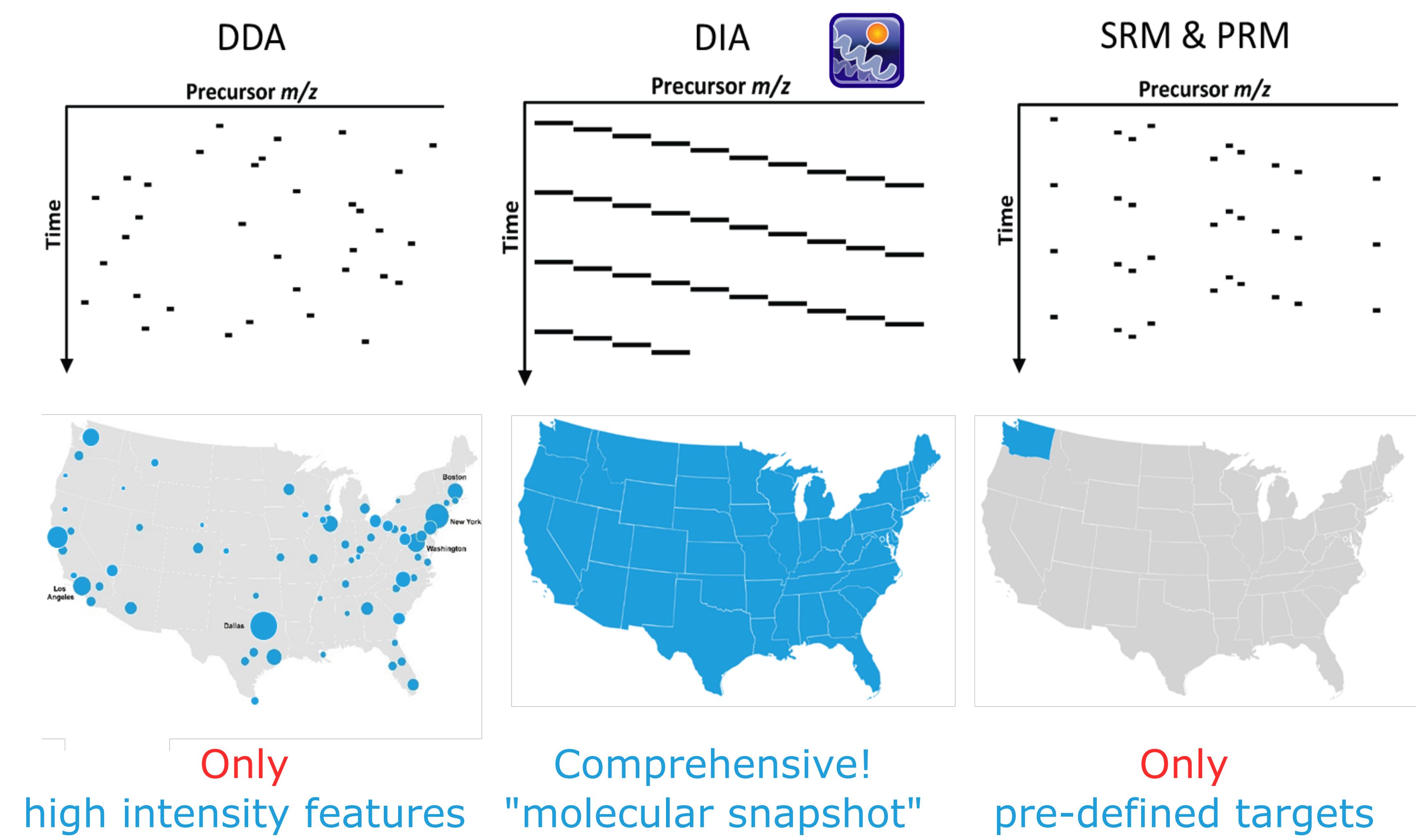# Pulling Back the Veil: Unlocking the Power of Comprehensive Proteomics in P100

Jarrett D. Egertson[1], Brian Searle[1], Jennifer Abelin[2], Ying S. Ting[1], Caitlin Feeney[2], Amanda Creech[2], Adam Officer[2], Jinal Patel[2], Xiaodong Lu[2], Dan Lam[2], Desiree Davison[2], Brendan X MacLean[1], Austin T. Keller[1], Michael J. MacCoss[1], Jacob Jaffe[2]
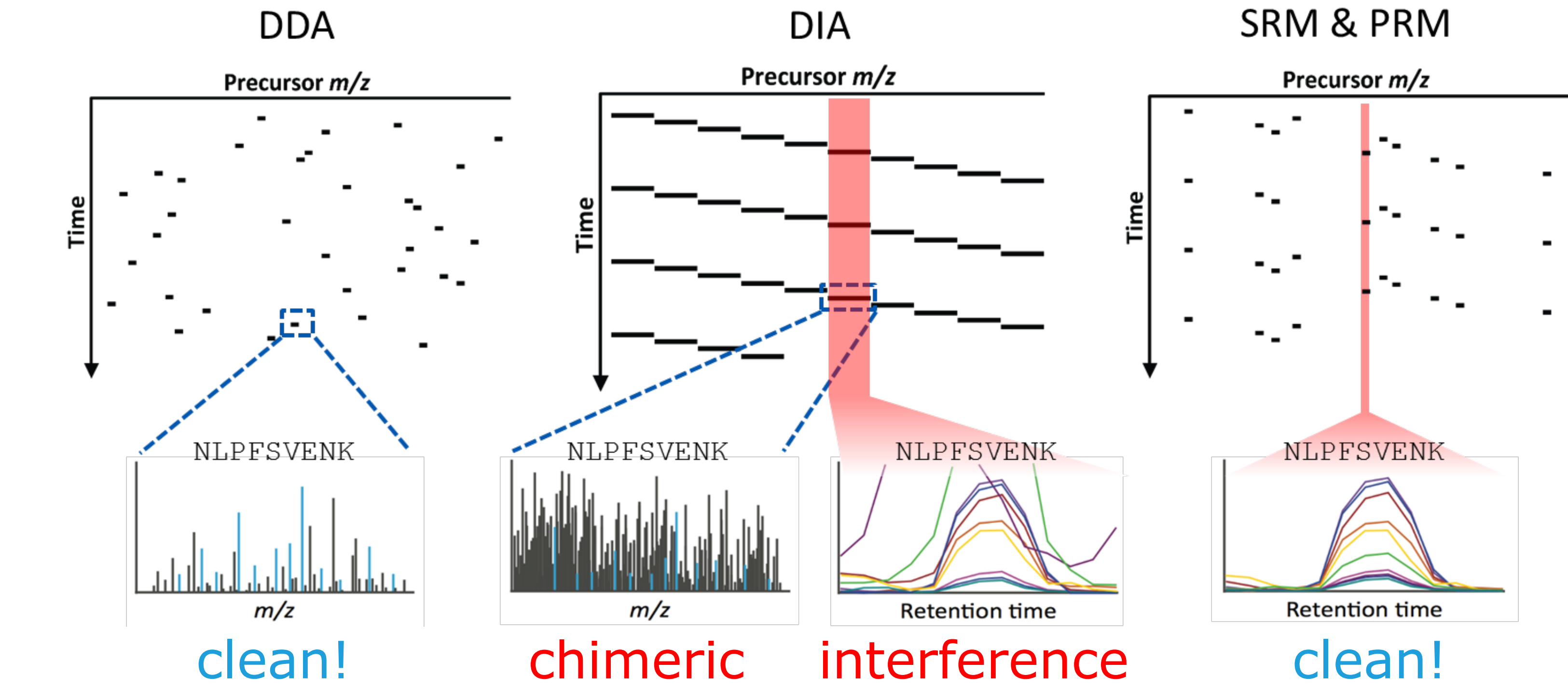
[1] University of Washington, Department of Genome Sciences, Seattle, WA   [2] Broad Institute of MIT and Harvard, Cambridge, MA

Data Independent Acquisition is Comprehensive

DDA
Precursor m/z

DIA
Precursor m/z

SRM & PRM
Precursor m/z

Only
high intensity features

Comprehensive!
"molecular snapshot"

Only
pre-defined targets

DIA Data is Challenging to Analyze

DDA
Precursor m/z

DIA
Precursor m/z

SRM & PRM
Precursor m/z

NLPFSVENK

clean!   chimeric   interference   clean!

Detection: MS/MS spectra are highly chimeric, how to interpret?
Quantification: Chemical noise interference is more prevalent

Primer: Targeted Data Analysis

MS/MS Analysis
Precursor Isolation

VLENTEIGDSIFDK++ (794.4 m/z)
M+1
M+2

20 m/z isolation

Fragmentation
y11 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1
V L E N T E I G D S I F D K
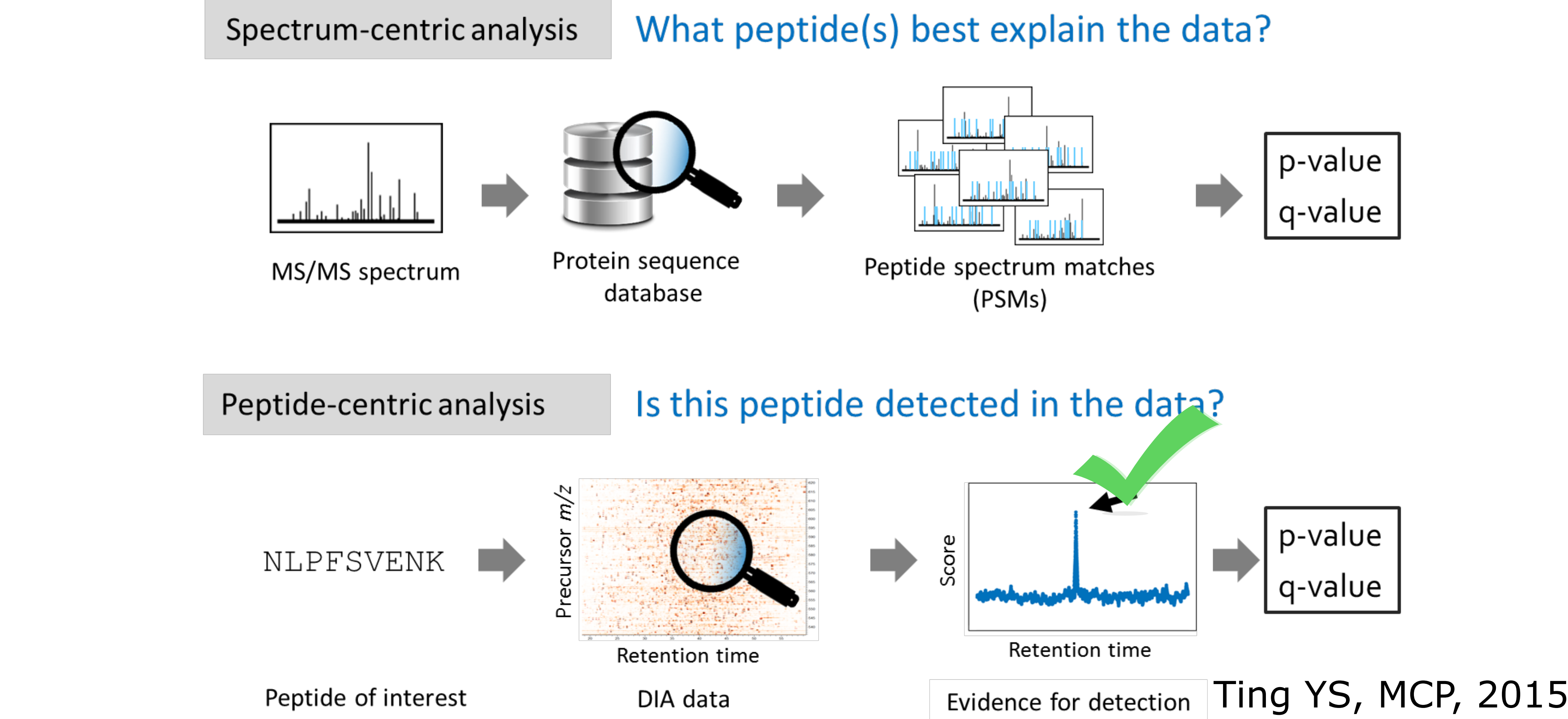b2 b3 b4 b5 b6 b7 b8 b9 b10 b11 b12 b13

Mass Analysis

1) A precursor m/z range is isolated (multiple precursors co-isolated)

2) Precursors are fragmented by collision-induced dissociation

3) Fragment ions are mass analyzed

4) Signal for theoretical fragment ions of a query peptide are extracted from the MS/MS spectra and used for quantification

Querying DIA Data by Peptide Centric Analysis

Spectrum-centric analysis   What peptide(s) best explain the data?

MS/MS spectrum → Protein sequence database → Peptide spectrum matches (PSMs) → p-value q-value

Peptide-centric analysis   Is this peptide detected in the data?

NLPFSVENK → Precursor m/z / Retention time (DIA data) → Score / Retention time → p-value q-value

Peptide of interest   DIA data   Evidence for detection   Ting YS, MCP, 2015

Pulling Back the Veil: From P100 to P6000 Overnight
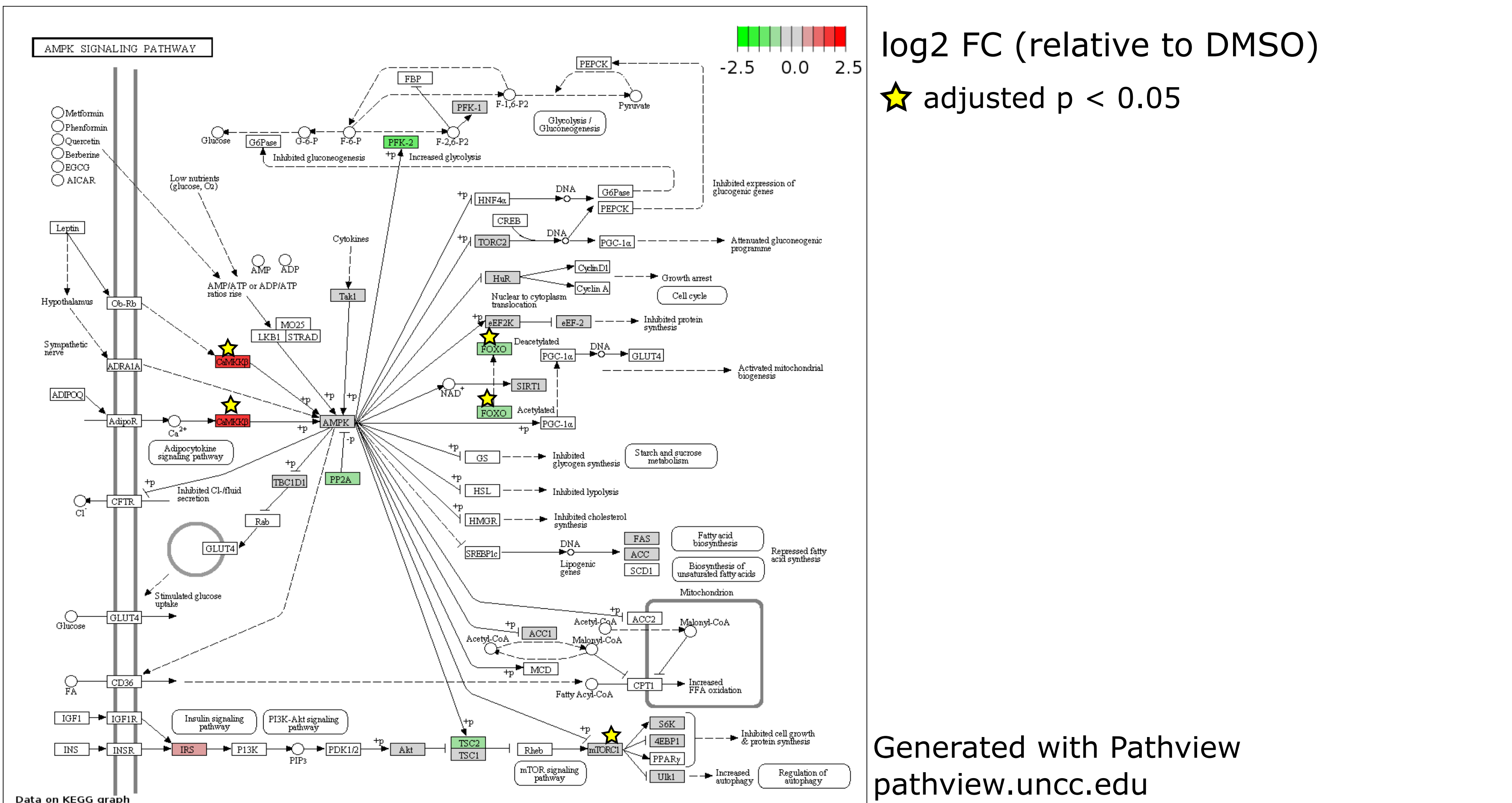
Query: 10,571 phosphopeptides (spectral library*)
Detect: 7,594 unique phosphopeptides (q<0.01)
Quantify: 6,357 (keeps sites specific to one protein)

* spectral library improves search sensitivity but is not required
software: EncyclopeDIA (w/ spectral library) or PECAN (w/o spectral library)
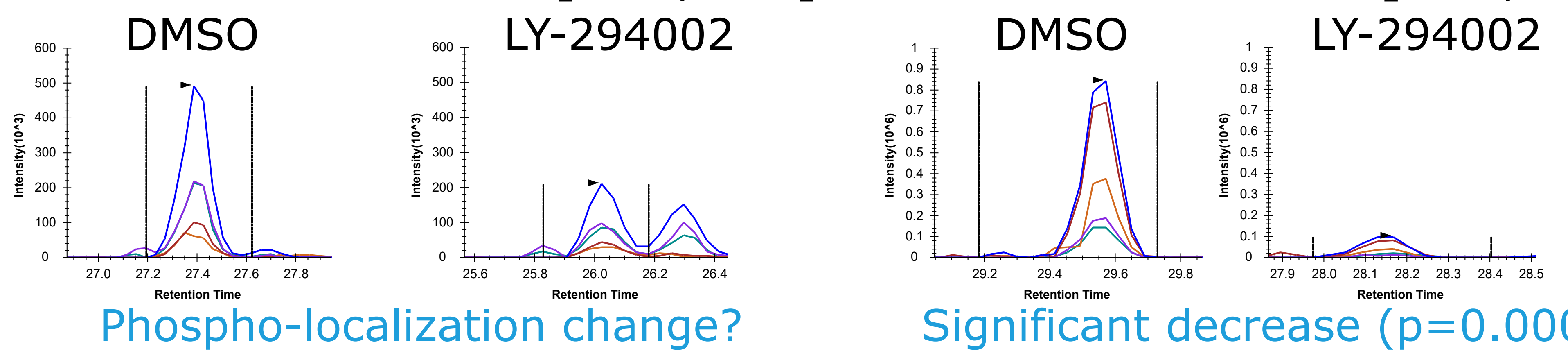
MAPK Signaling Pathway Coverage

MAPK SIGNALING PATHWAY

★ "P6000" only (35)
☆ P100 & P6000 (6)
★ P100 Only (1)
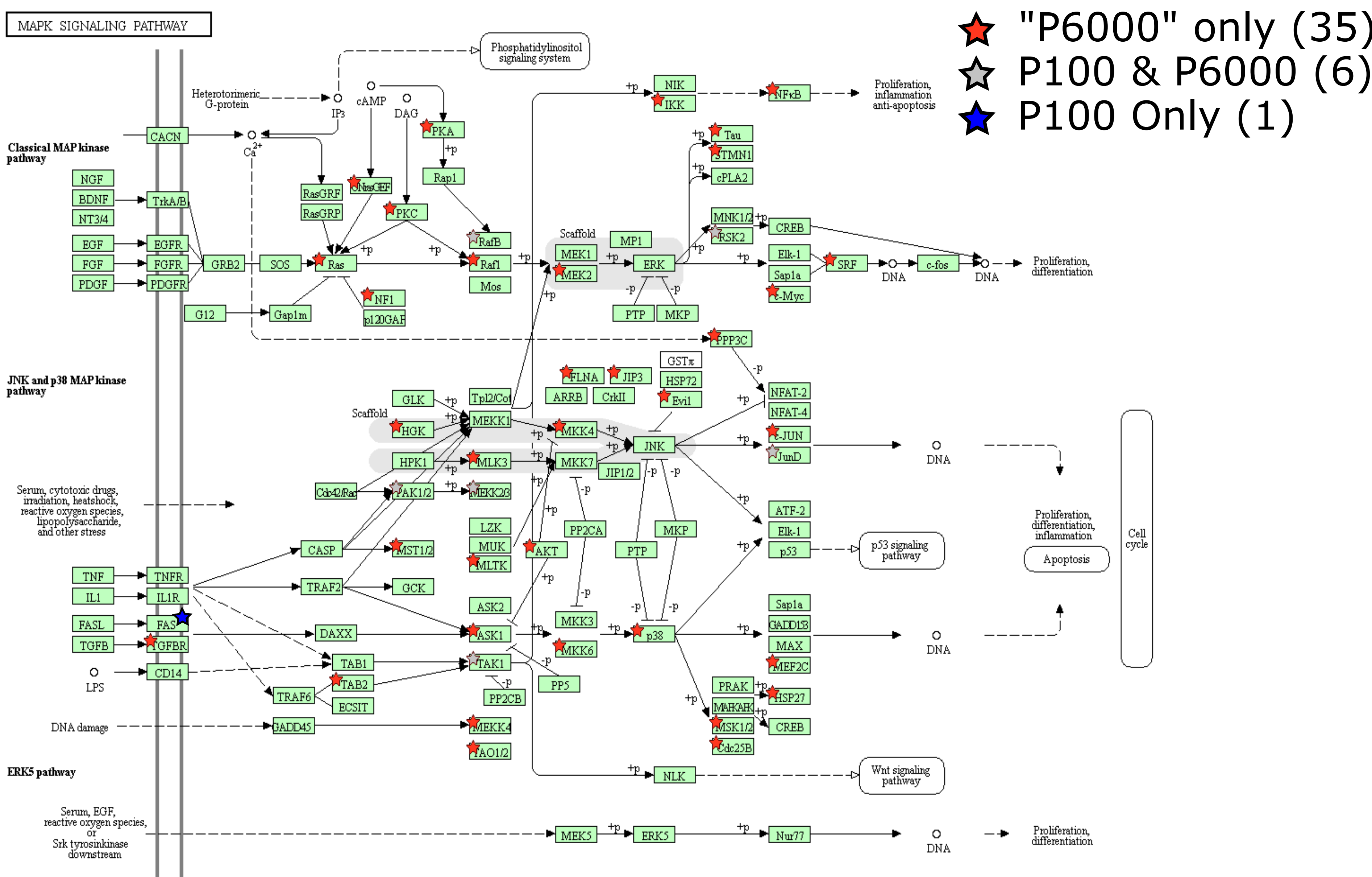
Plot created using DAVID: Huang DW *et. al.* Nature Protocols 2009

AMPK Signaling Pathway Response to LY-294002
(PC3 cell line)

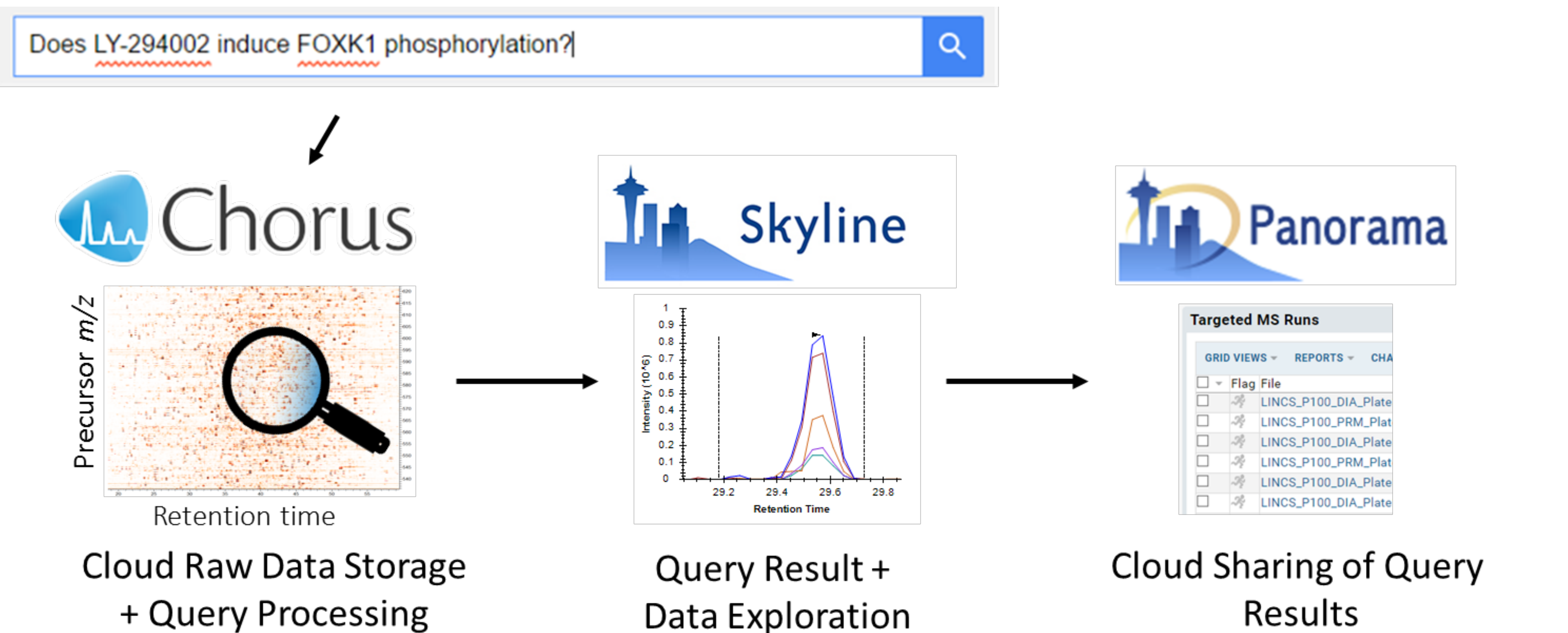AMPK SIGNALING PATHWAY

log2 FC (relative to DMSO)   -2.5 0.0 2.5
☆ adjusted p < 0.05

Generated with Pathview
pathview.uncc.edu

FOXK1 Phosphorylation Response to LY-294002

SAPA**S**PTHPGLMSPR [415,429]

DMSO   LY-294002

Phospho-localization change?

EG**S**PIPHDPEFGSK [442,455]

DMSO   LY-294002

Significant decrease (p=0.0007)

The Future

Provide a query engine on the cloud

Does LY-294002 induce FOXK1 phosphorylation?

Chorus → Skyline → Panorama

Cloud Raw Data Storage + Query Processing

Query Result + Data Exploration

Cloud Sharing of Query Results

UNIVERSITY of WASHINGTON