Electronic Supplementary Data

"Utility of High Resolution Accurate Mass Spectrometry (HRMS) in the Mass Isotopomer Distribution Analysis (MIDA) of CSF Proteins Modified by Stable Isotope Labeling in Mammals (SILAM) Methodology Applied to Neurodegenerative Diseases" by J. Cantone, C. Polson, C. Wei, V. Guss, M. Ahlijanian, J. Meredith and D. Drexler in Analytical Methods, 2017,

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To establish analytical figures of merit such as sensitivity, specificity, accuracy, precision, and reproducibility for the MIDA by LC-HRMS approach, the following studies were performed.

To institute the minimum MS signal intensity (AU) required for accurate and precise measurements of the isotopomers and specifically the M3 isotope, collected fractions from *in vivo* samples were serially diluted with ammonium carbonate buffer for ChrB (undiluted, 1:10, 1:50, 1:100, 1:500, 1:1000, 1:5000) and bTrace (undiluted, 1:10, 1:100, 1:500, 1:1000, 1:5000, 1:10000, 1:50000) analyses. The samples (n = 3) were analyzed by LC-HRMS and the mean of the M3/M0 ratio was determined. When compared to the theoretical M3/M0 ratio, a minimum MS signal intensity of $1.0x10^4$ AU of the M3 isotope (Figure S1 Panel A) was required so that the accuracy and precision of the measured data was in acceptable agreement (<10% CV, Figure S2 Panel B), which was implemented as a QC criterion.

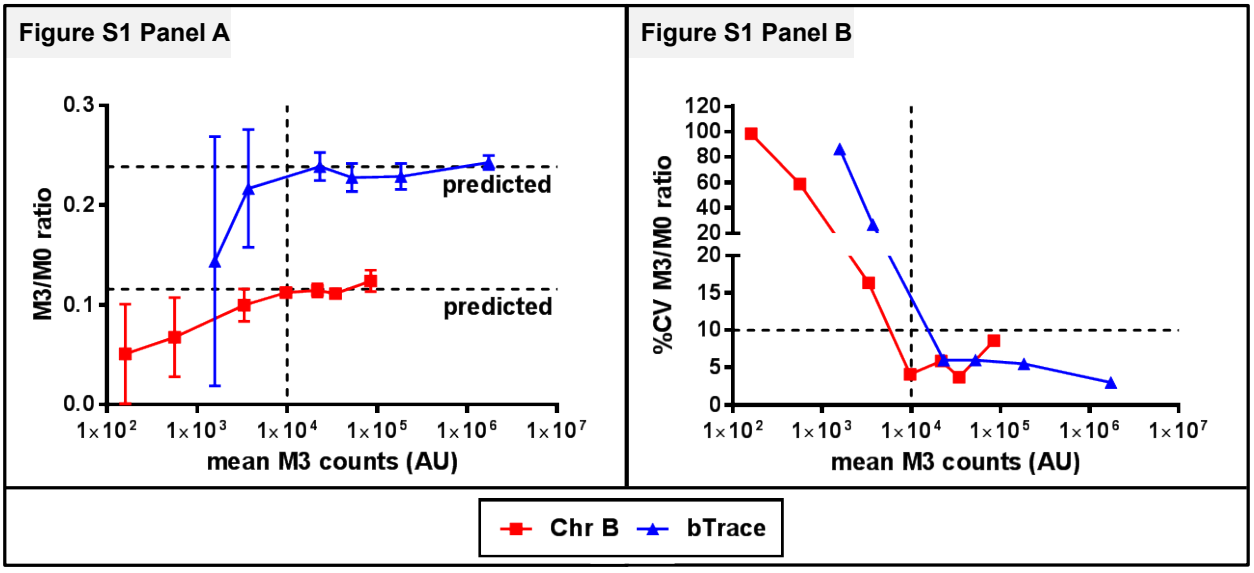

Figure S1: A minimum MS signal intensity of $1.0x10^4$ AU of the M3 isotope for ChrB and bTrace surrogate peptides (Panel A) results in an acceptable agreement (<10% CV) of the theoretical and measured M3/M0 ratios (Panel B).

To determine the lowest amount of detectable labeling, synthetic non-labeled and ^{15}N-labeled-L analogues (stable isotope label, SI-label) of the surrogate peptide of ChrB were mixed a various percentages (0, 0.5, 1, 2, 4, 6, 8, 10, 20, and 50) as neat solutions. The samples (n = 5) were analyzed by LC-HRMS and the mean of the M3/M0 ratio was determined. The %CV (coefficient of variance) was less than 2% at all levels. To reliably detect a significant change (delta) of the isotopomer distribution between labeling levels, it must be the mean plus 2-times SD (standard deviation), which is observed at 3% labeling and higher thus labeling less than 3% cannot be accurately measured (Figure S2 Panel A). Non-linearity was observed with >20% label. In summary, the M3/M0 ratio demonstrated a linear dynamic range from 3-20% label (Figure S2 Panel B).

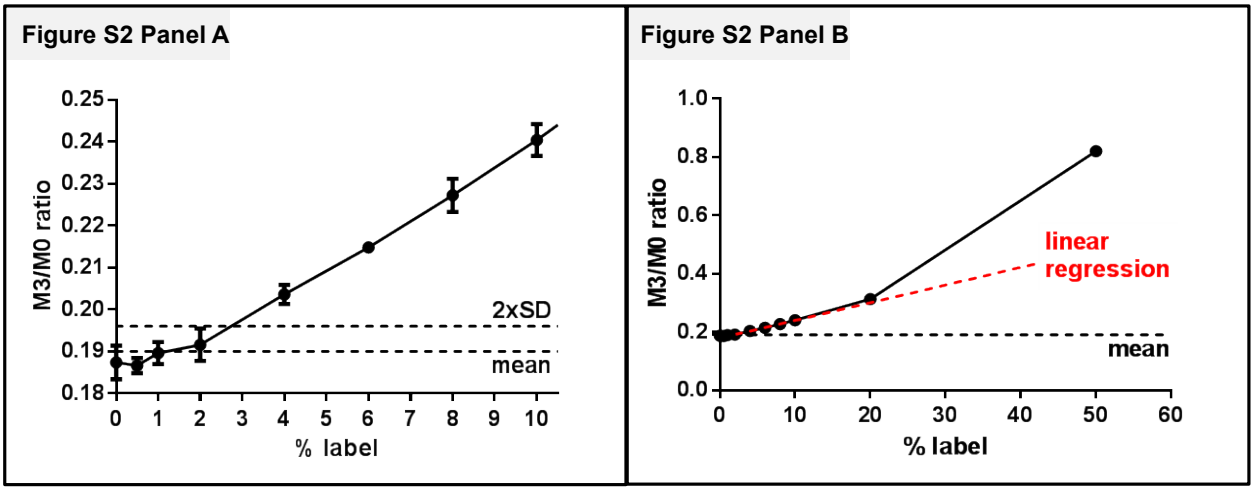

Figure S2: A minimum of 3% SI-label is required for an accurate measurement of the isotopomer distribution (Panel A). A linear dynamic range of the M3/M0 ratio of the ChrB surrogate peptide was observed between 3-20% SI-label (Panel B).