

TMT labeling for the masses: A robust and cost-efficient, in-solution labeling strategy

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Supplemental Data:

- Supplemental Figure 1
- Supplemental Figure 2

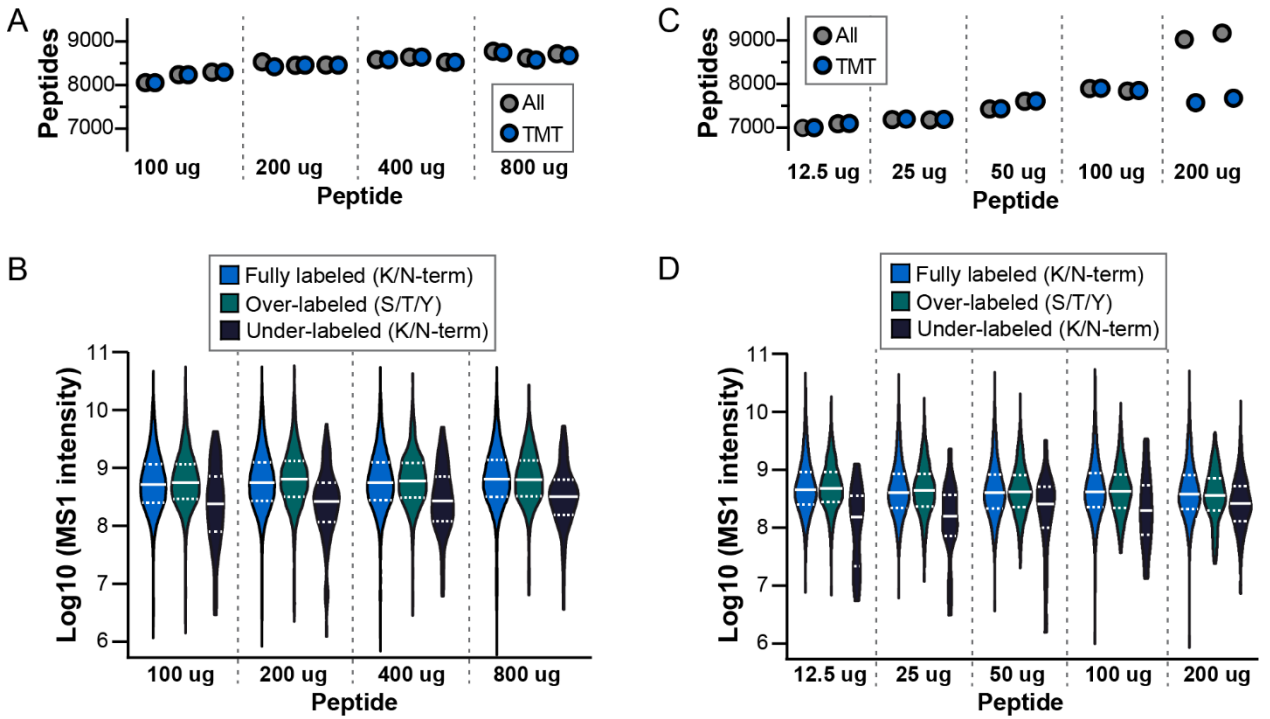


Fig. S1: **Peptide titration experiments using the vendor recommended (A, B) and a downscaled (C, D) TMT labeling protocol.** (A) The numbers of all peptides (grey circles, including fully, partially and non-labeled sequences) and only fully TMT labeled peptides (blue circles) are displayed for the labeling experiment series illustrated in main figure 1A. (B) MS₁ intensities of fully, over- and under-labeled peptides are shown for the labeling experiment series depicted in main figure 1A. Intensities of fully labeled peptides were extracted from the under-labeling search. (C) Same as (A) but for the labeling experiment series shown in main figure 1D. (D) Same as (B) but for the labeling experiment series illustrated in main figure 1D.

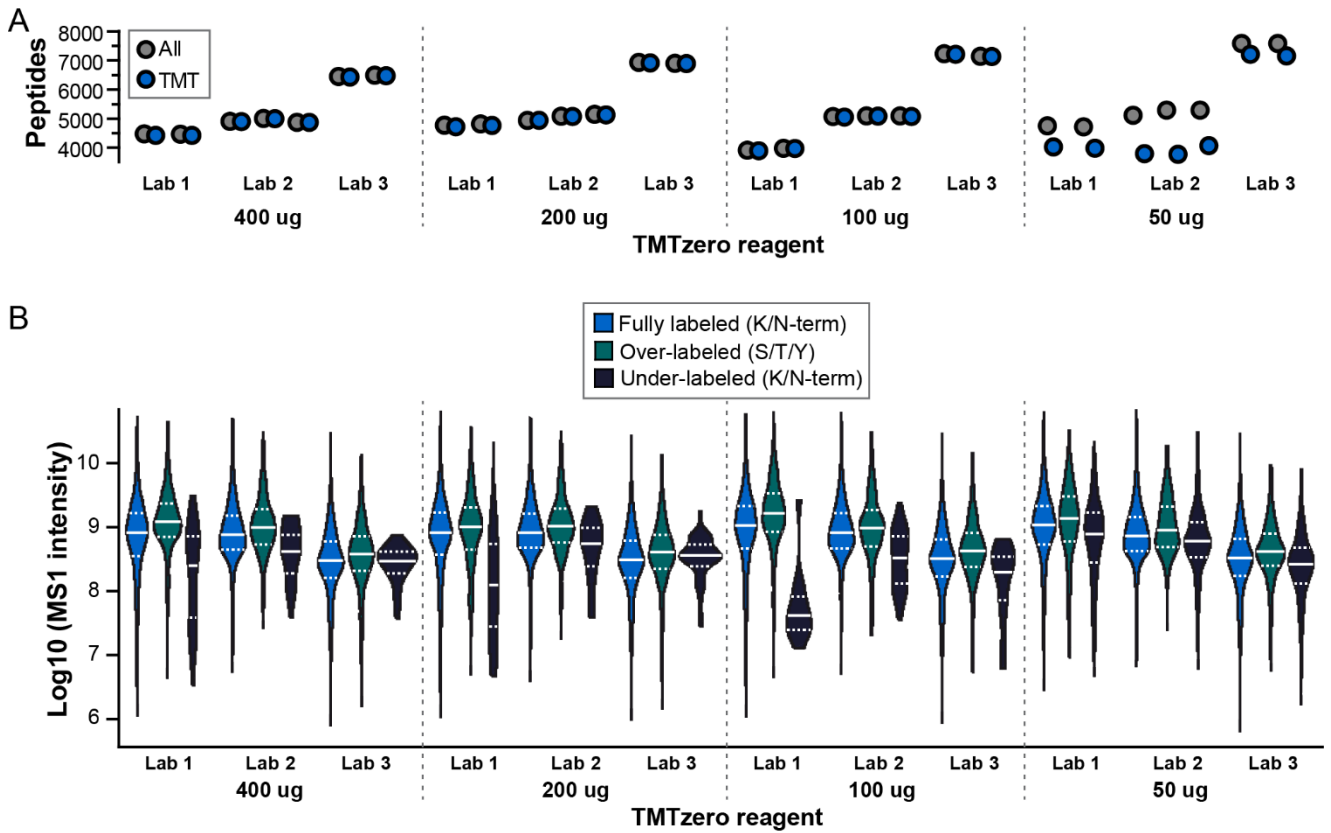


Fig. S2: **TMT titration experiments using the downscaled TMT labeling strategy across laboratories.** (A) The numbers of all peptides (grey circles, including fully, partially and non-labeled sequences) and only fully TMT labeled peptides (blue circles) are displayed for the labeling experiment series depicted in main figure 2A. (B) MS1 intensities of fully, over- and under-labeled peptides are shown for the labeling experiment series illustrated in main figure 2A. Intensities of fully labeled peptides were extracted from the under-labeling search.

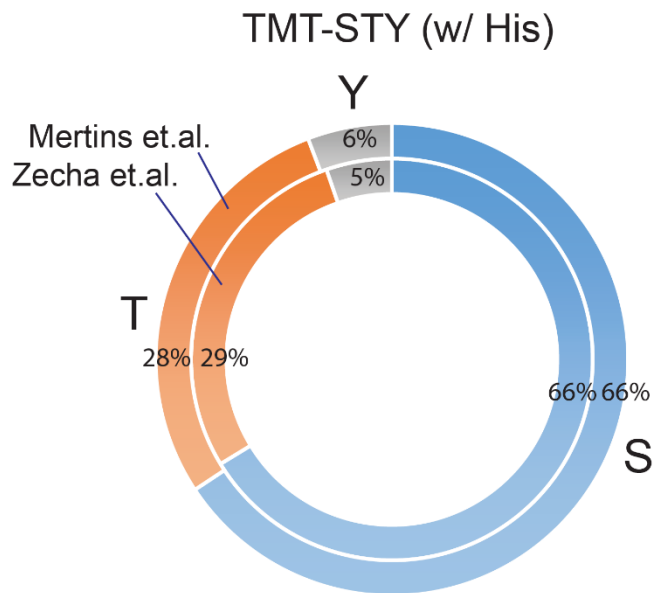


Fig. S3: **Benchmarking the optimized protocol for deep-scale proteomic analysis.** The fractions of TMT labeled serine (S), threonine (T) and tyrosine (Y) residues among over-labeled, histidine containing peptides are displayed.