Measuring Changes in Global Protein Expression

By Liquid Chromatography—Tandem Mass Spectrometry

Introduction

Mass spectrometry (MS) is the most powerful tool for proteomics to assess the relative abundance of proteins among biological samples. Numerous methodologies now support relative quantification measurements, providing a routine means to analyze protein expression patterns and post-translational modification states as a function of biological perturbation. Label-free quantification, SILAC (Stable isotope labeling by amino acids in cell culture) based quantification, and TMT (Tandem mass tag) isobaric multiplex (up to 18 plex) labeling quantification are available.

Poochon developed a standardized platform, PROMICMAPER, to measure changes in global protein expression in biological systems upon perturbations using isobaric mass tags (specifically the TMT multiplex labeling kit) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). PROMICMAPER allows for quantitative protein profiling up to 9,000 proteins, or deep quantitative protein profiling up to 12,000 proteins for one project. Additionally, the data generated by PROMICMAPER can be analyzed using Poochon's PROMICPATH data and pathway analysis tool, which uses ten groups of housekeeping protein complexes to evaluate the completeness and variability of a proteome profile. This evaluation ensures the integrity, reliability and reproducibility of a large set of protein profile data generated by PROMICMAPER. Pathway analysis is available for human and mouse origin samples.

Specifications

- → **Method** – TMT multiplex labeling based quantitative proteomics and LC-MS/MS
- → **Key Instruments** – Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer, Thermo Scientific™ UltiMate™ 3000 RSLCnano System
- → **Specificity** – Identify and quantify up to 9,000 different proteins from one set of 6 to 18 samples
- → **Acceptable Samples** – Frozen cell pellets (>1 million cells/sample), frozen tissue (≥100 mg/sample), protein lysate (≥100 µg/sample, ≥1mg/ml)
- → **Turnaround Time** – Typically, reports are available within 15 business days of sample receipt

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Schematic of Procedure Workflow

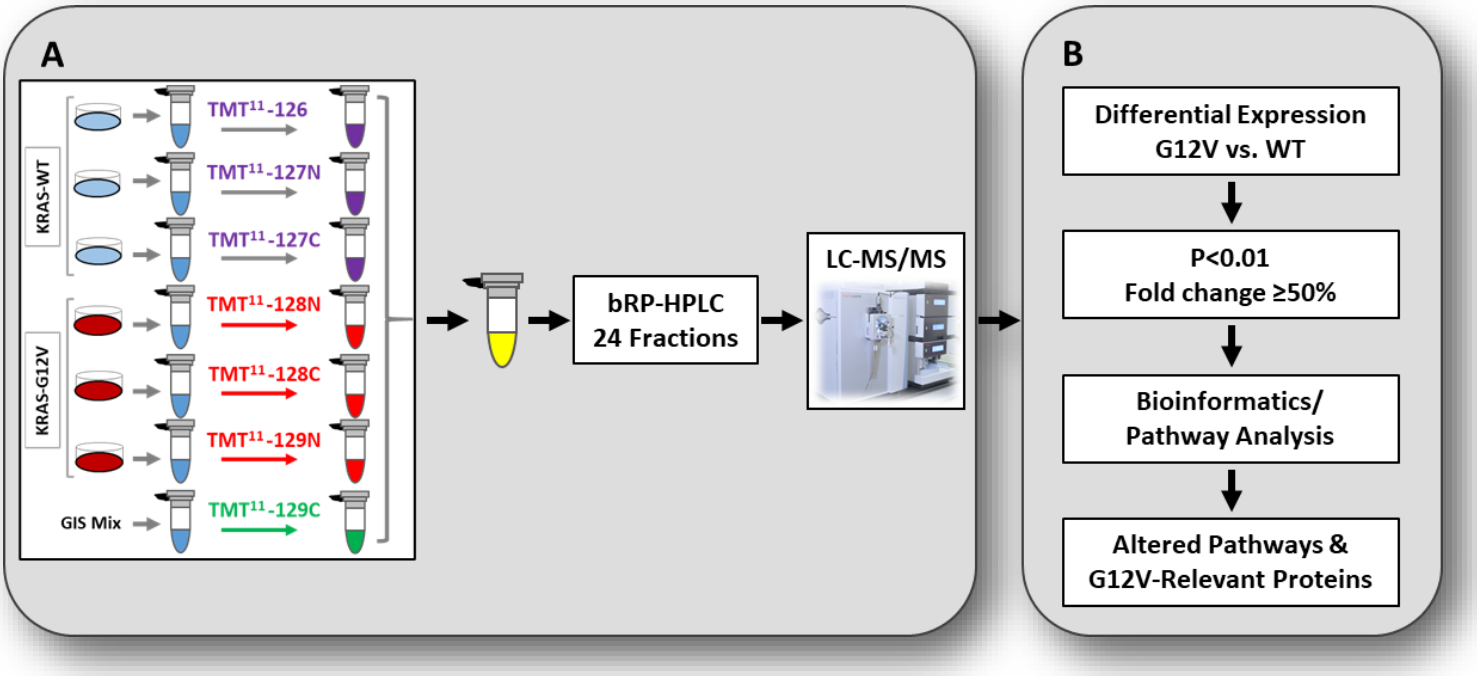

Figure 1:

A) Proteomic work-flow for TMT mass spectrometry-based quantification. *Note: GIS Mix = a pooled global internal standard (GIS), a mix of equal amounts of tryptic peptides from each of the six samples; bRP-HPLC = basic reverse-phase high performance liquid chromatography.*

B) Bioinformatic and statistical analysis approach used for differential expression analyses of datasets.

Note: *The newest Thermo Scientific™ TMTpro™ 18plex Label reagents can be used to analyze up to 18 samples in one assay. A larger group of samples can be analyzed using several sets of TMT-18plex kits.*

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Example One

A **Summary of Mass Spectrometry Data**

Quantified Proteins*	Total PSM#	Total Peptides	Unique Peptides	Proteins With Altered Abundance** in G12V	
				Abundance Increased	Abundance Decreased
6,659	224,031	58,418	54,392	411	394

** Proteins quantified across all TMT channels*

*** Proteins with altered expression levels (fold change ≥ **1.5**) with student test p-values ≤ **0.01***

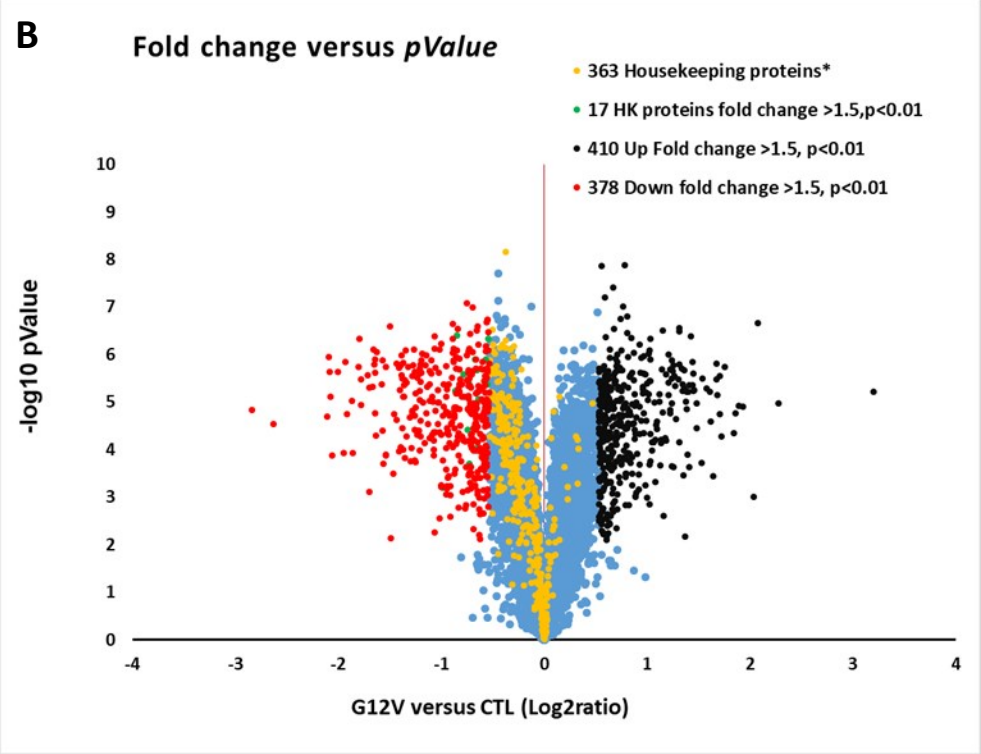

Figure 2: Proteomic characterization of the proteomes of MCF10 cell with Kras G12V expression and without G12V (CTL) expression. **A)** Summary of protein profiling data in G12V compared to WT. **B)** Volcano plots demonstrating the fold change of protein abundance between G12V and WT. The x-axis represents the log2 of fold changes and the y-axis represents the statistically significant p-value (-log10 of p-value, n=3).

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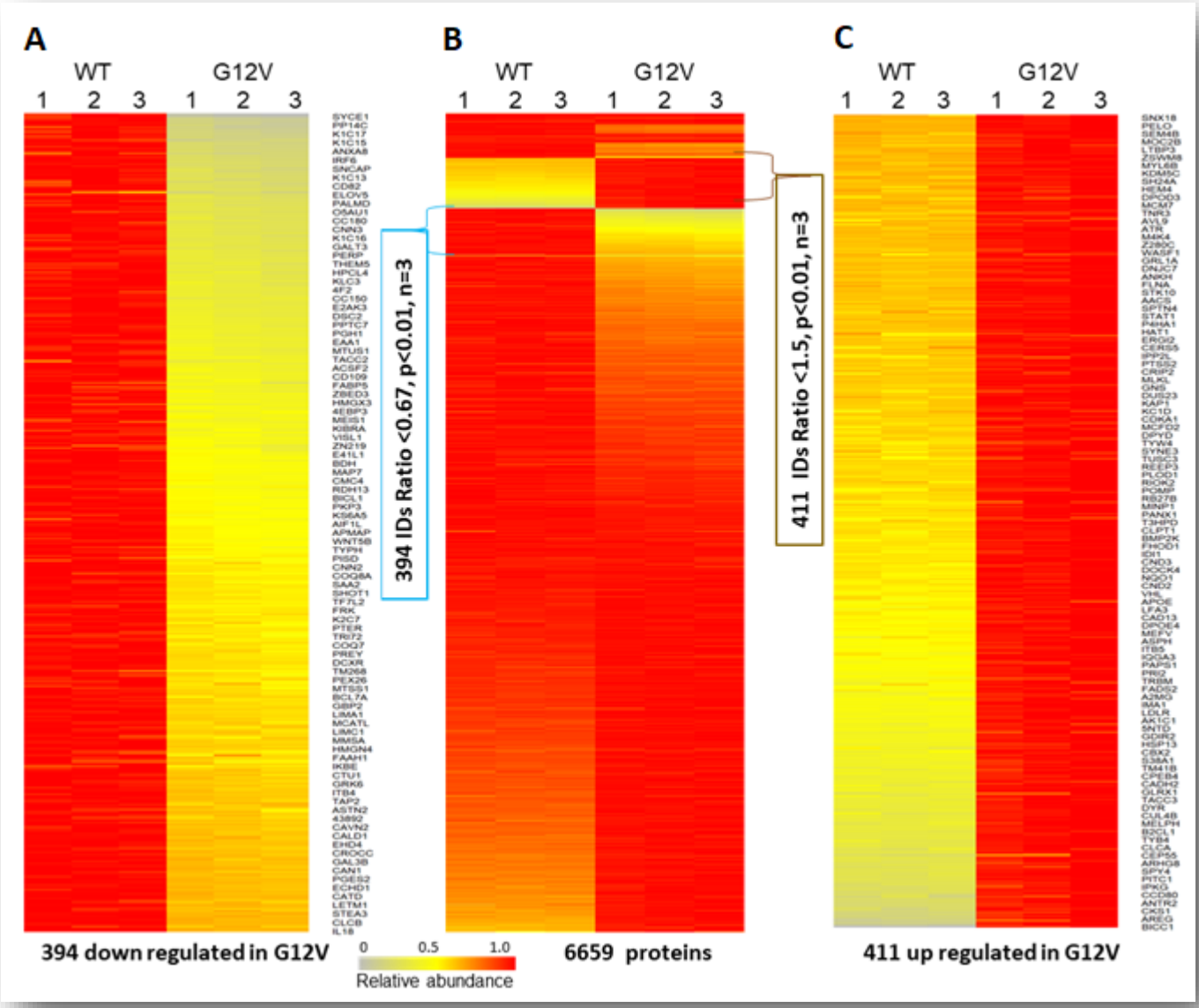

Figure 3: Proteomic characterization of the proteomes of cells transfected with Kras G12V mutant and without G12V mutant (WT) expression. **A)** Heat map showing the relative abundance of 394 proteins down-regulated in G12V mutant transfected cells. **B)** Heat map showing the relative abundance of 6659 proteins identified across 2 group of 6 samples. **C)** Heat map showing the relative abundance of 411 proteins up-regulated in G12V mutant transfected cells. *Note: The color key indicates the relative abundance of each protein (0 to 1.0) across 6 samples.*

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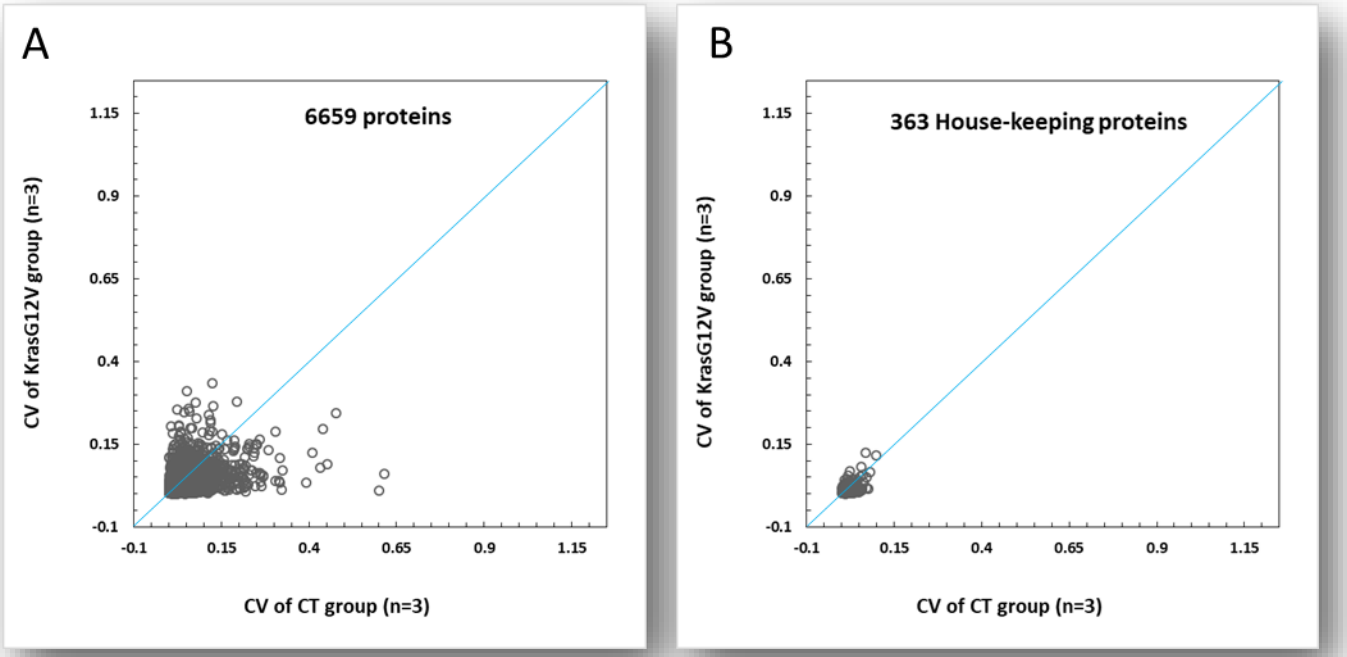

Figure 4: Evaluation of coefficients of variation (CVs) of protein abundance measurements. Two plots of CVs between two groups of samples, control and K-Ras G12V, n=3. The x-axis represents the CVs of 6659 proteins **(A)** and of 363 HK proteins **(B)** from control group (n=3), and the y-axis represents the 6287 proteins **(A)** and of 367 HK proteins **(B)** from K-Ras G12V group (n=3).

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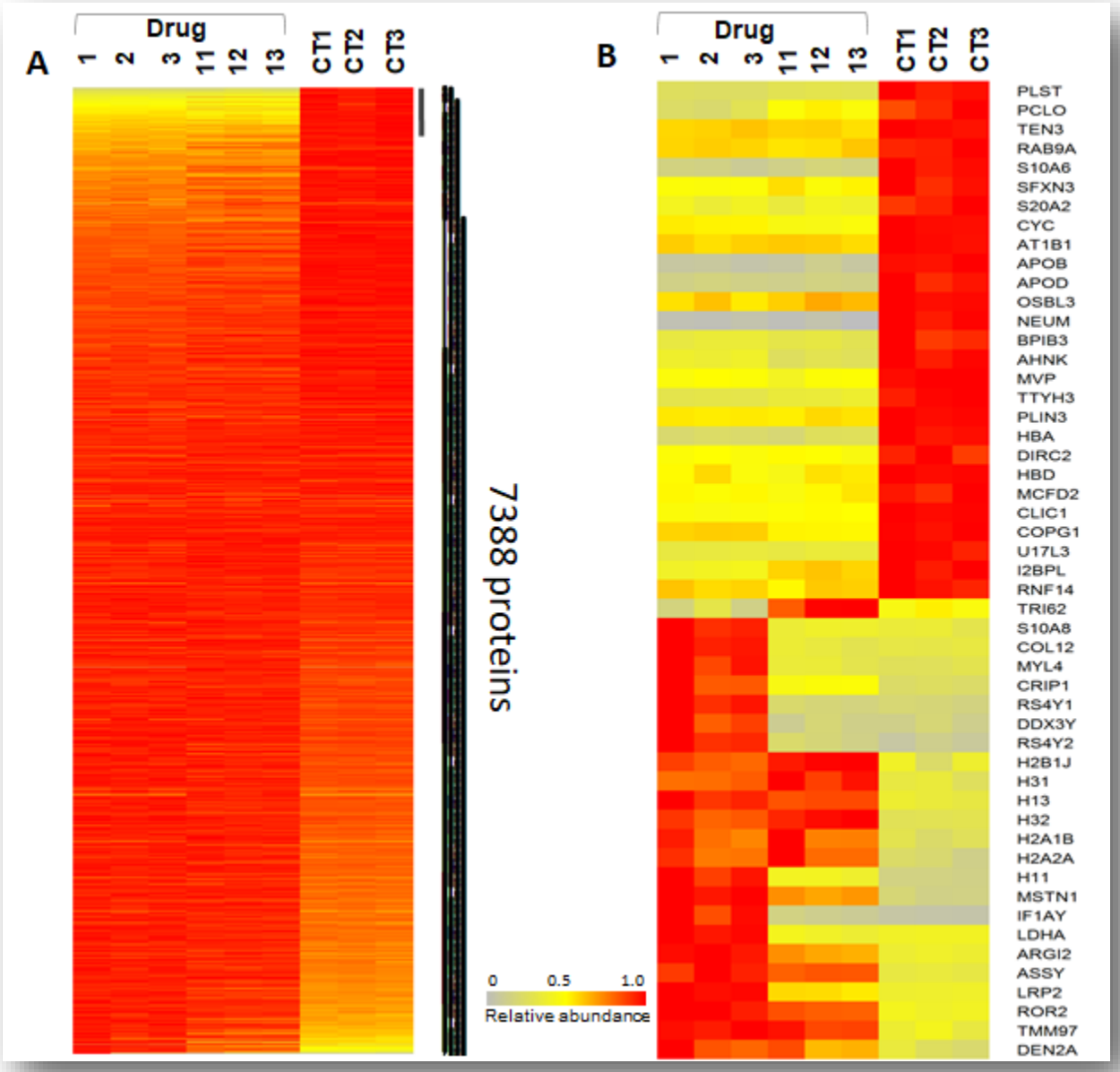

Figure 5: Proteomic characterization of the proteomes of a cancer cell line upon drug treatment. **A)** Heat map depicting the relative abundance of 7388 proteins identified across 3 group of 9 samples (triplicates/group). **B)** Heat map showing the relative abundance of significantly changed proteins identified in drug treated cells compared with untreated cells (fold change >300%, p<0.01, n=3). *Note: The color key indicates the relative abundance of each protein (0 to 1.0) across 9 samples.*

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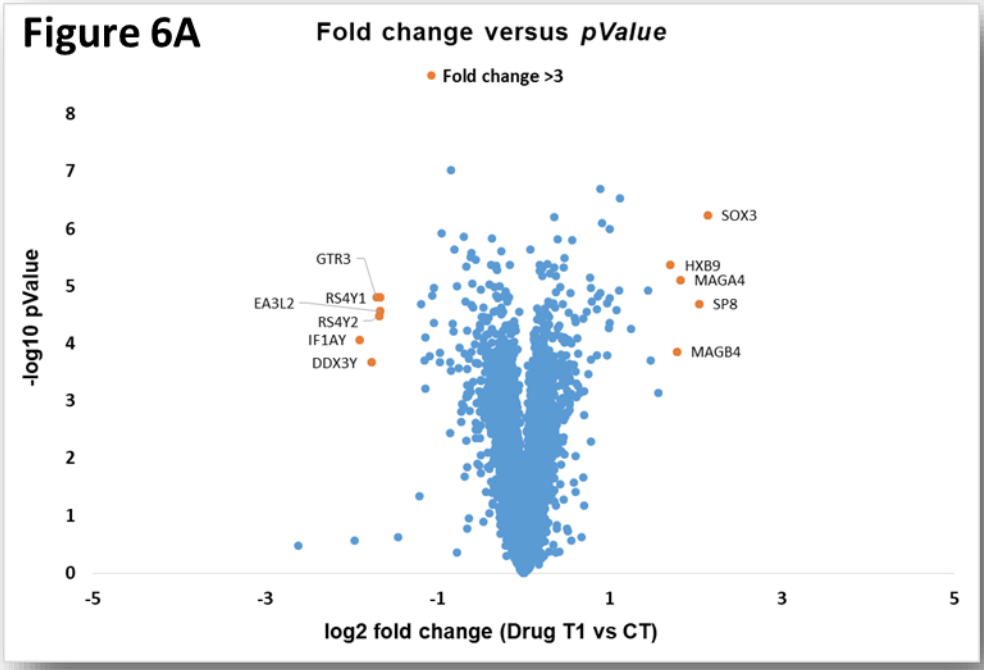
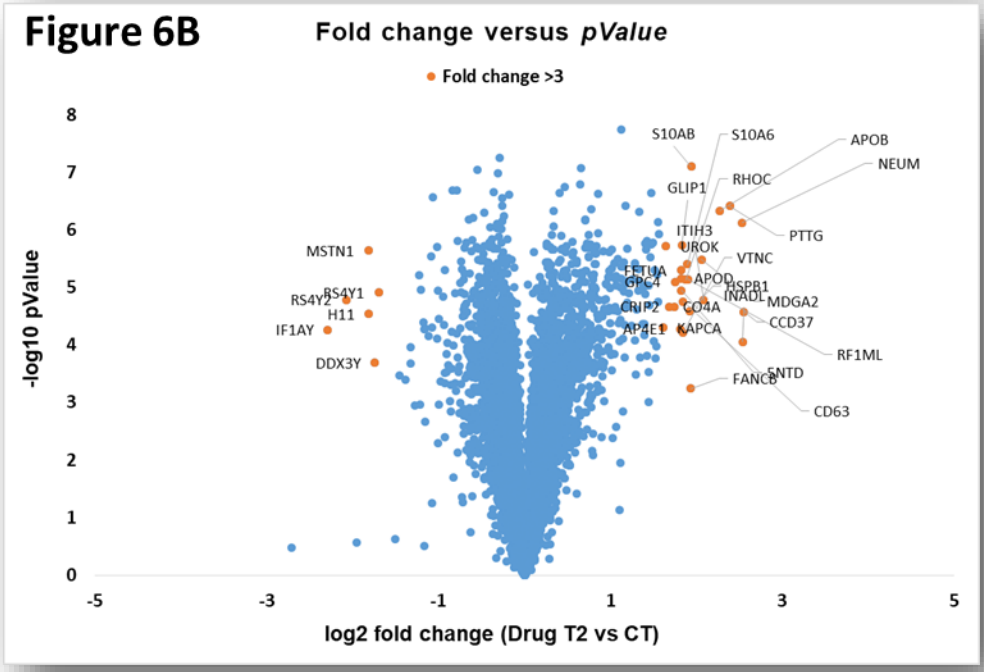
Example Two





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Example Two

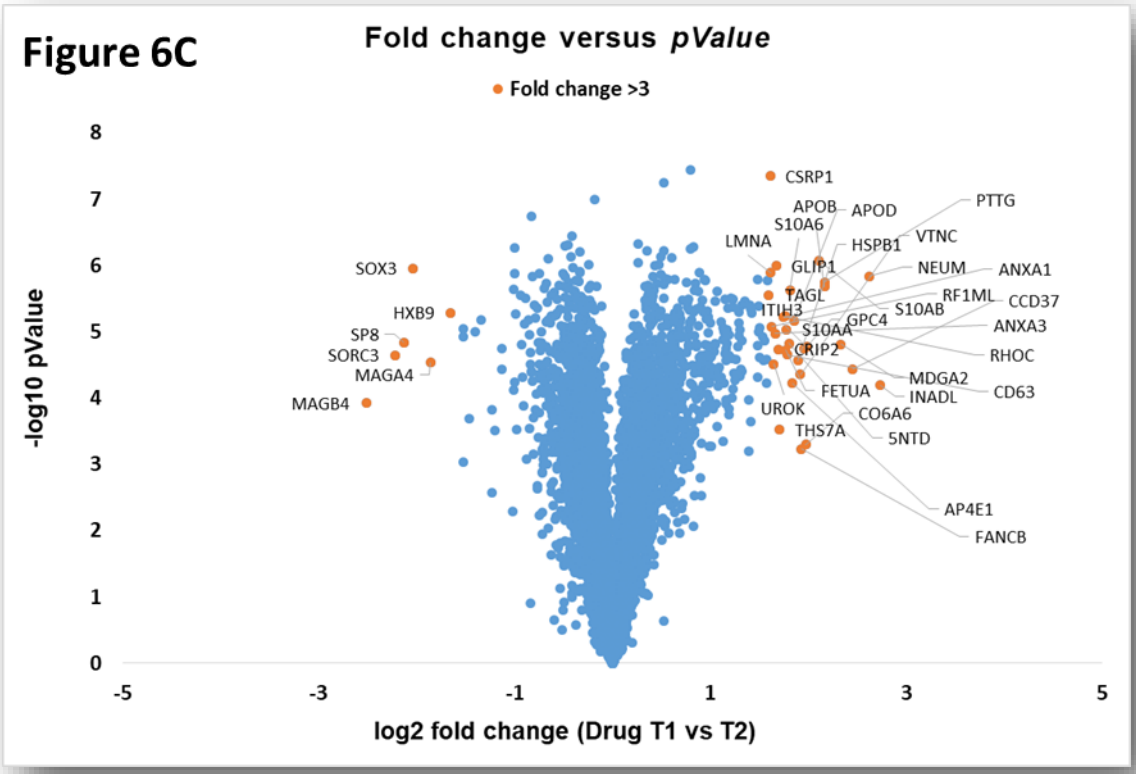

Figure 6: Proteomic characterization of the proteomes of a cancer cell line upon drug treatment. **A)** Volcano plot demonstrating the fold change of protein abundance between CT and Drug time-point 1. The x-axis represents the log2 of fold changes (Drug-1 versus CT), and the y-axis represents the statistically significant p-value (-log10 of p-value, n=3). **B)** Volcano plot demonstrating the fold change of protein abundance between CT and Drug time-point 2. The x-axis represents the log2 of fold changes (time-point 2 versus CT), and the y-axis represents the statistically significant p-value (-log10 of p-value, n=3). **C)** Volcano plot demonstrating the fold change of protein abundance between time-point 1 and time-point 2. The x-axis represents the log2 of fold changes (T1 versus T2), and the y-axis represents the statistically significant p-value (-log10 of p-value, n=3).

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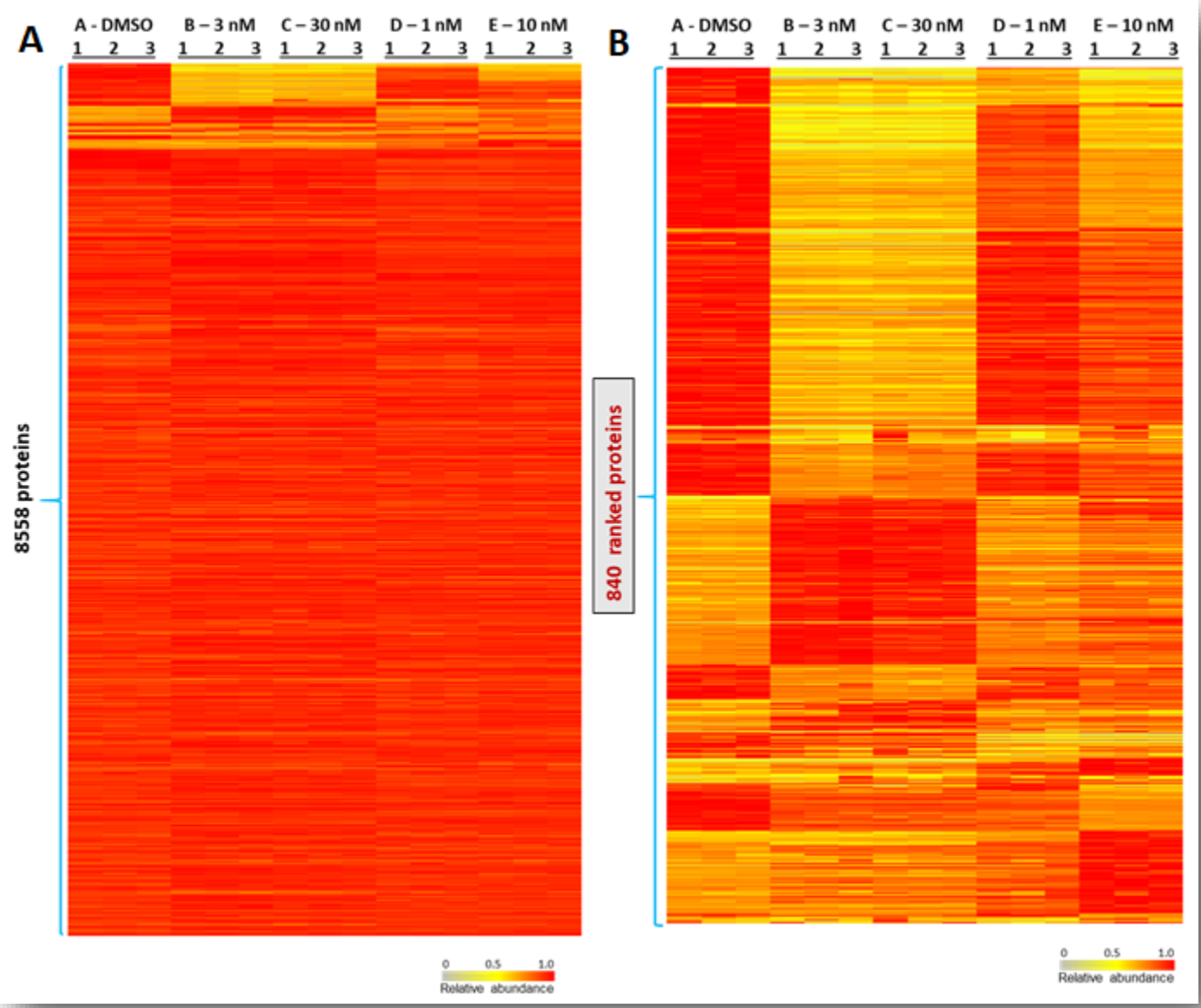

Figure 7: Proteomic characterization of 22W cancer cells treated with four drug doses. **A)** Heat map showing the relative abundance of 8558 proteins identified across 5 groups of 15 samples. **B)** Heat map showing the relative abundance of 840 ranked proteins identified across 5 groups of 15 samples (fold change >1.25, p>0.05, n=3). *Note: The color key indicates the relative abundance of each protein (0 to 1.0) across 15 samples.*

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Example Three

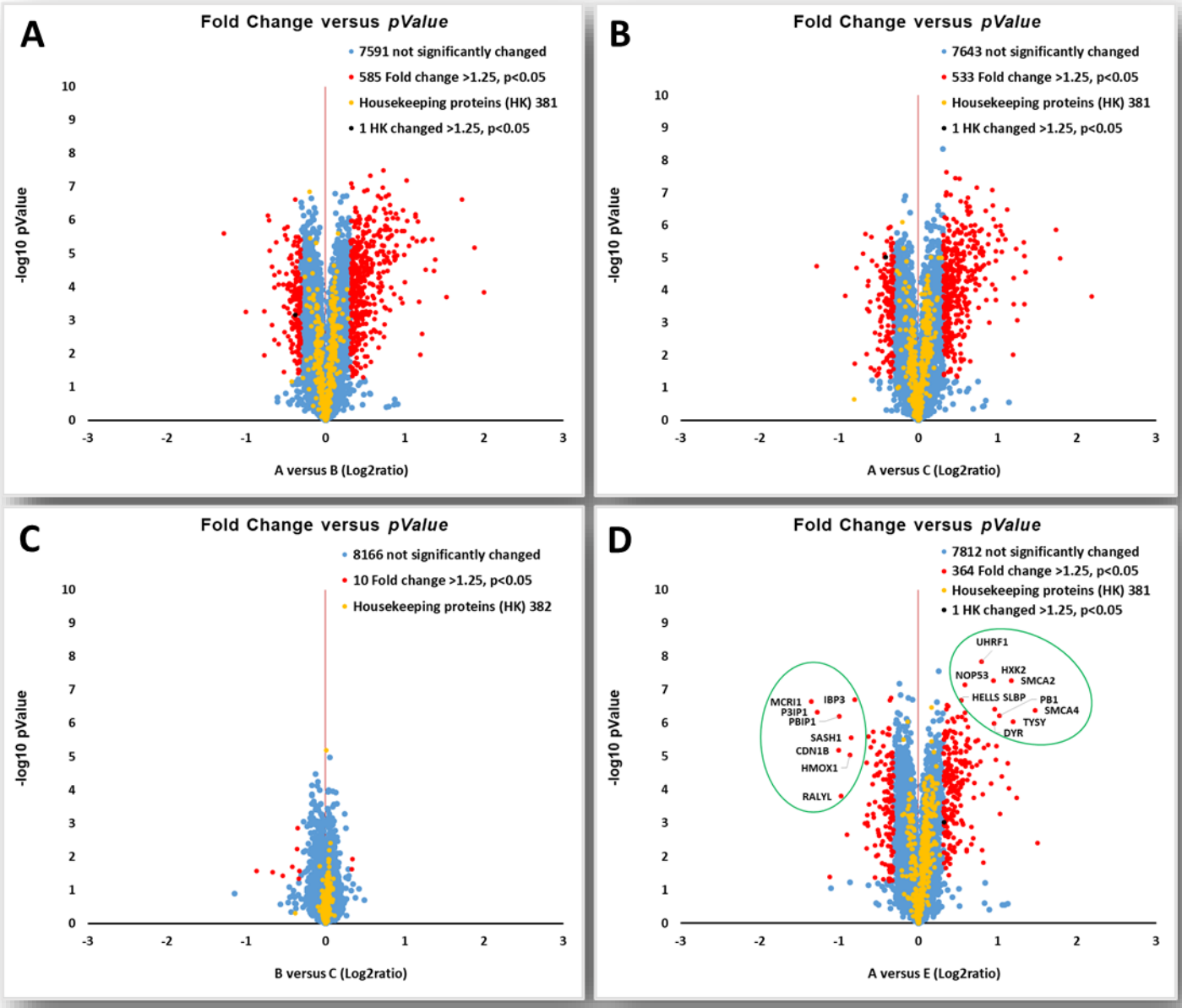

Figure 8: Proteomic characterization of 22W cancer cells treated with four drug doses. Volcano plots demonstrating the fold change of 8558 protein abundance between **(A)** A group and B group; **(B)** A group and C group; **(C)** B group and C group; **(D)** A group and E group. The x-axis represents the log2 of fold changes, and the y-axis represents the statistically significant p-value (-log10 of p-value, n=3). Blue dots represent proteins fold change <1.25, Red dots are proteins fold change >1.25, p>0.05. Orange dots are housekeeping protein (HK) not obviously changed.

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