Supplementary Information

Double Down Mass Spectrometry of Histone H4 Proteoforms: Tandem UV-Photon and Mobility-Selected Electron Capture Dissociations

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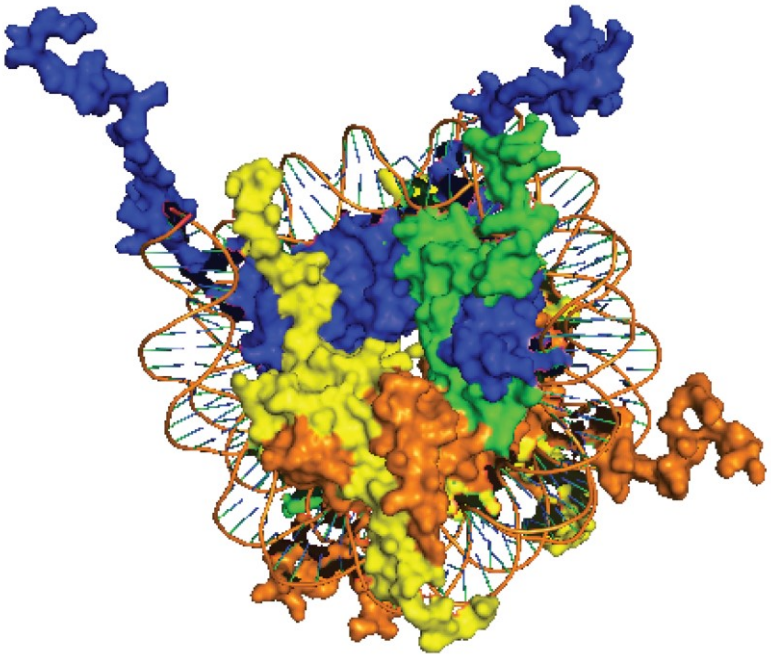

Figure S1. Three-dimensional structure of a nucleosome (PDB 1KX5). The histones H2A, H2B, H3.1 and H4.1 are illustrated in yellow, orange, blue and green, respectively. The DNA, wrapped around the octameric core histones, is also represented by cartoon lines.

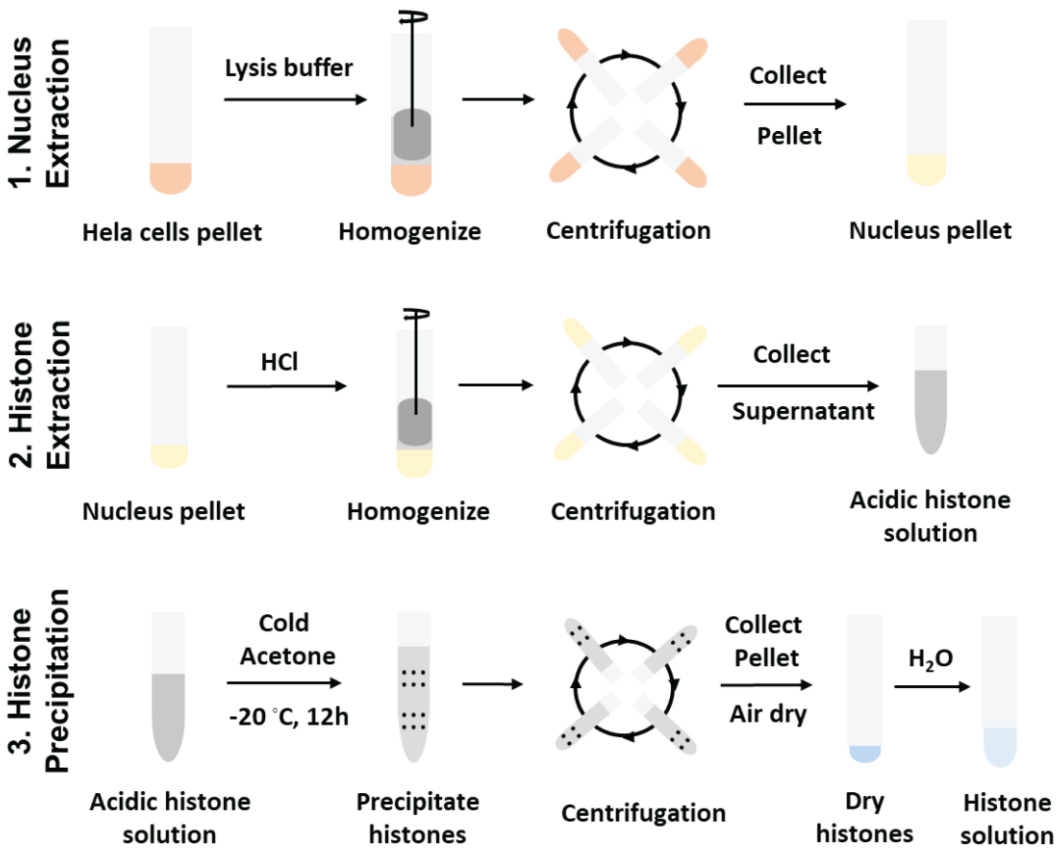

Figure S2. Histone extraction workflow from HeLa cells.

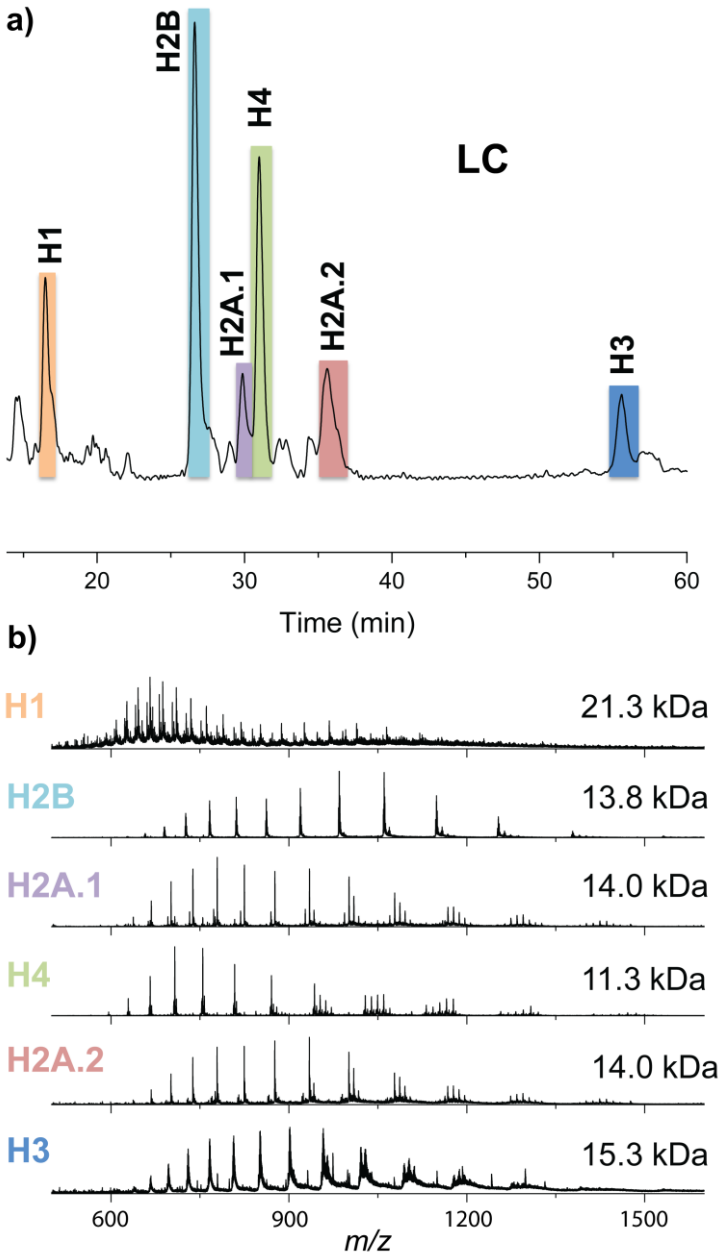

Figure S3. (a) LC profiles obtained from the pull-down histones of HeLa cells with resulting (b) mass spectra for each LC fraction. Fractions highlighted in orange, light blue, purple, green, red, and blue were assigned to histone H1, H2B, H2A.1, H4, H2A.2 and H3, respectively.

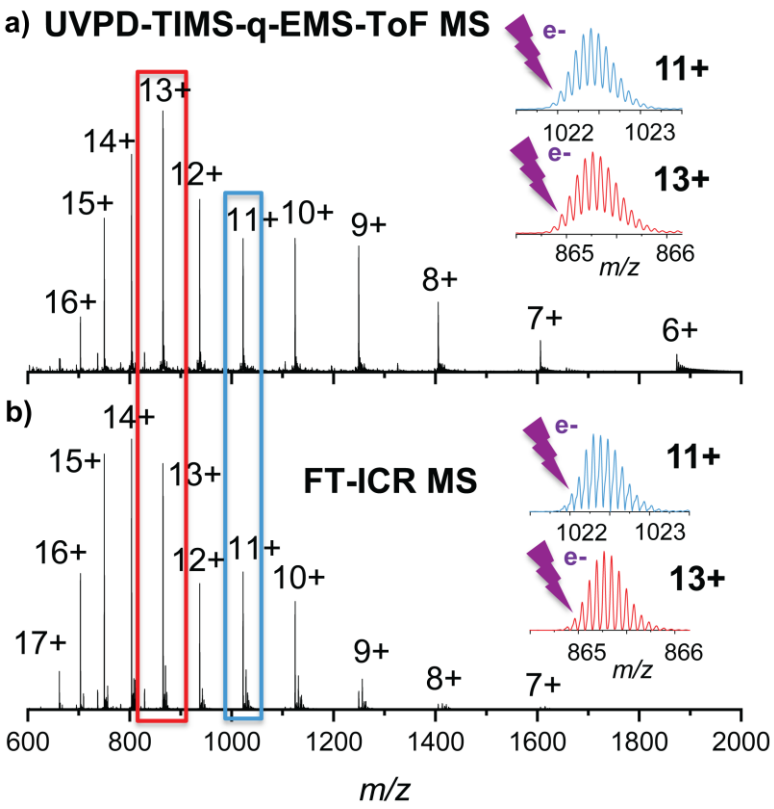

Figure S4. nESI-MS spectra of H_4 standard generated from (a) UVPD-TIMS-q-EMS-ToF MS and (b) FT-ICR MS platforms.

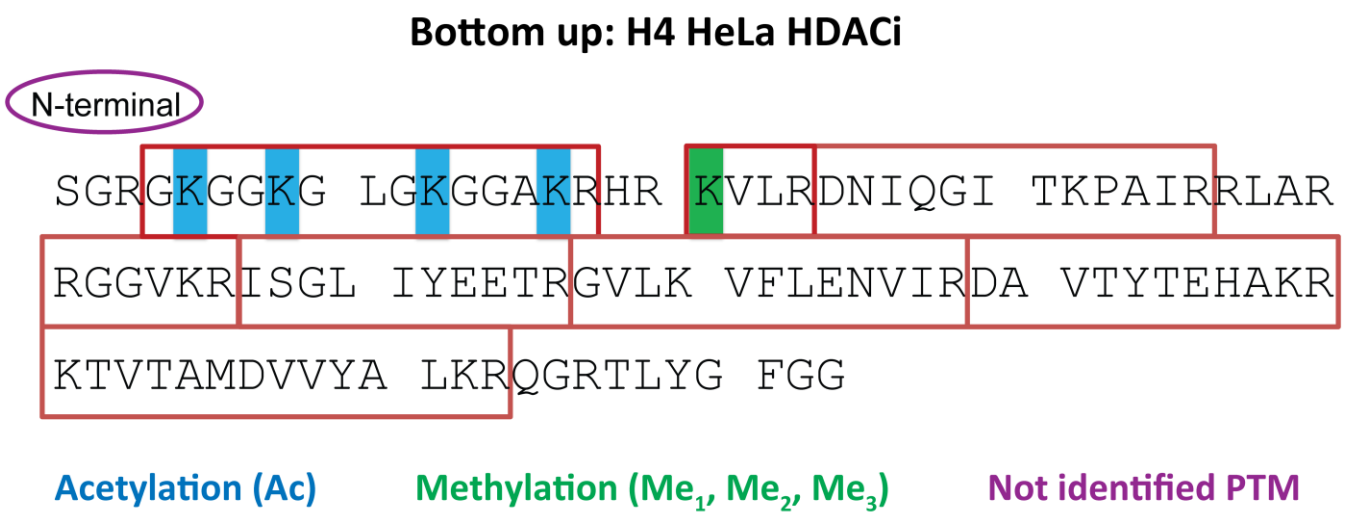

Figure S5. H4 sequence map showing the fragments observed using bottom-up experiments (red rectangles) in HeLa HDACi. PTMs containing acetylation and methylations are colored in blue and green, respectively. The PTM that could not be identified using this bottom-up approach is highlighted in purple.

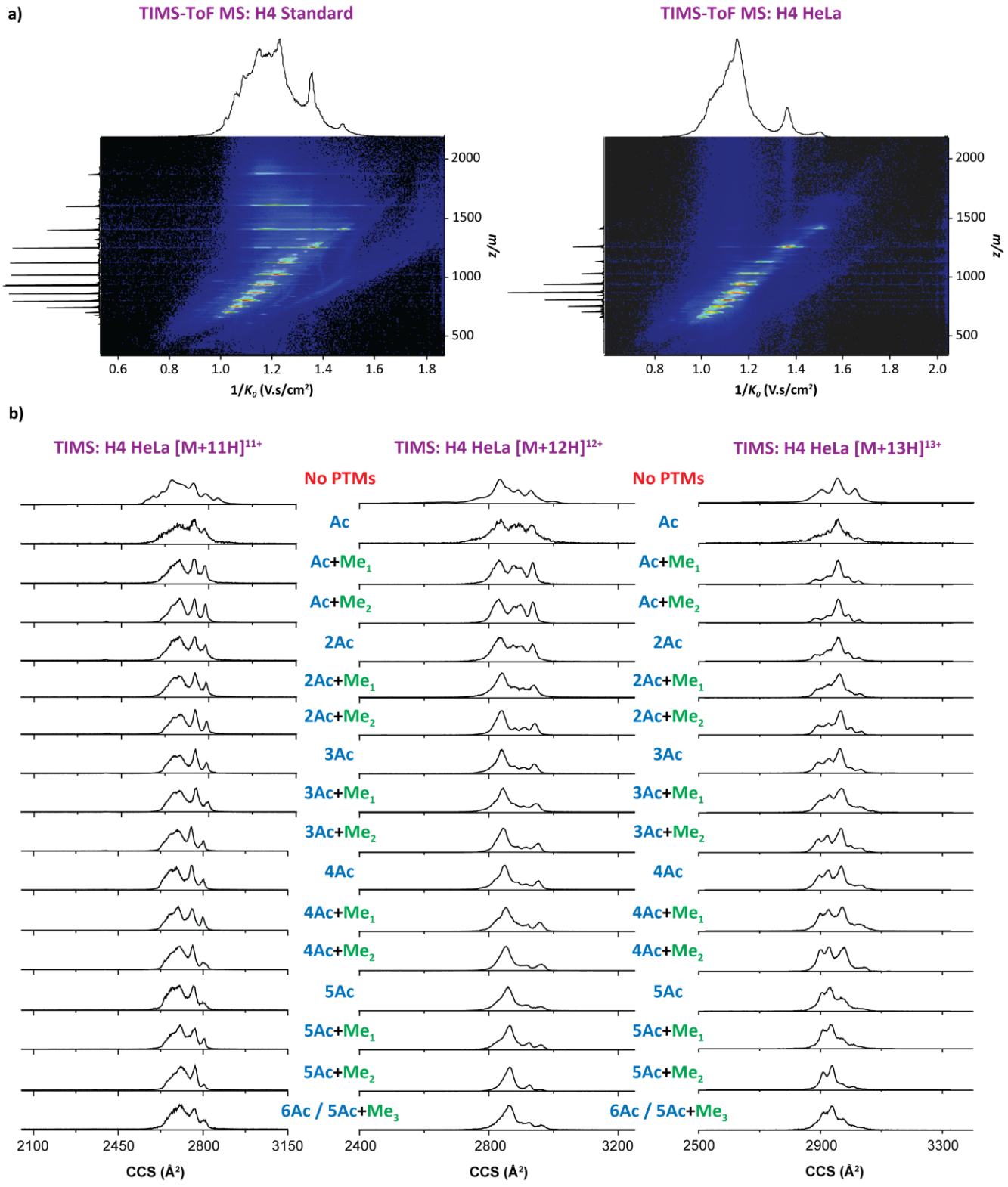

Figure S6. TIMS-ToF MS analysis showing (a) 2D TIMS-MS contour maps of H4 standard (left) and H4 HeLa (right) and (b) H4 TIMS distributions per PTMs for the $[M + 11H]^{11+}$, $[M + 12H]^{12+}$ and $[M + 13H]^{13+}$ ions.

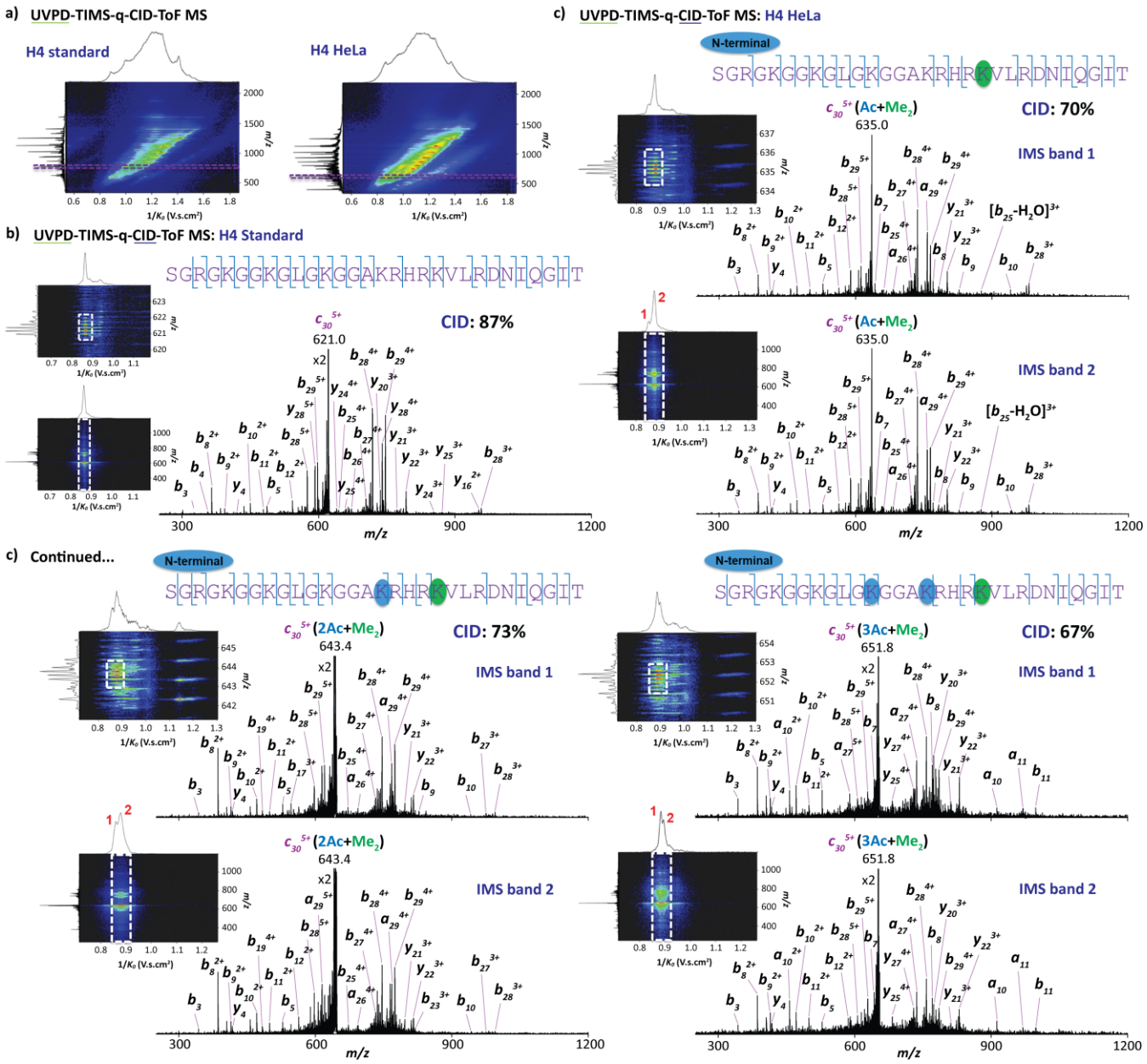

Figure S7. "Double down" mass spectrometry analysis showing (a) 2D UVPD-TIMS-ToF MS contour maps for both H4 proteins and 2D UVPD-TIMS-q-ECD-ToF MS contour maps of the c_{30}^{5+} fragments (left panel) together with the ECD spectra per IMS band (right panel) for the (b) H4 standard and (c) H4 HeLa.