Evaluating multiplexed quantitative phosphopeptide analysis on a hybrid quadrupole mass filter/linear ion trap/Orbitrap mass spectrometer

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Supporting Information

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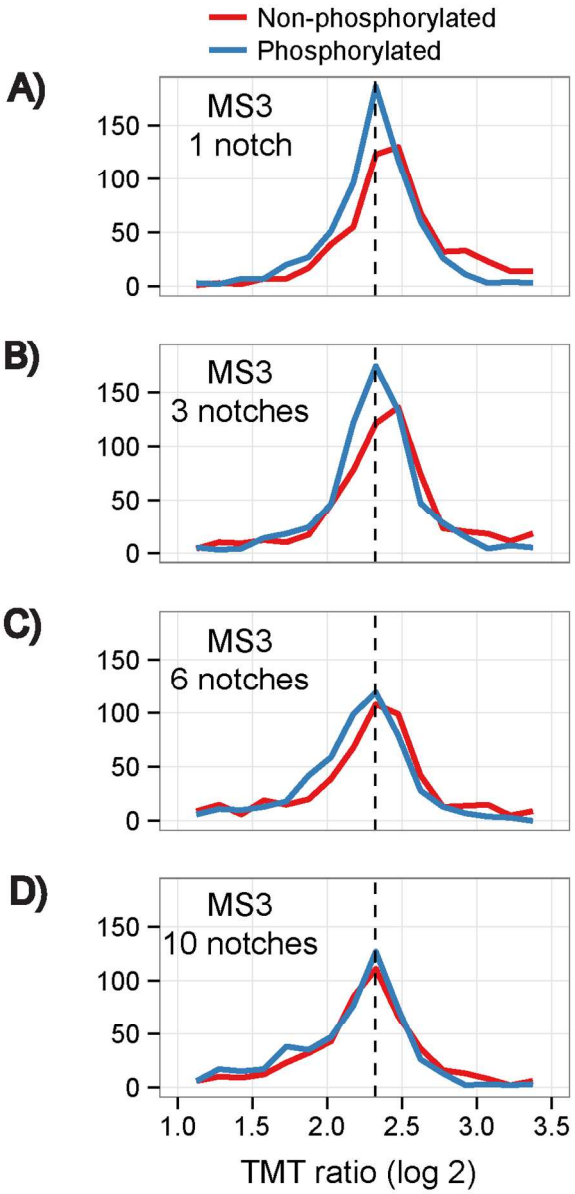
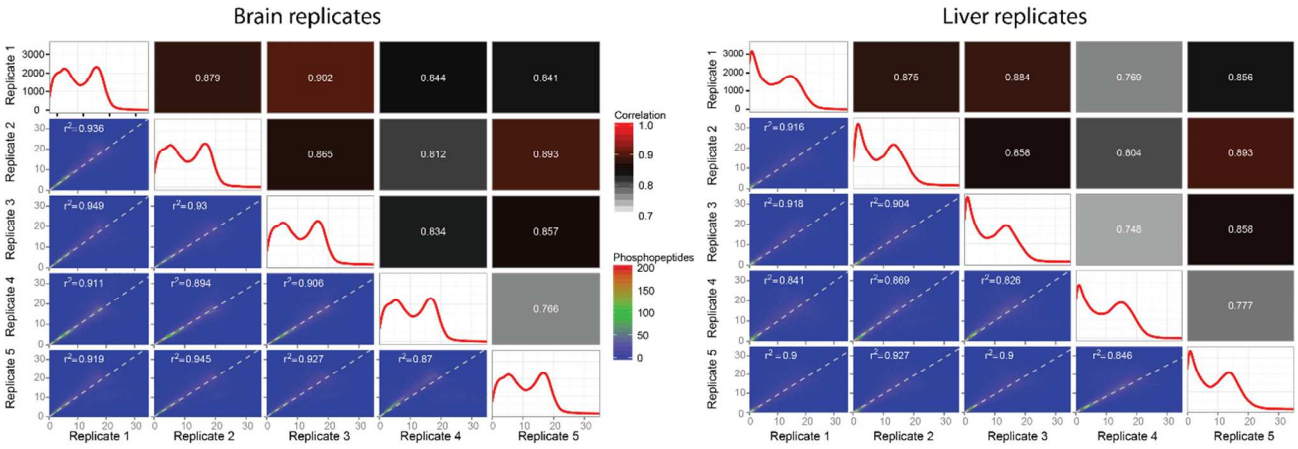
	Quantified
Phosphopeptides	38,247
Unique phosphopeptides	17,883
Proteins	4,048
Sites	11,015
Composite sites	2,958
Total Forms	**13,973**

Supplementary Table 1. Five mouse brains and five mouse livers were digested, phosphopeptide enriched, and labeled with 10-plex TMT. Following basic pH reverse phase fractionation, the resulting 24 fractions were analyzed by a SPS-MS3 method on the Orbitrap Fusion (see methods). Unique designates different sequence.



Supplementary Figure 1. Across the experiments where we varied the number of MS2 fragment ions included in the MS3 precursor population, we divided up the precursor peptides based on the presence or absence of a dominant (rank one intensity) neutral loss. If present, the peptide was classified as phosphorylated, if absent, the peptide was classified as non-phosphorylated. Histograms of ratios between channels corresponding to 5:1 ratio from each experiment are plotted above. Dashed lines represent the expected ratio of 5:1. **(A)** Phosphorylated peptides from the single notch MS3 experiment benefit from the significant retention of precursor intensity in the neutral loss, resulting in increased quantitative accuracy due to improved ion statistics. Conversely, non-phosphorylated peptides exhibited heterogeneous fragmentation resulting in perturbed ratios due to poor ion counts. As the number of notches increases **(panels B – D)**, and concurrently the proportion of MS2 ion current included in the MS3 spectrum, the accuracy of the non-phosphorylated peptides increases. The phosphorylated peptides displayed a slight tailing in their distributions as the number of notches increases; a consequence of a disproportionate increase in MS2 spectral space retained and the degree of intensity from the target precursor.



Supplementary Figure 2. 38,247 phosphopeptides from mouse brain or liver tissue were quantified. For all phosphopeptides, replicates within the tissues were plotted to assess reproducibility. In all cases, a strong linear relationship between biological replicates can be observed. The red trace provides a density plot of phosphopeptides for each replicate. Correlation coefficients for each replicate (Pearson) are indicated by the solid, color mapped boxes.