

Introduction

Bottom-up proteomics yields results at the protein, peptide, and PTM levels. However, these multi-level analyses require increasingly sophisticated computational and domain expertise to harmonize heterogeneous software outputs, link molecular entities, and implement appropriate quality control and downstream analyses, often slowing interpretation and limiting accessibility for many researchers.

Critically, these molecular layers are often analyzed in isolation, making it difficult to interpret site-specific PTM changes in the context of peptide consistency and protein-level regulation.

Mass Dynamics 3.0 (MD 3.0) provides a **web-based system for unified import, QC, statistics, and visualization across all entities**, accelerating the path from data to biologically contextualized insight without custom code.

Direct access to all levels of your proteomics data

Example Dataset

Description: The dataset consists of measurements from different mouse tissue regions: eye, heart, and brain collected using varying starting sample amounts¹.

Pre-processing: The raw data was downloaded from PRIDE repository PXD043370 (file Denis_uPhos_tissue.zip) and reprocessed using DIA-NN 2.0 to generate the parquet file containing PTM localization probabilities. The DIA-NN output was then uploaded to Mass Dynamics 3.0.

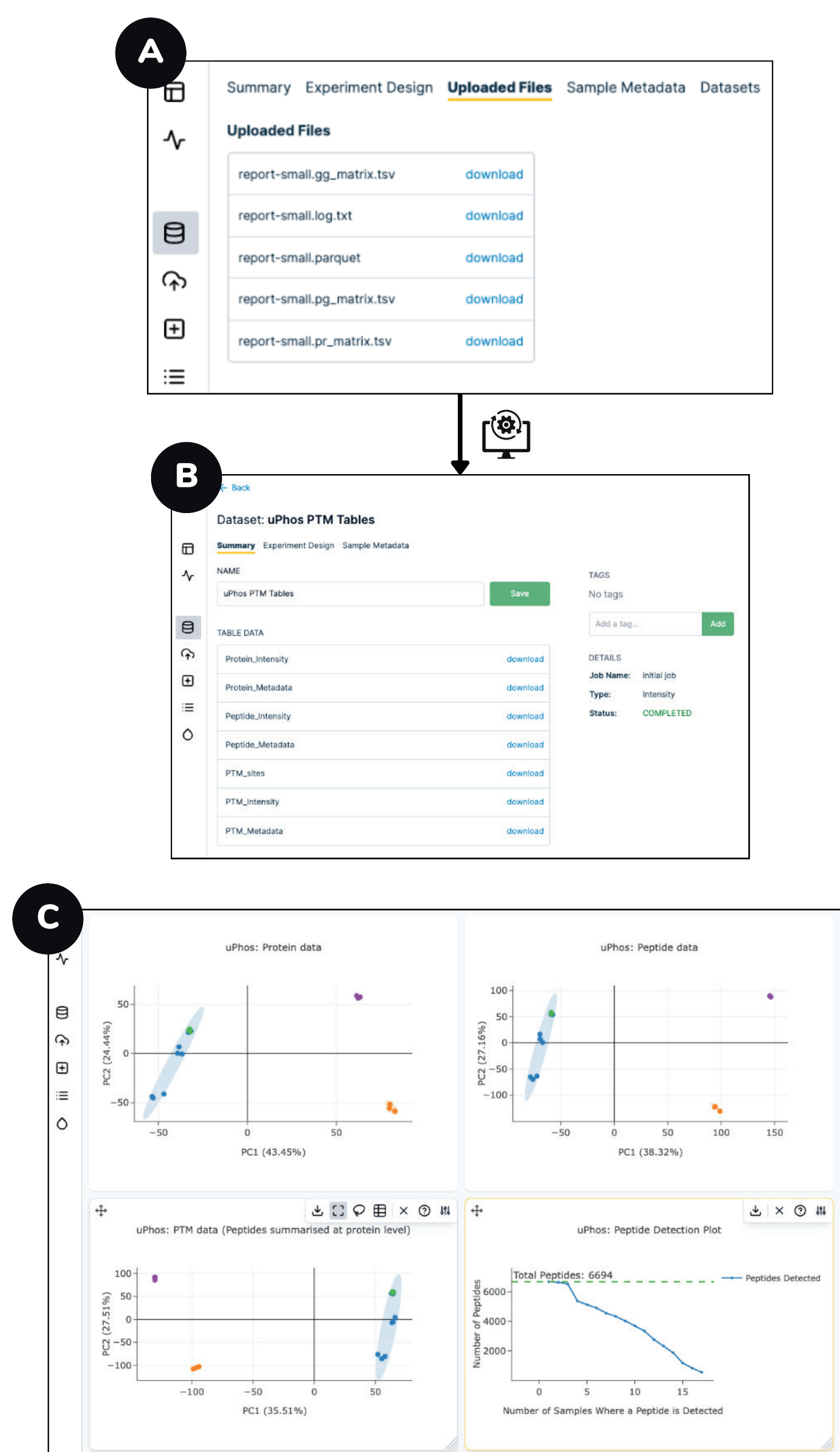


Figure 1. Upload and QC of DIA-NN protein and peptide demo data.
A) Uploaded DIA-NN files to MD 3.0.
B) All-level proteomics tables automatically generated by MD 3.0.
C) All omics level quality control directly accessible.

References

1. Oliinyyk et al., uPhos: a scalable and sensitive platform for high-dimensional phosphoproteomics. Mol Syst Biol (2024)
2. Turei D, et al., OmniPath: guidelines and gateway for literature-curated signaling pathway resources. Nature Methods (2016)

Conflicts of interest

The authors Giuseppe Infusini, Aaron Triantafyllidis and Andrew I. Webb are co-founders of Mass Dynamics, a for-profit enterprise delivering software as a service for the processing, analysis, and sharing of proteomics data. The authors Anna Quaglieri and Mark Condina are employees of Mass Dynamics.

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Filter and normalize peptide data before downstream analyses

Before downstream analysis like clustering, differential expression, or **PTM summarization**, peptide data can go through pre-processing through the normalization and imputation dataset, similarly to protein level data pre-processing.

Methods specific to peptides offer the ability to **create filtered peptide level** datasets:

- By modification type
- According to a localization probability threshold (when available) to exclude poorly localized PTM from further analyses

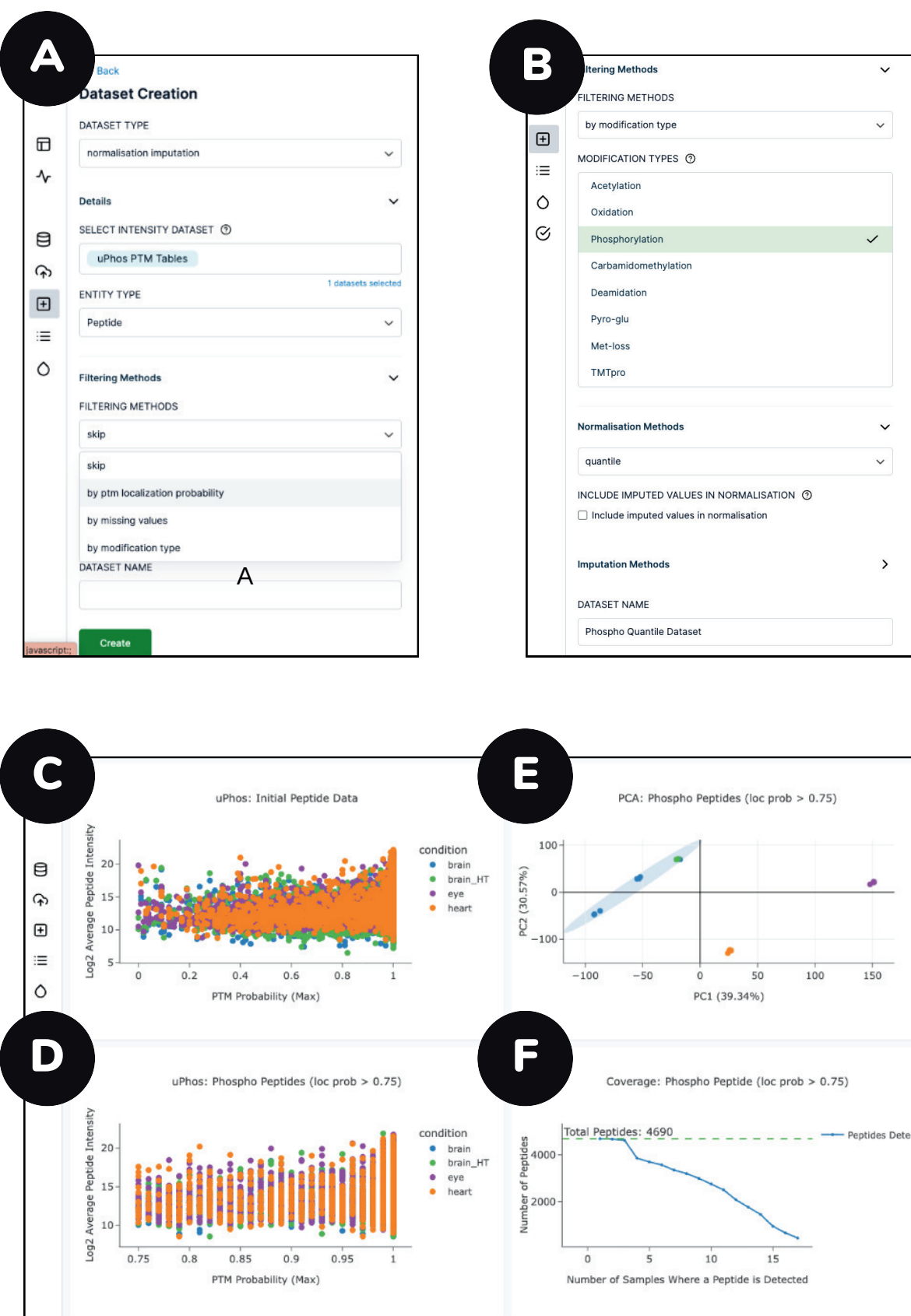


Figure 2. Peptide specific filtering: example for phosphopeptides

A) By localization probability threshold
B) By modification type
C-D) Localization probability vs intensity scatterplot of modified peptides before and after filtering.
E) PCA with the filtered phosphopeptides dataset.
F) Entity coverage plot with the filtered phosphopeptides.

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Summarize PTM intensities at the protein level

Statistical analysis of PTMs is typically performed at the protein-site level, where modified peptides mapping to the same site on the same protein are aggregated into a single quantity. MD 3.0 builds these **site-level tables by default** at upload and at request using the new **PTM Intensity Dataset**.

PTMs are fully supported for **DIA-NN**, **Spectronaut** and **MD Format** uploads.

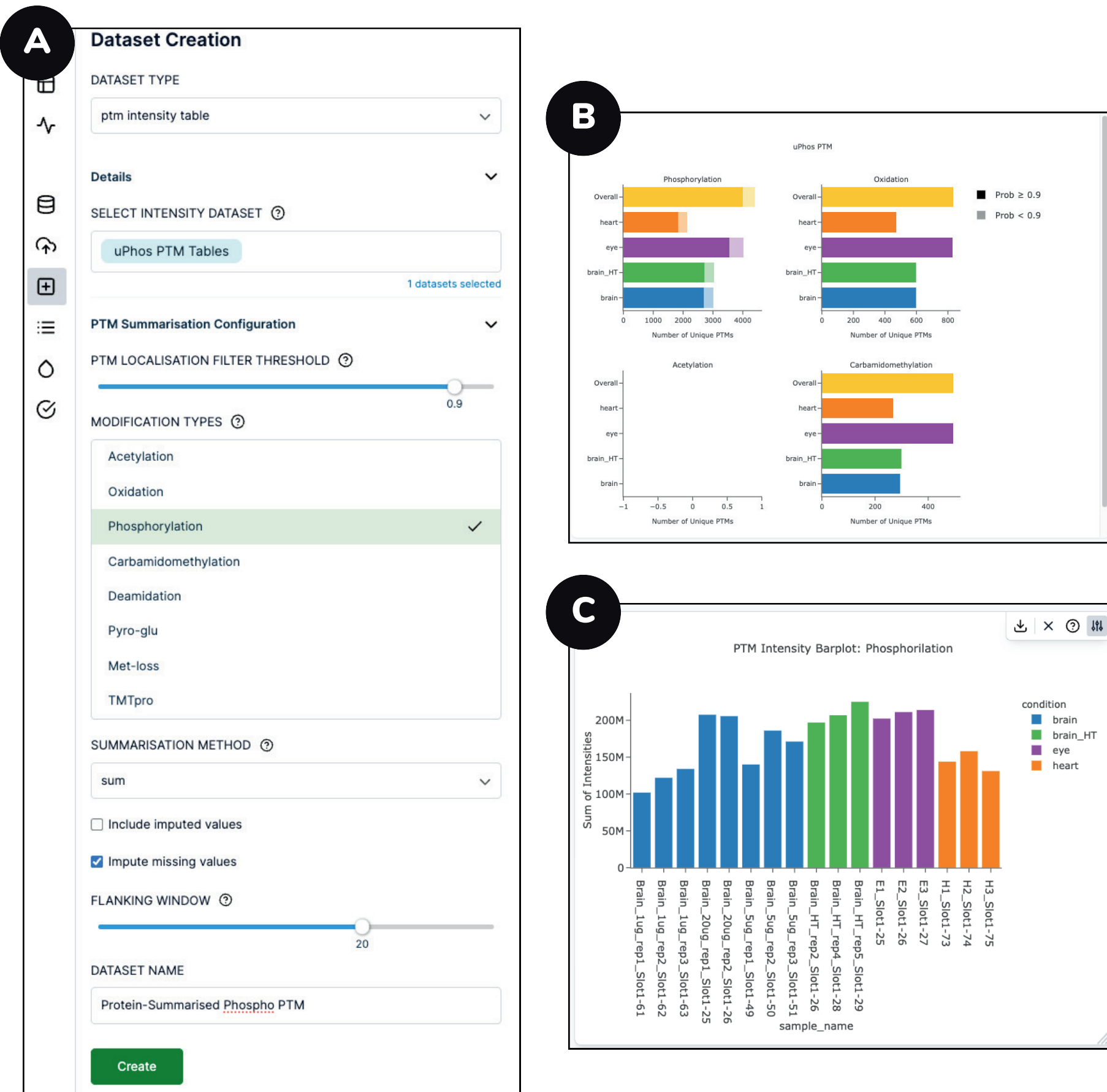


Figure 3. PTM intensities summarized at the protein level.

- A)** **PTM Intensity Dataset** to manually create a summarized PTM table allowing tweaking of parameter settings.
B) **PTM Identification Barplot** showing the distribution of the unique PTMs detected in the demo dataset separated by modification type and a selected condition of interest.
C) **PTM Intensity Barplot** showing the summed intensity of peptides/PTMs carrying the selected modification type and ordered and coloured by chosen groups.

PTM Analysis in Mass Dynamics



Find more detailed information about how PTM summarization is performed, as well as more broadly how to comprehensively analyse your PTM data in Mass Dynamics.

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Seamlessly connect and interpret your protein, peptide and PTM data

Differential expression and **Gene Set Enrichment Analysis (GSEA)** (when available for an entity type) was run between heart and brain tissues at the protein, peptide, and PTM levels. **Entity mapping** in MD 3.0 enables **seamless linking between protein and peptides**, so users can quickly check whether protein-level fold changes are supported by consistent peptide-level changes.

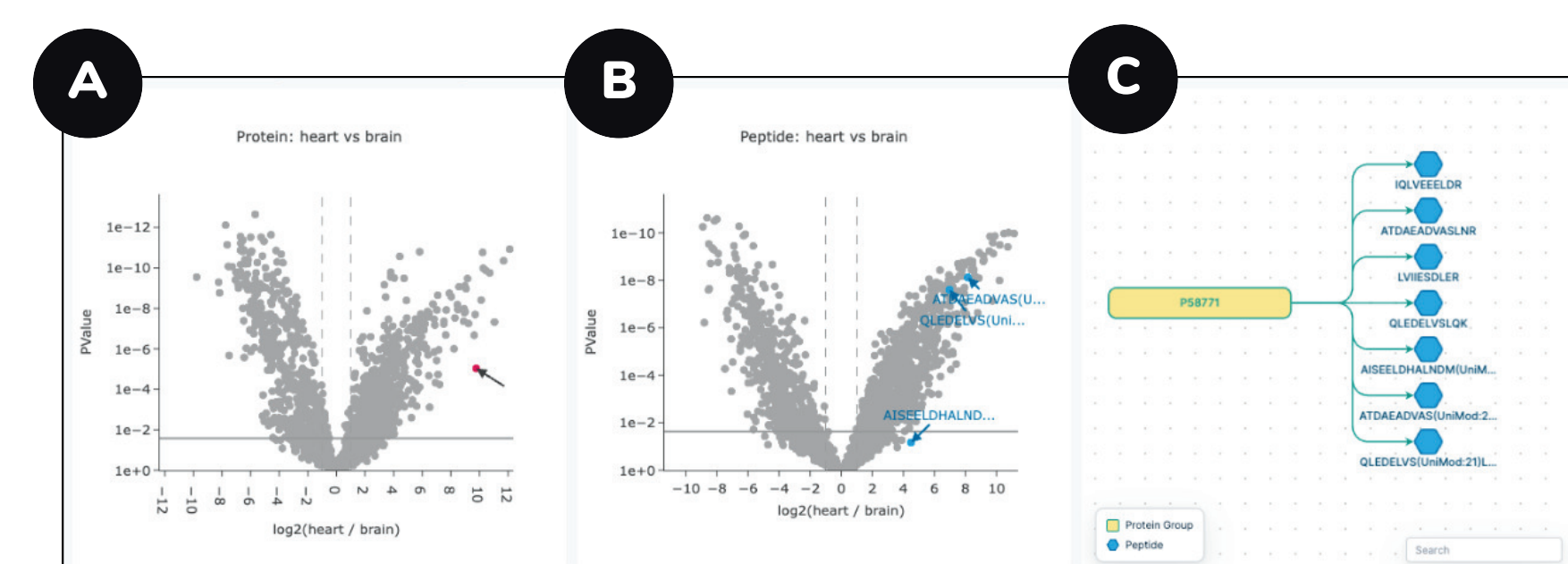


Figure 4. Mapping between protein and peptide level differential expression results.
A-B) Volcano plot for the heart vs brain pairwise comparison using the example data for protein and phosphopeptide data respectively. A protein is highlighted in the protein volcano plot and **all its peptides automatically highlighted** in the peptide volcano plot via MD 3.0 **Entity Mapping service**.
C) Graph showing the **entity mapping link** between the protein selected and the peptides identified in the same dataset.

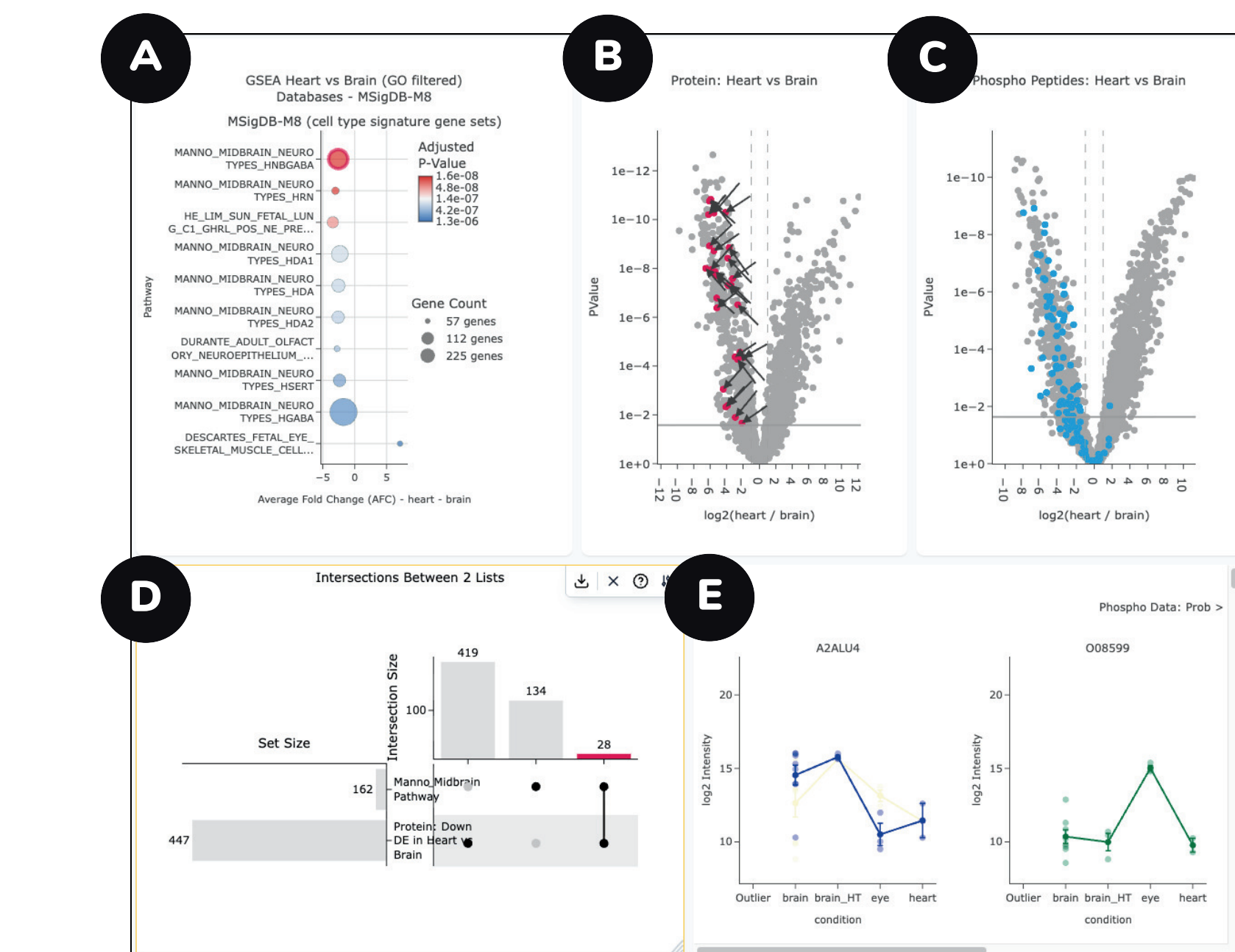


Figure 5. From Gene Sets to Proteins to Peptides.

- A)** GSEA bubble plots for the top MSigDB C8 protein pathway. Proteins in the MANNO_MIDBRAIN_NEUROTYPES_HNBGABA sets were saved by selecting the pathway bubble.
B) UpSet plot showing overlap between the saved pathway from A) and the downregulated proteins from the Heart vs Brain comparison with intersection bar selected.
C-D) Protein- and peptide-level volcano plots highlighting intersecting proteins from B).
E) Lineplots of the peptides mapped to the proteins selected by the intersection in B) showing the trend across the remaining conditions in the dataset.

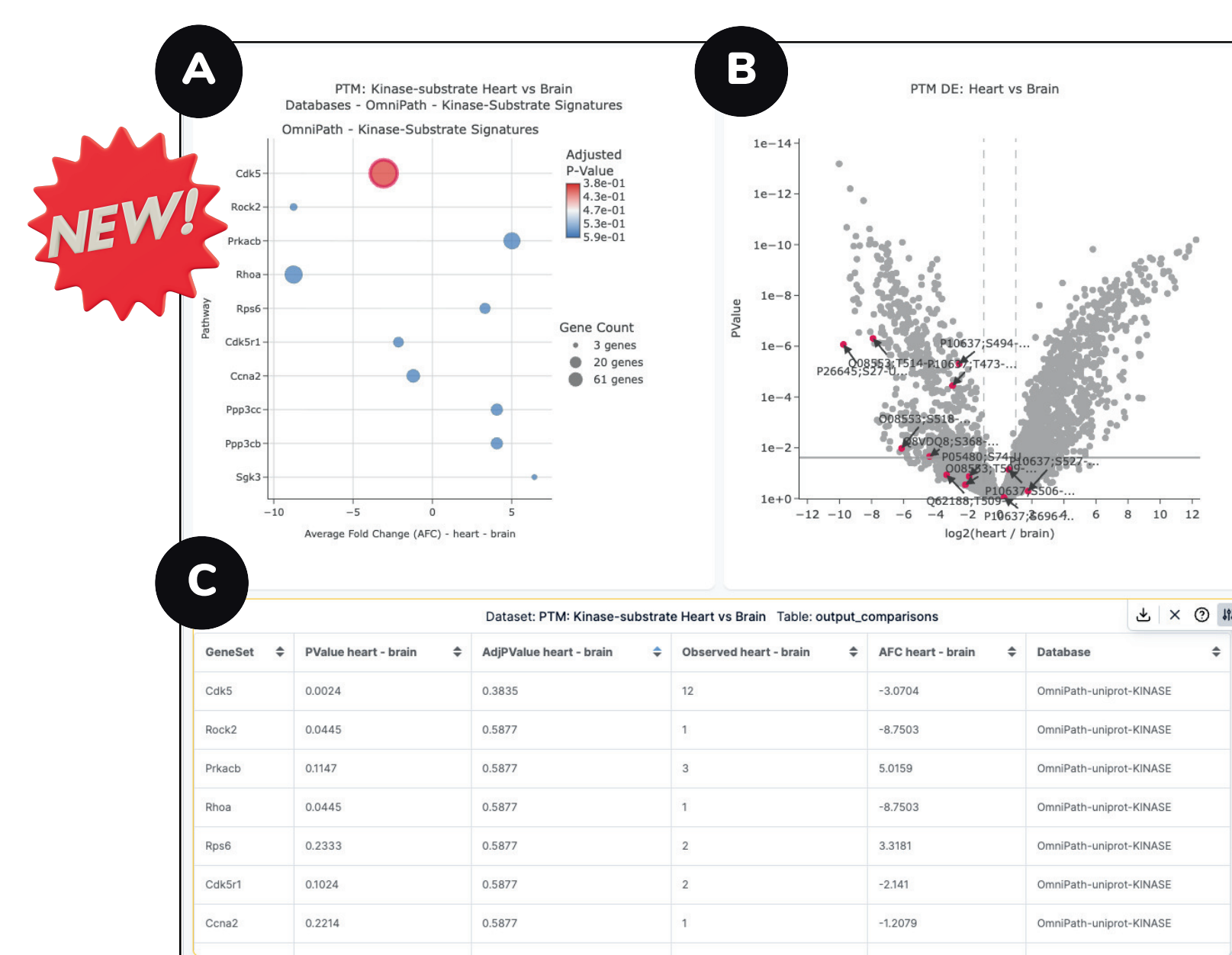


Figure 6. PTM level Differential Expression and Enrichment analysis.
A) GSEA of PTMs using the Kinase-substrate database from OmniPath² for the Heart vs Brain comparison.
B) Volcano plot of Heart vs Brain comparison using PTM level data.
C) Table showing the top statistical results of the GSEA in A).

Keen to try this with your own data?



Simply scan the QR code to get started.