

Precision Phosphotyrosine Proteomics



Deep Coverage · Site-Level Quantification · Kinase Signaling Insights

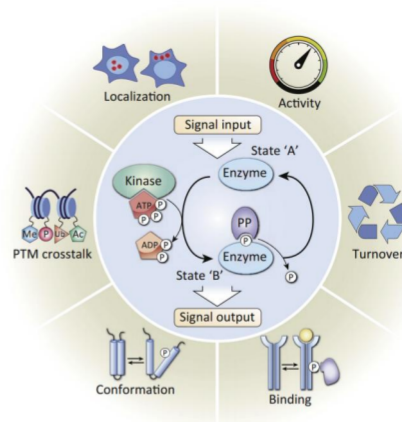
Overview of Phosphoproteomics

Protein phosphorylation is an enzyme-catalyzed process in which protein kinases transfer the γ -phosphate of ATP to specific amino acid residues—primarily serine, threonine, and tyrosine—on substrate proteins.

As one of the most widespread post-translational modifications (PTMs), phosphorylation is estimated to occur in more than 30% of cellular proteins. It represents one of the most fundamental and important mechanisms for regulating protein activity and function.

Protein phosphorylation plays a central role in a wide range of physiological and pathological processes, including cell proliferation, development, differentiation, and apoptosis. Accordingly, phosphoproteomic analysis is widely used to investigate signal transduction, developmental regulation, cancer biology, disease mechanisms, and therapeutic response.

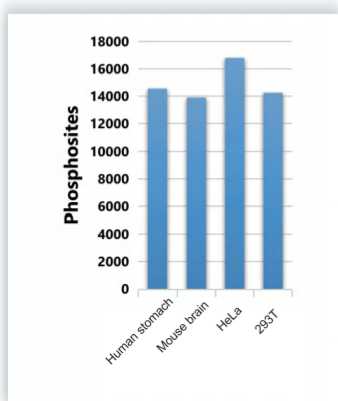
PTM BIO has developed a deep, quantitative phosphotyrosine (pY) profiling workflow that uses proprietary antibodies to selectively enrich phosphotyrosine peptides and reduce sample complexity. By integrating rigorous two-tier quality control with comprehensive bioinformatics analysis, this workflow enables sensitive characterization of tyrosine phosphorylation and its regulatory roles across diverse physiological and pathological processes.



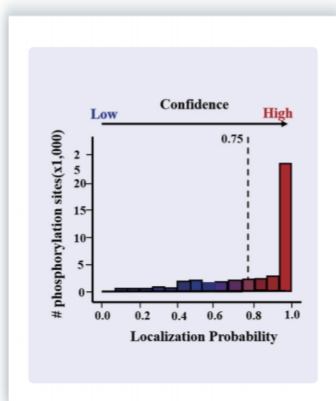
Phosphoproteomic Data Analysis

Technical Advantages

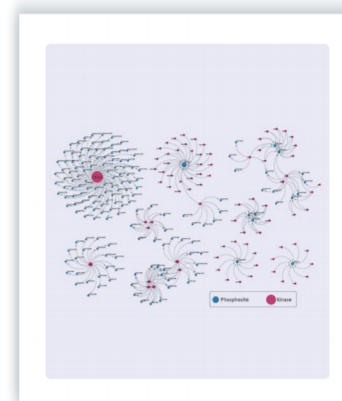
Deeper Phosphosite Coverage



Enhanced Quality Control



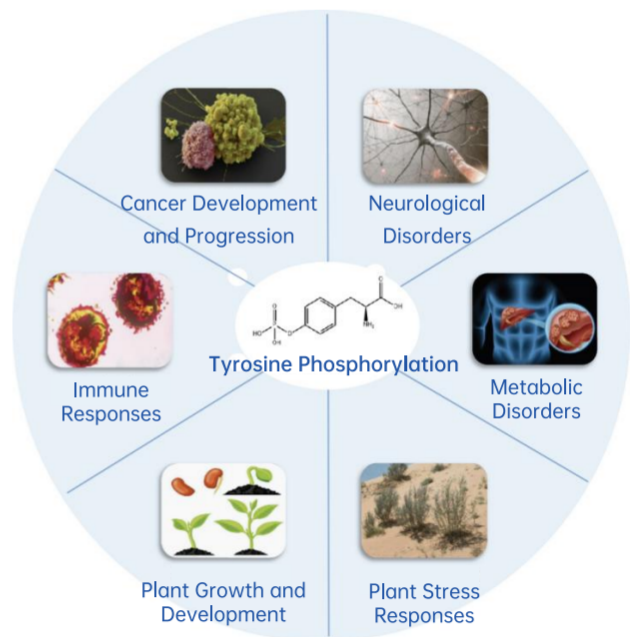
Advanced Bioinformatics



Tyrosine Phosphorylation: An Overview

Tyrosine phosphorylation (pY) is the covalent addition of a phosphate group to the hydroxyl group of a tyrosine residue. By modulating protein activity and protein-protein interactions, it coordinates multilevel signaling networks and plays essential roles in cellular signal transduction, proliferation, differentiation, and disease progression, including cancer and neurodegenerative disorders.

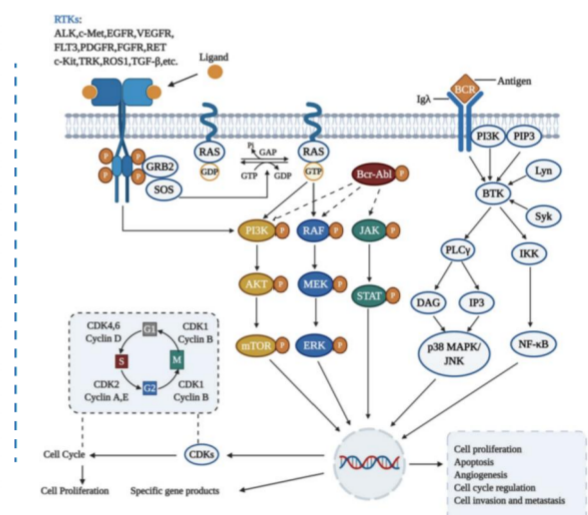
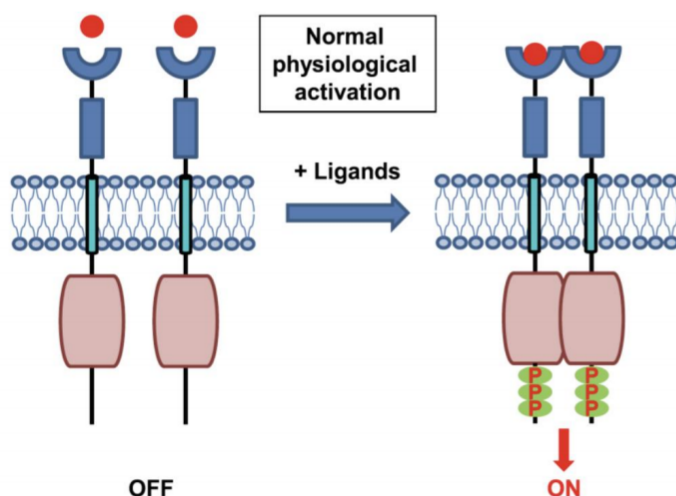
Tyrosine phosphorylation also has substantial therapeutic relevance, as tyrosine kinases constitute the largest target class among FDA-approved small-molecule kinase inhibitors. Consequently, tyrosine phosphorylation has become an increasingly important focus of biological and biomedical research.



Why Study Tyrosine Phosphorylation?

1. A Critical Regulator of Cell Signaling

- Ligand-induced autophosphorylation activates many receptor tyrosine kinases and creates docking sites for downstream signaling proteins.
- Tyrosine phosphorylation regulates key components of major signaling pathways, including JAK/STAT, p53, PI3K, EGFR, TGF- β , and ABL.
- Although less abundant than serine/threonine phosphorylation, tyrosine phosphorylation plays essential roles in cell-cycle regulation, proliferation, differentiation, apoptosis, invasion, and metastasis.

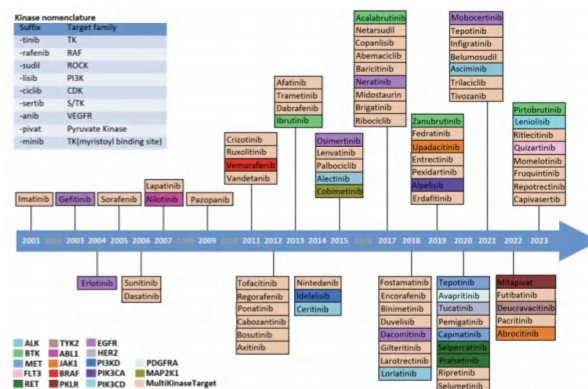


2. Broad Biological and Clinical Relevance

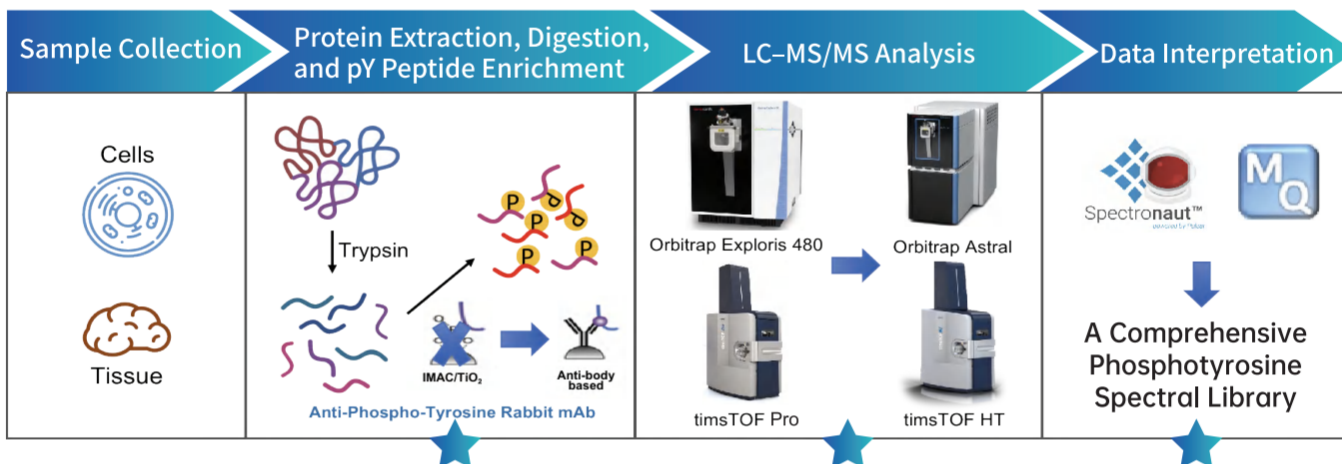
· Dysregulated tyrosine phosphorylation contributes to cancer, neurological disorders, infectious diseases, and other pathological conditions.

· Many FDA-approved small-molecule kinase inhibitors target receptor or nonreceptor tyrosine kinases, highlighting the therapeutic importance of tyrosine phosphorylation.

· As of the end of 2025, 74 of the 94 FDA-approved small-molecule protein kinase inhibitors targeted receptor or nonreceptor tyrosine kinases.



Analytical Platform: High-Resolution LC-MS/MS



Five Key Advantages of PTM BIO's Phosphotyrosine Proteomics Platform

PTM BIO's Phosphotyrosine Proteomics 2.0 platform delivers deeper pY coverage, stronger analytical confidence, and streamlined support for studying tyrosine phosphorylation.

01 Enrichment: Proprietary Recombinant pY Affinity Reagents

Reliable Lot-to-Lot Performance

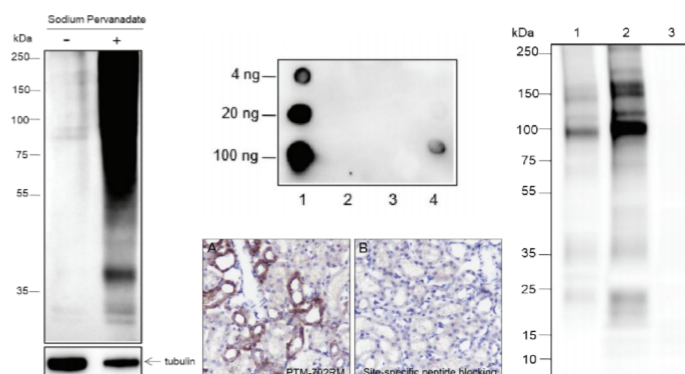
Recombinant antibody production minimizes lot-to-lot variation and improves reproducibility.

High Specificity and Sensitivity

Engineered monoclonal antibodies enable efficient, specific, and sensitive pY peptide enrichment.

Expanded Phosphosite Coverage

A pan-pY affinity resin increases phosphosite coverage and quantitative depth.



02 Analytical Platform: High-Resolution LC-MS/MS

Advanced High-Resolution Mass Spectrometry

Our platform includes more than 20 high-resolution mass spectrometers, including five Orbitrap Astral and three timsTOF HT systems.

Deeper Coverage with Greater Confidence

Advanced instrumentation delivers high sensitivity, throughput, and phosphotyrosine coverage.



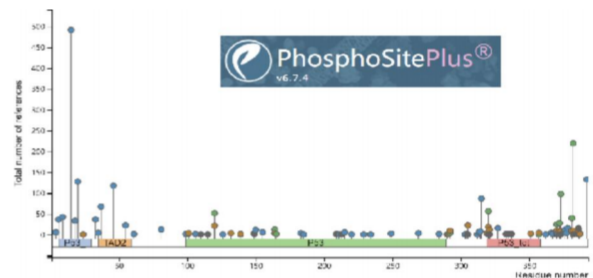
03 Data Analysis: In-House pY Spectral Library with 50,000+ Spectra

Broad Coverage of pY Signaling Networks

Our in-house library contains more than 50,000 pY spectra, improving phosphosite identification across receptor tyrosine kinase families.

Identification of Candidate Sites for Functional Follow-Up

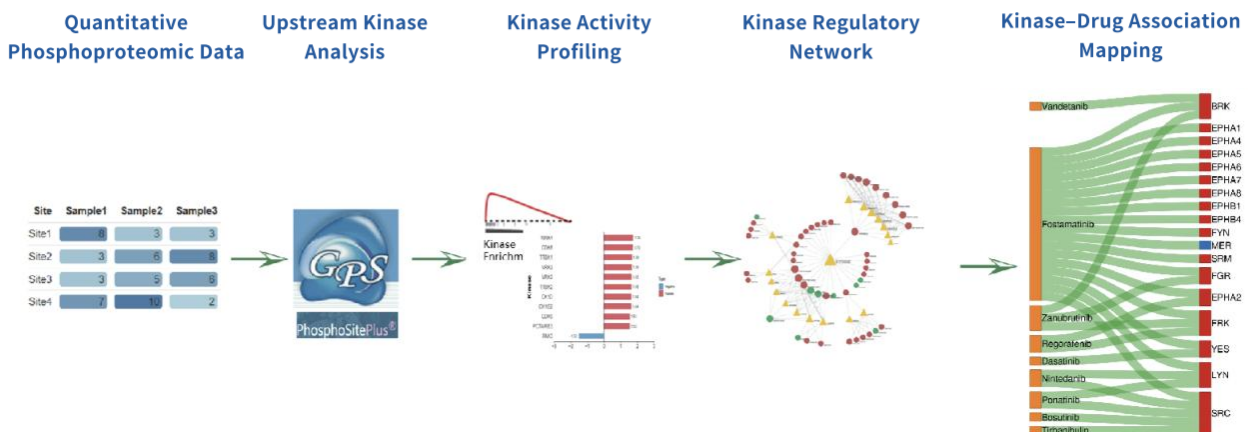
Coverage of key signaling proteins, including p53 and JAK1, supports the discovery of previously unreported candidate phosphosites, such as p53 Tyr103 (Y103), for subsequent functional validation.



04 pY-Focused Bioinformatics for Phosphotyrosine Research

Mechanistic Interpretation of pY Signaling

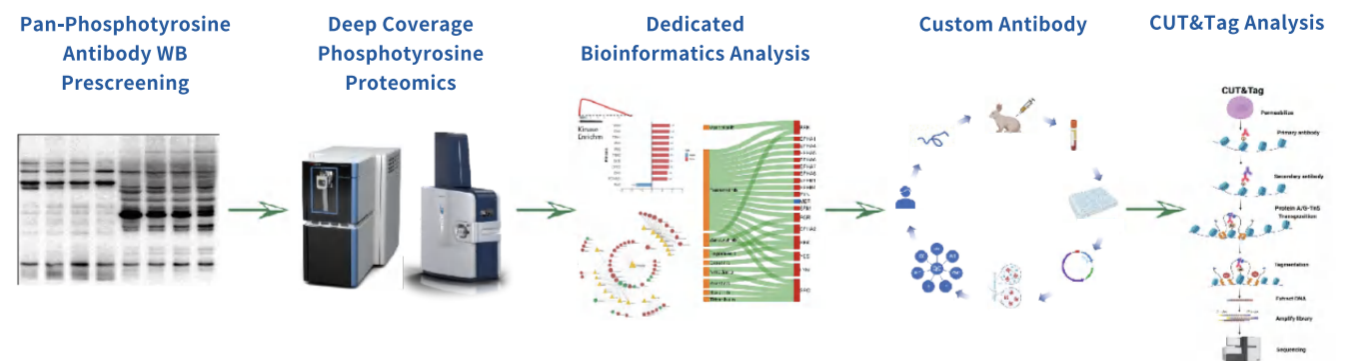
Our workflow links phosphosite changes to upstream kinases, regulatory networks, and drug associations, supporting both mechanistic and translational research.



05 Performance: Deep Phosphotyrosine Site Coverage

From Screening to Mechanistic Validation

Our integrated service combines pan-phosphotyrosine Western blot prescreening, high-depth pY profiling, dedicated bioinformatics, site-specific antibody development, and CUT&Tag analysis.



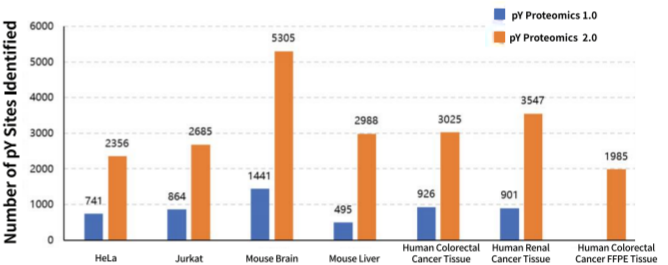
Deep Phosphotyrosine Site Coverage

Across benchmark samples, the platform identifies an average of more than 3,000 pY sites per LC-MS/MS run, with more than 5,000 sites identified in selected samples—representing a 3- to 6-fold improvement over version 1.0.

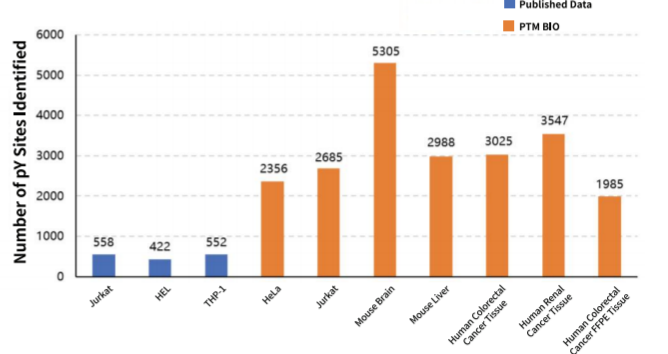
For both endogenous and stimulus-induced tyrosine phosphorylation, benchmarking against published datasets demonstrated 3- to 10-fold greater pY site coverage despite substantially reduced sample input. In stimulated samples, up to 13,663 pY sites were identified in a single LC-MS/MS run.

(Results vary by sample type, sample input, stimulation condition, LC gradient, instrument platform, acquisition method, database, FDR threshold, and site-localization criteria.)

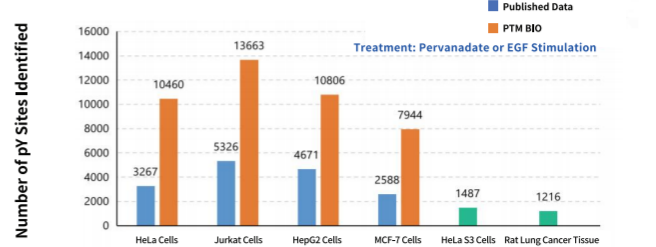
Identification Depth: pY Proteomics 1.0 vs. 2.0



Endogenous pY Site Coverage: Comparison with Published Data

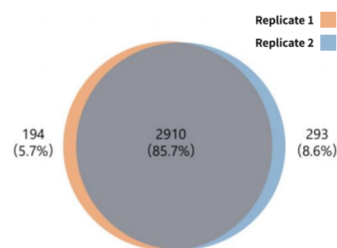


Stimulus-Induced pY Site Coverage: Comparison with Published Data

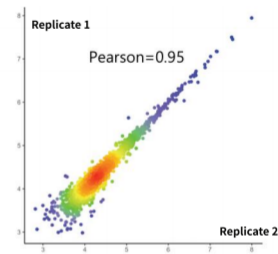


High Reproducibility and Quantitative Consistency in pY Profiling

- Technical replicates showed an **85.7%** overlap in identified pY sites.
- Quantitative measurements were highly correlated between replicates, with Pearson $r = 0.95$.



pY Site Overlap Between Technical Replicates



Quantitative Correlation Between Technical Replicates

Selected Publications in Phosphotyrosine Proteomics

Cancer Cell

Molecular targets of glucocorticoids that elucidate their therapeutic efficacy in aggressive lymphomas



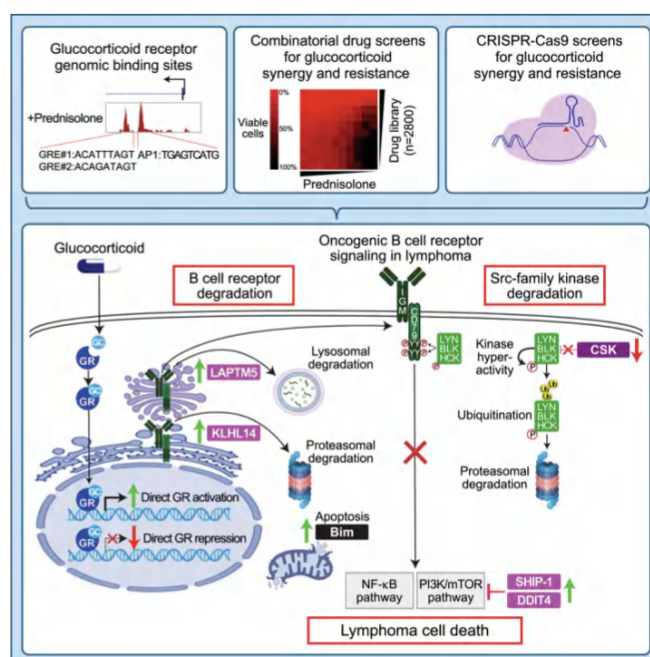
Research Overview

High-throughput drug and CRISPR-Cas9 screens revealed that glucocorticoids suppress oncogenic B-cell receptor (BCR) signaling, a recurrent dependency in aggressive B-cell malignancies, including diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL).

Integrated RNA-seq and CUT&RUN analyses identified 58 direct glucocorticoid receptor (GR) target genes involved in glucocorticoid-induced cytotoxicity. Proteomics identified LAPTM5 as the most upregulated protein and CSK as the most downregulated kinase.

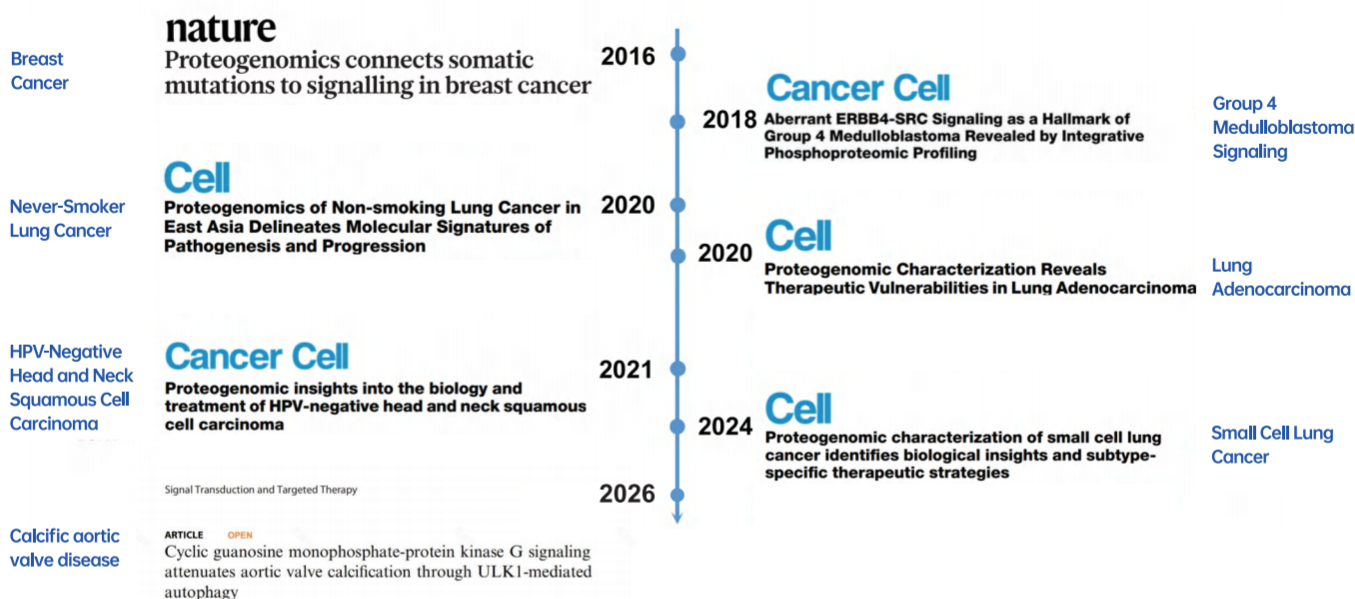
Phosphotyrosine proteomics and ubiquitinomics showed that GR-mediated CSK repression hyperactivates BCR-proximal Src-family kinases, promoting their ubiquitination and proteasomal degradation and ultimately suppressing oncogenic BCR signaling.

Combining glucocorticoids with pharmacological CSK inhibition may offer a promising therapeutic strategy for BCR-dependent aggressive lymphomas.



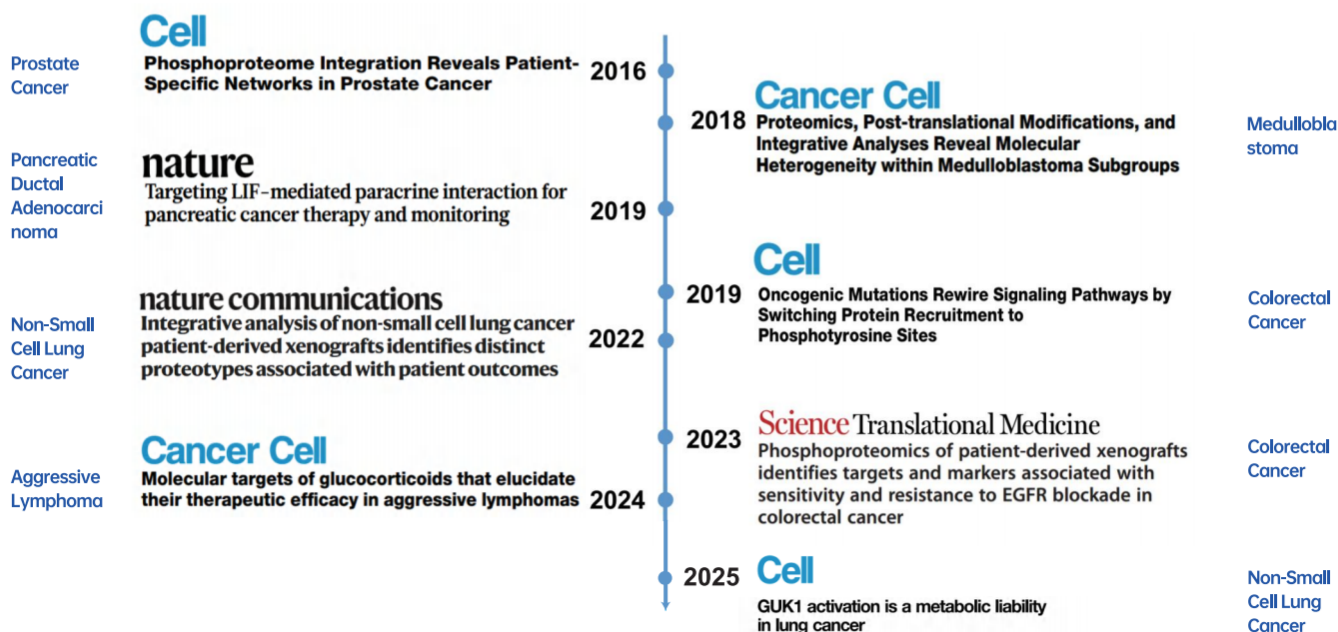
Phosphoproteomics in Precision Oncology: From Molecular Discovery to Clinical Translation

Global phosphoproteomics has generated important discoveries in large-cohort clinical proteogenomic studies and supported numerous high-impact publications. However, phosphotyrosine sites typically account for only 1–3% of the phosphosites identified by conventional workflows. Increasing the depth of pY profiling can reveal additional disease-associated signaling nodes and druggable targets, helping accelerate clinical translation.



Selected Oncology Studies Using Global Phosphoproteomics and pY Profiling

Global phosphoproteomics and dedicated phosphotyrosine profiling enable deeper characterization of kinase signaling, identification of additional pY-associated targets, and discovery of clinically relevant therapeutic vulnerabilities.



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