Supplementary Methods

Yeast samples

Proteins were precipitated by the addition of 0.4 N H_2SO_4 to 300 µL of 10 mg/mL yeast lysate and incubated at 4 °C on a rotator overnight. Precipitated protein was pelleted by spinning at 16,000 X g at 4 °C for 10 min. The supernatant was removed before 1 part 100% TCA was added to 3 parts supernatant and cooled on ice for 1 hour to precipitate proteins that remained in solution. Proteins of interest were pelleted by spinning at 16,000 X g at 4 °C for 10 min. The supernatant was discarded and the pellet washed with acetone followed by spinning at 16,000 X g at 4 °C for 5 min for a total of three wash cycles. The resulting pellet was air dried for 20 min before the sample was resuspended in 0.2 % formic acid and passed through a 30 kDa molecular weight cutoff filter to remove larger proteins.

Online reverse-phase chromatography was performed using a Nano-Acuity UPLC system (Waters, Milford, MA). Peptides were loaded onto a precolumn (75 µm ID, packed with 5 cm of 3 µm, 300 Å Magic C18 particles, Bruker, Michrom) for 10 min at a flow rate of 1 µL/min. Samples were then eluted over an analytical column (50 µm ID, packed with 15 cm of 3 µm, 300 Å Magic C18 particles, Bruker, Michrom) at 300 nL/min using a 120 min gradient of solvent A (99.8% water, 0.2% formic acid) and solvent B (99.8% acetonitrile, 0.2% formic acid): 8% to 35% B from 0 to 60 min and 35% to 85% B from 60 to 120 min.

Data were collected on an LTQ Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA). The nitrogen flow to the Orbitrap chamber was altered such that the increase in pressure (as measured by a Penning ionization gauge) was ~0.15 X 10^{-10} Torr, as compared to the pressure in the absence of nitrogen. Medium resolution survey scans (30,000 resolving power; 3 microscans) were used to guide data dependent sampling of the top two most intense peaks. Three scan functions were performed for each precursor: (1) isolation and analysis

at 30,000 resolution (4 microscans), (2) isolation and analysis at 480,000 resolution (4 microscans), and (3) HCD activation (NCE = 30) of the precursor followed by FT analysis (480,000 resolution; 4 microscans) of product ions with m/z values > 500. Target ion accumulation values were set to 3 x 10^6 and 1 x 10^5 for MS^1 and MS^2 scans, respectively. For all scan functions the precursor ions were isolated ±2.5 Th and peaks with assigned charge states of 1-3 were excluded from analysis. A maximum of 500 precursors were dynamically excluded for 20 s with a window -1.5 Th and +1.5 Th surrounding the precursor.

Myoblast/myotube samples

C2C12 cells were grown in DMEM dropout culture media (Cambridge Isotopes) supplemented with K_{602} or K_{080} lysine. Cells were harvested after six passages, with the exception of the cells grown on K_{602} lysine, which were placed in differentiation media and allowed to differentiate for an additional five days before harvesting.

The cells were resuspended in Nuclei Isolation Buffer (NIB; 15 mM Tris-HCl pH 7.5, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 250 mM sucrose) with 0.2% NP-40 at a final ratio of 10:1 buffer:sample, and the NeuCode channels were combined 1:1 based on total protein amounts (BCA, Pierce). Nuclei were isolated and histones extracted and separated as previously described.[1]

Intact histone variants were resuspended in 50% ACN/H2O with 0.2% formic acid and infused via a static nanospray capillary into an LTQ Orbitrap mass spectrometer at 3 μL/min. Spectra were collected and averaged for approximately 2 minutes. Analysis was performed at 480,000 resolution (2 microscans), and target ion accumulation values were set to 2 x 10^6 and 8 x 10^5 for MS^1 and MS^2 scans, respectively. For MS^2 analysis, the species at 776 Th was isolated (10 Th window) and fragmented with ETD (10 ms reaction time).

To determine protein identity, the mass shift (36 mDa) induced by the presence of isotopically labeled lysine was used to determine the number of lysines within a precursor. The masses of all yeast proteins were then calculated assuming lysines were isotopically labeled with eight deuterium atoms, and candidate proteins were filtered based on the deviation of the intact protein mass of the isolated precursor ion. The initial tolerance was 10 Th, with the closest match being used (Elongin-C). Corresponding MS^2 spectra were then manually validated to confirm protein sequence.

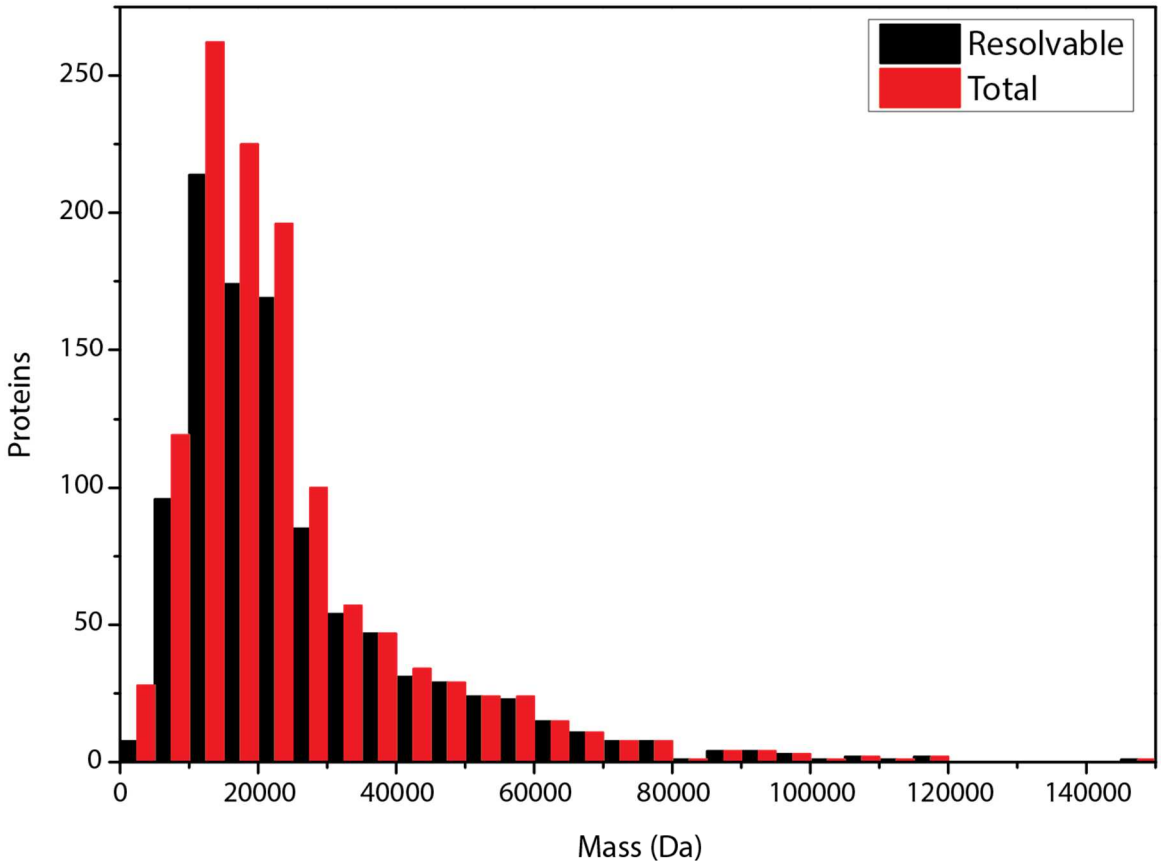

Figure 1. Histogram of the molecular mass of all of the proteins from the Kelleher data set and the proteins that could be resolved. The resolved proteins were determined by using 36 mDa spaced lysine isotopologues and 480,000 resolution. This demonstrates that we can resolve a large majority of the identified proteome in yeast regardless of molecular weight.

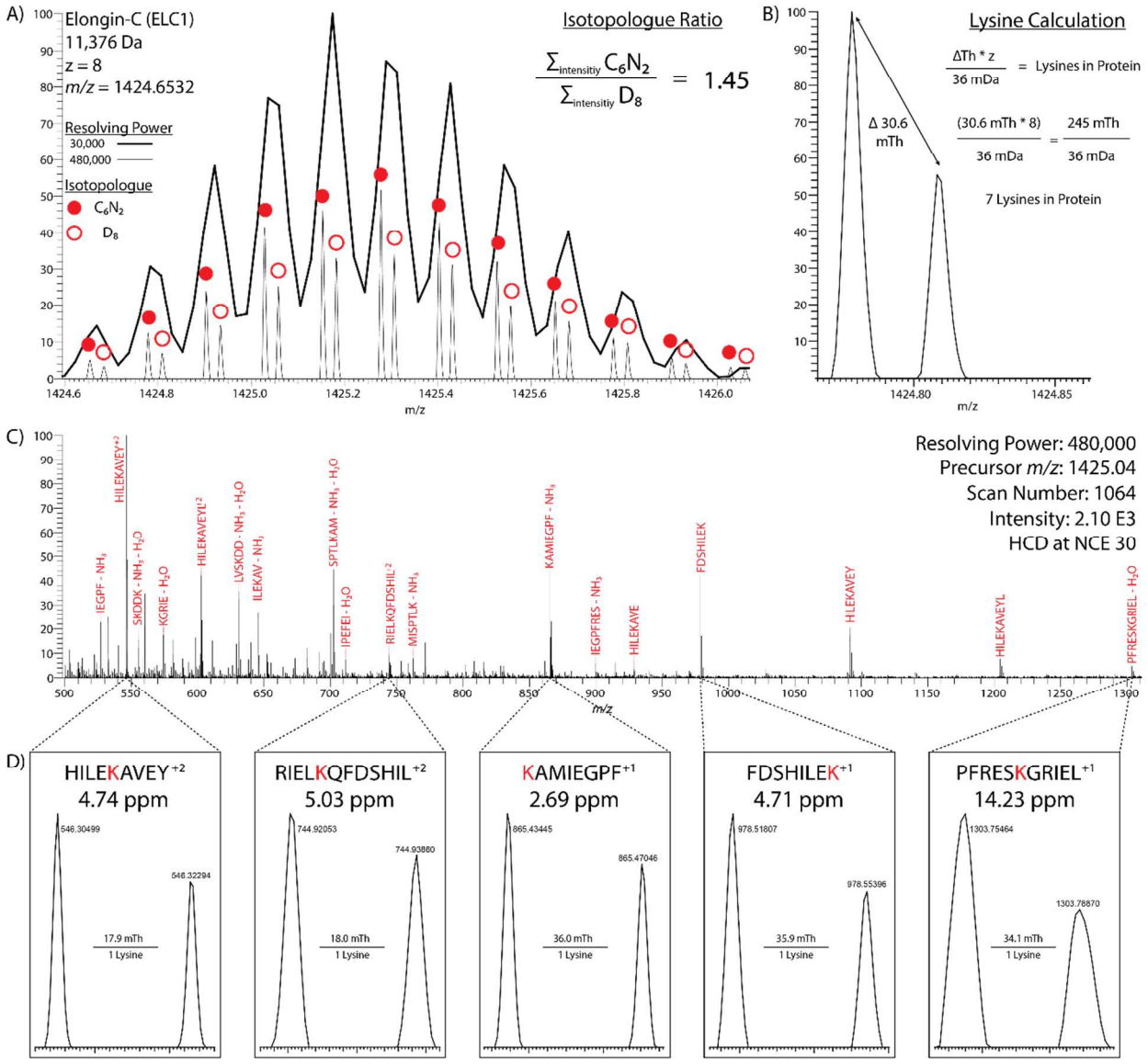

Figure 1. **Identification and Quantitation of Elongin-C from yeast using NeuCode.** A. The +8 charge state of Elongin-C (Uniprot Q03071) was isolated and analyzed first at 30,000 resolution which shows one distinct isotope distribution. A scan at 480,000 resolution, however, reveals the presence of two forms of the protein. The K_{602} form of lysine is represented by a closed circle, while the K_{080} form is represented by an open circle. The sum of the intensities of the two different forms provides the quantitative data, yielding a ratio of 1.45:1. B. The spacing between the isotopologue peaks can be used to calculate the number of lysines present in the protein. The protein is carrying 8 charges and the peaks are spaced 30.6 Th apart, indicating 7 lysines. C. Annotated fragmentation

spectrum of the Elongin-C precursor. The sequence of each product ion peak (within 20 ppm) is listed above the peak. D. Selected examples of product ions containing a lysine, showing both isotopologue peaks.

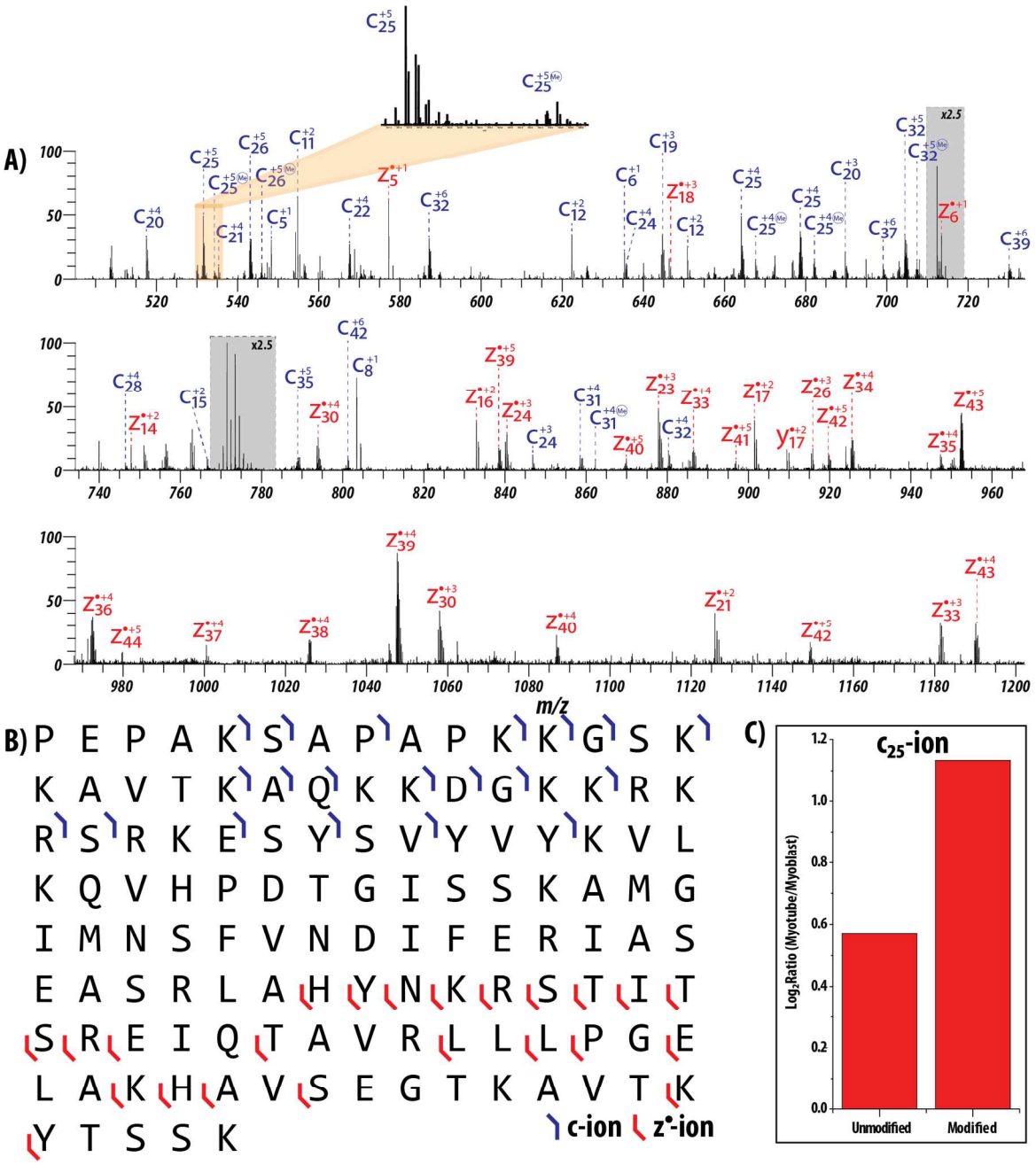

Figure 2. **Identification and Quantification of histone H2B from an ETD fragmentation spectrum.** Histone H2B (Uniprot P70696) purified from differentiating murine myoblasts was infused into the mass spectrometer and fragmented with ETD. A. The annotation of the fragments from 500-1200 Th. The inset displays the resolved isotopologues of the modified and unmodified forms of the c25 fragment used to derive the quantitation in C. B. Sequence of H2B, with the coverage represented by carrots at each fragment site (blue for c-ions, red for $z^{\bullet}$ ions). C. Integration of the two forms of the c25 ion demonstrate a change due to differentiation.

References

(1) Young, N. L.; DiMaggio, P. A.; Plazas-Mayorca, M. D.; Baliban, R. C.; Floudas, C. A.; Garcia, B. A. *Molecular & cellular proteomics : MCP* **2009**, *8*, 2266-84.