Supporting Information:

High-Throughput Database Search and Large-Scale Negative Polarity LC-MS/MS with Ultraviolet Photodissociation for Complex Proteomic Samples

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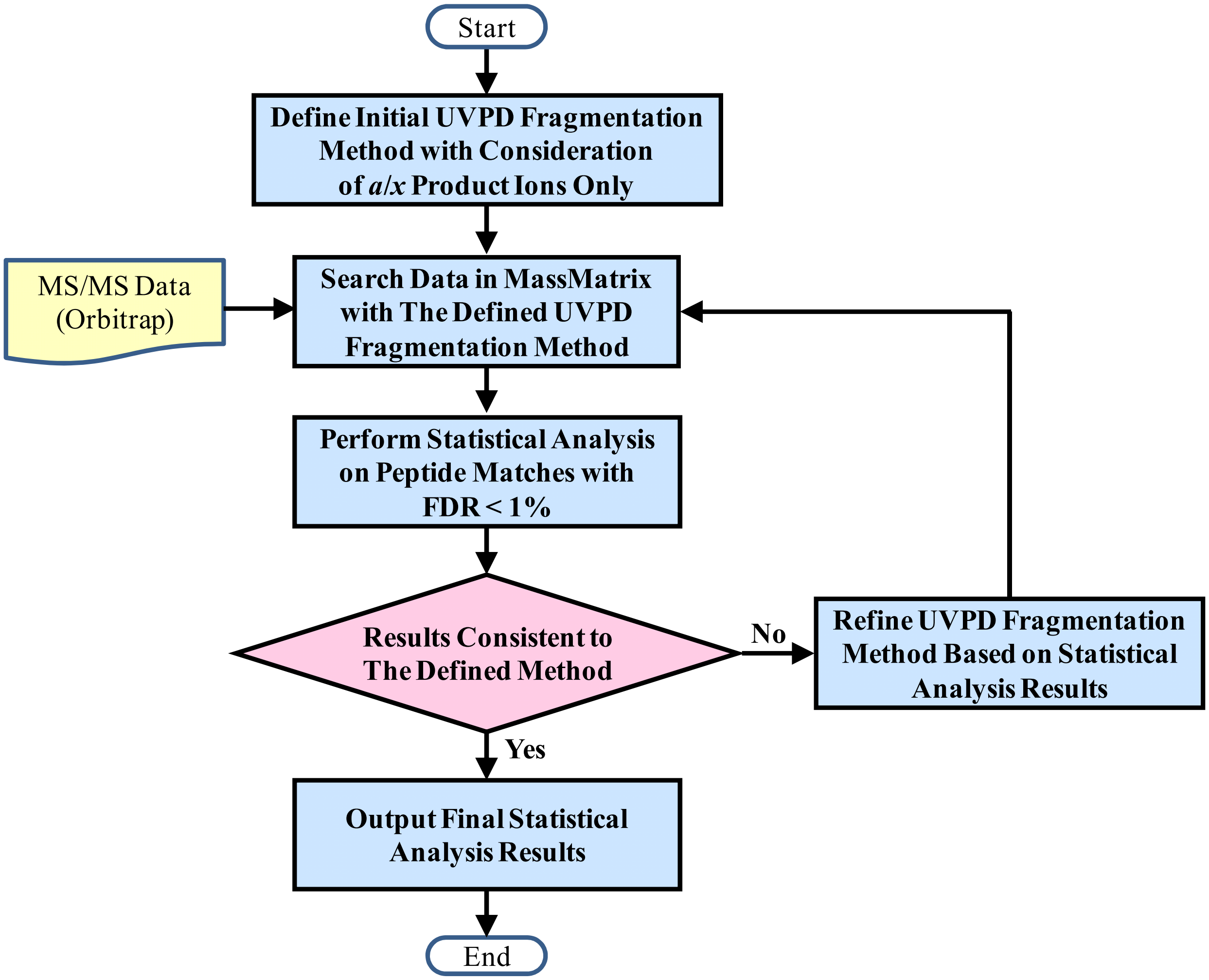
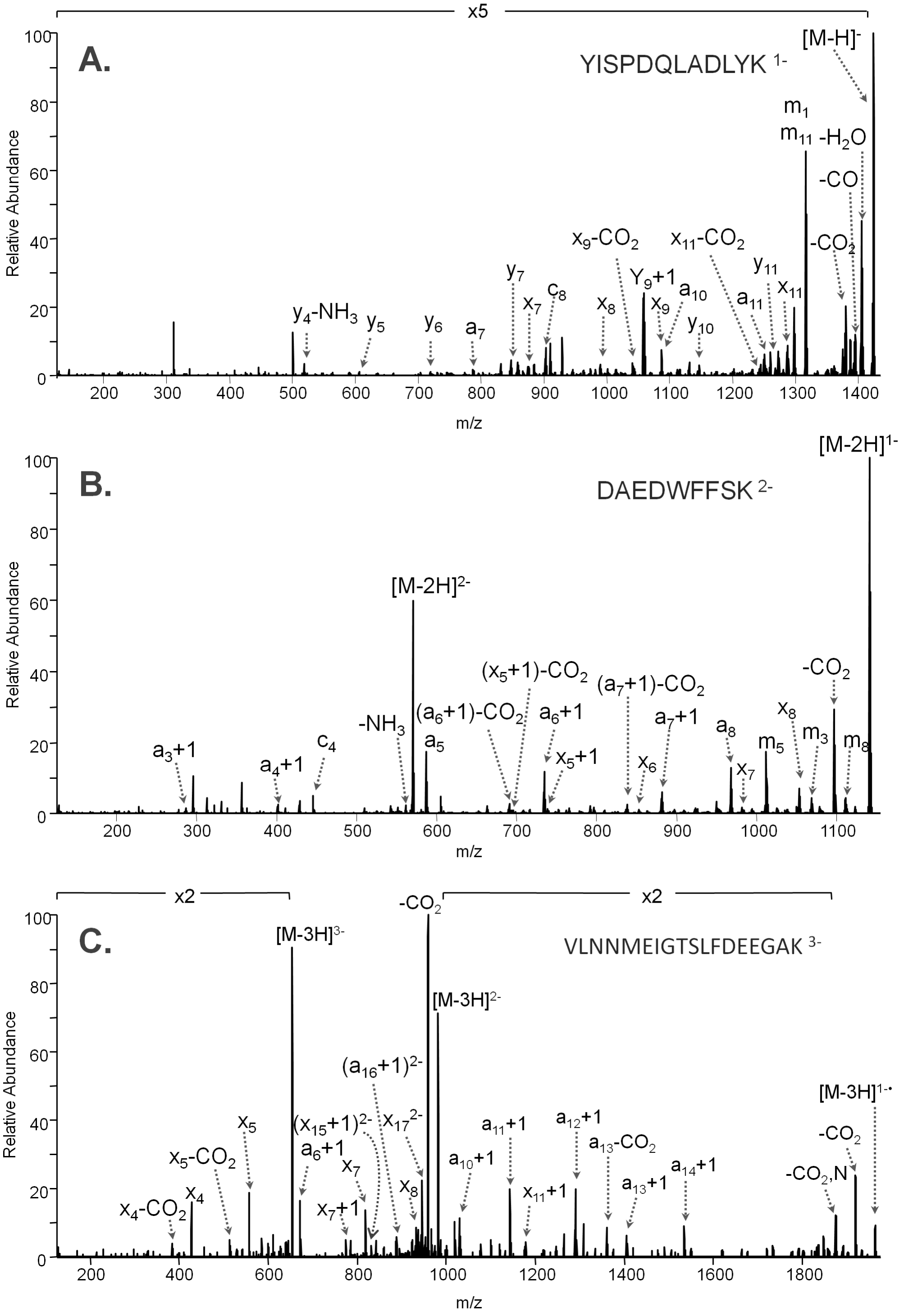
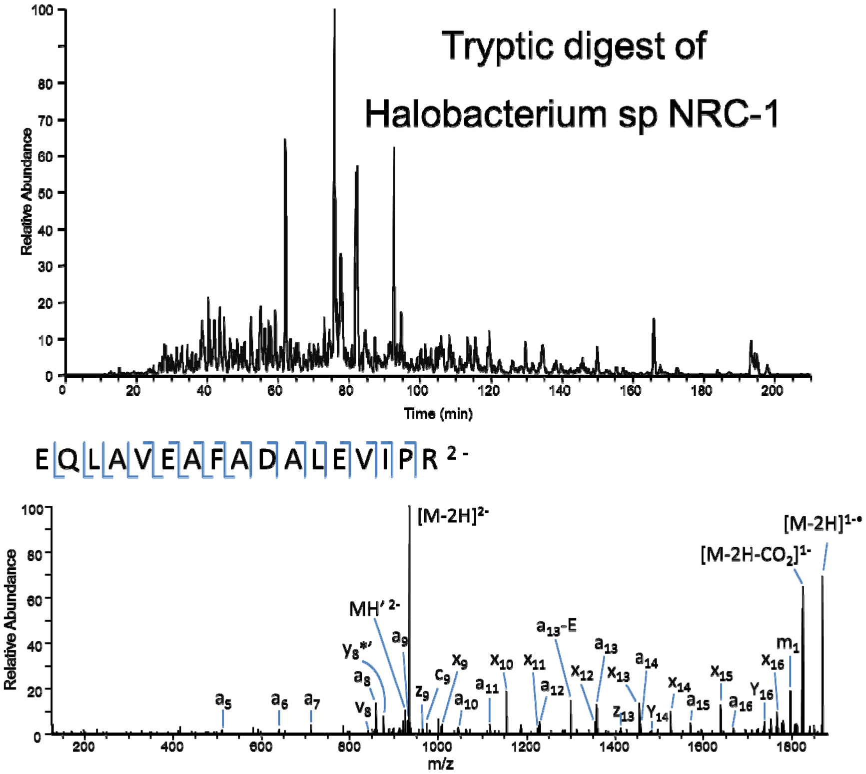
Scheme 1. Flow diagram of the optimization of the *in silico* fragmentation pathways for MassMatrix UVPD search algorithm.



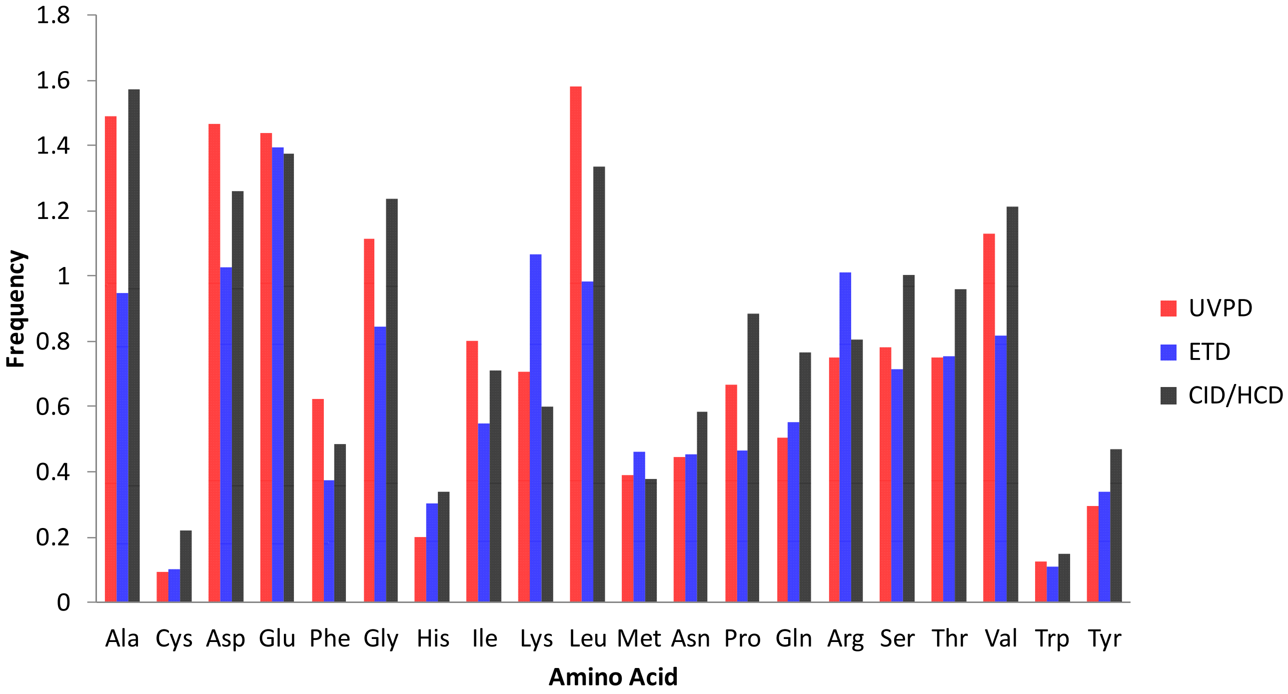
Supplemental Figure 1. Example spectra for MS/MS of tryptic HeLa peptide anions acquired on an Orbitrap Elite mass spectrometer. 193 nm UVPD of (A) Isoform alpha-enolase of Alpha-enolase peptide YISPDQLADLYK, 1- (*m/z* 1423.71), (B) KRT13 42 kDa protein peptide DAEDWFFSK, 2- (*m/z* 570.74), and (C) Phosphoglycerate kinase 1 peptide VLNNMEIGTSLFDEEGAK, 3- (*m/z* 654.64).



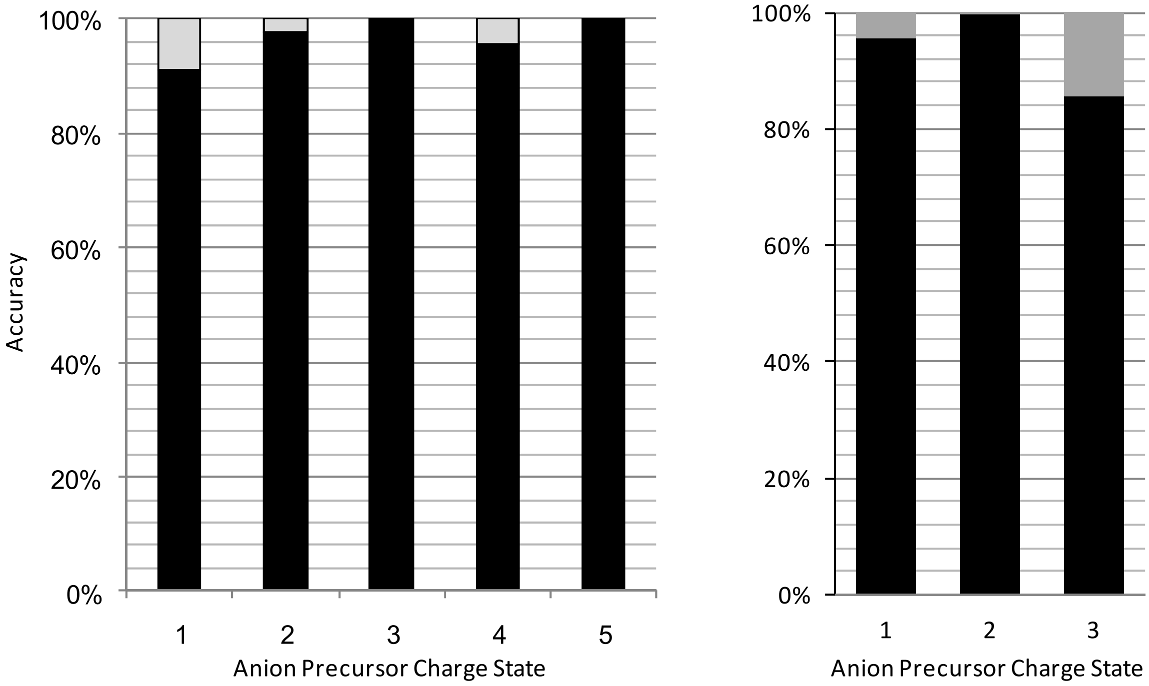
Supplemental Figure 2. LC chromatographic trace and representative UVPD spectrum of a tryptic peptide anion from a *Halobacterium* lysate. The peptide (retention time 145.5 min) maps to the Thermosome subunit alpha protein.



Supplemental Figure 3. Amino acid composition analysis for peptides unique to different fragmentation methods for HeLa and Halo digests.

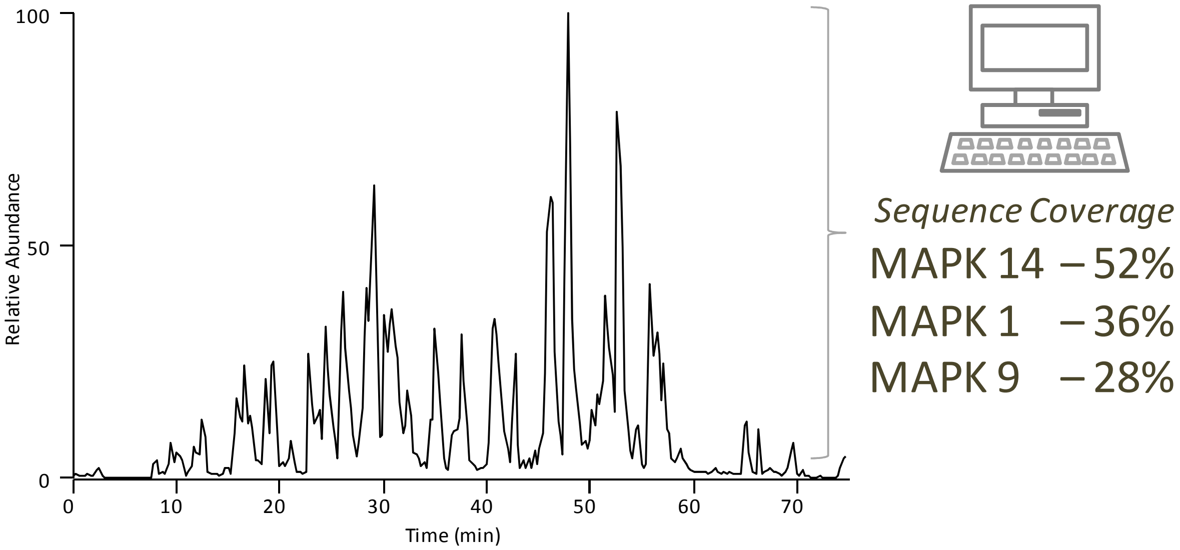


Supplemental Figure 4. Accuracy of charge state assignment algorithm for (A) charges 1- through 5- of a known mixture of three mitogen-activated kinases (MAPKs) and (B) charges 1- through 3- of a complex mixture of a HeLa tryptic digest.

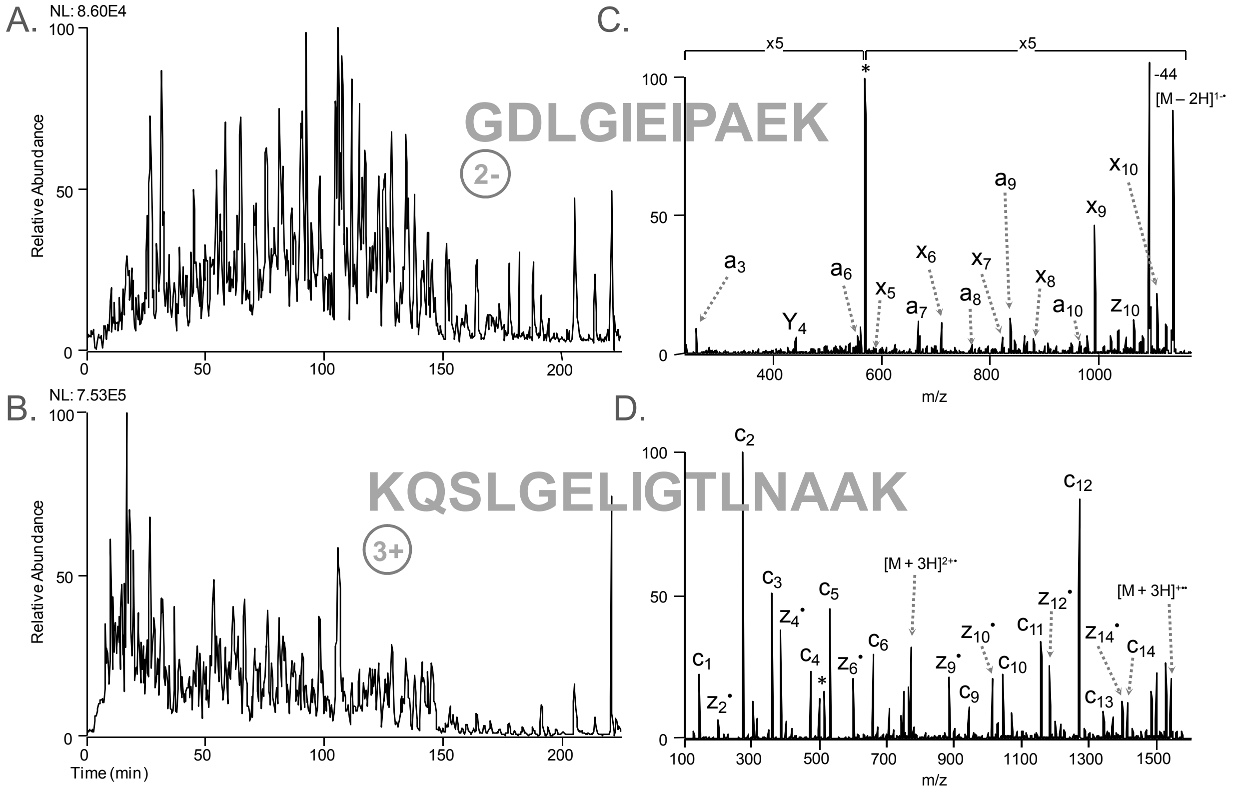


(A) **(B)**

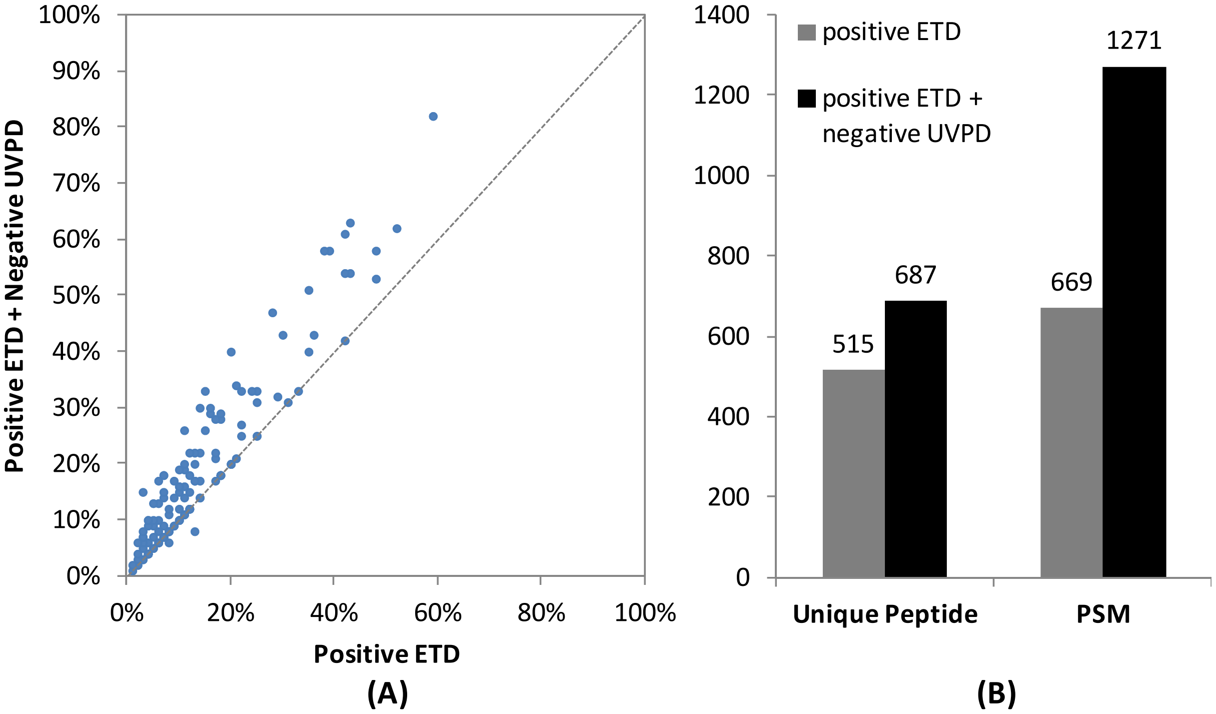
Supplemental Figure 5. Base peak chromatogram of tryptic peptides from a known mixture of three mitogen-activated kinases (MAPKs) (left portion of figure). The sequence coverage of the MAPKs after MassMatrix analysis (right portion of figure).



Supplemental Figure 6. Polarity switching nanoLC-MS/MS for sequencing of cation (ETD) and anion (193 nm UVPD) tryptic HeLa peptides in a single run. (A) Extracted negative polarity base peak chromatogram. (B) Extracted positive polarity base peak chromatogram. (C) 193 nm UVPD of pyruvate kinase isozyme peptide GDLGIEIPAEK, 2-, retention time 71.18 min. (D) ETD of triosephosphate isomerase peptide KQSLGELIGTLNAAK, 3+, retention time 100.05 min.



Supplemental Figure 7. Polarity switching nanoLC-MS/MS for sequencing of cation (ETD) and anion (193 nm UVPD) tryptic HeLa peptides in a single run. (A) Sequence coverage of proteins identified by positive ETD only versus positive ETD plus negative UVPD. (B) Number of unique peptides and peptide spectral matches (PSM) identified by positive ETD only versus positive ETD plus negative UVPD.



Supplemental Table 1. Links to Peptide Atlas Data Archive for Orbitrap raw datasets:

Dataset tag	Link to dataset	Identifier
HeLa_1_NUVPD	http://www.peptideatlas.org/PASS/PASS00163	PASS00163
HeLa_2_NUVPD	http://www.peptideatlas.org/PASS/PASS00164	PASS00164
HeLa_3_NUVPD	http://www.peptideatlas.org/PASS/PASS00165	PASS00165
HeLa_1_CID	http://www.peptideatlas.org/PASS/PASS00166	PASS00166
HeLa_2_CID	http://www.peptideatlas.org/PASS/PASS00167	PASS00167
HeLa_3_CID	http://www.peptideatlas.org/PASS/PASS00168	PASS00168
HeLa_HCD_1	http://www.peptideatlas.org/PASS/PASS00148	PASS00148
HeLa_HCD_2	http://www.peptideatlas.org/PASS/PASS00149	PASS00149
HeLa_HCD_3	http://www.peptideatlas.org/PASS/PASS00150	PASS00150
HeLa_1_ETD	http://www.peptideatlas.org/PASS/PASS00169	PASS00169
HeLa_2_ETD	http://www.peptideatlas.org/PASS/PASS00170	PASS00170
HeLa_ETD_3	http://www.peptideatlas.org/PASS/PASS00171	PASS00171
Halo_NUVPD_1	http://www.peptideatlas.org/PASS/PASS00160	PASS00160
Halo_NUVPD_2	http://www.peptideatlas.org/PASS/PASS00161	PASS00161
Halo_NUVPD_3	http://www.peptideatlas.org/PASS/PASS00162	PASS00162
Halo_CID_1	http://www.peptideatlas.org/PASS/PASS00151	PASS00151
Halo_CID_2	http://www.peptideatlas.org/PASS/PASS00152	PASS00152
Halo_CID_3	http://www.peptideatlas.org/PASS/PASS00153	PASS00153
Halo_HCD_1	http://www.peptideatlas.org/PASS/PASS00154	PASS00154
Halo_HCD_2	http://www.peptideatlas.org/PASS/PASS00155	PASS00155
Halo_HCD_3	http://www.peptideatlas.org/PASS/PASS00156	PASS00156
Halo_ETD_1	http://www.peptideatlas.org/PASS/PASS00157	PASS00157
Halo_ETD_2	http://www.peptideatlas.org/PASS/PASS00158	PASS00158
Halo_ETD_3	http://www.peptideatlas.org/PASS/PASS00159	PASS00159

Supplemental Table 2. Links to Peptide Atlas Data Archive for LTQ raw datasets:

Dataset tag	Link to dataset	Identifier
HeLa_ETD_UVPD_switch	http://www.peptideatlas.org/PASS/PASS00179	PASS00179
MAPKmix_CID_1	http://www.peptideatlas.org/PASS/PASS00180	PASS00180
MAPKmix_CID_2	http://www.peptideatlas.org/PASS/PASS00181	PASS00181
MAPKmix_CID_3	http://www.peptideatlas.org/PASS/PASS00182	PASS00182
MAPKmix_UVPD_1	http://www.peptideatlas.org/PASS/PASS00183	PASS00183
MAPKmix_UVPD_2	http://www.peptideatlas.org/PASS/PASS00184	PASS00184
MAPKmix_UVPD_3	http://www.peptideatlas.org/PASS/PASS00185	PASS00185

Supplemental Table 3. Links to Peptide Atlas data archive for peptide and protein match lists from MassMatrix searches

Dataset tag	Link to dataset	Identifier
Halo_match_lists	http://www.peptideatlas.org/PASS/PASS00186	PASS00186
HeLa_orbi_match_list	http://www.peptideatlas.org/PASS/PASS00187	PASS00187
All for LTQ HeLa	http://www.peptideatlas.org/PASS/PASS00199	PASS00199
All for MAPK	http://www.peptideatlas.org/PASS/PASS00200	PASS00200