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#RESEARCHNEVERSTOPS

PhosphoScout®

FOR FURTHER INFORMATION:

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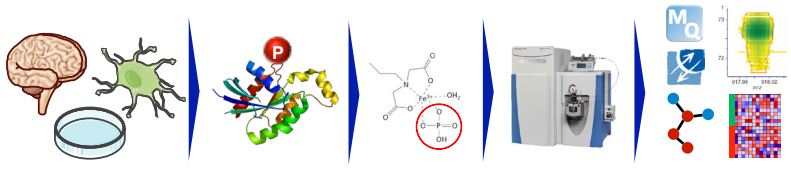
Mode-of-Action Analysis Using Global Quantitative Phosphoproteomics

- *PhosphoScout® is a global, quantitative phosphoproteomic platform that can identify regulated protein phosphorylation sites in response to treatments or genetic modifications.*
- *PhosphoScout® yields an un-precedented depth and a high number of quantified phosphorylation sites (>20,000) from variety of input materials including living cells, animal models and patient samples.*
- *PhosphoScout® facilitates identification of pharmacodynamics and drug response biomarkers.*

PHOSPHOSCOUT® TECHNOLOGY

PhosphoScout can be applied to any given cell line, tissue or patient sample. To identify phosphopeptides, a highly optimized workflow is combined with custom nano-flow liquid chromatography and state-of-the art mass-spectrometry. The resulting mass spectrometric data is processed using MaxQuant and further analyzed using ScoutExplorer – a in-house developed tool box designed for statistical and bioinformatics analysis of quantitative proteomics data.



PHOSPHOSCOUT® WORKFLOW

Protein extracts from cells or tissues are enzymatically cleaved. High-pH reverse phase chromatography in combination with IMAC metal affinity chromatography is used to enrich the phosphopeptides from the respective cells or tissues. Subsequent mass spectrometry and bioinformatic analysis allow quantification and comparison of phosphopeptide patterns between different samples.

CASE STUDY: PHOSPHODIESTERASE 10A INHIBITION

PhosphoScout can quantify phosphorylation patterns in response to drug administration. It also facilitates monitoring of signaling integration and points to additional therapeutic applications. This was illustrated by investigating the effect of the PDE10A inhibitor MP-10 on the phosphorylation pattern in the Huntington disease mouse model Q175 (Beaumont et al., 2016).

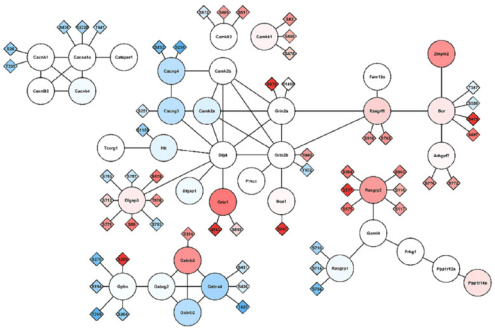
NUMBER OF DETECTED PROTEINS AND PHOSPHORYLATION SITES

To gain statistically validated data, 9-10 biological replicates per condition were performed. Only phosphorylation sites that could be localized within the peptide sequence with high confidence were considered for further analysis.

	Count
Total number of detected P-sites	26,108
Average number of P-sites per sample	10,405
Number of regulated sites	769
Number of detected proteins	8,490
Average number of proteins per sample	5,539

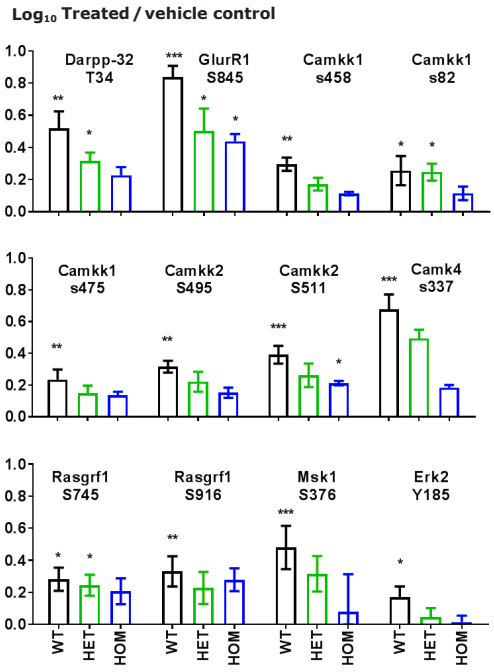
INTEGRATING DATA WITH PROTEIN INTERACTION NETWORKS

The Subextractor algorithm combines phospho-proteomic data with information from the STRING protein-protein interaction database. It identified phosphorylation changes in scaffolding and membrane proteins important for the regulation of synaptic transmission.



INFLUENCE OF MP-10 ON SIGNALING PROTEINS

PDE10 inhibition is known to increase cAMP levels and to activate downstream signaling pathways. We detected a strong phosphoproteomic signature indicating CREB activation through Darpp-32, CamK and MAPK/ERK pathways. Even though phosphorylation response is attenuated inhomozygous Q175 mice, CREB activation was still apparent.



Reference: *Beaumont, V. et al. Phosphodiesterase 10A Inhibition Improves Cortico-Basal Ganglia Function in Huntington's Disease Models. Neuron 92, 1220–1237 (2016).*