-presented peptides annotated to the respective HLA allele (ligands). **j**, Ranked normalized area of TOF_{IMS} exclusive (top), Orbitrap exclusive (bottom) and shared (line) HLA class I ligands identified primary samples (n=10). The relative rank is separated into thirds (vertical lines). Percentages of ligands included in the 1st, 2nd and 3rd third relative rank are indicated in the table. **k**, HLA class I and II peptide yields identified in samples (n=10) using TOF_{IMS} and Orbitrap MS. Boxes represent median and 25th to 75th percentiles, whiskers minimum to maximum, paired Kruskal-Wallis t-test. **l, m**, Number of unique HLA class I (l) and II peptide (m) identifications by TOF_{IMS} in all samples (n=10) across the chromatographic gradient. **n, o**, GRAVY score of exclusive TOF_{IMS}, Orbitrap and shared unique HLA class I (e) and II peptides (f) across samples (n=10). Violin plots with median, 25th to 75th percentiles and unpaired Kruskal-Wallis t-test. Abbreviations: RT, retention time; Da, Dalton; norm, normalized; RCC; renal cell carcinoma; HNSCC, head and neck squamous-cell carcinoma; PBMC, peripheral blood mononuclear cell; CLL, chronic lymphocytic leukemia; GRAVY, grand average of hydrophobicity. Source data are provided as a Source Data file.



Supplementary Fig. 3 | TOF_{IMS} technology enables increased tumor-associated HLA class I and HLA class II-presented peptide identifications. **a**, pie chart of tissue origin of rejected tumor-associated antigens of the comparative immunopeptidome profiling of published HLA class I (inner circle) and HLA class II (outer circle) for OvCa, CLL and CML. **b, c**, UpSet plots showing HLA class I ligand (b) and HLA

class II peptide (c) intersection size between peptides identified from one head and neck squamous cell carcinoma (HNSCC) primary sample using TOF_{IMS} and Orbitrap mass spectrometers and their overlap with benign_{TOFIMS} dataset and published benign Orbitrap dataset. Abbreviations: HLA, Human leukocyte antigen; OvCa, ovarian carcinoma; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HNSCC, head and neck squamous cell carcinoma; publ, published; Orbi, Orbitrap. Source data are provided as a Source Data file.



Supplementary Fig. 4 | Characterization of TOF_{IMS}-based CLL dataset. **a, b, c**, Comparison of HLA-A (a), HLA-B (b) and HLA-C (c) allotypes included in the CLL sample cohort with allotypes included in the benign_{TOFIMS} and in published benign repositories. **d**, HLA class I and HLA class II ligand yields of the primary CLL samples (n = 22). **e, f, g, h, i**, overlap analysis of CLL, benign_{TOFIMS} and published benign repositories identified HLA class I (e), HLA class II (f), HLA-A*02 (g), HLA-B*35 (h) and HLA-C*07 (i) ligands. Abbreviations: HLA, Human leukocyte antigen; CLL, chronic lymphocytic leukemia; freq, frequency. Source data are provided as a Source Data file.