Supporting Information Coversheet

Determining the Mitochondrial Methyl Proteome in *Saccharomyces cerevisiae* using Heavy Methyl SILAC

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Table of Contents

Supplementary Table 3. Raw data tables from ProLuCID searches through the IP2 pipeline.

Supplementary Figure 1. Extracted precursor ion chromatograms and fragmentation patterns of candidates from cytoplasmic ribosomal protein fraction.

Supplementary Figure 2. Extracted precursor ion chromatograms and fragmentation patterns of candidates from mitochondrial ribosomal protein fraction.

Supplementary Figure 3. Extracted precursor ion chromatograms and fragmentation patterns of candidates from mitochondrial fraction.

Supplementary Figure 4. Structural models of newly identified methylation sites.

Supporting Information Legends

Supplementary Table 1. Summary of the methyl peptides identified in this study as well as function and localization of the resulting methyl protein. Contains Supplementary Table 1.1: Table of Candidate Methylated Proteins; Supplementary Table 1.2: All Peptides Identified as Methylated; Supplementary Table 1.3: Candidate Methyl Peptides Across All Three Fractions; Supplementary Table 1.4: Candidate Methyl Peptides From the Cytoplasmic Ribosomal Protein Fraction; Supplementary Table 1.5: Candidate Methyl Peptides From the Mitochondrial Protein Fraction; Supplementary Table 1.6: Candidate Methyl Peptides From the Mitochondrial Ribosomal Protein Fraction.

Supplementary Table 2. Summary of overlapping methylated open reading frames from known and current studies used to generate Figure 7. Contains Supplementary Table 2.1: Overlapping Open Reading Frames from Known Studies; Supplementary Table 2.2: List of Open Reading

Frames Identified as Methylated in Known and Current Studies; Supplementary Table 2.3: Methylated Yeast Proteins Curated from UniProt.

Supplementary Table 3. The raw data from the ProLuCID searches as analyzed through the IP2 pipeline. Contains Supplementary Table 3.1: Raw data for MRP replicate 1 from ProLuCID searches through the IP2 pipeline; Supplementary Table 3.2: Raw data for MRP replicate 2 from ProLuCID searches through the IP2 pipeline; Supplementary Table 3.3: Raw data for Mitochondria replicate 1 from ProLuCID searches through the IP2 pipeline; Supplementary Table 3.4: Raw data for Mitochondria replicate 2 from ProLuCID searches through the IP2 pipeline; Supplementary Table 3.5: Raw data for Mitochondria replicate 3 from ProLuCID searches through the IP2 pipeline; Supplementary Table 3.6: Raw data for Cytoplasmic Ribosomes replicate 1 from ProLuCID searches through the IP2 pipeline; Supplementary Table 3.7: Raw data for Cytoplasmic Ribosomes replicate 2 from ProLuCID searches through the IP2 pipeline; Supplementary Table 3.8: Raw data for Cytoplasmic Ribosomes replicate 3 from ProLuCID searches through the IP2 pipeline.

Supplementary Figure 1. Extracted precursor ion chromatograms and fragmentation patterns of candidates from cytoplasmic ribosomal protein fraction. For each candidate peptide, in each replicate, of the cytoplasmic ribosomal protein fraction, extracted precursor ion chromatograms for the light (top) and heavy (bottom) peptide are displayed first. The zero, +1 and +2 isotopes are shown in blue, magenta, and red, respectively. The methylated residue is highlighted in blue in the peptide sequence. Fragmentation patterns of the corresponding peptide follow. The methylated residue is highlighted in yellow in the peptide sequence.

Supplementary Figure 2. Extracted precursor ion chromatograms and fragmentation patterns of candidates from mitochondrial ribosomal protein fraction. For each candidate peptide, in each replicate, of the mitochondrial ribosomal protein fraction, extracted precursor ion chromatograms for the light (top) and heavy (bottom) peptide are displayed first. The zero, +1 and +2 isotopes are shown in blue, magenta, and red, respectively. The methylated residue is highlighted in blue in the peptide sequence. Fragmentation patterns of the corresponding peptide follow. The methylated residue is highlighted in yellow in the peptide sequence.

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Supplementary Figure 4. Structural models of newly identified methylation sites. Structural models of methylated protein candidates made using Protein Homology/analogy Recognition Engine V 2.0 (Phyre[2]) and visualized in PyMol. The methylated residue reported in Tables 1, 2, and 3 is highlighted in orange.