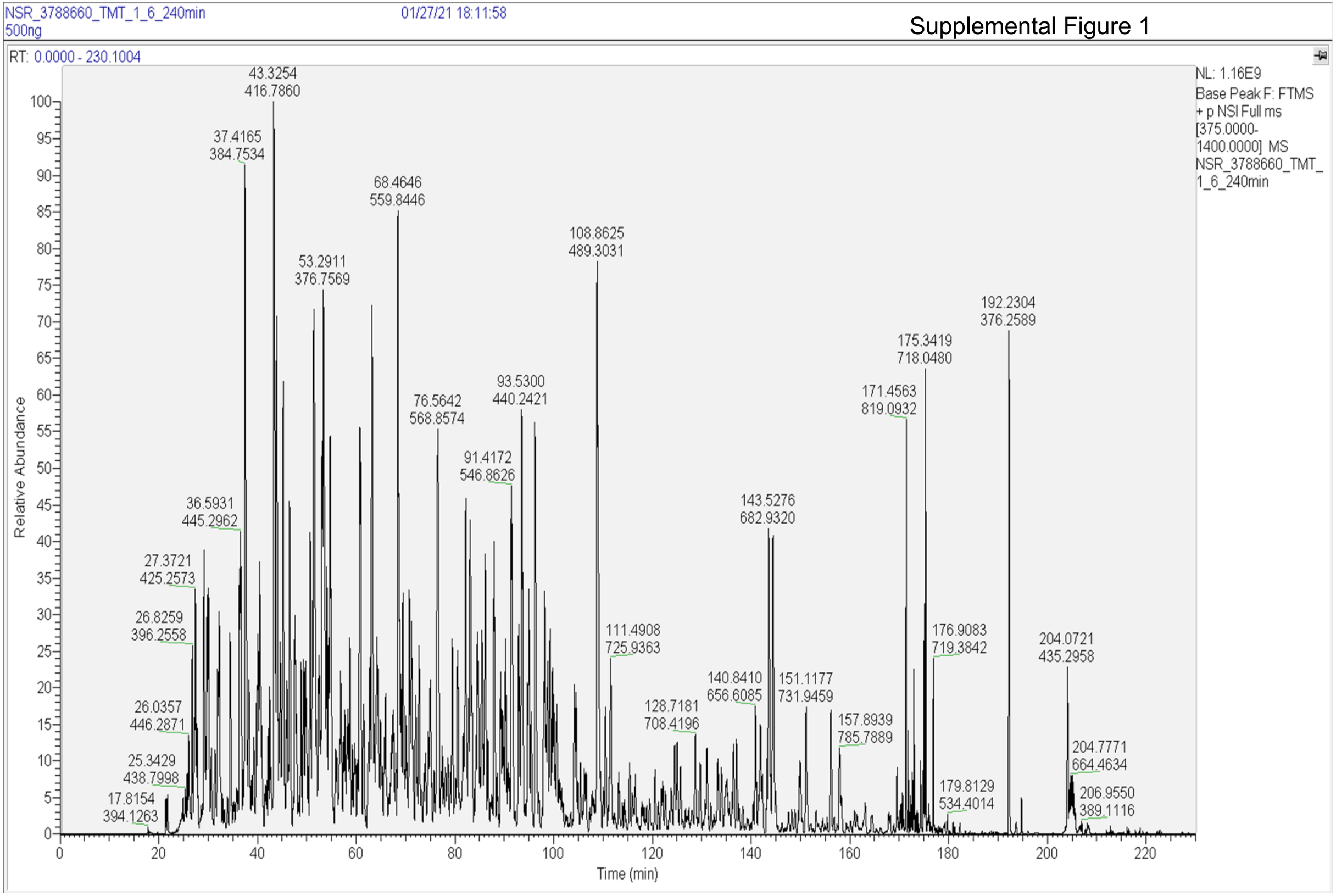
A




Supplemental Figure 1

B

Experiment Methods

| Parameter | Value |
|---|---|
| Experiment: | NSR_3788660 (1) |
| Peak List Generator: | |
| Version: | |
| Charge States Calculated: | |
| Deisotoped: | |
| Textual Annotation: | |
| Database Set: | 1 Database |
| Database Name: | Contaminants.fasta; Mus_Musculus_Uniprot_Rev_17042_07292020.fasta |
| Version: | |
| Taxonomy: | All Entries |
| Number of Proteins: | 17263 |
| Does database contain common contaminants?: | |
| Search Engine Set: | 1 Search Engine |
| Search Engine: | Sequest |
| Version: | IseNode in Proteome Discoverer 2.4.0.305 |
| Samples: | All Samples |
| Fragment Tolerance: | 0.020 Da (Monoisotopic) |
| Parent Tolerance: | 10.0 PPM (Monoisotopic) |
| Fixed Modifications: | +229 on K (TMT6plex) |
| Variable Modifications: | -131 on M (Met-loss), -89 on M (Met-loss+Acetyl), +16 on M (Oxidation), +... |
| Database: | Contaminants.fasta; Mus_Musculus_Uniprot_Rev_17042_07292020.fasta (... |
| Digestion Enzyme: | Trypsin |
| Max Missed Cleavages: | 2 |
| Probability Model: | |
| NSR_3788660_TMT_1_6_240min_Static: | Use Previously Calculated Probability (No analysis) [all charge states] |
| NSR_3788660_TMT_7_12_240min_Static: | Use Previously Calculated Probability (No analysis) [all charge states] |
| Scaffold: | Version: Scaffold_4.11.1 |
| Modification Metadata Set: | 2334 modifications |
| Source: | C:\Program Files\Scaffold 4\parameters\unimod.xml |
| Comment: | |
| Protein Grouping Strategy: | Experiment-wide grouping with protein cluster analysis |
| Peptide Thresholds: | 95.0% minimum |
| Protein Thresholds: | 95.0% minimum and 2 peptides minimum |
| Peptide FDR: | 0.0% (Decoy) |
| Protein FDR: | 0.1% (Decoy) |
| GO Annotation Source(s): | |
| Pathway Annotation Source(s): | |
| Alternate ID Source(s): | FASTA:UniProt/Swiss-Prot Alt. Accession (UniProtKB) |

DATABASE SEARCHING-- Tandem mass spectra were extracted by [unknown] version [unknown]. Charge state deconvolution and deisotoping were not performed. All MS/MS samples were analyzed using Sequest (Thermo Fisher Scientific, San Jose, CA, USA; version IseNode in Proteome Discoverer 2.4.0.305). Sequest was set up to search Contaminants.fasta; Mus_Musculus_Uniprot_Rev_17042_07292020.fasta (unknown version, 17263 entries) assuming the digestion enzyme trypsin. Sequest was searched with a fragment ion mass tolerance of 0.020 Da and a parent ion tolerance of 10.0 PPM. TMT6plex of lysine was specified in Sequest as a fixed modification. Met-loss of methionine, met-loss+Acetyl of methionine, oxidation of methionine, acetyl of the n-terminus and nethylmaleimide of cysteine were specified in Sequest as variable modifications.

CRITERIA FOR PROTEIN IDENTIFICATION-- Scaffold (version Scaffold_4.11.1, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability by the Percolator posterior error probability calculation (Käll, L et al, Bioinformatics, 24(16):i42-i48, Aug 2008). Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii, Al et al Anal. Chem. 2003;75(17):4646-58). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Proteins sharing significant peptide evidence were grouped into clusters.