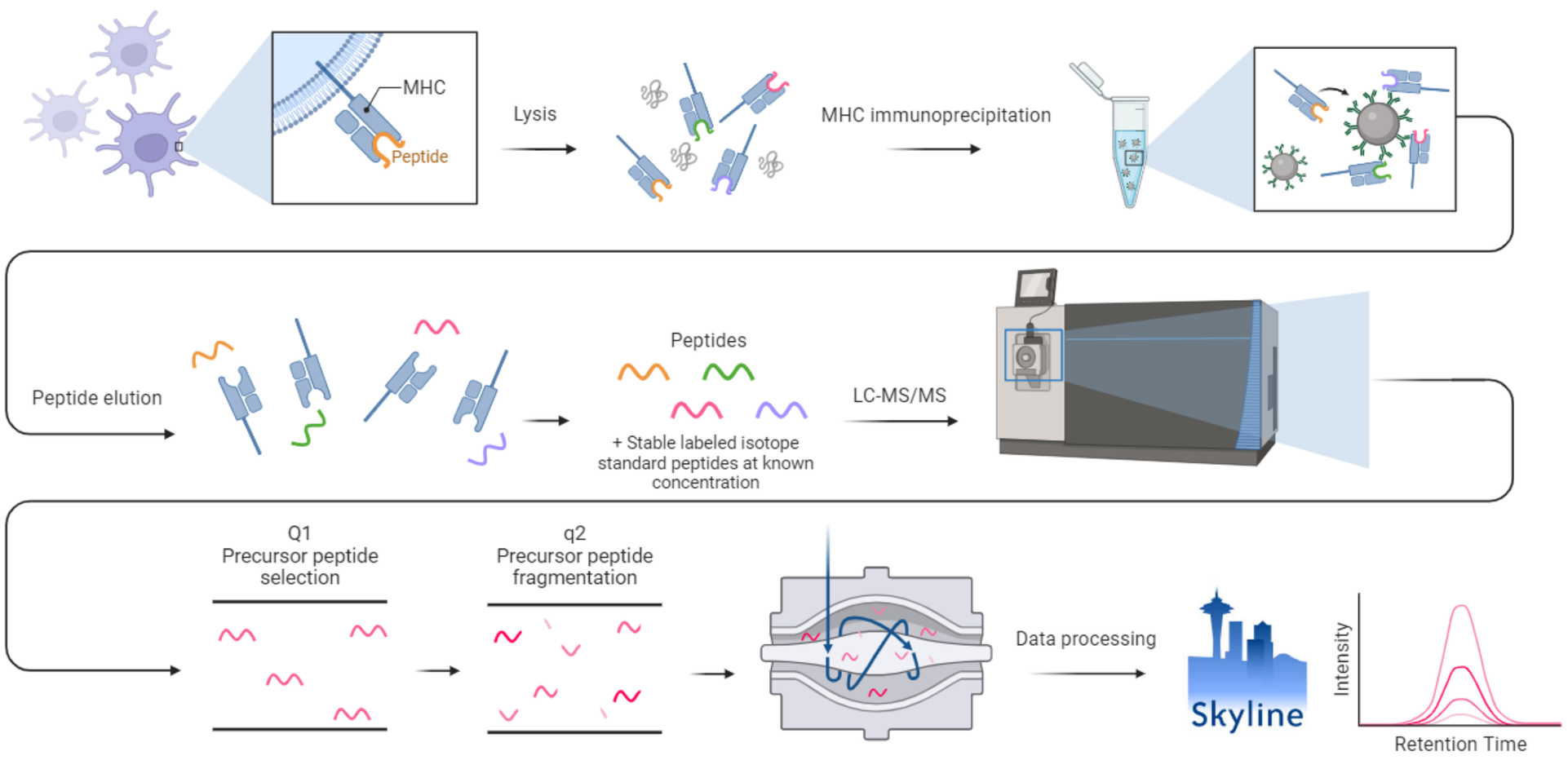
Neoantigen quantitation

Neoantigens are unique, mutated proteins that arise from genetic alterations in cancer cells.

The immune system can sometimes recognize these altered proteins as they are presented by the MHC class I complex, triggering an immune response to target and destroy the cancer cells producing them. Immunotherapies, such as cancer vaccines or adoptive T-cell therapies, can be designed to specifically target and activate the immune system against these tumor-specific neoantigens, sparing healthy tissues and minimizing side effects. By understanding the range and diversity of neoantigens, researchers can improve the effectiveness and precision of cancer treatments, offering new hope for patients with various types of cancer.

Our workflow for neoantigen quantitation is described below.

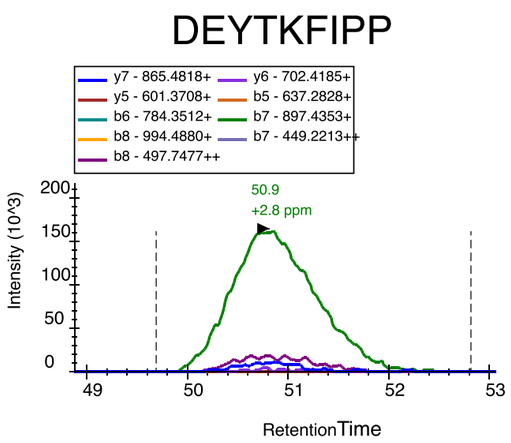


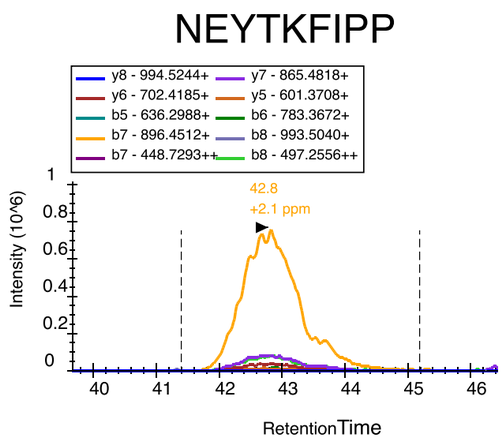
Parallel Reaction Monitoring (PRM)

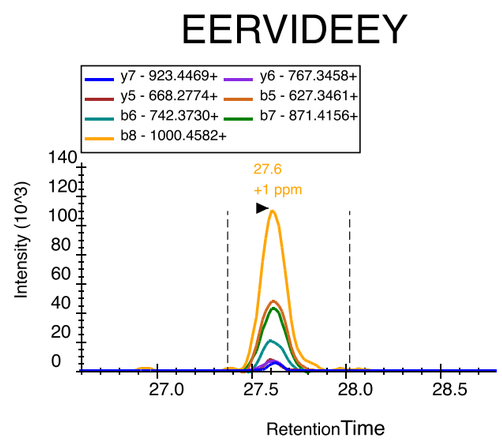
Using human colon cancer cell line, HCT116, we showcase our ability to identify specific neoantigens and quantify their number of copies per cell. In this experiment 100M HCT116 cells were immunoprecipitated (IP) in triplicate. Peptides were eluted from enriched MHC and loaded on a C18 plate, reduced, and alkylated on column, followed by elution. 25% of each IP was analyzed on a nano LC/MS/MS using a Waters NanoAcquity system interfaced to an Orbitrap Fusion Lumos Tribrid mass spectrometer for a 2h gradient. All resulting data were processed using Skyline. Here we quantify the RBB7 N61D and PDP1 N379D neoantigens and provide copy number per cell for both WT and mutant.

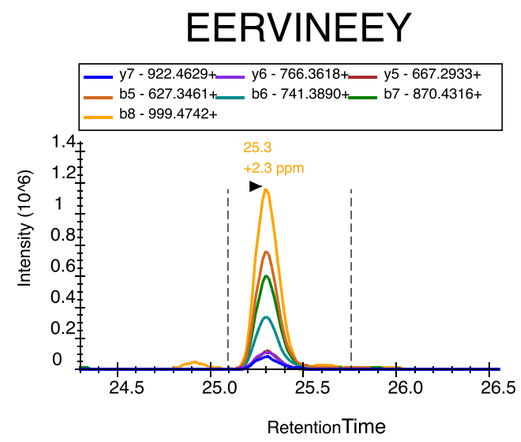
This work is an extension of the following publication: Becker JP, Helm D, Rettel M, Stein F, Hernandez-Sanchez A, Urban K, Gebert J, Kloor M, Neu-Yilik G, von Knebel Doeberitz M, Hentze MW, Kulozik AE. NMD inhibition by 5-azacytidine augments presentation of immunogenic frameshift-derived neoepitopes. iScience. 2021 Apr 1;24(4):102389.

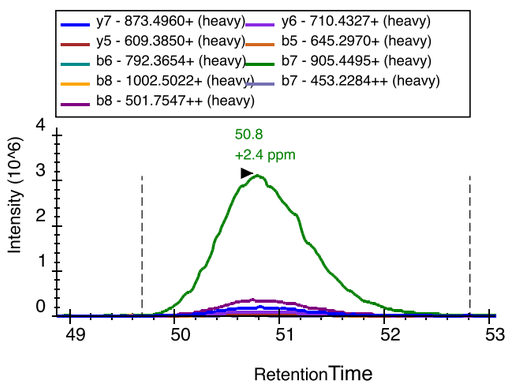
Mass chromatograms for the endogenous and internal standard peptides are shown below as stacked plots.

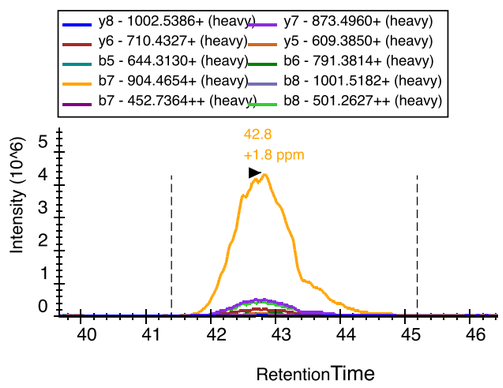


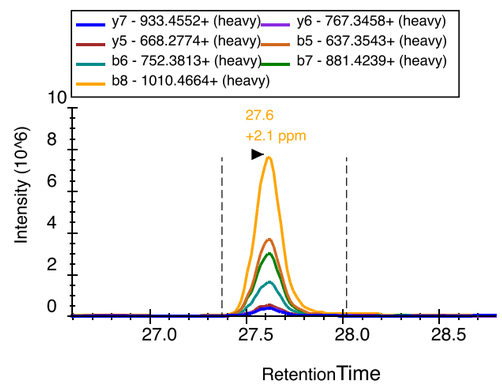


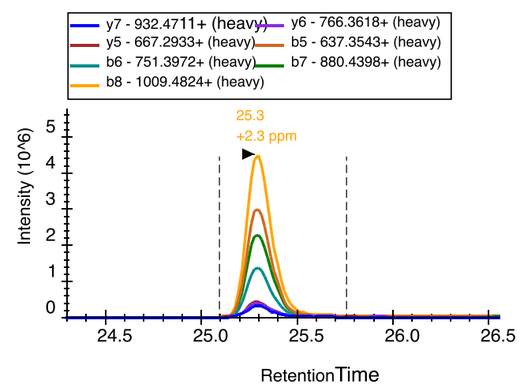




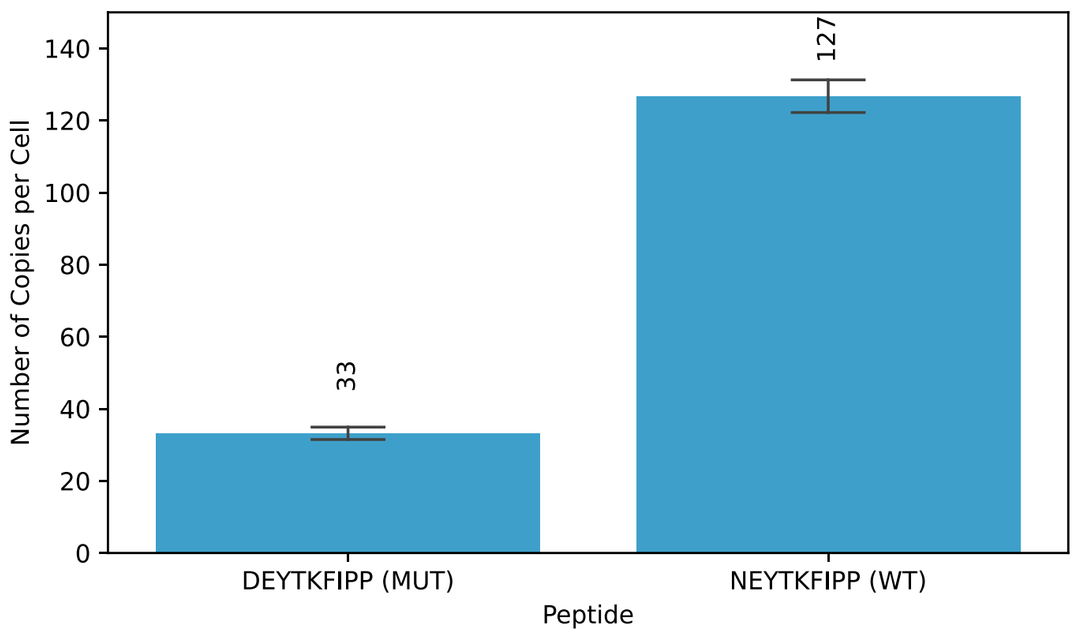


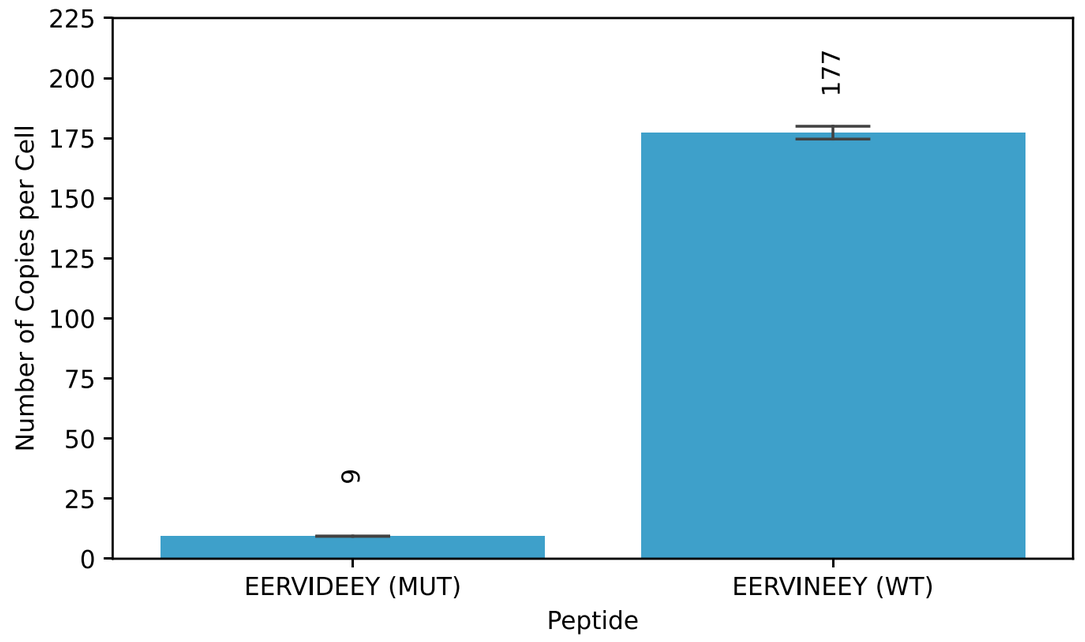






Neoantigen (MUT) vs Wild (WT) quantitative data are summarized in charts below.

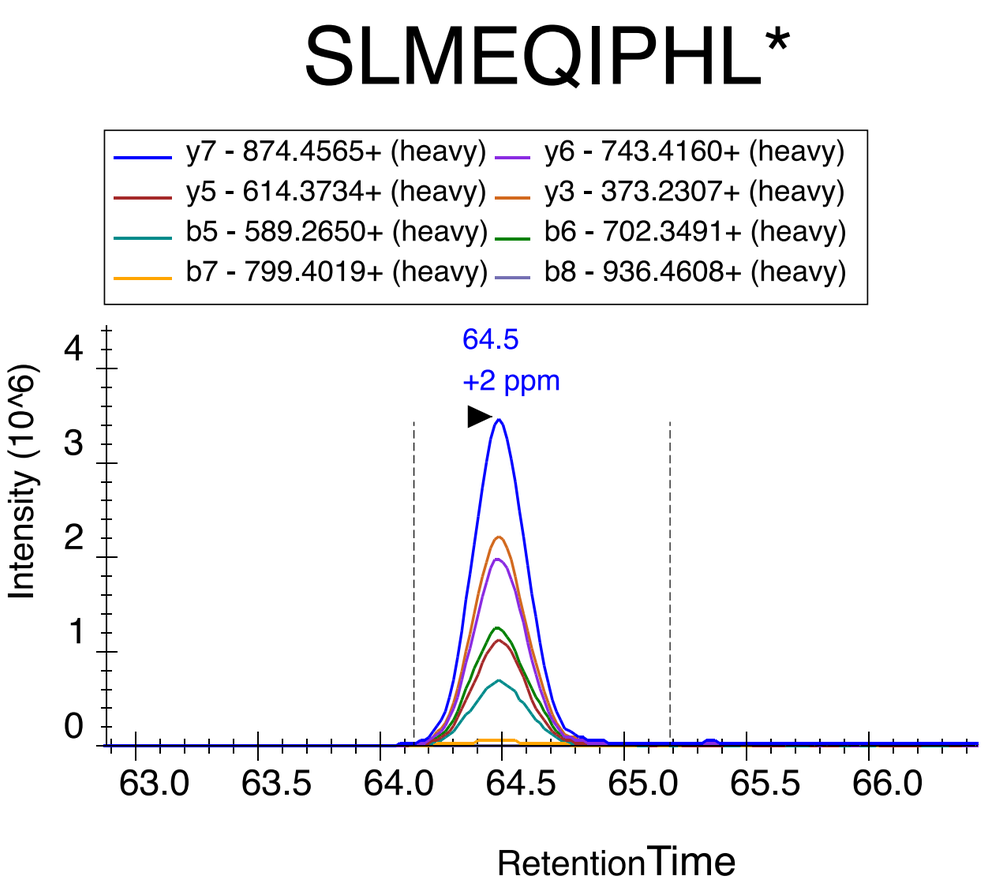


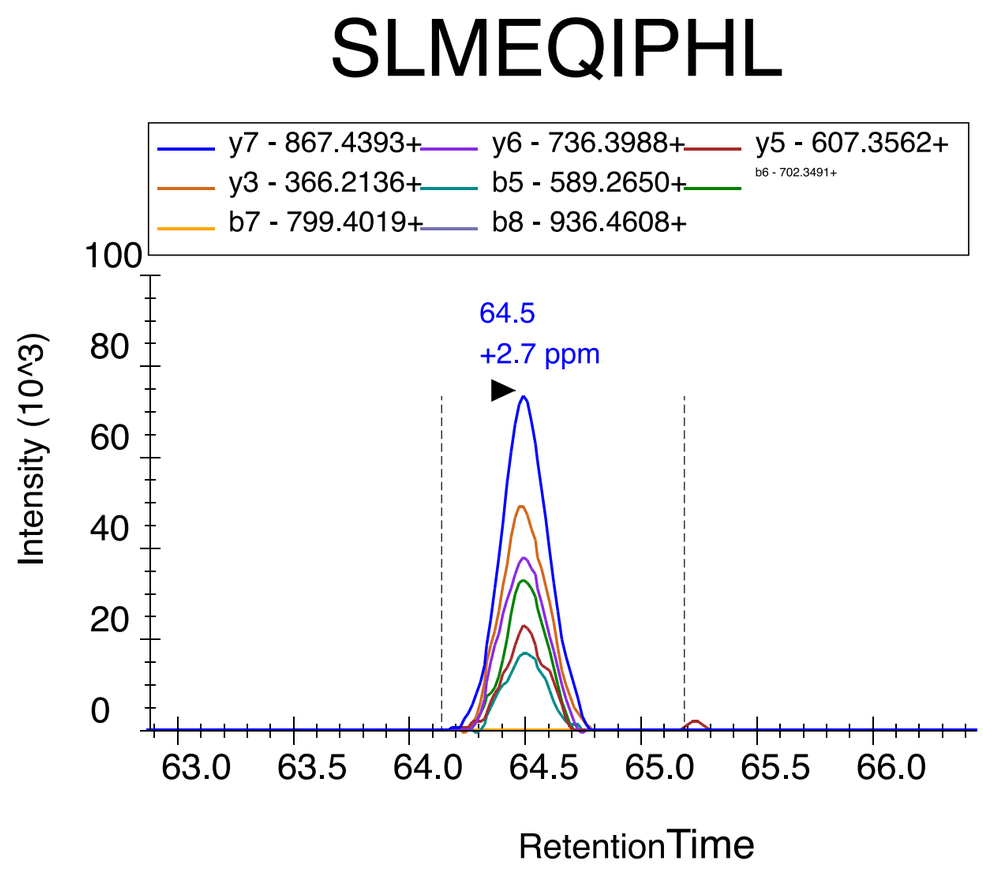


Frameshift-derived InDel Neoepitope Quantitation

Human leukocyte antigen (HLA) class I-presented peptides derived from frameshifted protein sequences are termed InDel neoepitopes (Becker et al). They allow patrolling CD8+ T cells to identify and target tumor cells presenting such InDel neoepitopes. Here we quantify the CKAP2 derived InDel peptide SLMEQIPHL in HCT116 cells and calculate the number of inDel presented peptides per cell.

Mass chromatograms for the endogenous and internal standard peptides are shown below as stacked product ion chromatograms.





InDel peptide data are summarized in the plot below.