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)



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).



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

Method Editor Global Paramet		ers Scan Parameters Su		Summary	ummary	
ocument View Tree View						
Method Summary		Orbitrap Resolution: 120000 Mass Range: Normal			Low: 0.05 High: 0.05	
		Use Quadrupo	le Isolation: True			
Method Settings		RF Lens (%): 35			Data Dependent	
Application Moder Intact Protein		AGC Target: 1.0e6 Maximum Injection Time (ms): 50			Data Dependent Mode: Cycle Time Time between Master Scans (sec): 30	
Method Duration (min): 155		Microscans: 2	2 rofile			
Global Parameters		Polarity: Posi	tive		Scan Event Type 1:	
Globar Parameters		Source Fragme Energy (V): 5	entation: Enabled		Sort by Intensity	
Ion Source		Scan Descripti	on:		Precursor Priority: Most Intense	
Ion Source Type: NSI	Fil	ters:			Scan	
Spray Voltage: Static		racted Mass			Scan:	
Negative Ion (V): 600		rgeted Mass			ddMS ² OT ETD	
Positive Ion		ass List			Isolation Mode: Quadrupole	
		Include Start/End Times: False			Isolation Window (m/z): 1.2 Isolation Offset: Off	
Positive Ion		Include Intensity Threshold: True Mass List Type: m/z			Activation Type: ETD	
Time (min) Voltag	e (V)				ETD Reaction Time (ms): 30	
Negative Ion		Compound	m/z	Intensity	ETD Reagent Target: 2.0e5 Max ETD Reagent Injection Time (ms): 1000	
				Threshold	ETD Supplemental Activation: False	
Negative Ion			535,0137	100000	Scan Range Mode: Define m/z range	
Time (min) Voltag	e (V)		527.9169	100000	Orbitrap Resolution: 60000 Scan Range (m/z): 200-2000	
Sweep Gas (Arb): 0 Ion Transfer Tube Temp (°C): 75 Use Ion Source Settings from Tune: False FAIMS Mode: Not Installed			AGC Target: 1.0e5	AGC Target: 1.0e5		
			540.7199	100000	Injections for All Available Parallelizable Time: Paise Maximum Injection Time (ms): 5000 Microscans: 3 Data Type: Profile	
			542.1207	100000		
MS Global Settings			543.5214	100000	Use EASY-IC**: False	
Pressure Mode: Low Pressure Default Charge State: 1 Internal Mass Calibration: Off			544.9216	100000	Scan Description:	
			546.3216	100000		
			547.7221	100000		
Experiment#1 [MS]			549.1228	100000		
Start Time (min): 0			550.5233	100000		
End Time (min): 155			551.9238	100000		
Cycle Time (sec): 30			553.3239	100000		
Master Scan:			554.7245	100000		
MS OT			556.1255	100000		
MS OT						

Detailed MS instrument method



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



The LC/MS ion signal heat maps acquired at $E_{\rm C}$ of 87.5, 100, and 132.5 V/cm (top to bottom).