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#RESEARCHNEVERSTOPS

Integrating Proteomics and PTM Data for Comprehensive Multi-Omics Analysis

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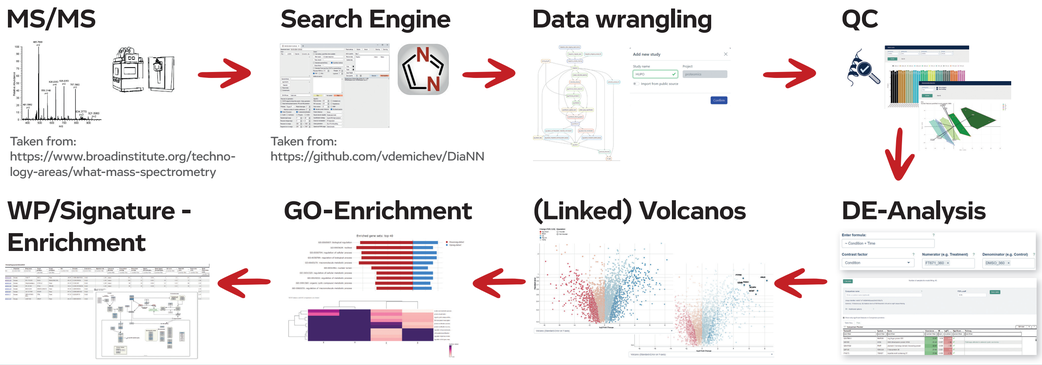
INTRODUCTION

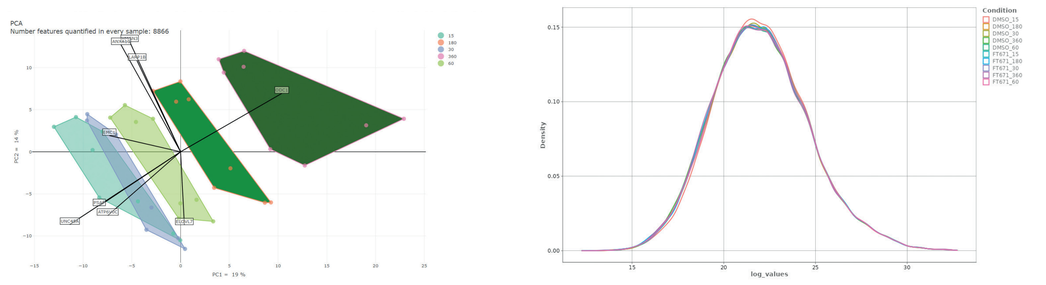
Multi-omics data analysis, especially when integrating information from different modalities such as post-translational modification analysis and global transcriptomics, can be a time-consuming and difficult process. We introduce PanHunter™, an all-in-one omics data analysis platform that enables everyone including non-coding researchers and disease experts to generate reliable and easy-to-interpret results from complex data sets. We demonstrate its power and intuitiveness by generating visualizations and drawing conclusions from a publication by Steger et al. that studies the effects of the ubiquitin protease inhibitor FT671 on human colon cells[1]. By generating a few key visualizations in PanHunter with little effort, it was possible to reproduce selected results and conclusions of the paper within a short period of time: Application of FT671 leads to increased ubiquitination (at PTM level) and thus to degradation of its substrates MDM2 (at protein expression (Px) level). MDM2 is degraded only slightly despite heavy ubiquitination, as mentioned in the paper, likely due to p53 stimulation. This stimulation can also be seen in the depicted p53 network pathway (bottom left).

[1] Steger et al., 2021, https://doi.org/10.1038/s41467-021-25454-1

PANOMICS WORKFLOW

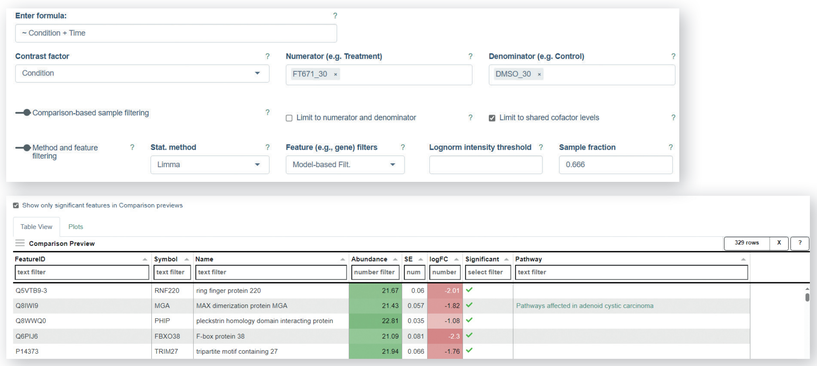


IS THE DATA READY FOR STATISTICAL TESTS?



- There do not seem to be extreme outliers in the PCA
- Intensity values are normally distributed, no hint towards aberrant samples

ARE SOME FEATURES DIFFERENTIALLY EXPRESSED?

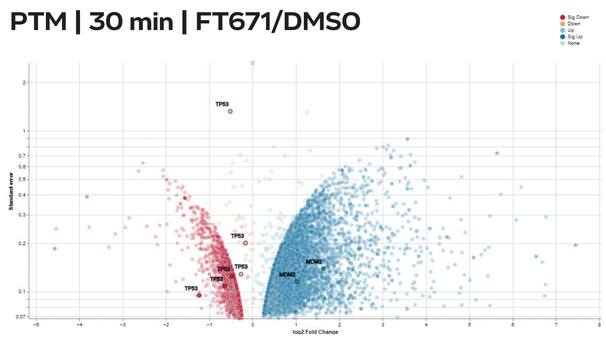


Differential expression (DE) analysis identifies proteins that are expressed at different levels between conditions in a statistically robust manner. Pan Hunter carries out these analysis for proteomics and PTM data using the Bioconductor R package Limma[2]. Reasonable default values are set for all parameters in PanHunter. However, more advanced users have the option to adjust parameters. All comparison settings are saved as metadata, ensuring that results are always reproducible. For the analysis presented, features were excluded from Limma calculation when found in less than 2/3 of the samples of one condition. Additionally, the moderated t-test was based on samples with shared cofactor levels only.

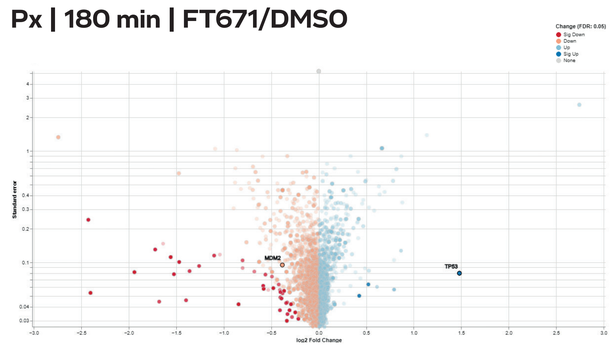
[2] Ritchie et al., 2015 https://doi.org/10.1093/nar/gkv007

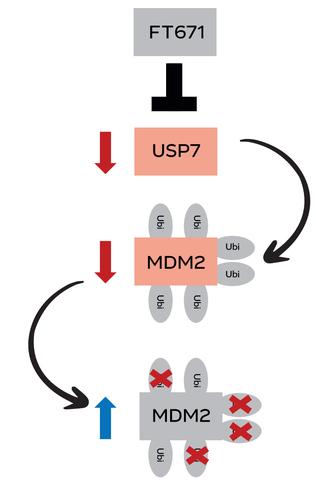
HOW ARE PX AND PTM CHANGES INTERLINKED?

PTM | 30 min | FT671/DMSO



Px | 180 min | FT671/DMSO

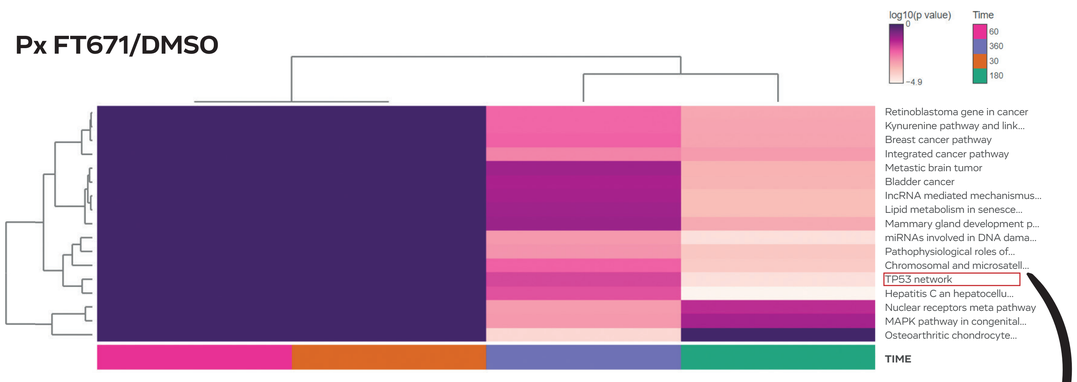




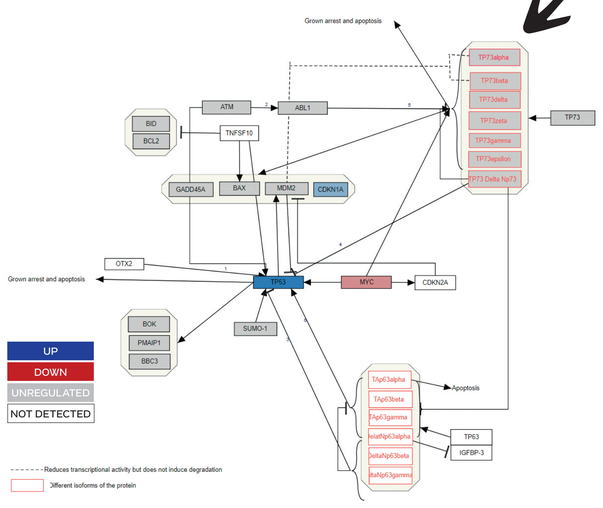
PTM level changes arise earlier (30 min), MDM2 gets increasingly ubiquitinated in response to the FT671 treatment. Consequently, MDM2 is slightly downregulated on PX level (at 180 min). Inverted observations can be made for p53.

WHICH FUNCTIONS DO DIFFERENTIALLY EXPRESSED PROTEINS FULFIL?

Px FT671/DMSO



Px FT671_180/DMSO_180

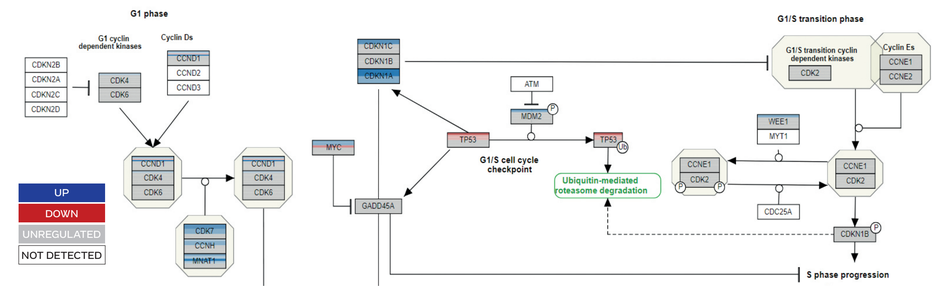


Top: Enriched pathways for the Px data are shown. As expected only the later timepoints (180, 360 min) show significant regulation.
Bottom: TP53 network from Wikipathways is displayed. TP53 is highlighted as being upregulated as well as CDKN1A (p21). The stimulating effect of p53 on MDM2, counteracting the increased ubiquitination, can also be seen[3].

[3] Steger et al., 2021, https://doi.org/10.1038/s41467-021-25454-1

WHICH FUNCTION DO THE PROTEINS FULFIL WITH DIFFERENTIALLY REGULATED PTM ACTIVITY?

PTM FT671/DMSO



Top: Most enriched pathways for the PTM data are shown. All 5 timepoints show significantly regulated pathways. Many are in relation to cancer.
Bottom: Cytosolic part of the G1 to S1 cell cycle control pathway from Wikipathways. Tp53 takes a central role also here. Several colours for one protein indicate multiple detected PTMs.