Supporting Information

Middle-Down Proteomic Analyses with Ion Mobility Separations of Endogenous Isomeric Proteoforms

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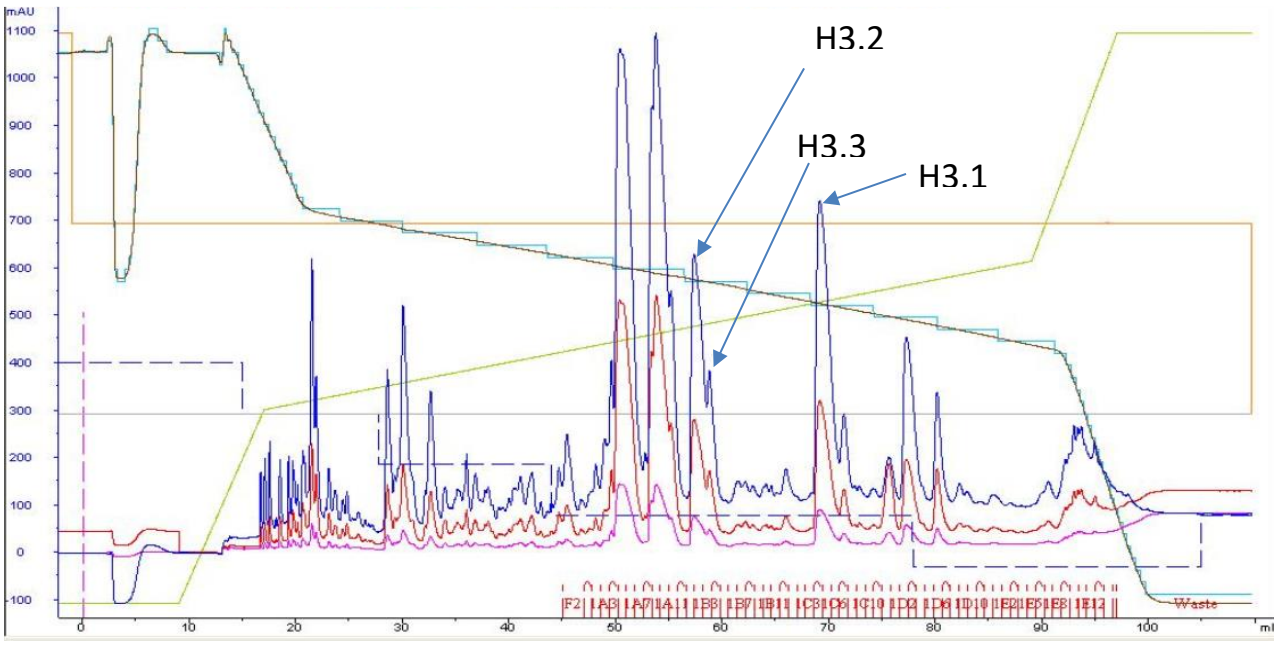
Figures 1- 5: in this document

Table S1 (in separate Microsoft Excel File)

(a) List of 526 presently discovered proteoforms

(b) Subset showing the 40 forms comprising the five most abundant for each of eight selected chemical compositions (in Figure 4)

Figure S1



Present RPLC separation of the histone complement from mESC cells: UV traces at 215 nm (blue), 225 nm (red), and 235 nm (pink) with the peaks for H3 histones labeled. Also shown are the concentration of buffer B (green line), flow rate (orange line), and solvent conductivity (brown line).

Figure S2

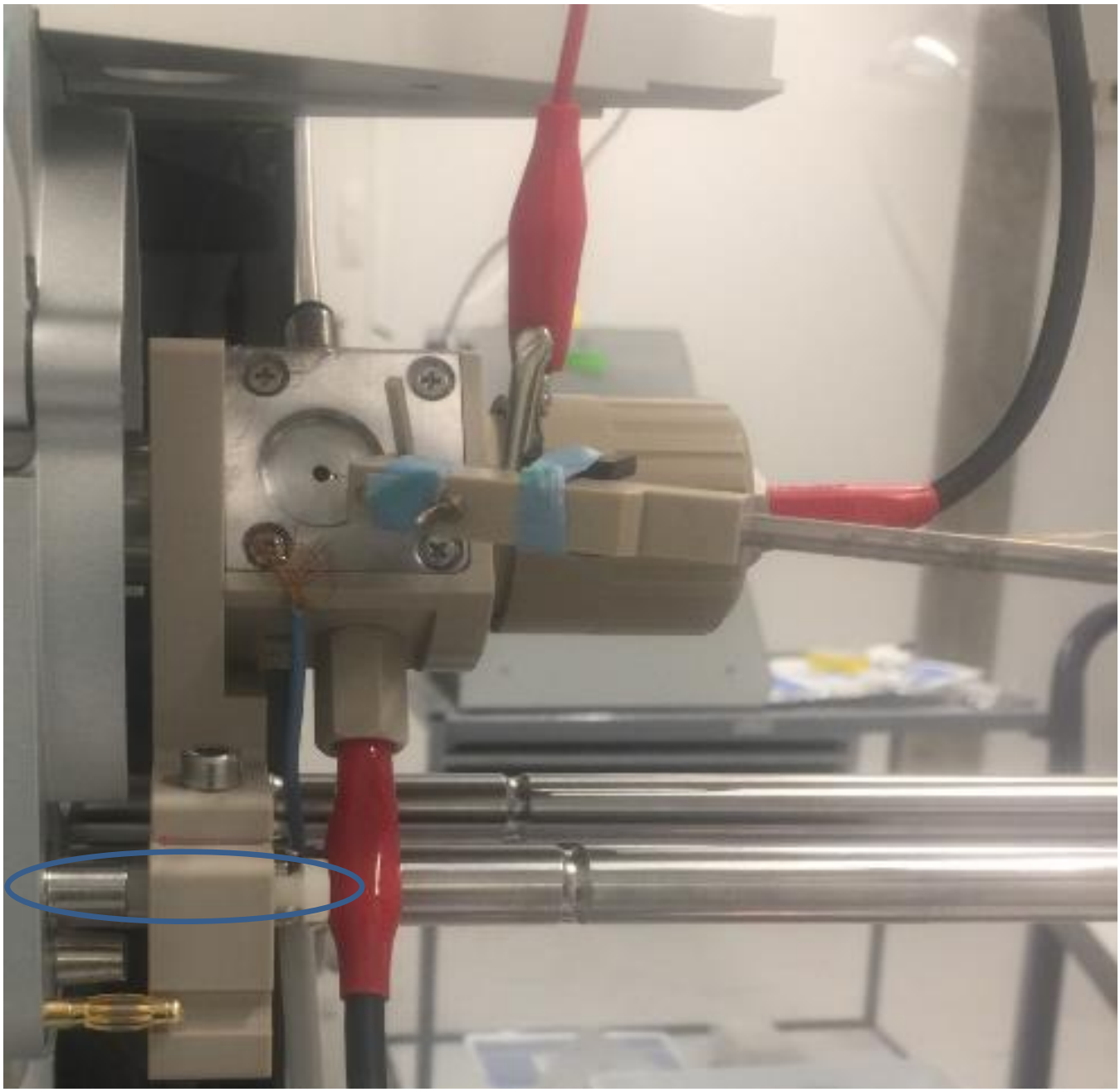
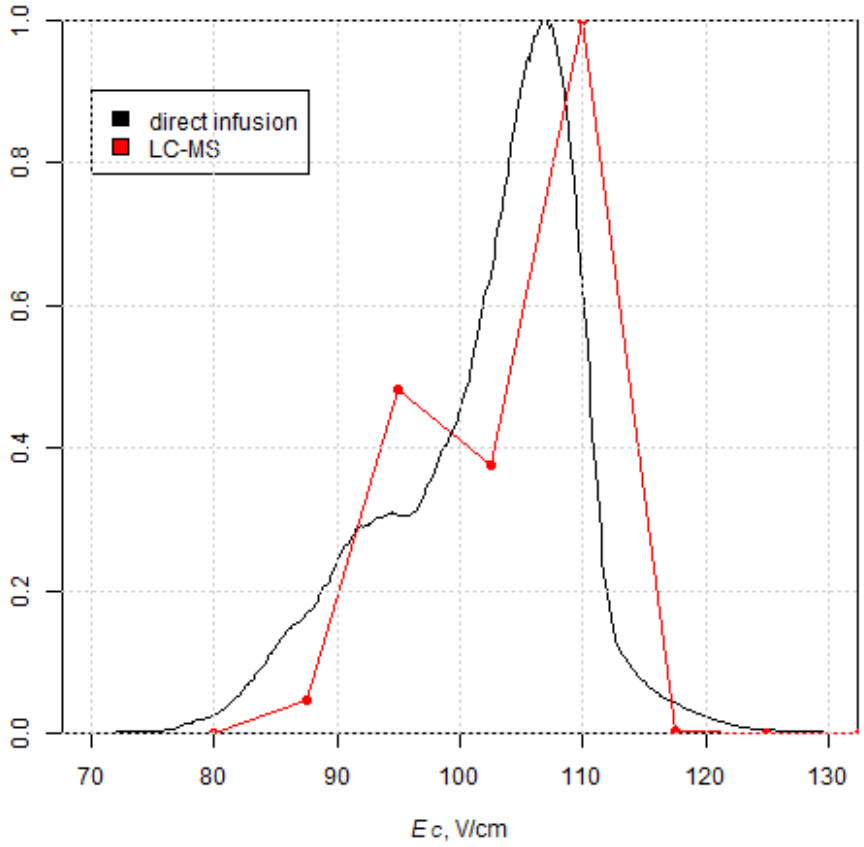


Photo of FAIMS unit attached to the inlet of Thermo Orbitrap Fusion Lumos mass spectrometer

Figure S3

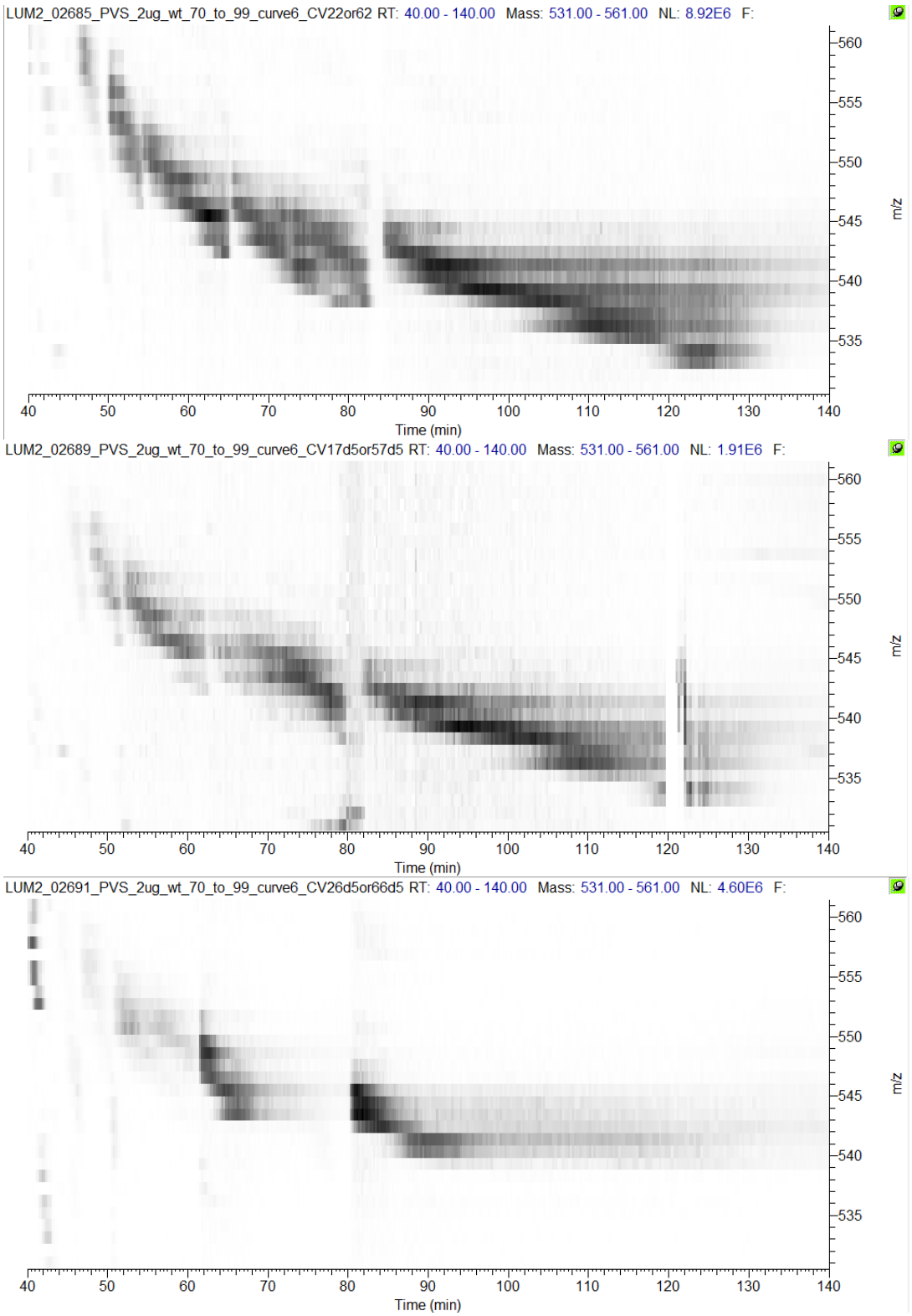
Detailed MS instrument method

Figure S4



Normalized FAIMS spectra for the unmodified proteoform: measured by scanning during the direct infusion and reconstructed from LC/MS data at selected E_C values. The two spectra were acquired on different days, and small systematic E_C shift between them likely reflects the day-to-day variation of DV and/or ambient gas pressure & temperature ubiquitous in FAIMS operation.

Figure S5



The LC/MS ion signal heat maps acquired at E_C of 87.5, 100, and 132.5 V/cm (top to bottom).