SUPPLEMENTAL FIGURES

Sup FIG. 1 Distribution of heavy/light ratios and unique peptide pairs. The signal ratio of $[^{13}C_6]$Lysine-Labeled peptides to unlabeled peptides was calculated. A, shows the distribution of ratios for peptides unique to a single protein group. B, shows the distribution of Unique $[^{13}C_6]$Lysine-Labeled peptides observed per protein.

Sup FIG. 2. Technical precision of peptide measure. To assess technical precision in peptide measure, four 30 μg aliquots of a human IM fraction were individually mixed with 15 μg $[^{13}C_6]$brain ISTD and analyzed by LC-SRM/MS with triplicate injections. A, Peptide measure CV was calculated and the average of these taken for the four repeated preparations. A, shows this average CV number was plotted against the average integrated area of each peptide from all preparations and injections. While CV correlates with peak area ($R^2 = .71$), the CV values for all but four peptides are under 20%. B, shows the distribution of CV of the four repeated preparations by light/heavy ratios and light only intensities.

Sup FIG. 3. Comparison of LC-SRM/MS and LC-MS/MS analysis. To assess technical precision in peptide measure, four 30 μg aliquots of a human total homogenate were individually mixed with 15 μg $[^{13}C_6]$-brain ISTD and analyzed by LC-SRM/MS with triplicate injections. A 60 μg aliquot of the same human total homogenate fractions was mixed with 30 μg $[^{13}C_6]$-brain ISTD, separated by SDS PAGE in to 5 fractions, and analyzed by LC-MS/MS on a Q-Exactive with triplicate injections. A, of 1408 unique gene symbols were identified in the Q-Exactive analysis. Of these, 131 were monitored by LC-SRM/MS on the TSQ Vantage. B, Shows the L/H ratios for 84 peptides quantified in both experiments.
Sup FIG. 4. **Comparison of manual and Pinpoint flagging SRM evaluation.** Peptide SRM peaks were evaluated by both manual inspection and Pinpoint flagging algorithms. The peptides were then divided into two groups: those included in protein quantification calculations (*Included*) and those excluded from quantification calculations (*Excluded*) and plotted by the L/H (A) and Apex score (B) as calculated by Pinpoint. In both cases, the majority of the *Excluded* peptides had higher scores, indicating agreement between manual evaluation and Pinpoint flagging.

Sup FIG. 5. **Distribution of variance in protein quantification by fraction.** CVs for protein enrichment values were calculated for each fraction, and the distribution plotted in 5 unit bins up to 30%. A, Hom. B, Syn. C, Ves. D, Para. E, PSD. The distribution shape is often similar between the two species, but the center is often lower in preparations from mouse tissue.

Sup FIG. 6. **Heat Map of Hierarchical clustering, mouse fractions.** To sort out protein groups and fractions by enrichment value unsupervised hierarchical clustering, with Pearson's Correlation as the similarity metric, was utilized. This figure shows this clustering analysis of the mouse fractions as a heat map. Note that even though mouse parasympathetic 3 (MP 3) shows a slightly different pattern of enrichment from MP 1 and MP 2, the fraction still clusters with the other parasympathetic preparations. *synaptosomal (MSF), vesicular (MV), parasympathetic (MP) and PSD (MD).*

Sup FIG. 7. **Heat Map of Hierarchical clustering, human fractions.** To sort out protein groups and fractions by enrichment value unsupervised hierarchical clustering, with Pearson's Correlation as the similarity metric, was utilized. This figure shows this clustering analysis of the
human fractions as a heat map. *synaptosomal (HSF), vesicular (HV), parasympathetic (HP) and PSD (HD).*

Sup FIG. 8. **Heat Map of Hierarchical clustering, mouse and human fractions.** To sort out protein groups and fractions by enrichment value unsupervised hierarchical clustering, with Pearson's Correlation as the similarity metric, was utilized. This figure shows this clustering analysis of the human and mouse fractions as a heat map. A, Kinases, scaffolding proteins and glutamate receptors were enriched in PSD fractions. B, SNARE complex proteins and vesicular amino acid transporters were enriched in vesicular fractions. C, guanine nucleotide binding protein subunits and voltage-dependent ion channels were enriched in parasympathetic fractions. D, Synaptosomal fractions were rich in cytosolic enzymes. E, a group of proteins were enriched in human PSD fractions, but not in mouse fractions. F, septin proteins measured were enriched in mouse PSD and parasympathetic fractions, but not in those fractions prepared from human tissues. *Human: synaptosomal (HSF), vesicular (HV), parasympathetic (HP) and PSD (HD).* Mouse, synaptosomal (MSF), vesicular (MV), parasympathetic (MP) and PSD (MD).

Sup FIG. 9. **Postmortem interval and contamination in PSD fractions.** We compared enrichment values for the “contaminant” proteins identified by the clustering analysis found to be differentially enriched in human and mouse D fractions. A, shows enrichments in human (HD) and mouse (MD) postsynaptic density (D) fractions and B, shows enrichments in human (HSF) and mouse (M SF) synaptosomal fractions (SF) and B. Error bars are standard deviation of three samples. To investigate if these differences could be explained by postmortem interval (PMI), D fractions were prepared from mouse brain tissue with simulated PMIs of 0 to 16 hours. C, shows measures of “contaminant” proteins over PMI and D, shows glutamate receptors and postsynaptic scaffolding proteins.
Sup FIG. 10. **Differences in septin family protein enrichments between mouse and human are not explained by postmortem interval.** We compared enrichment values for septin proteins in three fractions. A show enrichment in human parasynaptic fractions (H P) postsynaptic density (H D) and synaptosomal (H SF). B, shows the same for fractions prepared from mouse, M P, M D and M SF. C, shows measures for septin proteins over increasing postmortem interval (PMI) in D fractions prepared from mouse tissues.

Sup Table. 1. **Results of LC-MS/MS analysis from all gel fractions.** B. Protein description, C. Total protein coverage (percent), D. Total number of proteins in group, E. Total number of unique peptides observed, F. Total number of peptides observed, G. Total number of peptide spectral matches (PSMs), H. Protein family identifiers, I. Number of amino acids, J. molecular weight (kilodaltons), K. Calculated isoelectric point.

Sup Table. 2. **Peptide SRMs.** Columns: A. Gel Fraction, B. Protein Common Name, C. Peptide, D. Precursor, E. Fragment.

Sup Table. 3. **Light/heavy averages for each fraction.** Columns: A. Protein common name, B. Gene Name, D. – H. Human fraction averages, J. – N. Human fraction standard deviations, P. – T. Human fraction CV%\$s, W – AA, Mouse fraction averages AC – AG, Mouse fraction standard deviations AI – AM Mouse fraction CV%\$s. Homogenate (H), Synaptosomal Fraction (SF), Vesicular (V), Parasynaptic (P), Postsynaptic Density (PSD).
Sup FIG. 1.

A.

B.
Sup FIG. 2.

A.

B.

Sup FIG. 3.

B.

A. 20 131 1287
Sup FIG. 4.
Sup FIG. 5.
Sup FIG. 6.
Sup FIG. 7.
Sup FIG. 9.

Sup FIG. 10.