

quantms, ms<sup>2</sup>rescore and multiple search engines  
enables deep proteome coverage across protein  
quantification, immunopeptidomics, and  
phosphoproteomics experiments

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**Abstract**

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The exponential growth of public proteomics datasets has surpassed the analytical capacity of traditional desktop tools, particularly for large-scale automated reanalysis. To address this challenge, we present an integrated workflow that combines quantms, a cloud-native pipeline, with MS2Rescore, a machine learning-based rescoring tool. This workflow enables deep and scalable reanalysis of massive proteomic datasets. Powered by the Nextflow engine for parallel computing, the pipeline incorporates fragment ion intensity predictions from MS2PIP and retention time predictions from DeepLC, improving peptide-spectrum match reliability through Percolator. We applied this approach to four representative datasets covering label-free quantification, TMT labeling, immunopeptidomics, and phosphoproteomics. Compared to traditional methods, our workflow achieved a 16–22.8% increase in identified spectra, along with the quantification of hundreds of additional proteins and phosphosites. These improvements demonstrate that integrating multiple search engines with machine learning-derived features not only enhances identification sensitivity but also deepens quantitative insights for downstream biological interpretation. Overall, this workflow offers a reproducible and scalable solution for the reanalysis of public proteomics data, advancing FAIR principles by promoting scientific transparency, accessibility, and data reuse.

**Keywords:** Proteomics, Reanalysis, Workflow, Machine learning.

## 1 Introduction

In recent years, the field of proteomics has experienced rapid growth in the availability of publicly accessible datasets, accompanied by a shift toward studies analyzing larger sample cohorts. As of June 2025, over 40,000 datasets have been submitted to PX repositories, including a substantial increase in large-scale submissions comprising more than 100 instrument files [1]. However, conventional desktop tools such as MaxQuant [2], pFind [3], MSFragger [4], and Proteome Discoverer are limited in their capacity to perform automated, large-scale quantitative analyses in cloud or distributed environments, hindering the reanal-

ysis of extensive experiments on standard workstations.

To address this limitation, we recently developed quantms, an open-source, cloud-based pipeline designed for massively parallel reanalysis of quantitative proteomic datasets [5]. The pipeline is highly modular and flexible, accommodating a wide range of quantitative proteomics approaches. quantms automatically distributes computations using the Nextflow workflow engine across one or more computing nodes, depending on the number of instrument files and samples [6]. To ensure traceability and reproducibility, the pipeline is built entirely on standardized open file formats and reproducible execution environments [7] [8], adhering strictly to the FAIR (Findability, Accessibility, Interoperability, and Reusability) principles [9].

With the integration of machine learning (ML) into proteomics, various models have been developed to accurately predict peptide properties, such as MS2PIP [10] for fragment ion intensities and DeepLC [11] for retention time prediction. Early approaches employed decision trees and single-layer neural networks, while more recent deep learning models such as Prosit [12] achieve significantly improved accuracy for predicting fragment ion intensities and retention times. These highly accurate predictions enable superior matching of experimental data to theoretical expectations and have reinvigorated rescored strategies in proteomics. MS2Rescore represents a modular package that generates multiple features assessing the similarity between observed and predicted MS/MS spectra utilizing MS2PIP. Furthermore, MS2Rescore calculates RT-dependent features, e.g., delta RT, utilizing DeepLC [13].

Previously, quantms did not leverage measurable peptide properties such as fragment ion intensities and retention times. To overcome this limitation, we integrated MS2Rescore into quantms and incorporated customized features following Nextflow and nf-core best practices. We demonstrate that the enhanced pipeline supports in-depth analysis of large-scale public proteomics datasets across diverse experimental designs, including label-free quantification (LFQ), tandem mass tag (TMT)-based quantification, immunopeptidomics, and phosphoproteomics studies.

## 2 Methods

### 2.1 MS/MS Data and quantms Search Results

To develop and evaluate the performance of the quantms-integrated MS2Rescore workflow, we selected four publicly available benchmark datasets. Three were obtained from the PRIDE Archive under the identifiers PXD001819, PXD019643, and PXD026824, and one from the CPTAC data portal under PDC000127. The PXD001819 dataset contains 48 Sigma UPS1 proteins spiked into a background of yeast cell lysate at nine different concentrations: 0.05, 0.125, 0.25, 0.5, 2.5, 5, 12.5, 25, and 50 fmol/ $\mu$ L to evaluate quantification performance. We evaluated five different quantms workflow settings: (1) Comet, (2) Comet and MSGF+, (3) Comet with MS2Rescore, (4) Comet and MSGF+ with MS2Rescore, and (5) Comet, MSGF+, and SAGE with MS2Rescore to explore multiple search engines and their integration with MS2Rescore for improved identification and quantification results. All search parameters were the same as described in the previous publication. A stringent FDR at 1% was applied at peptide spectrum match (PSM) and protein level at the dataset level. The search results from quantms and MaxQuant at the PSM and protein group levels are provided in Supplemental File S1.

### 2.2 Rescoring Features and Postprocessing

The quantms-rescoring Python package integrates MS2Rescore and calculates a wide range of features based on the DeepLC-predicted and observed retention times, and the MS2PIP-predicted and observed MS2 peak intensities. Signal-to-noise ratio (SNR) features are also calculated for specific cases in the quantms-rescoring Python package. Then, the quantms workflow calculate a posterior (error) probability for each PSM using Percolato. This is performed under three different feature configurations (1) a baseline model using only search engine-derived features, (2) the baseline model plus MS2Rescore-derived features, and (3) the above combined with SNR features. To merge results from multiple search engines, Con-

sensusID aggregates PSMs into unified scores. Final PSM-level q-values are then obtained either directly from Percolator or calculated using OpenMS' target-decoy approach based on the predicted probabilities. For phosphoproteomics datasets, LuciPHOr2 is employed to assign site-level localization scores and estimate the associated false localization rate using tools from the OpenMS toolkit.

## 3 Results

### 3.1 quantms with MS2Rescore enhancing identification and quantification in label-free experiments

To systematically evaluate the performance of the quantms-integrated MS2Rescore workflow at both identification and quantification levels, we first analyzed the public benchmark dataset PXD001819. For identification benchmarking, five different workflows configurations were designed and compared, including (1) Comet with Percolator, (2) two search engines with Percolator, (3) Comet with MS2Rescore features and Percolator, (4) two search engines with MS2Rescore features and Percolator, and (5) three search engines with MS2Rescore features and Percolator to determine whether features from intensity-based and retention time-based predictors enhanced the identification and quantification process.

Significant PSMs were filtered based on q-values, and the FDR was used as a key metric to compare workflows. As shown in Figure 1, using consensus scores from two search engines significantly increased identification rates at a fixed FDR threshold. Specifically, combining Comet and MSGF+ improved identified spectra by 17% over Comet alone, and incorporating MS2PIP and DeepLC features through MS2Rescore led to an additional 16% increase. At the quantification level, including MS2Rescore-derived features allowed more low-abundance UPS1 proteins to be quantified (Figure 2B), highlighting the contributions of the integrated workflow.

To better understand the contribution of individual features, we extracted the top 20 fea-

ture weights from Percolator (Supplemental Figure 1). Over half of the top-weighted features were derived from MS2Rescore. Notably, SpecPearsonNorm had a strong positive weight, indicating that a better correlation between predicted and observed intensities improves confidence. Conversely, RtDiffBest had a negative weight, suggesting that large deviations in retention time reduce match quality. Interestingly, peptide length also emerged as a significant positive feature after MS2Rescore integration, likely because longer peptides generate more fragment ions and thus are more reliably identified.

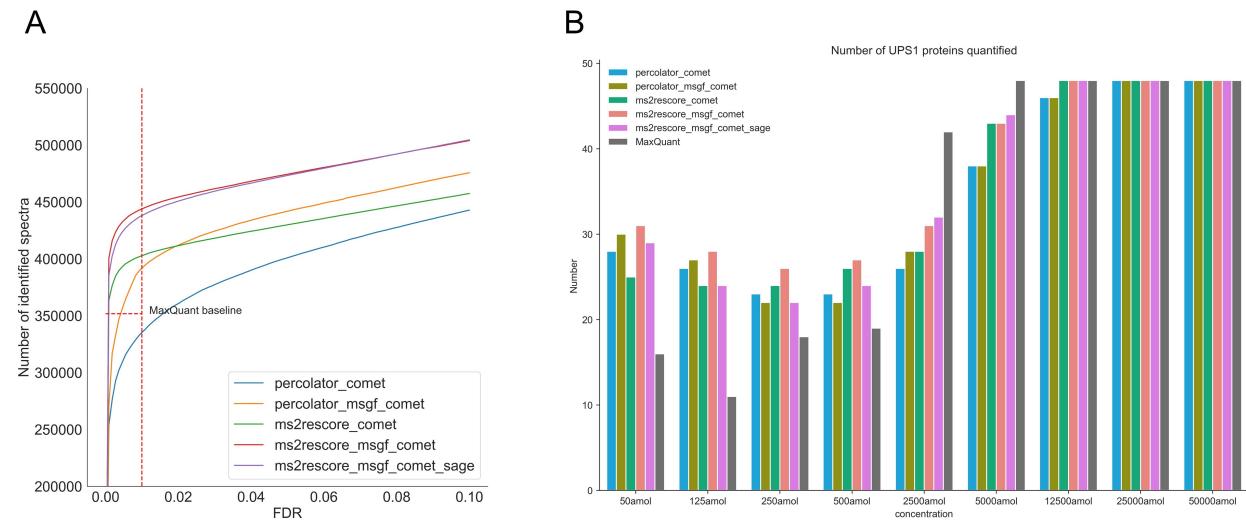


Figure 1: The number of identified spectra as a function of differing FDR levels for different workflow settings. (A) Results for the PXD001819 dataset. (B) Results for the PXD001819 dataset showing protein quantification. Five different workflow settings are shown: the combination of Comet search engine with Percolator rescore, the combination of Comet with MSGF+, the combination of two search engines with MS2Rescore features, the combination of two search engines with MS2Rescore and SNR features, and the combination of three search engines with MS2Rescore and SNR features. Multiple search engines consensus identification, additional intensity-based and retention time-based features, and SNR features lead to both higher identification rates and high-quality identification.

### 3.2 quantms integrated with MS2Rescore improves identification and quantification for large-scale TMT experiments

We next applied the workflow to a large-scale TMT-labeled dataset from CPTAC (PDC000127). The integration of multiple search engines and MS2Rescore features led to marked improve-

ments in both identification and quantification (Figure 2). The PSM identification rate increased by 3.6%, and 921 proteins were newly quantified compared to the workflow without MS2Rescore (Figure 2A, 2B). Among these, 59 proteins had abundance levels within the top 10% (Figure 2C). To assess the biological impact, we conducted differential expression analysis using the MS2Rescore-enhanced workflow. As shown in Figure 2D, 27 newly quantified proteins were significantly differentially expressed. Notably, FOXG1—one of these proteins—has been associated with prognosis in Clear Cell Renal Cell Carcinoma, as reported by Yang et al. [14]. These findings illustrate that quantms incorporating MS2PIP and DeepLC-derived features enhances not only identification sensitivity but also enables deeper biological insights.

We further examined the rescore contribution of individual features by analyzing the top 20 SVM weights from Percolator (Supplemental Figure S2). For Comet-based results, SpecPearsonNorm, DotProdIonYNorm, and RtDiffBest were highly weighted. The interpretations of these weights were consistent with those from the label-free dataset. Importantly, peptide length again emerged as a key feature. Similar weight distributions were observed for MSGF+ and SAGE results, confirming the generalizability of these feature effects across different search engines.

### 3.3 Evaluation of quantms integrated with MS2Rescore on HLA Class I Peptides

To further assess the performance of the quantms-integrated MS2Rescore workflow, we analyzed the HLA Class I immunopeptidomics dataset PXD019643. Four workflow configurations were evaluated: (1) Comet + Percolator, (2) Comet + MSGF+ + Percolator, (3) Comet + MSGF+ + MS2Rescore features, and (4) the combination of two search engines with MS2Rescore and SNR features. These were compared in terms of the total amount of identifications as well as the number of unique identifications based on sequence. Overall, multiple search engines and rescore with MS2Rescore substantially improved the spectrum

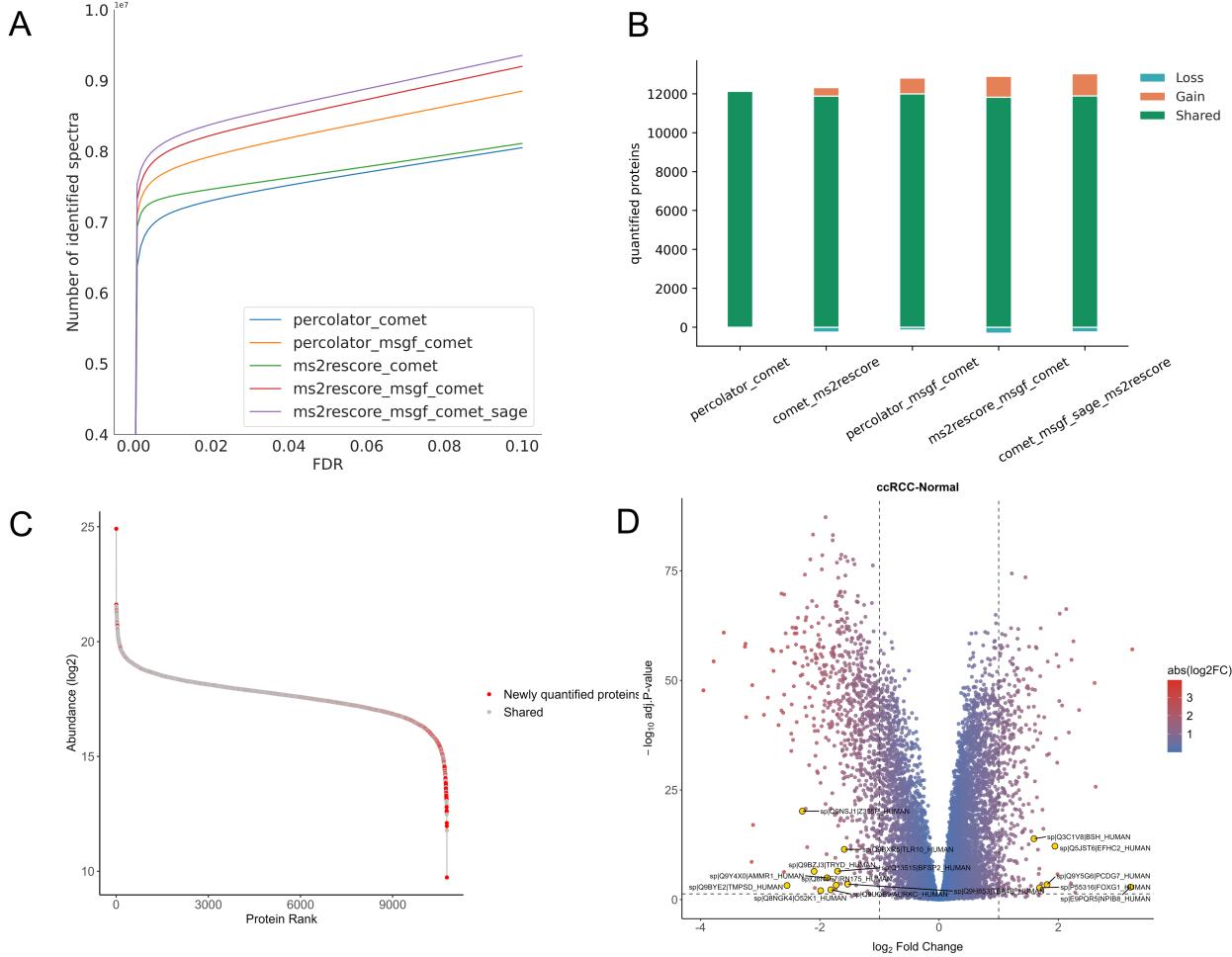


Figure 2: Comparison of identification and quantification results for different workflow settings. (A) The number of identified spectra as a function of differing FDR levels for different workflow settings. (B) The number of quantified proteins. The green part indicates the intersection between a workflow and Comet results. (C) The rank of protein abundance from Comet and MSGF+ with MS2Rescore. The red dots represent proteins quantified only in the Comet and MSGF+ with MS2Rescore workflow compared to the Comet and MSGF+ workflow without MS2Rescore. (D) The volcano plot for differential expression analysis from MSstatsTMT. The red dots represent proteins quantified only in the Comet and MSGF+ with MS2Rescore workflow compared to the Comet and MSGF+ workflow without MS2Rescore.

identification rate compared to using only the Comet search engine or no rescoring at both 1% and 0.1% FDR. Multiple search engines achieved an 11.7% increase in the number of identified spectra compared to using only Comet search engines, and MS2Rescore features further increased the number of identified spectra by 22.8% compared to multiple search

engines without MS2Rescore features, as shown in Figure 3.

The power of providing these predictions to Percolator is further illustrated when visualizing the distributions for decoy PSMs, rejected target PSMs, and accepted target PSMs from individual support and consensus supports (Figure 3C, D). The accepted target PSMs are clearly separable from the decoy and rejected target PSMs using only the Pearson correlation coefficient (PCC). The distributions for accepted targets from individual support and consensus supports are highly similar in that they both exhibit low retention time errors and high PCC values. This further demonstrates that quantms is effective for integrating the identification scores of multiple search engines.

### **3.4 quantms integrated with MS2Rescore facilitates the identification of phosphorylated peptides**

In addition, we investigated the performance of our quantms workflow with MS2Rescore on post-translational modification experiments (PXD026824). For phosphoproteomics analyses, different workflow settings were evaluated: (1) Comet alone, (2) Comet combined with MSGF+, and (3) multiple search engines combined with MS2Rescore features. These were processed using Percolator to determine whether multiple search engines and MS2Rescore facilitated the identification and quantification process. In Figure 4, it is clearly visible that the introduction of multiple search engines consensus identification and MS2Rescore features extensively improves the stringency with which Percolator can classify PSMs. This is illustrated by the remarkable shift to the left compared with Comet alone. In addition, there is also a gain in identification rate, which is shown in the vertical direction of this figure. The two search engines consensus identification combined with MS2Rescore features enabled a 19% increase in the number of identified spectra compared to consensus identification without MS2Rescore (Figure 4A). The top 20 weights from Percolator are shown in Supplemental Figure S4. We can observe the same features as in the above results, such as the DotProdIonYNorm feature.

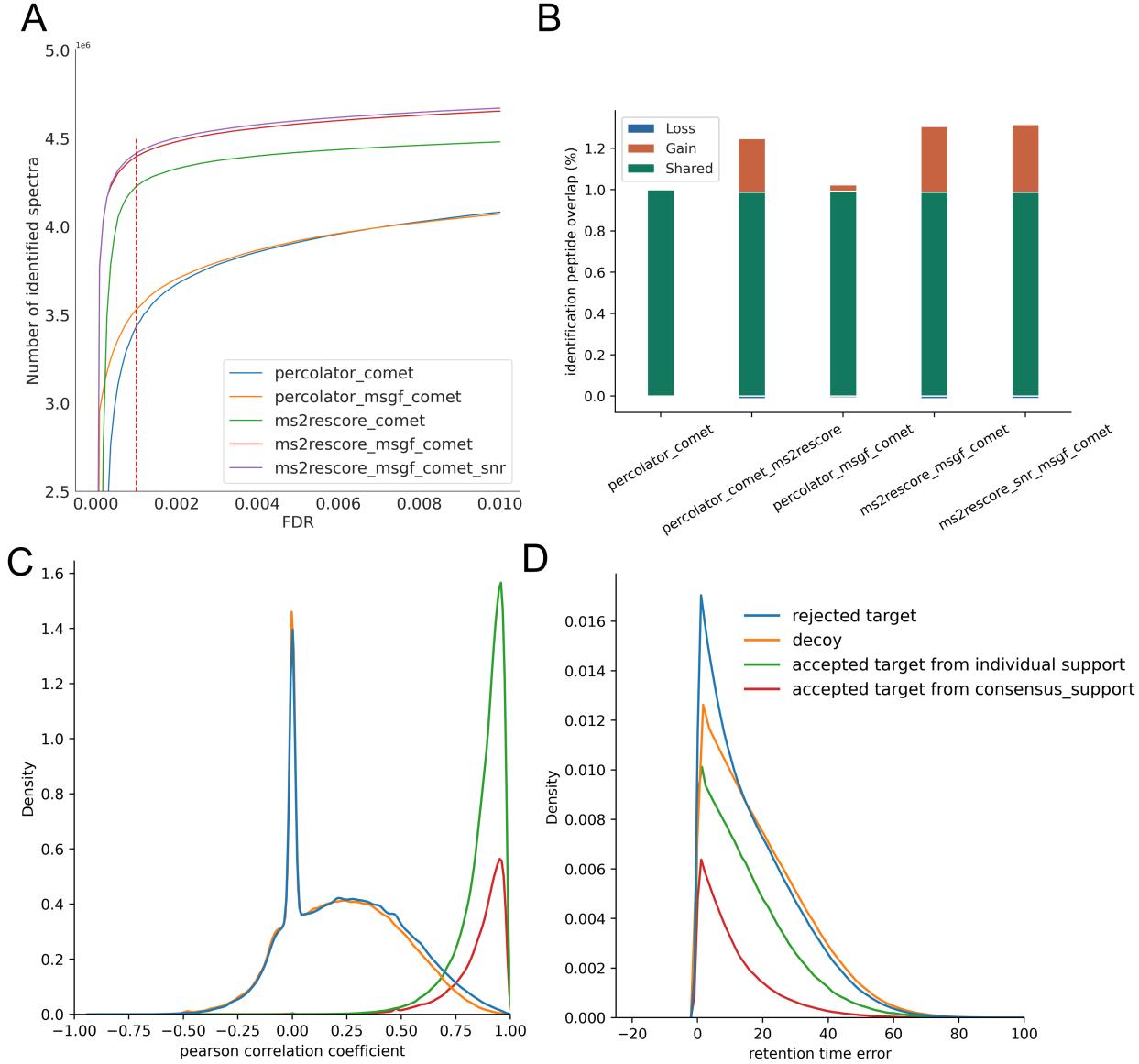


Figure 3: Comparison of immunopeptides identification results for different workflow settings. (A) Line plot showing the number of spectra identified for four workflow settings at different PSM FDR levels. (B) Percentage of unique identified peptides using different workflow settings. Density plots showing the distribution of the smallest retention time error between observed and predicted retention time (C) and the Pearson correlation between observed and predicted peak intensities (D) for each PSM split into decoys (red), rejected targets with q-value  $>0.01$  (blue), and accepted targets from individual support and consensus support with q-value  $<0.01$  (green). Note that the rejected targets distribution coincides with the decoy distribution.

Protein phosphosite false localization rate (FLR) control is necessary for phosphoproteomics analysis. Therefore, we also investigated the effect of different workflow settings on

phosphorylated peptides at different FLR levels (Figure 4B, C). The search engines consensus identification combined with MS2Rescore features increased the number of phosphorylated peptide identifications by 17% at 0.01 local FLR compared to the approach without MS2Rescore. There are 1,345 phosphorylated peptides quantified only in the consensus identification combined with MS2Rescore process. Considering protein phosphosites, consensus identification combined with the MS2Rescore process newly reports 350 protein phosphorylation sites (Figure 4D). Altogether, these results show that quantms integrated with MS2Rescore can boost performance in phosphoproteomics.

## 4 Discussion

Advancements in deep learning-based tools have significantly improved the accuracy and sensitivity of peptide-spectrum match (PSM) rescoring, yet their integration into streamlined, reproducible, and quantitative proteomics workflows has remained limited, especially for large-scale public data analysis. In this study, we demonstrate the systematic incorporation of MS2Rescore into the open-source quantms pipeline and evaluate its impact across various experimental settings, including label-free quantification (LFQ), tandem mass tag (TMT)-based quantification, immunopeptidomics, and phosphoproteomics. Our results show that enhanced feature sets derived from MS2PIP and DeepLC not only improve identification rates but also translate into gains in quantification depth. Importantly, we provide evidence that the improved identifications via rescoring propagate downstream into quantification, leading to increased numbers of proteins with reliable abundance estimates, as well as enhanced detection of differentially expressed proteins.

To demonstrate the applicability and advantages of quantms integrated with MS2Rescore in quantitative proteomics, we performed a series of reanalyses using publicly available large-scale datasets with well-established benchmarks. In label-free experiments, MS2Rescore-enhanced workflows achieved significant increases in PSM and peptide identification while

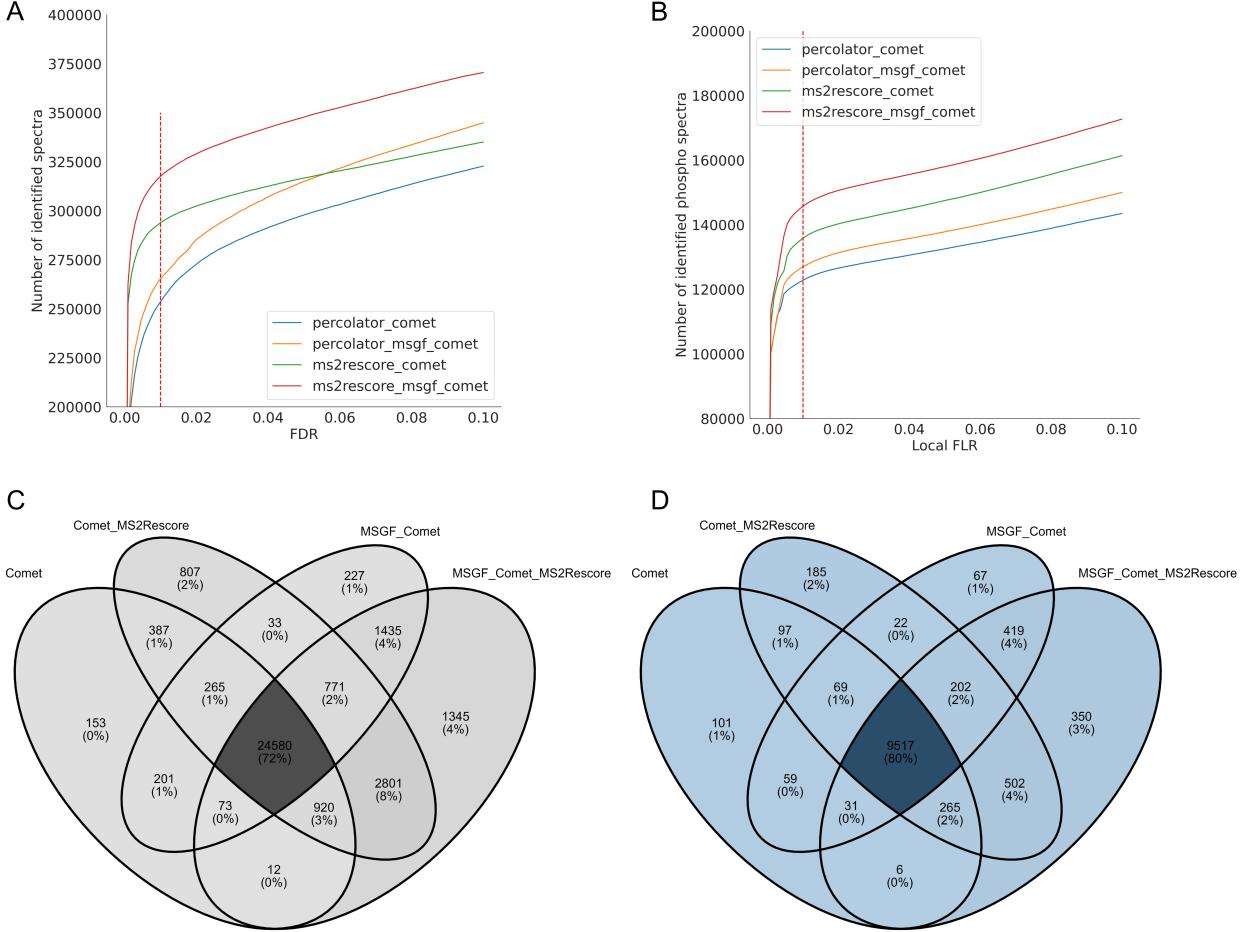


Figure 4: Comparison of phosphorylated peptide identification results for different workflow settings. (A) Line plot showing the number of spectra identified for four workflow settings at different PSM FDR levels. (B) Line plot showing the number of phosphorylated spectra identified for four workflow settings at different local false localization rate levels after an FDR of less than 0.01 at the PSM level. (C) Venn diagram of peptides quantified for four settings. (D) Venn diagram of protein phosphosites at protein FDR 0.01 and FLR 0.01 for four settings.

maintaining or improving quantification reproducibility across replicates. For TMT experiments, the integration of MS2Rescore into quantms increased the number of quantifiable proteins. In more challenging applications, such as immunopeptidomics and phosphoproteomics, the deep learning-based rescore pipeline consistently yielded higher identification rates and improved sensitivity. These improvements contribute meaningful biological insights, such as phosphosite discovery and the identification of differentially expressed proteins with potential clinical relevance.

Overall, the results in this publication highlight the synergistic benefits of integrating machine learning-based features within end-to-end, cloud-based proteomics workflows. By embedding MS2Rescore into the quantms pipeline, we provide a practical and accessible solution for the community to leverage cutting-edge spectral prediction and retention time modeling without the need for complex manual configuration. This integrated approach enables reproducible reanalysis of large public datasets, increases identification confidence, and strengthens quantitative conclusions across diverse experimental designs. Future work will focus on expanding this approach to other types of post-translational modifications and exploring the integration of additional machine learning tools to further enhance proteomics data analysis capabilities.

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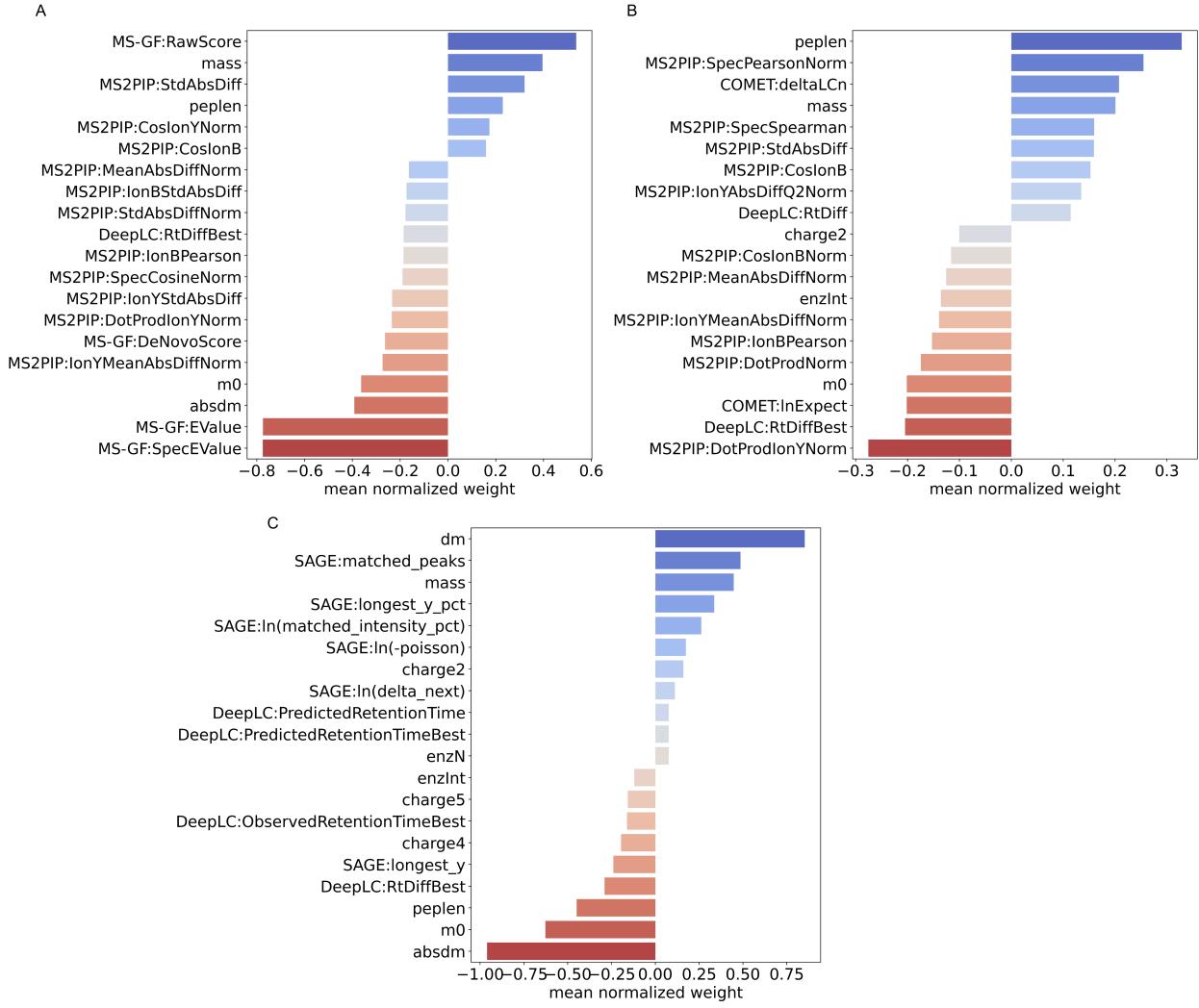


Figure S1: The weights that Percolator assigns to different features of the search engine feature vector in MSGF+ (A), Comet (B), and SAGE (C) combined with Percolator settings from PXD001819. The more different from zero a weight is (both positive and negative), the more important that feature is in the final Percolator classification.

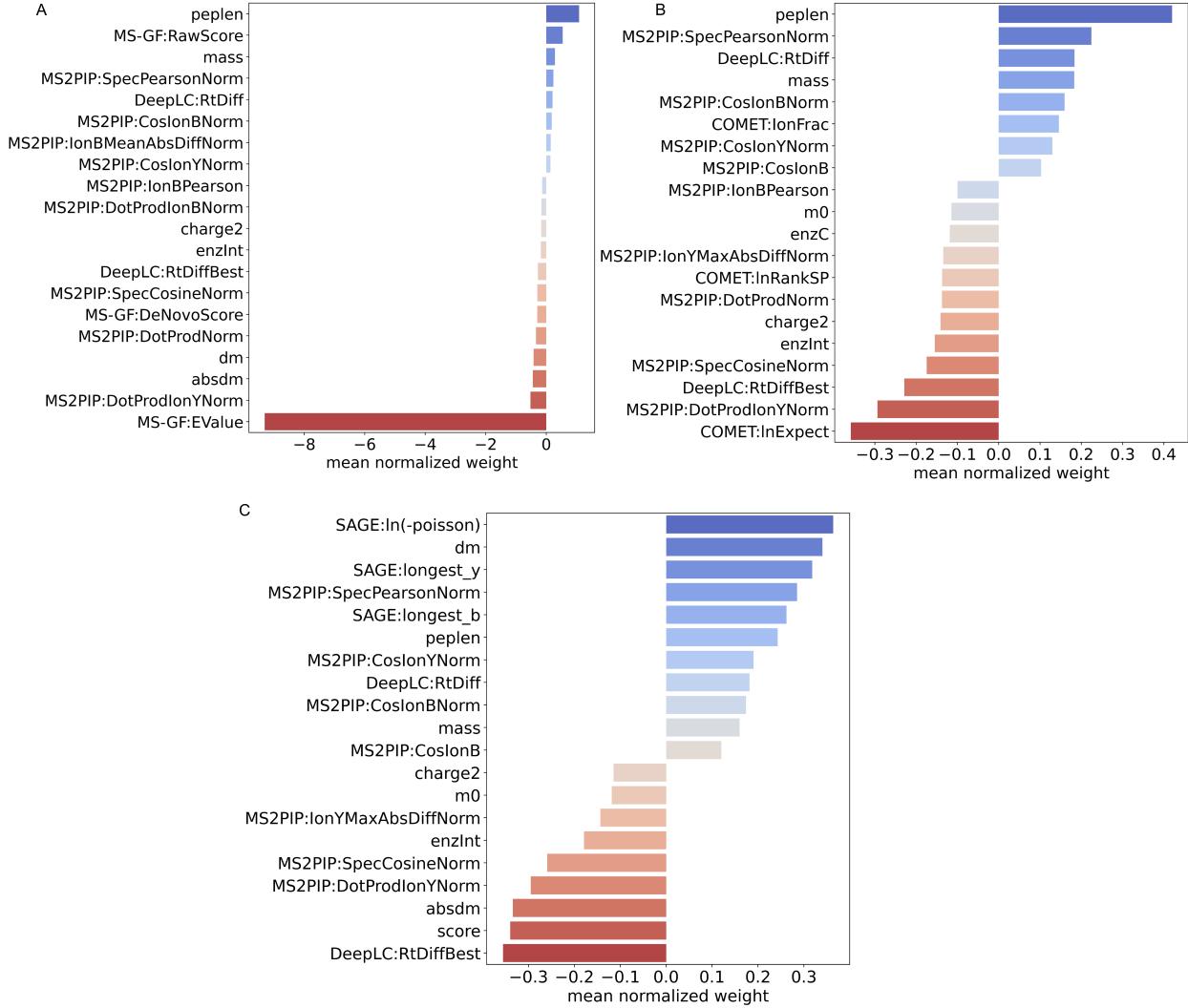


Figure S2: The top 20 normalized weights from Percolator for MSGF+ (A), Comet (B), and SAGE (C) identification results from PDC000125.

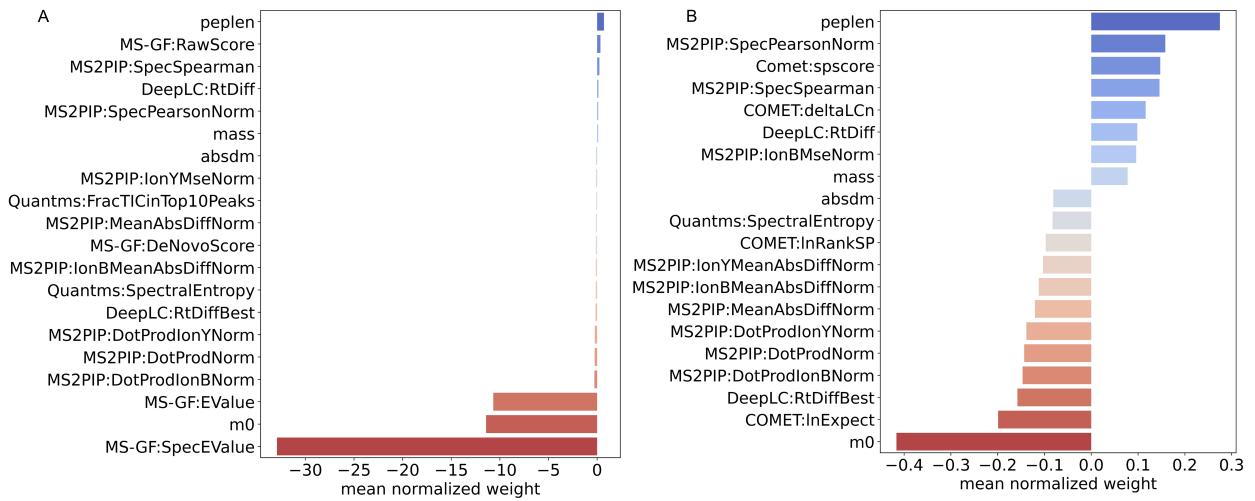


Figure S3: The top 20 normalized weights from Percolator for MSGF+ (A) and Comet (B) identification results in the PXD019643 dataset.

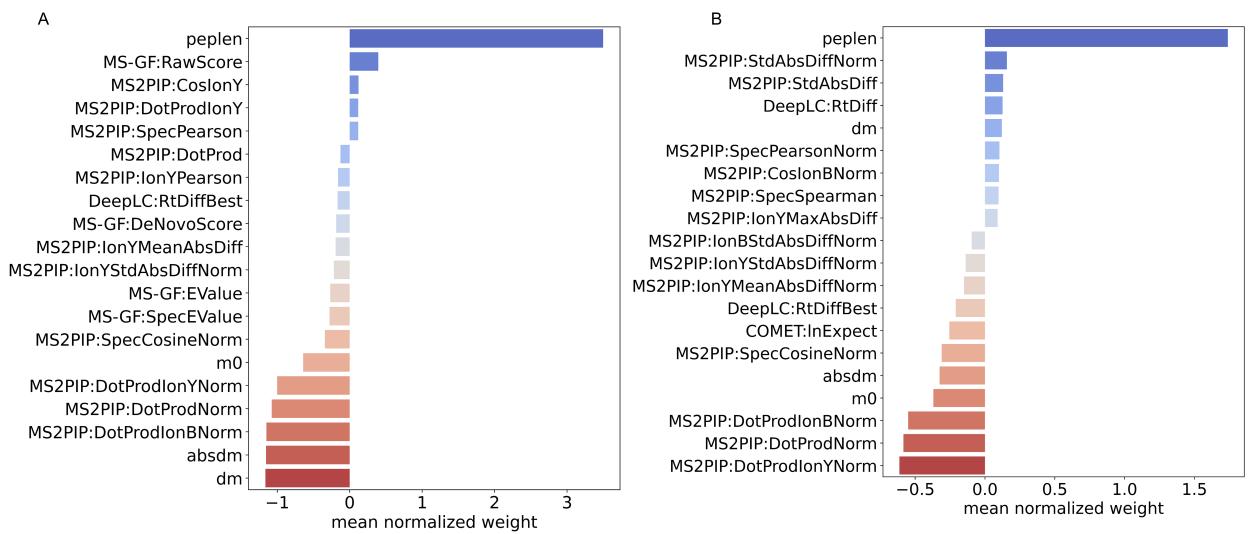


Figure S4: The top 20 normalized weights from Percolator for MSGF+ (A) and Comet (B) identification results in the PXD026824 dataset.