**Supplementary figures and tables**

**Supplementary Fig. S1: Structure of plasmid construct used for transfection.**

**Supplementary Fig. S2: Transfection efficiency assessment.** Transfection efficiencies were calculated through flow cytometry by calculating the percentage of cells that are expressing GFP.

**Supplementary Fig. S3: Sanger sequencing to confirm G12D mutation from *KRAS* G12D-knockin cell line.**

**Supplementary Fig. S4: Optimization of immuno-precipitation-based neo-antigen enrichment.** A) Evaluation of different crosslinking conditions through assessment of the enrichment efficiencies of crosslinked antibodies. B) Three different cell lysis and IP procedures were compared for their yields of MHC molecules. C) Optimization of the elution conditions. D) Enrichment efficacy of IP steps in MANA-SRM through capturing sequentially diluted MHC tetramers spiked into a SW480 cell lysate.

**Supplementary Fig. S5: MS/MS analysis of the contamination.** A characteristic 44 Da-interval between adjacent major MS2 peaks is obvious.

**Supplementary Fig. S6: HILIC-based cleanup to reduce the ion-suppression from MANA-SRM samples.** 16 out of 33 peptides were co-eluted together with a significant contamination composed of PEG-derivatives. SRM signal intensities for the affected peptides were examined before (red column) and after (green column) the HILIC-based cleanup and compared to the signal strength observed from the pure peptide alone (black column).

**Supplementary Fig. S7: Peptide recovery ratios.** Standard heavy isotope labeled peptides were spiked into an enriched SW480 MHC sample at different abundances (1 femtomole to 625 femtomole) after the elution but before filtration. The recovery ratios for all the peptides were measured by comparing the signal strengths detected from MANA-SRM approach to the strengths detected from standard peptide alone.

**Supplementary Table S1. Sequences used for plasmid construction.**

**Supplementary Table S2. MANA-SRM parameters for peptide targets.**

**Supplementary Table S3. MANA-SRM sensitivity improvement through the D-Red approach.** Peptide abundances measured with and without reduction treatment, or with only one step in the D-Red treatment, were compared. A significant increase in the detectable signals was observed for the samples treated by the complete D-Red approach.

**Supplementary Table S4. MAMA-SRM quantitative performance measured by tetramers.** MHC tetramers with bounded mutant peptides were mixed together with heavy peptides and heated to dissociate the peptides from the monomer, followed by filtration. Peptide abundances were measured. The same initial amount of MHC tetramers with bound peptides were spiked into COS-7 cell lysates where there are no HLA molecules and no mutant proteins present (clear background), and a standard MANA-SRM pipeline was followed. The peptide abundances were measured. The ratio of the peptide abundances measured after the spike-in and MANA-SRM to the value measure before spike-in is the recovery ratio for each of the tetramer-bounded peptides in the MANA-SRM assay.