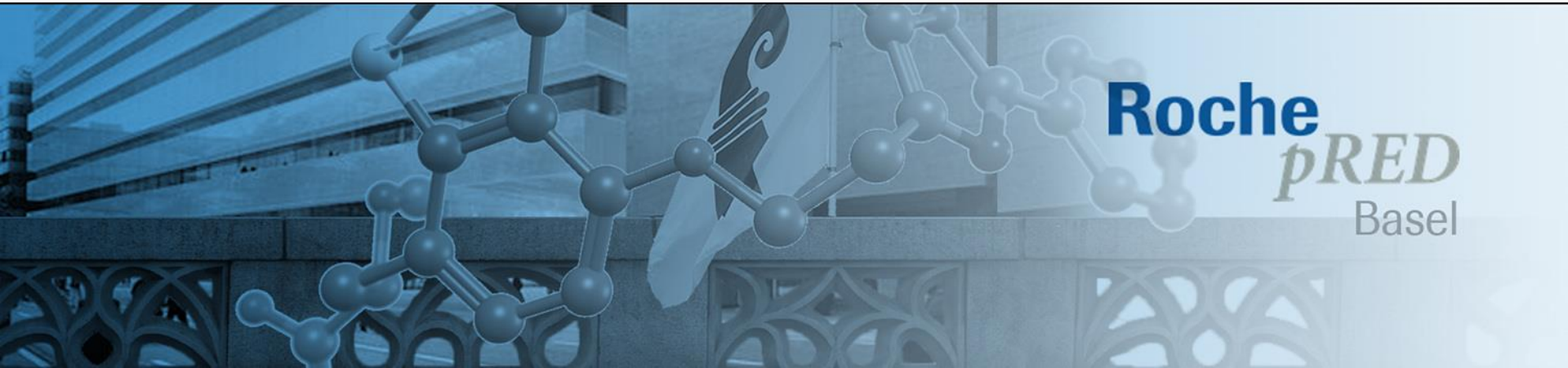
Targeted proteomics (and Skyline) to characterize an *in vitro* model of human neuronal development

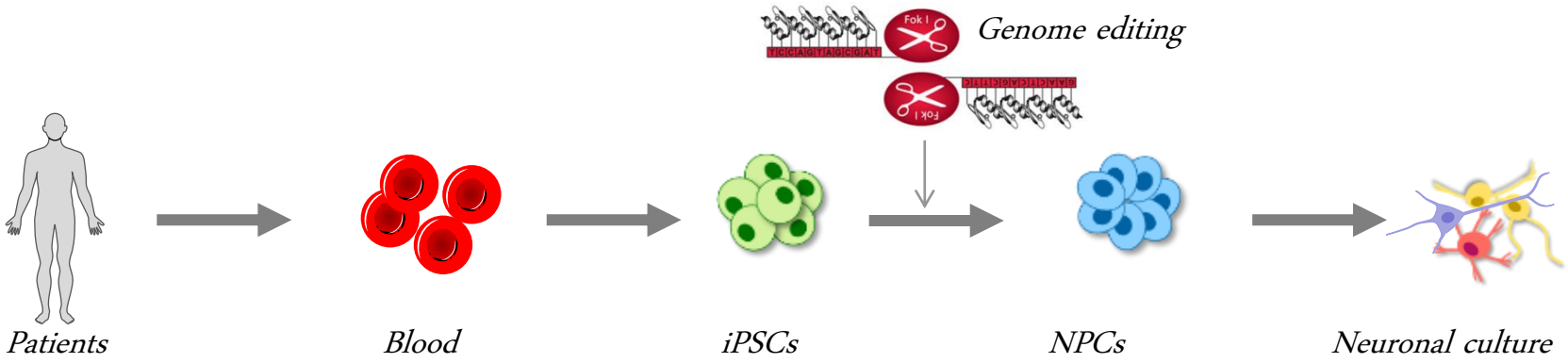
Skyline User Meeting May 31st 2015

Tom Dunkley
Roche Innovation Center Basel

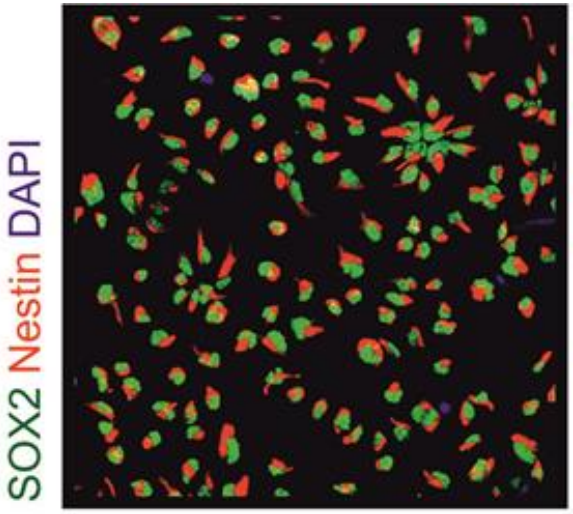


Neurons in a dish to model disease

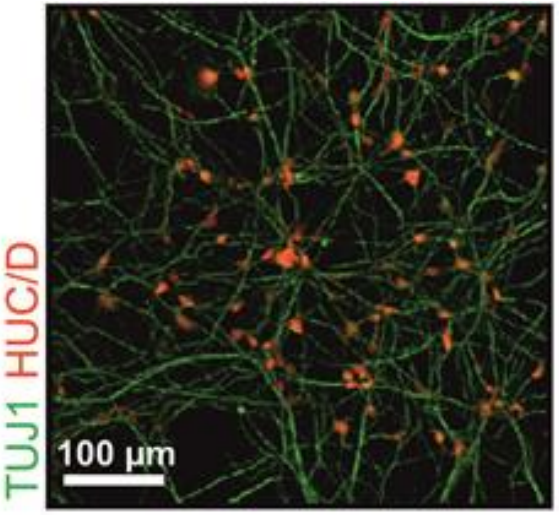
Enabled by induced pluripotent stem cells & genome editing



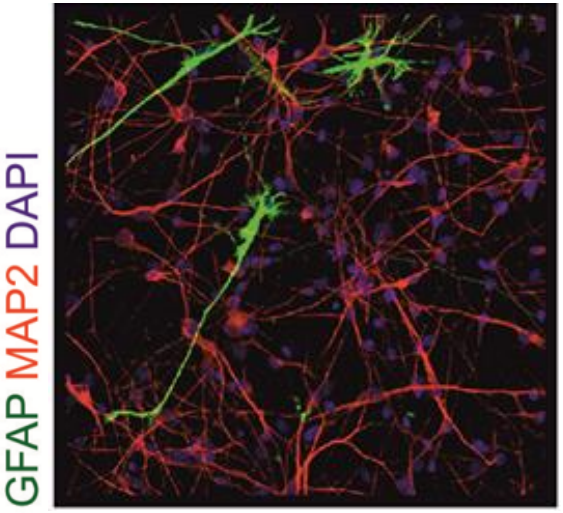
NPC – day 0



day 14



Neurons - day 41

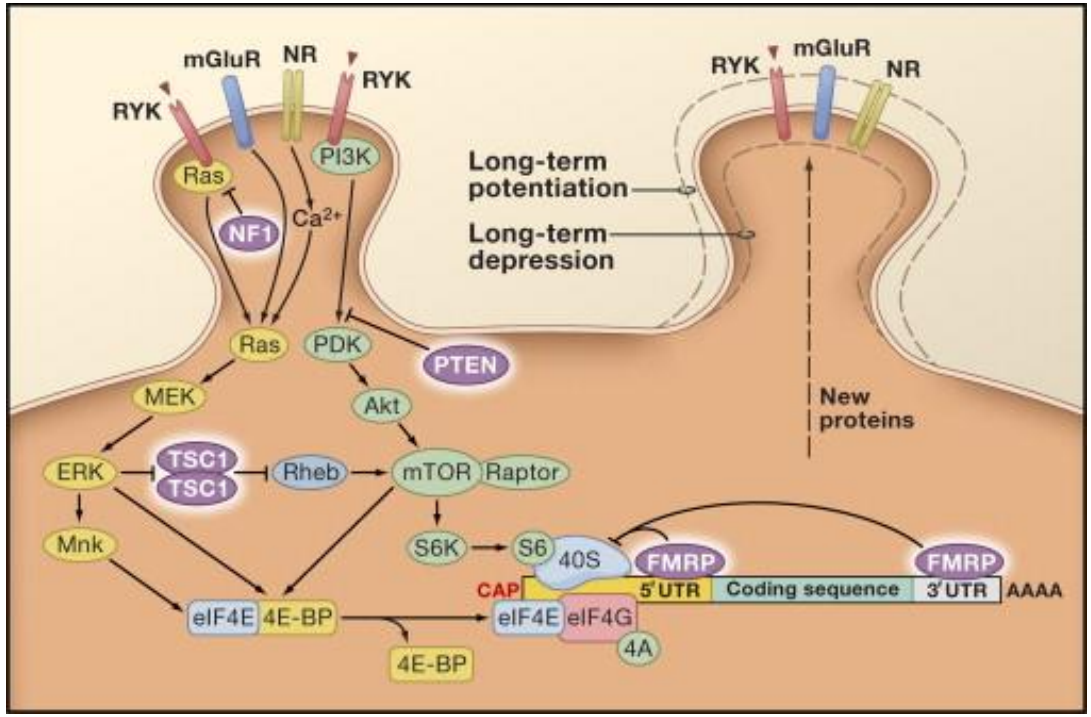


Protein regulation in synapse function

…and dysregulation in neurodevelopmental disease

Table 1. Single-Gene Disorders with High Rates of Autism

Gene	Disorder	Rate of Autism	Rate in Autism	MR	Gene Function
FMR1	Fragile X syndrome	15%–30%	2%–5%	+	Translational repressor
TSC1/2	Tuberous sclerosis complex	25%–60%	1%–4%	+	Inhibitor of mTOR
PTEN	PTEN hamartoma syndrome (ASD with macrocephaly)	ND	1%	+	Inhibitor of PI3K/mTOR signaling
NF1	Neurofibromatosis type I	4%	0%–4%	+	Ras GAP
MECP2	Rett's syndrome	100%	2%	+	Global transcriptional repressor
UBE3A	Angelman's syndrome	40%	1%	+	E3 ubiquitin ligase

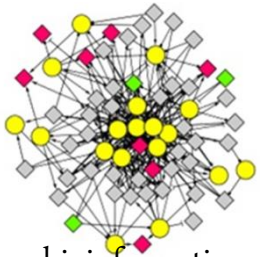


Understanding protein dysregulation in neurodevelopmental disease enables:

- Target identification
- Mechanism-of-action biomarker ID
- Identification of endpoints for phenotypic screens

Kelleher R. J. & Bear M. F. The Autistic Neuron: Troubled Translation? Cell (2008) 135, 401–406

proteomics

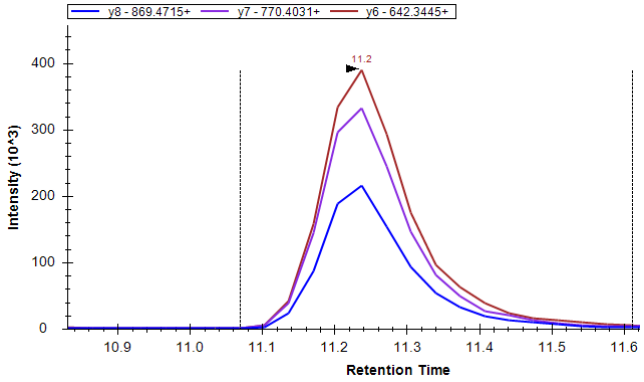


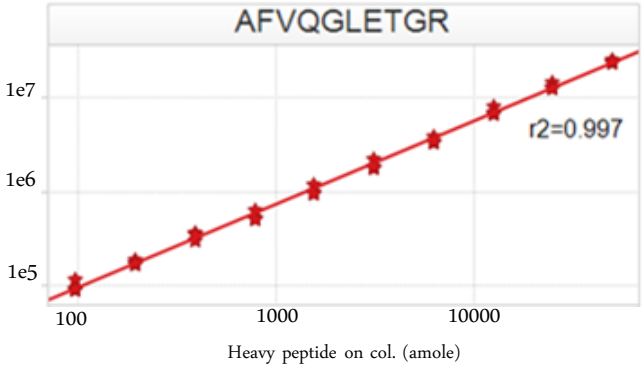
bioinformatics

genomics

literature

- sp|Q5VV42|CDKAL_HUMAN
- sp|Q6UWB1|I27RA_HUMAN
- sp|Q6UXK5|LRRN1_HUMAN
- sp|Q6WRI0|IGS10_HUMAN
- sp|Q8IWK6|GP125_HUMAN
- sp|Q8N158|GPC2_HUMAN
  - VDASGPDVPTR
    - 557.2804++
    - 562.2845++ (heavy)
  - GYSLNLIPPALISGEHLR
    - 650.6984+++
    - 654.0345+++ (heavy)
  - AFVQGLETGR
    - 539.2880++
    - 544.2921++ (heavy)
- sp|Q8TAT2|FGFP3_HUMAN
- sp|Q8TB73|NDNF_HUMAN
- sp|Q8TE99|ACPL2_HUMAN
- sp|Q8WUH6|CL023_HUMAN
- sp|Q93045|STMN2_HUMAN





Hypothesis generation:

- Proteins in disease biology
- Proteins as predictive biomarkers
- Proteins in pharmacodynamics & pharmacokinetics

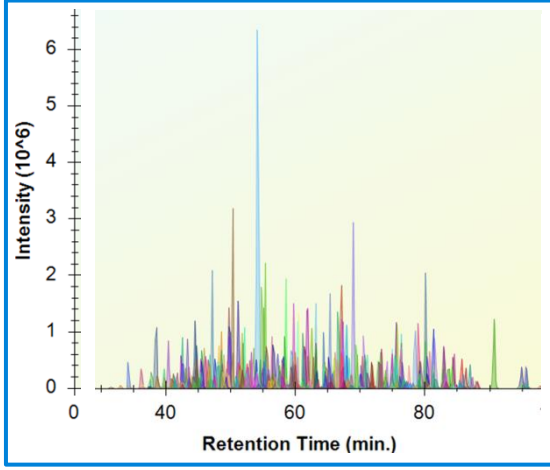
Targeted proteomics

(SRM/MRM/PRM)

Testing of protein hypotheses:

- Antibody independent
- Precise quantification
- High multiplex

246-protein neuroSRM panel



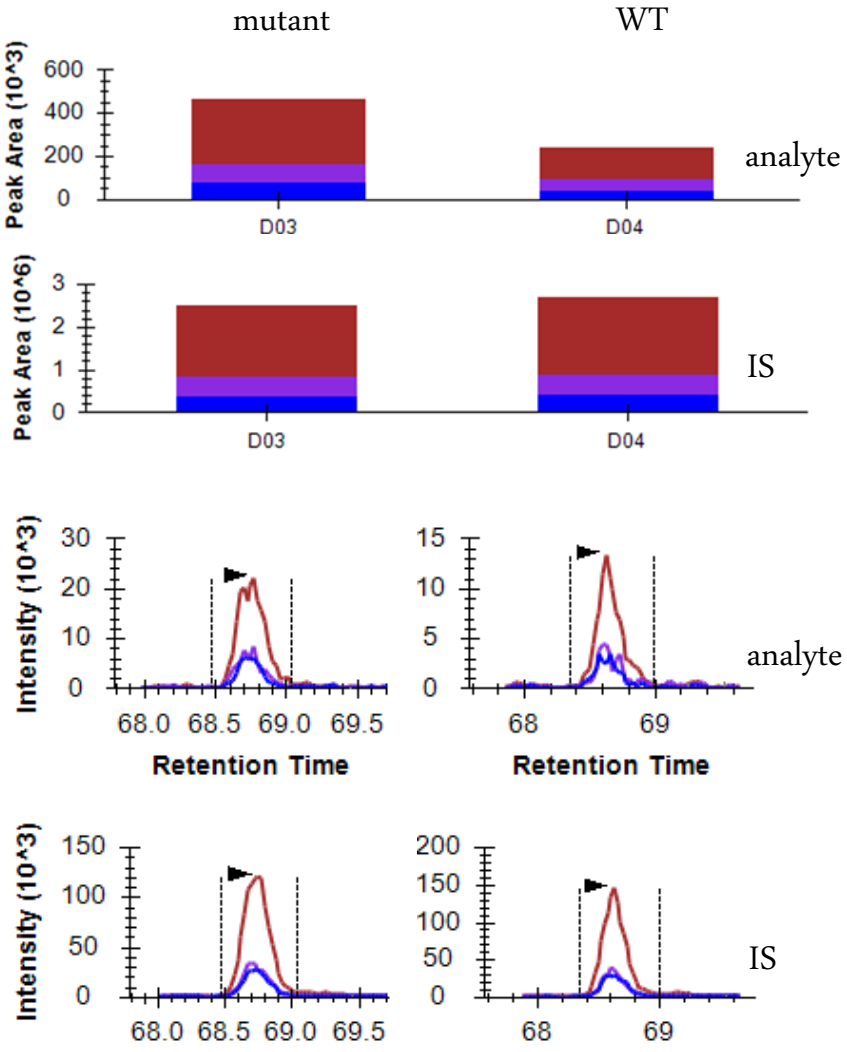
- Multiplexed SRM method
- Internal standards for all peptides
- 246 proteins, 478 peptides, 2,868 tran.
- 2 h per sample

Delorme R. *et al.* Nature Medicine 19, 685–694 (2013)

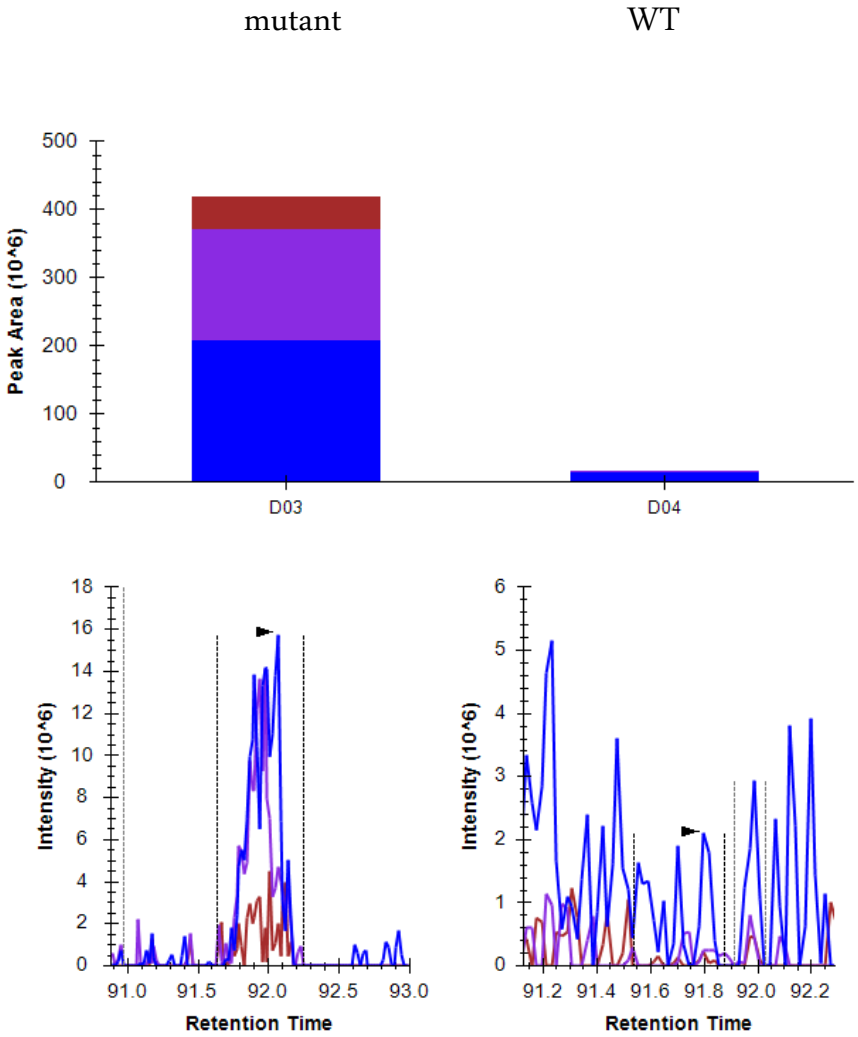
Targeted proteomics: *precise quant, confident ID*

UBE3A - VDPLETELGVK

Targeted - SRM, Vantage (2868 tran.)

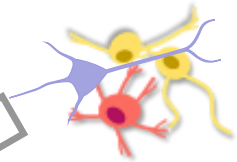


Discovery – MS1, QE (70k res.)



... but don't miss the bigger picture

Combine targeted and discovery proteomics



Test hypotheses

Generate new hypotheses

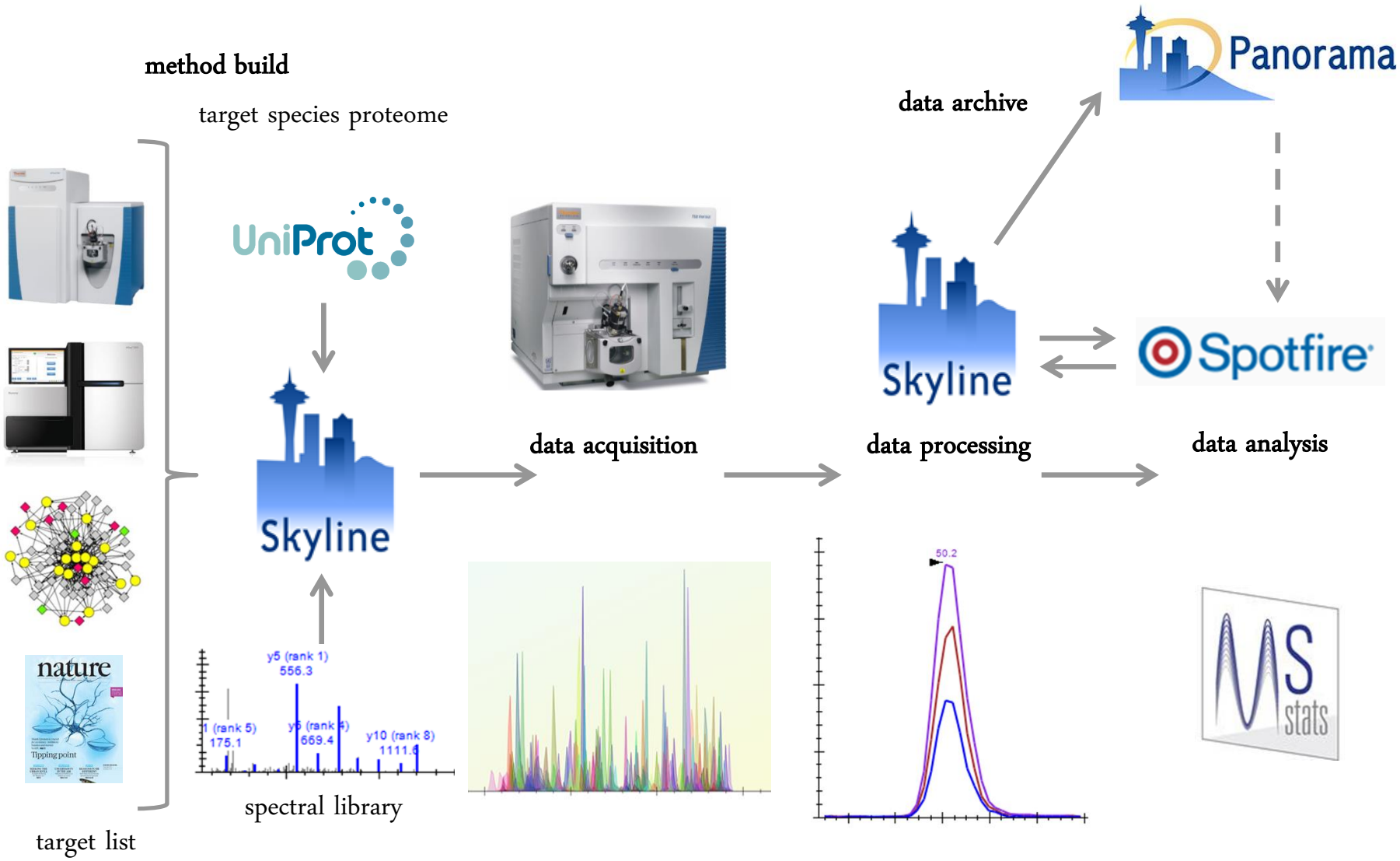
Targeted proteomics (SRM/PRM)

Discovery proteomics

- Precise quantification
- High confidence in specificity with internal standards

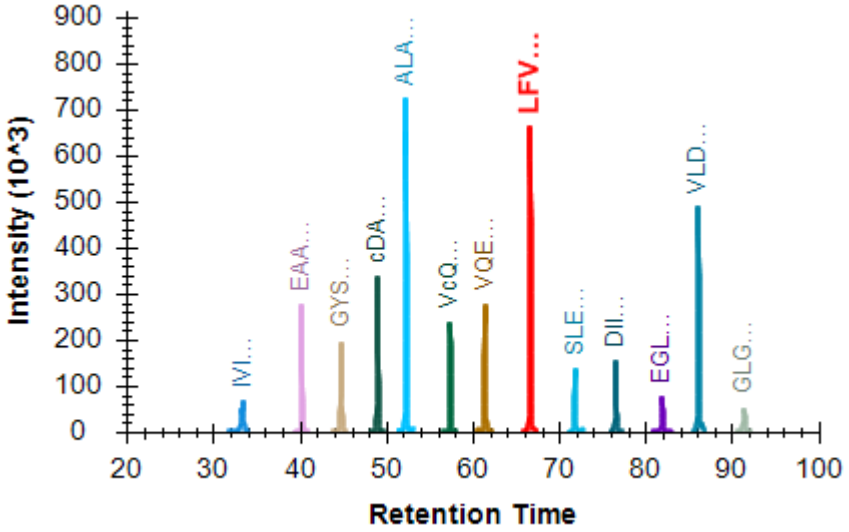
VALIDATE

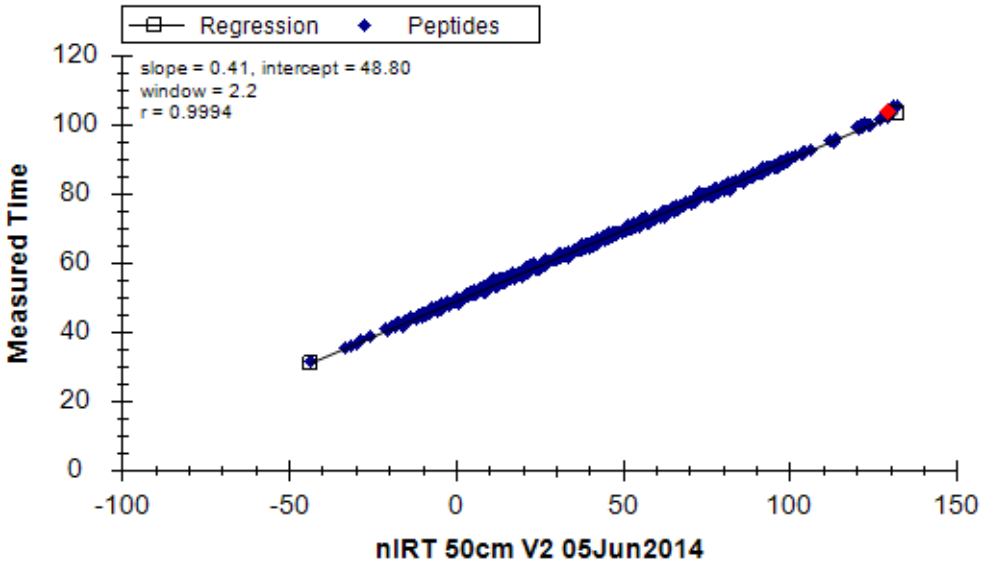
Targeted proteomics (SRM) workflow

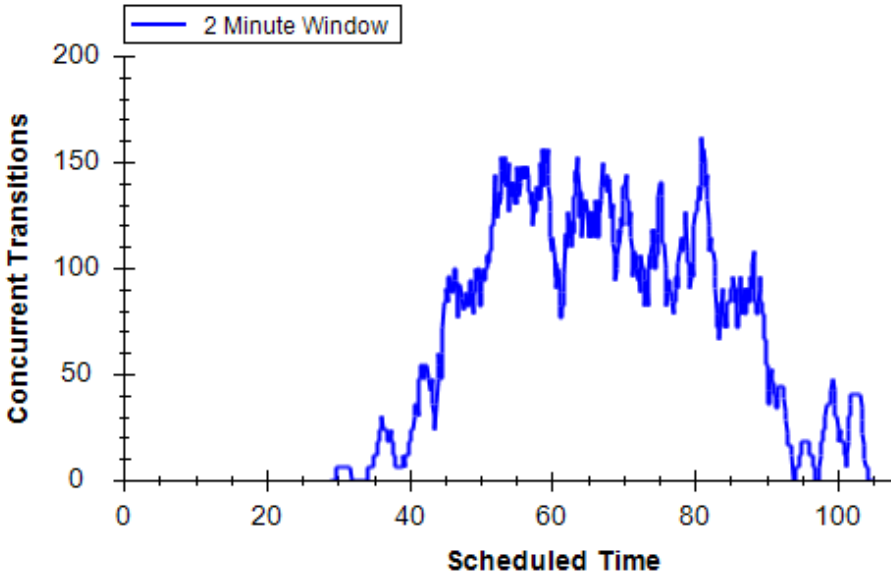


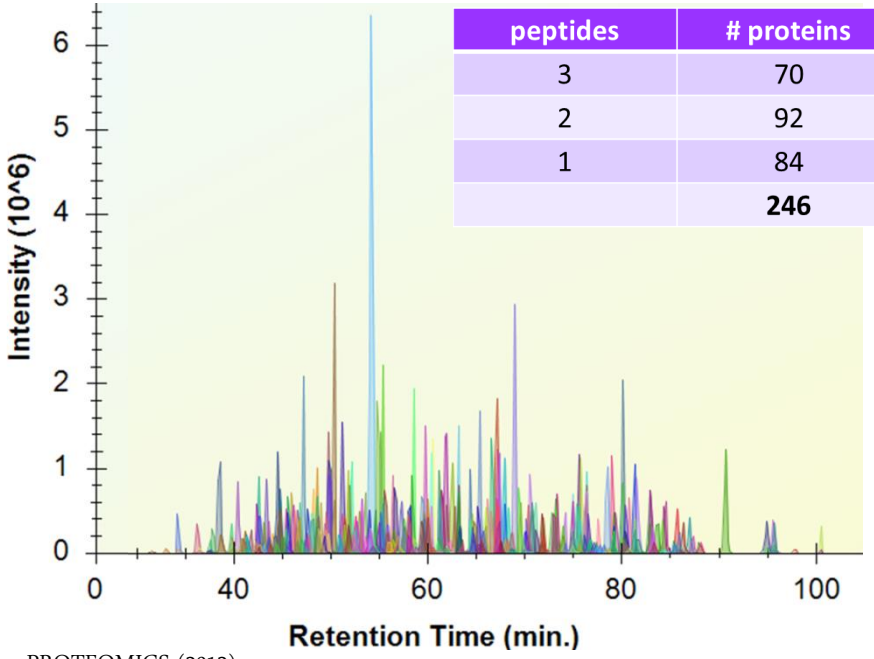
Retention time scheduling enables 2,868 transitions/run

Simplified using iRT







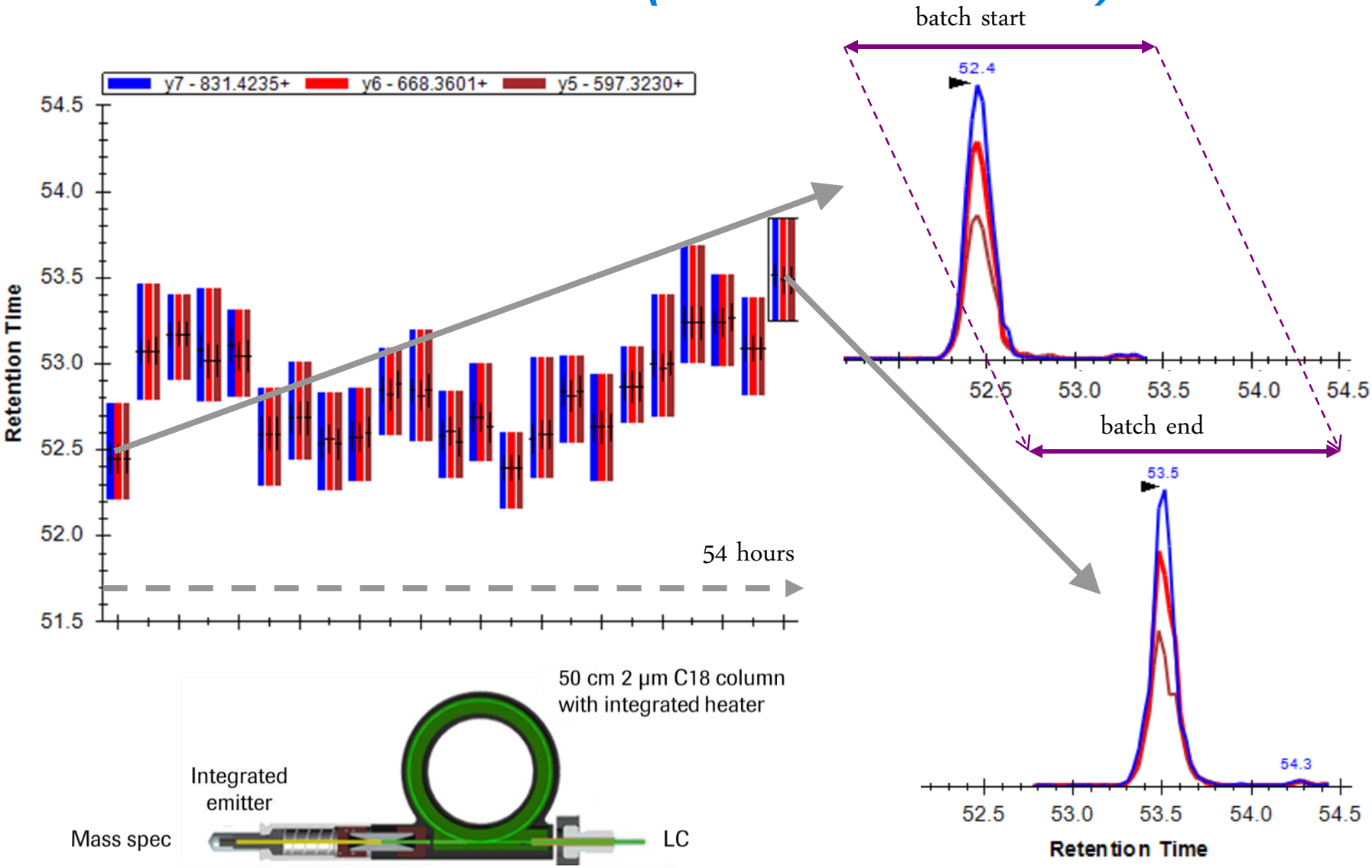


peptides	# proteins
3	70
2	92
1	84
	246

Escher C. *et al.*, Using iRT, a normalized retention time for more targeted measurement of peptides. PROTEOMICS (2012)

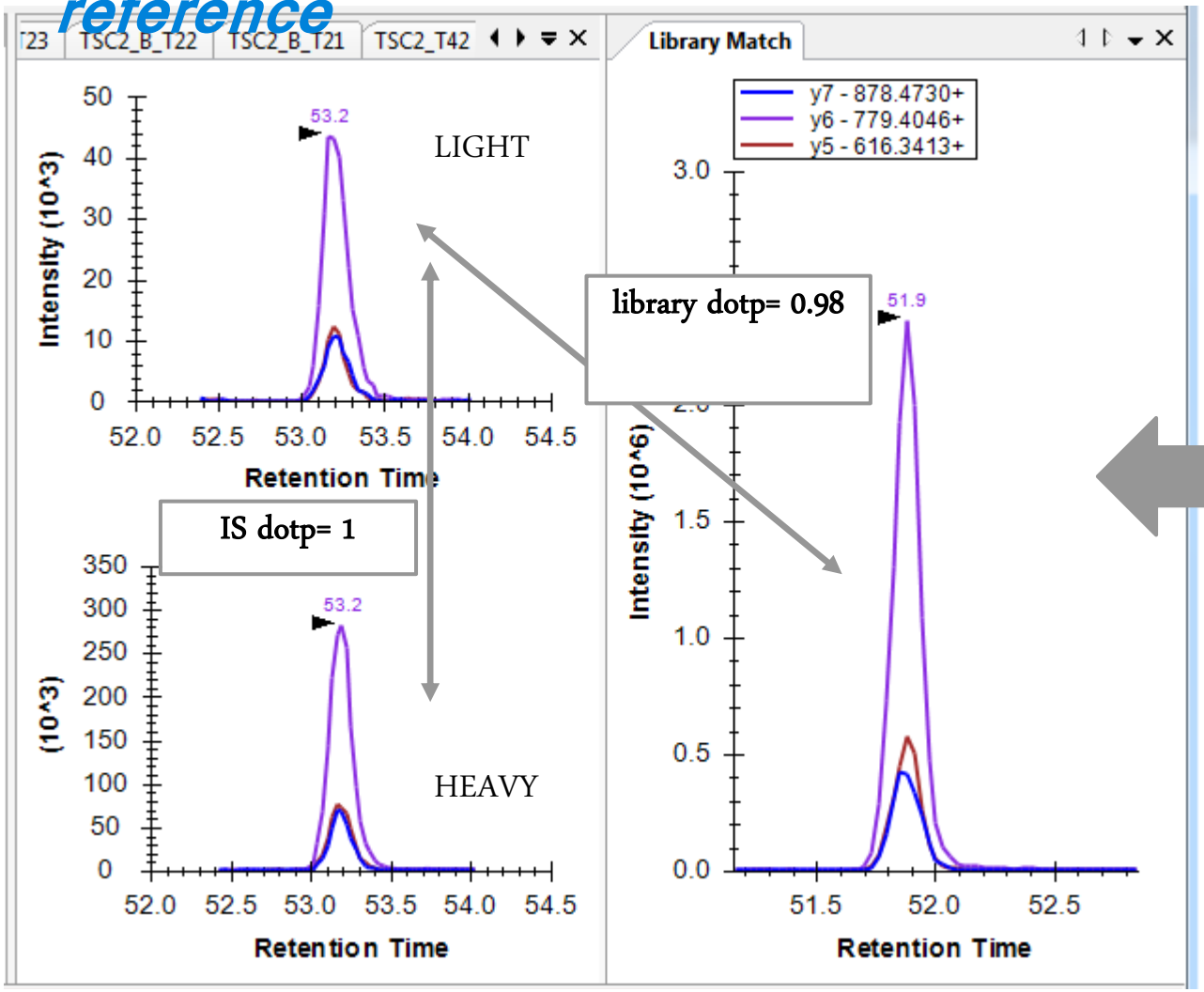
Dynamic scheduling adjusts for RT drift

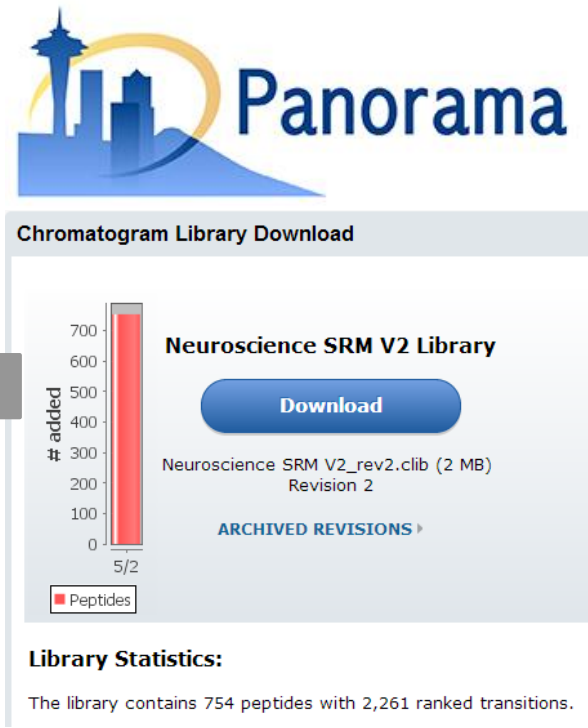
Enables 2 min windows (and weekends off)



Gallien S. *et al.*, Highly multiplexed targeted proteomics using precise control of peptide retention time. PROTEOMICS (2012)

Chromatogram libraries (Panorama) provide a useful reference





Tracking instrument performance

Panorama QC folder

Genentech
A Member of the Roche Group

Roche

LabKey Software
PARTNERS IN SCIENCE

pRED

Vantage 68 120min. gradient 50cm Easyspray column SRM Pepmix

Poster #134 (Tue): Performing quality control on targeted proteomics assays using Skyline and Panorama.

QC Summary

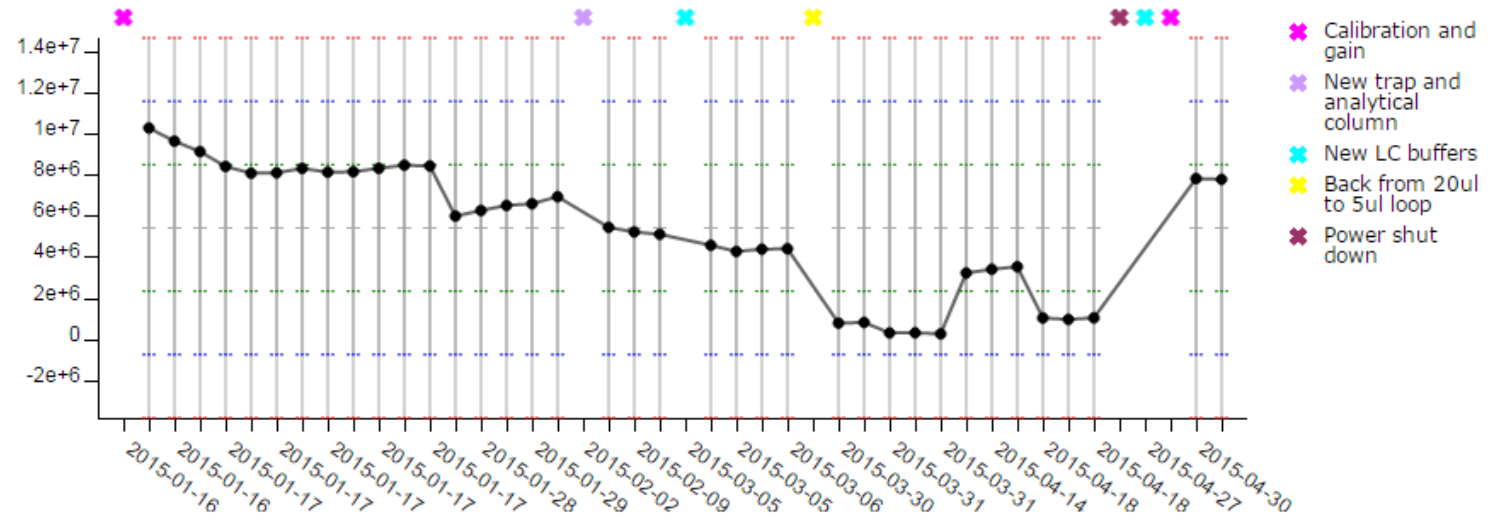
10 Skyline documents uploaded containing 37 sample files
9 precursors tracked

QC Plots

Chart Type: Peak Area | Start Date: 2015-01-16 | End Date: 2015-04-30 APPLY

Y-Axis Scale: Linear | Group X-Axis Values by Date

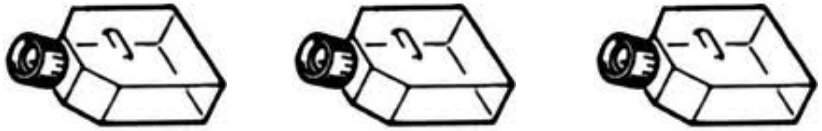
DAVTPADFSEWSK



Characterization of the neuronal development model

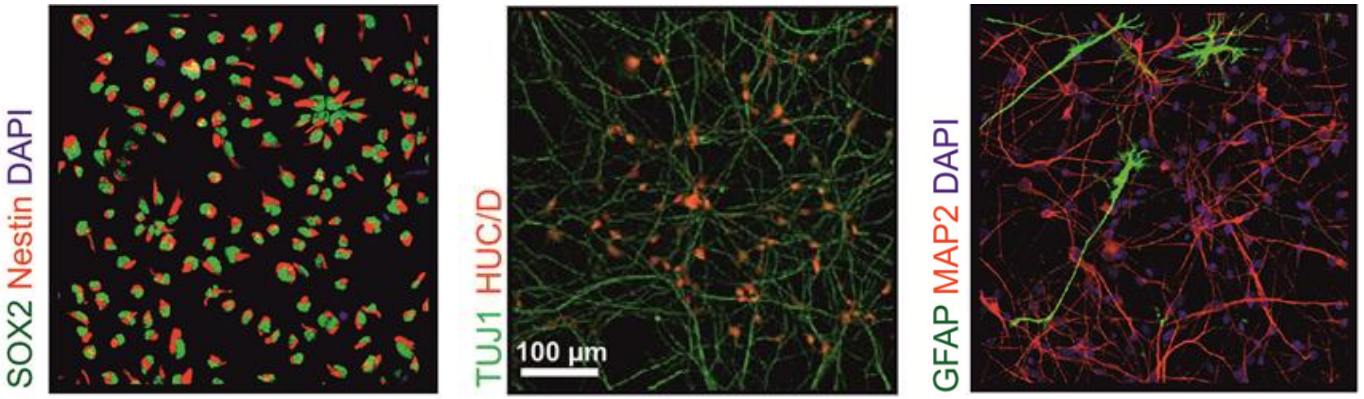
Experimental design

SA001 SA001 GE1 SA001 GE2

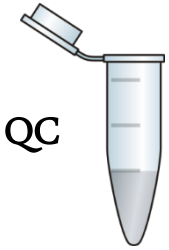


- 3 hPSC-derived neural precursor cell (NPC) lines
- Parental (SA001) & two lines with 'silent' genome editing (GE)
- 4 GE lines with disease-relevant mutations also analyzed (not reported here)

NPC – day 0 day 14 Neurons - day 41



- 3 developmental stages
- 5 to 9 independent replicate differentiations



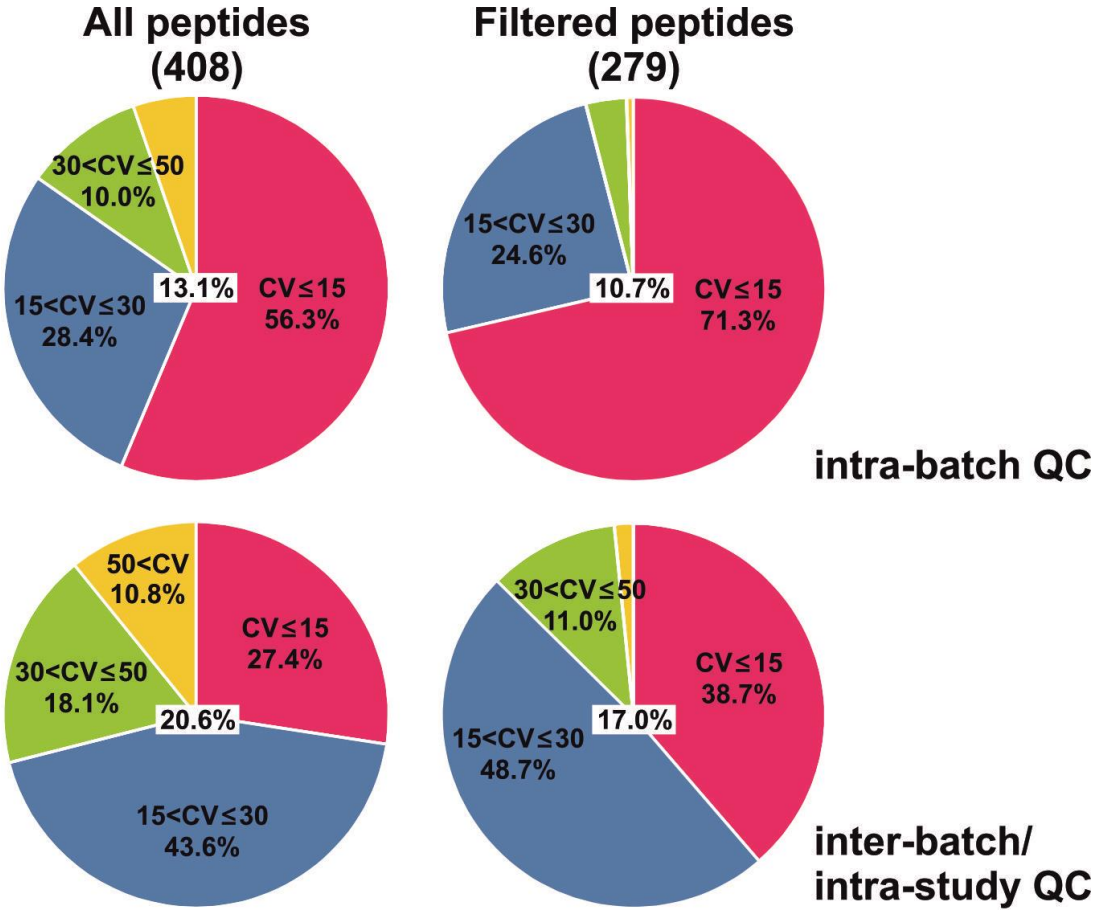
QC

- Pooled QC prepped and analyzed 3-5 times / batch
- Assess technical variation for whole process (except cell lysis)

- **177 samples**
- **8 batches analyzed over ~ 2 months**

Technical performance of the SRM assay

Robust, reproducible measurements over a 2-month experiment



'All peptides'

- Skyline output after manual peptide + transition exclusion

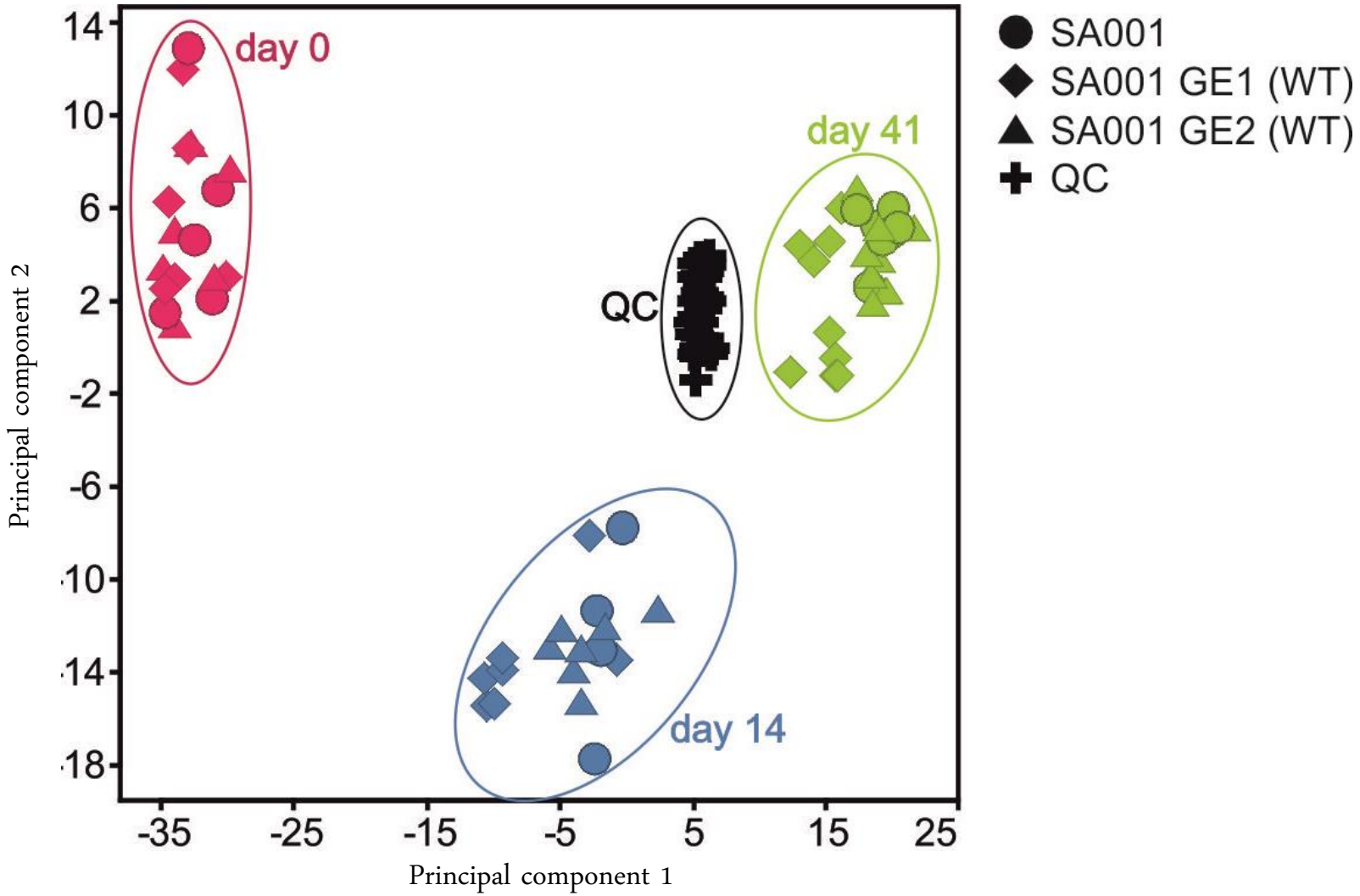
'Filtered peptides'

- In >70% samples from any day:
  - <30% intra-batch CV
  - Within linear range (<30% relative error)

Methods for automated feature selection needed

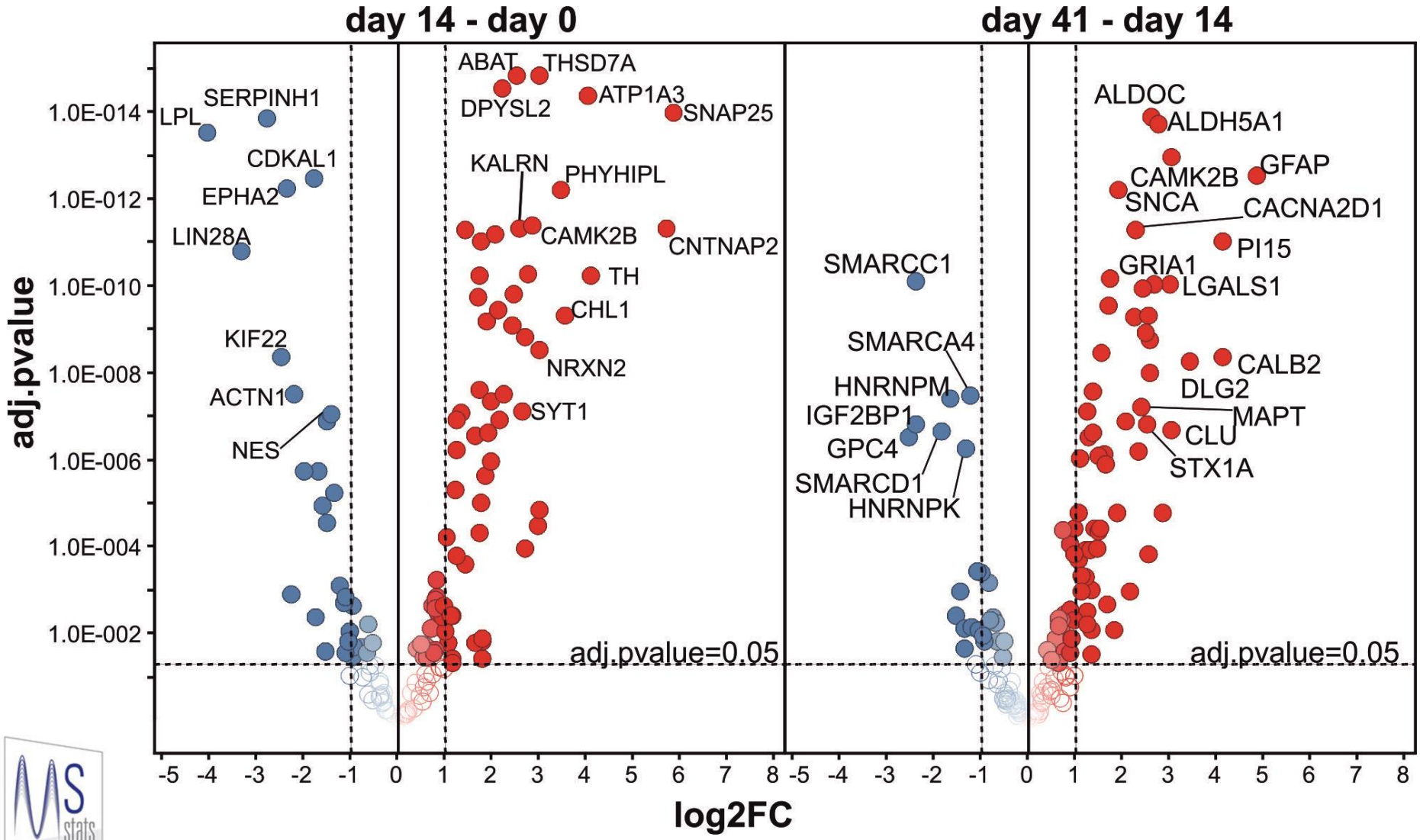
Clustering of samples based on peptide data (PCA)

Protein regulation over time is the most significant source of variation



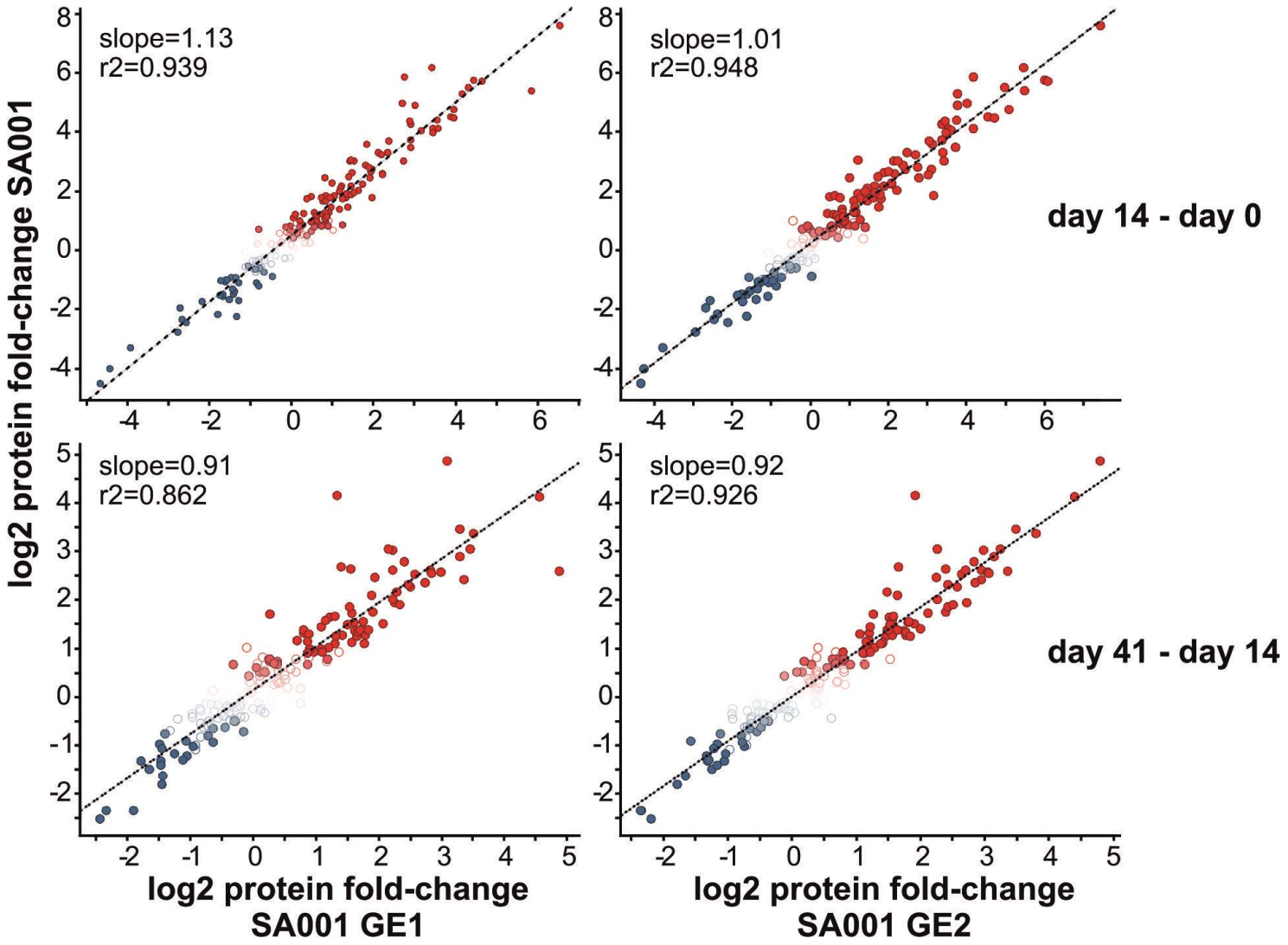
Roche

Majority of NeuroSRM proteins regulated between 3 developmental stages



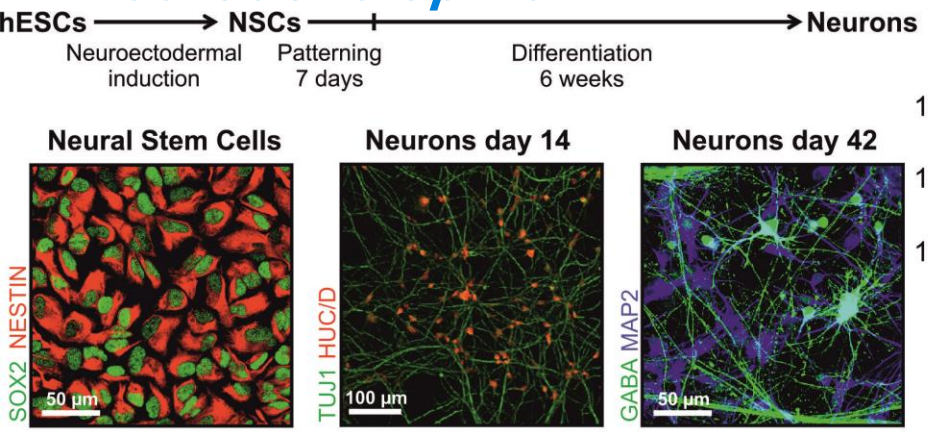
Correlation between the isogenic cell lines

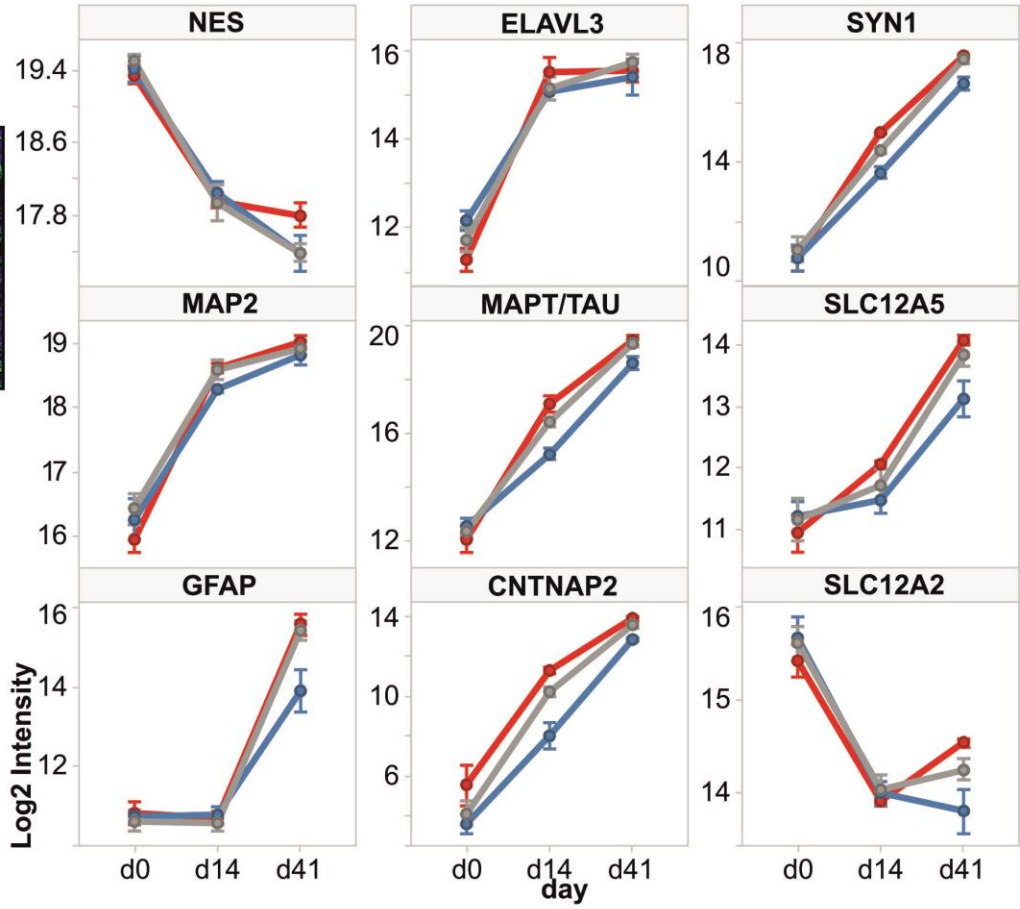
Pattern of protein regulation consistent across the WT & 2 'silent' GE lines



Regulation of key developmental marker proteins

In vitro hESC model recapitulates in vivo neurodevelopment



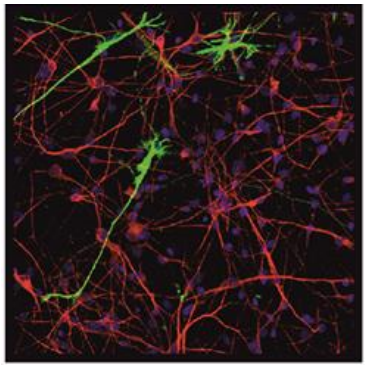


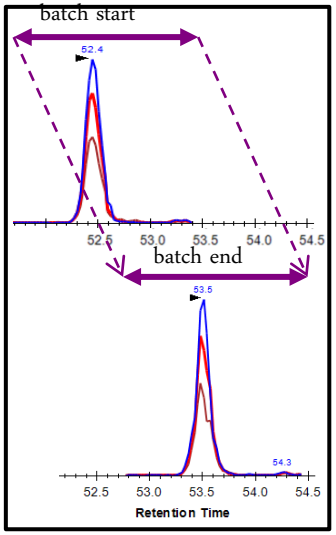
- Comparison Human Brain Transcriptome (HBT) mRNA data:
  - Significant match (p-value = 3.8e-11)
  - 104/165 mRNAs/proteins (63%) having identical modulation
  - 17 proteins (10%) modulated in opposite directions in human brain (mRNA) & hPSC-derived neuronal development model (protein)

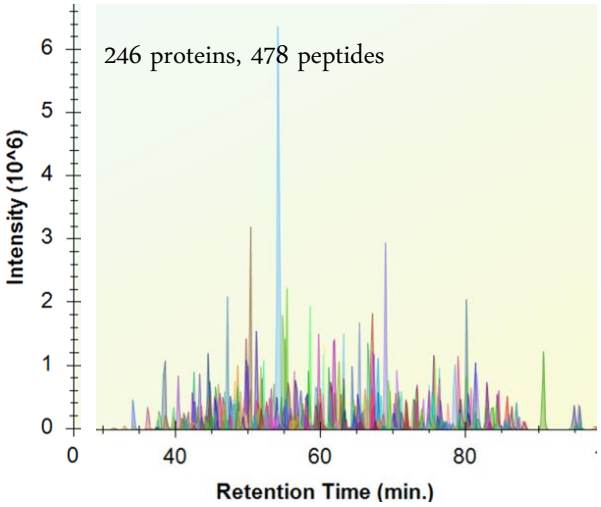
Dunkley T. *et al.* Proteomics Clinical Applications (2015)

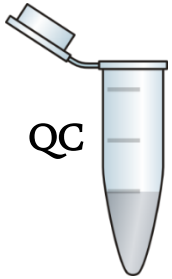
Summary

hESC-derived neurons characterized through targeted proteomics







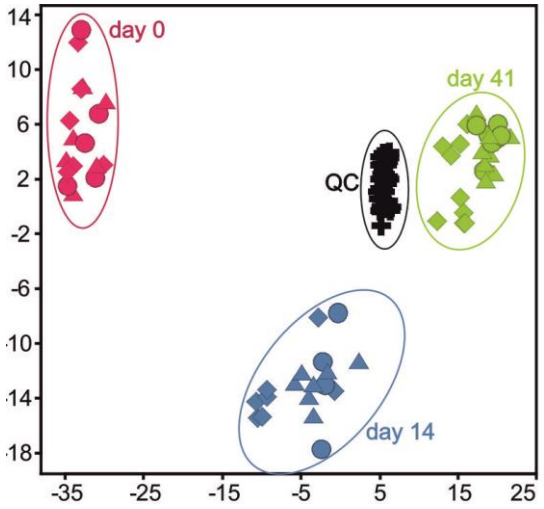


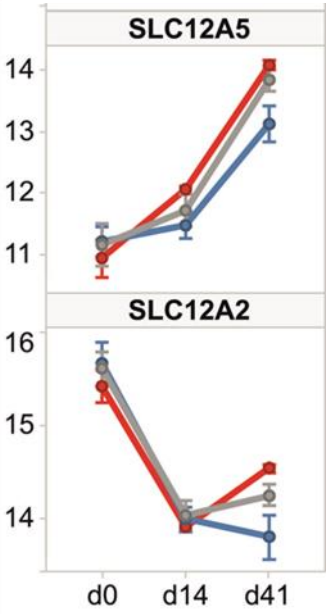
data archive

Panorama

Skyline

Spotfire





Acknowledgements

- **Arno Friedlein**
- Peter Jakob
- Sabine Kux van Geijtenbeek
- Axel Ducret
- Carine Steiner
- Hanno Langen
- Paul Cutler
- Michel Petrovic
- Ignacio Fernandez Garcia
- Ravi Jagasia
- **Veronica Costa**
- Sebastian Lugert
- Stefan Aigner
- Martin Ebeling
- Meghan T. Miller
- Christoph Patsch
- Paolo Piraino

Genentech
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- Kristin Wildsmith

- Josh Eckels

Northeastern University

- Olga Vitek
- Lin-Yang (Mike) Cheng

- Mike MacCoss
- Brendan MacLean
- Jarrett Egertson
- Vagisha Sharma

Doing now what patients need next