



SUPPLEMENTARY FIG. S3. Percolator improves the identification of both iodoTMT- and cystTMT-labeled peptides. (A) Mascot score and (B) charge state distributions of peptide spectrum matches for iodoTMT-labeled, cystTMT-labeled, IAM-labeled, and non-Cys-containing peptides. More than 80% of iodoTMT-labeled peptides bore 3+ or higher charges, compared to only 20% for peptides without Cys residue or 48% for peptides with IAM alkylated Cys. About 40% of iodoTMT-labeled peptides were identified by a Mascot score <20, compared with only 7.6% of non-labeled peptides identified at such a low score. (C) The PEP of each iodoTMT-labeled PSM as calculated by the Mascot Percolator was plotted against its Mascot ion score to demonstrate its correlation and derived orthogonality. Dash lines indicate the threshold based on Mascot scores or PEPs for determining the confidence of peptide identification (FDR < 0.01). (D) Venn diagram summary of identified iodoTMT- or cystTMT-labeled peptide hits under 1% FDR, as determined by Mascot score alone or with additional PEP considerations provided by Mascot Percolator. *Note:* The Percolator module uses a semi-supervised method to train a machine-learning algorithm to discriminate between correct and incorrect PSMs (5, 34, 68). A set of features related to the quality of the match (*e.g.*, search engine scores, charge state, precursor mass deviation, and average fragment mass deviation (Supplementary Table S1) were computed through a given FDR to train a robust classifier for re-scoring and reporting associated *q*-value and PEP of each PSM in the data set. Therefore, all PSMs from both forward and decoy databases were sorted by PEP and the identification threshold was set to reach the designed FDR. FDR, false discovery rate; IAM, iodoacetamide; PEP, posterior error probability; PSM, peptide spectrum match.