

# **Just - Evotec Biologics**

**Multi-Omic Characterization of  
CHO media and lysate during the  
production of biotherapeutics**

## Agenda

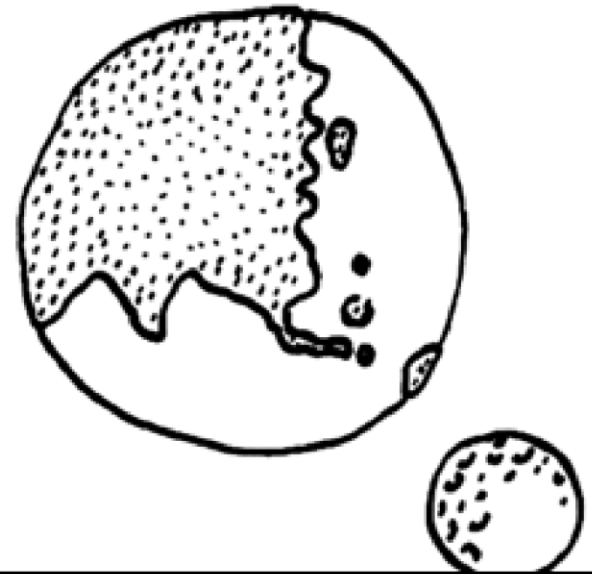
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### Just - Evotec Biologics - Why Omics?

Inducible Cell line and Experimental Design

Results

Conclusion



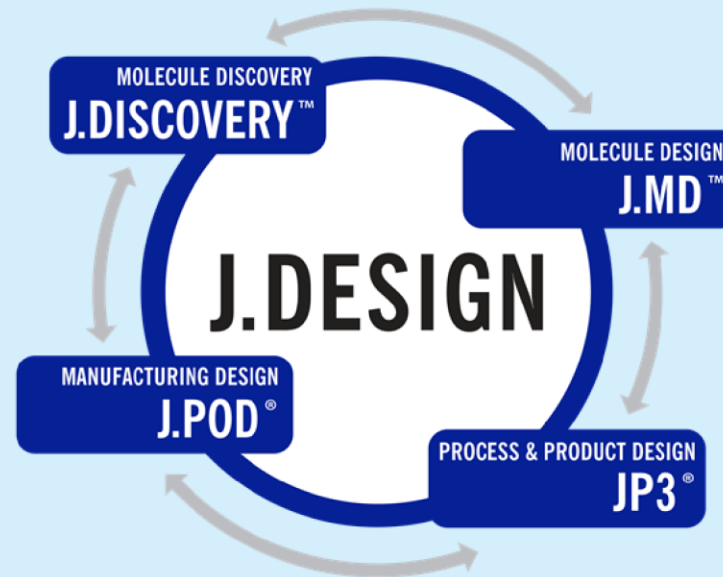
# Just - Evotec Biologics is a technology design company

Integrated data support to harness machine learning

**the Just. mission**  
design and apply innovative technologies to dramatically expand global access to biotherapeutics

Superior Molecule Discovery

Clinical and Commercial Manufacturing

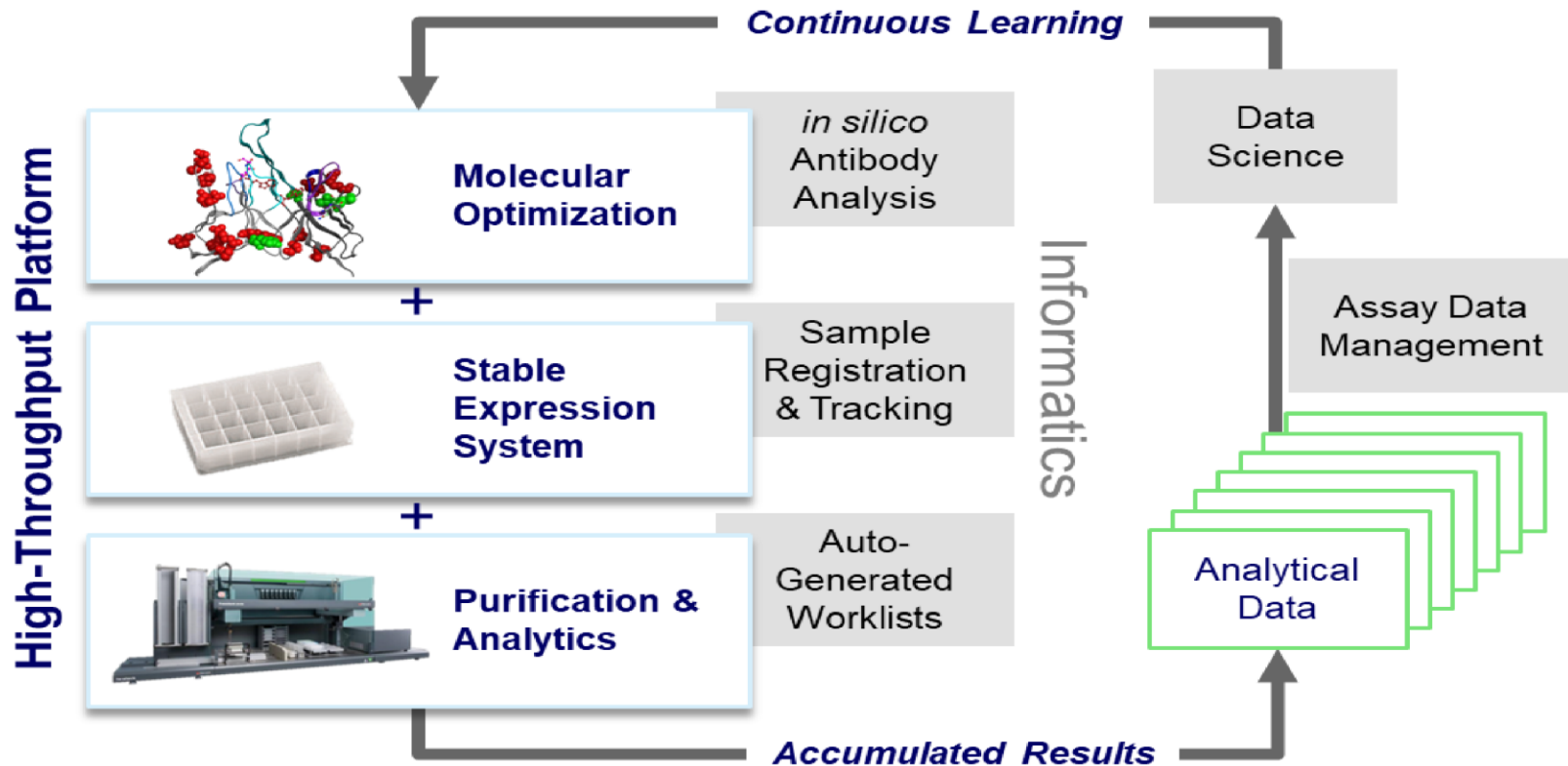


Sequence optimized for developability

Cell line, process and formulation development

# Platform optimization requires large data input

Analytical data drives platform improvement

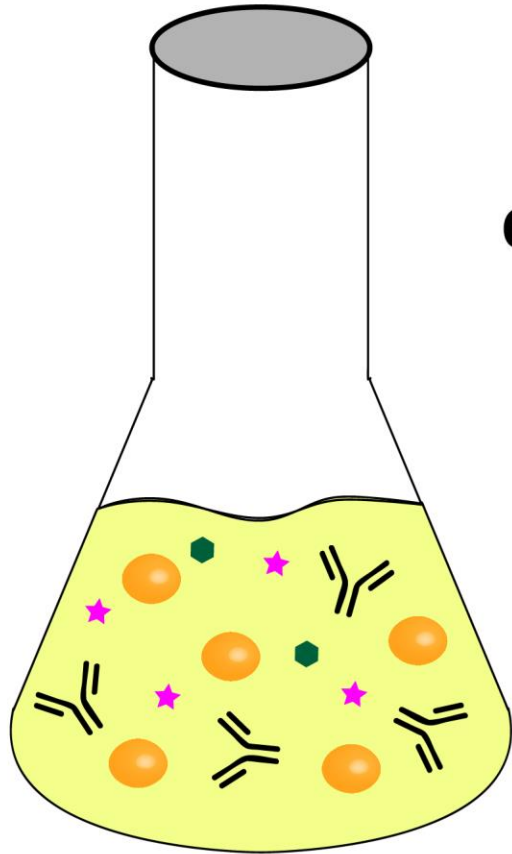




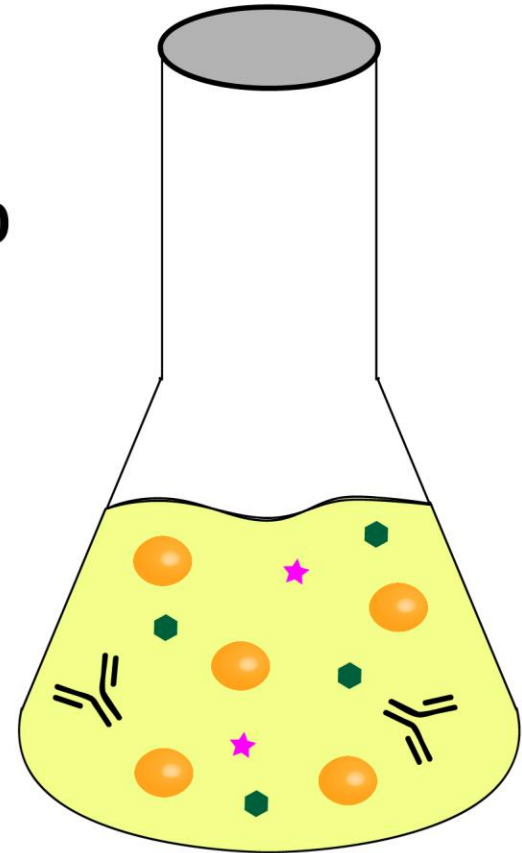
## Cell metabolism is perturbed by high expression of biologics

Biomarkers of high expression allow for optimization across platform

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**Biomarkers can be  
tools to select  
clone and media to  
optimize viability  
and titer**



## Agenda

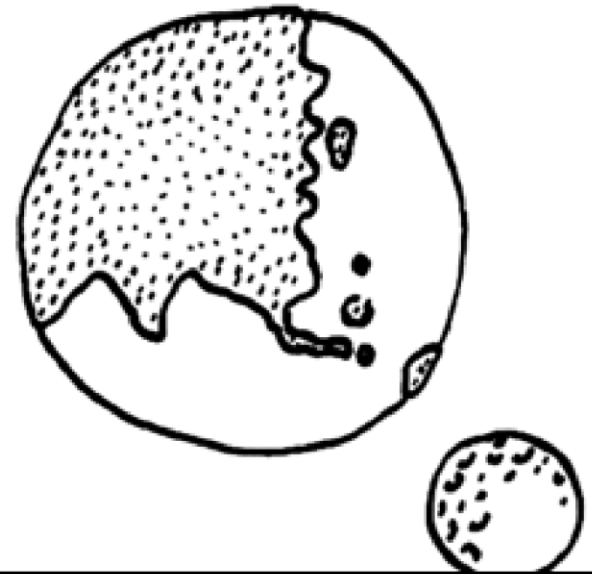
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Just - Evotec Biologics - Why Omics?

**Inducible Cell line and Experimental Design**

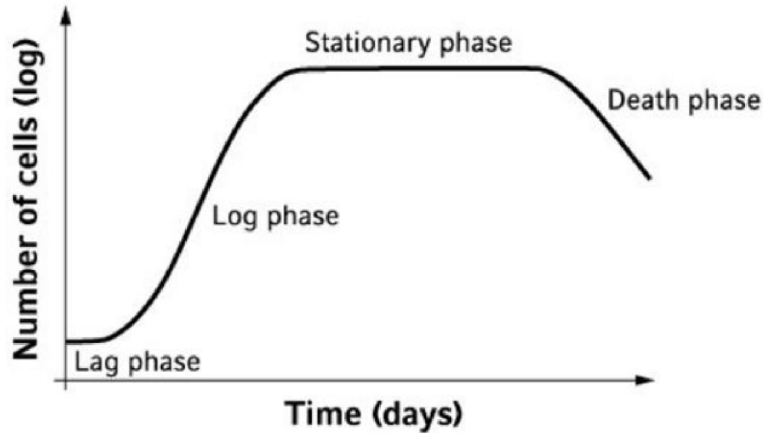
Results

Conclusion

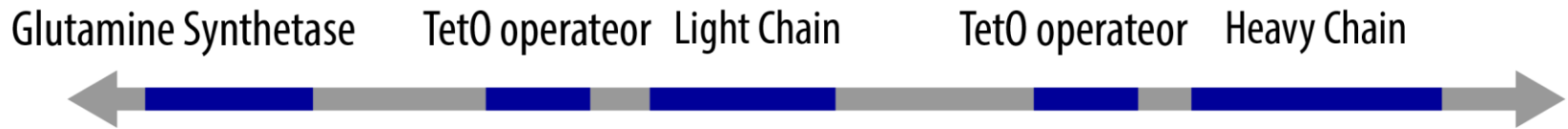


# Inducible Cell Line allows separation of growth and production phases

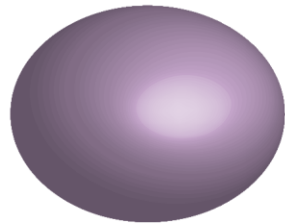
6F5 Inducible Cell Line



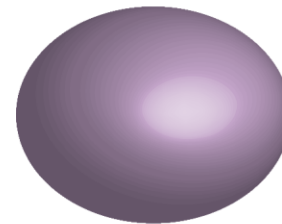
Selecting pools without addition of doxycycline allows cells to recover more effectively without needing to produce protein



rtTA

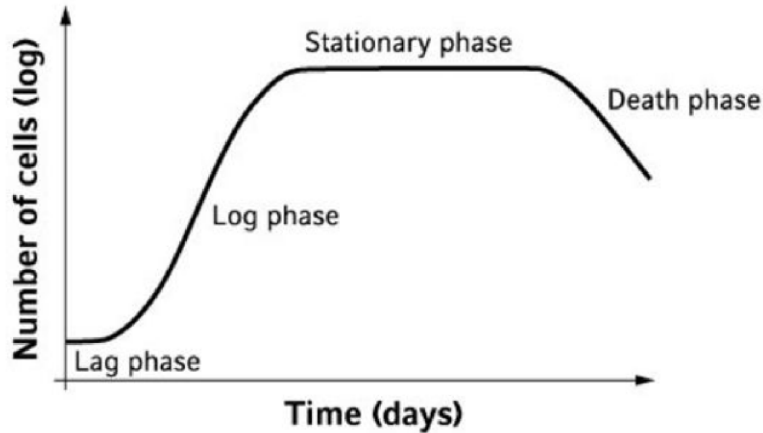


rtTA

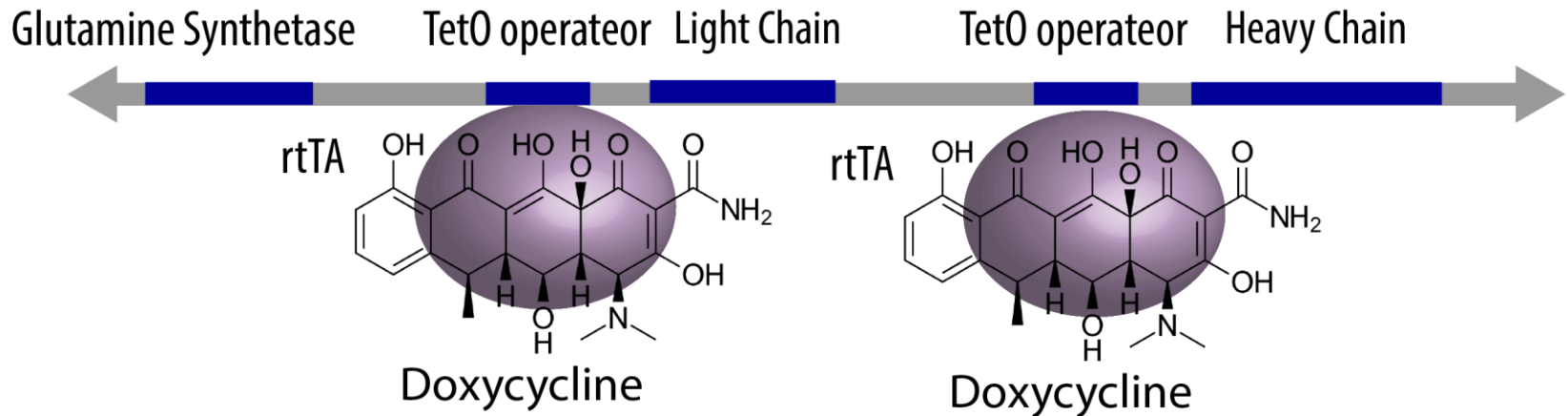


# Inducible Cell Line allows separation of growth and production phases

6F5 Inducible Cell Line



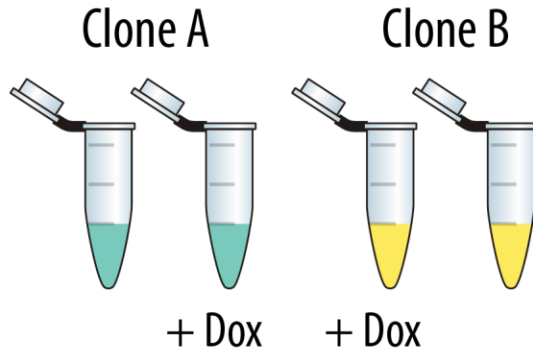
Selecting pools without addition of doxycycline allows cells to recover more effectively without needing to produce protein



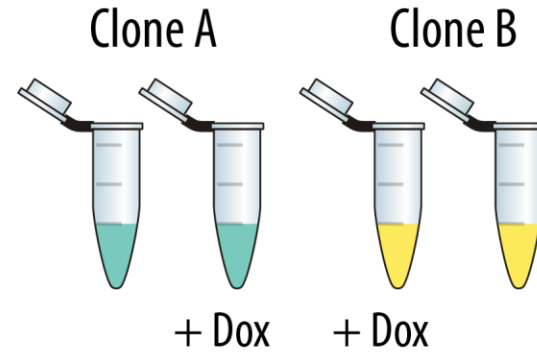
## Experimental Design

16 Media and cell pellet samples collected for proteomic and metabolomic analysis

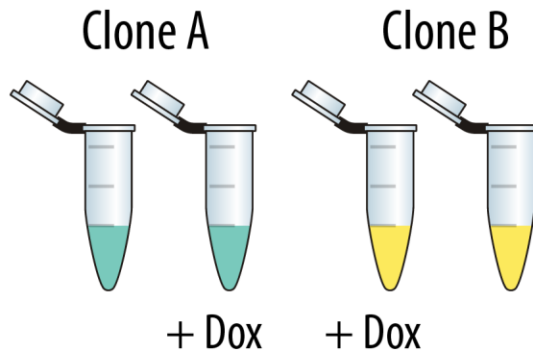
**Day 3**



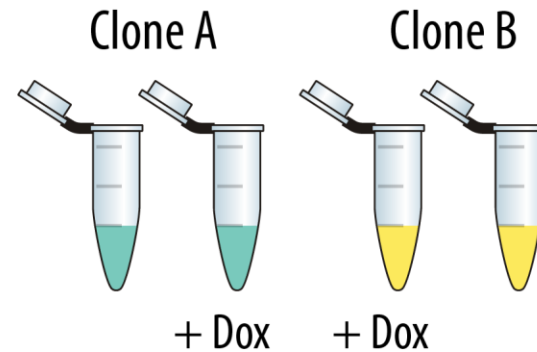
**Day 6**



**Day 8**

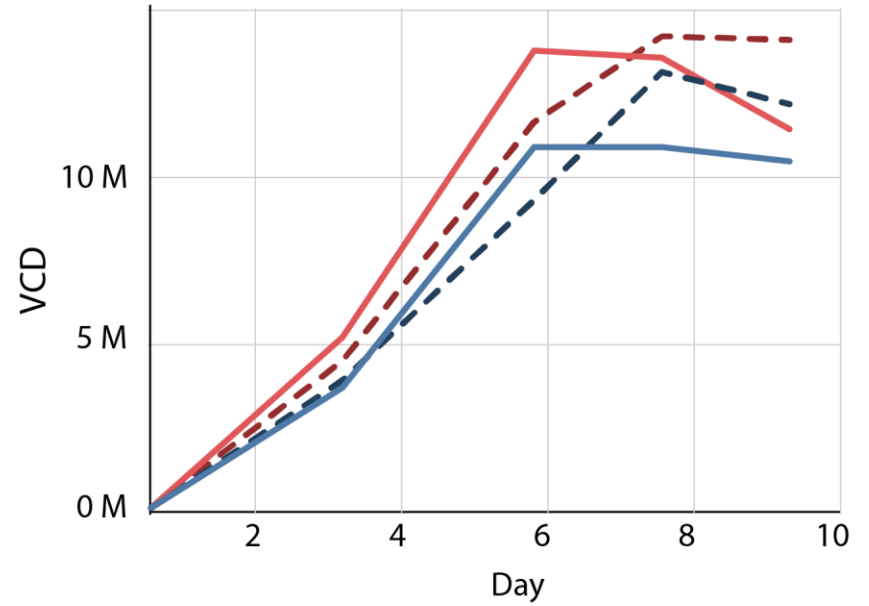
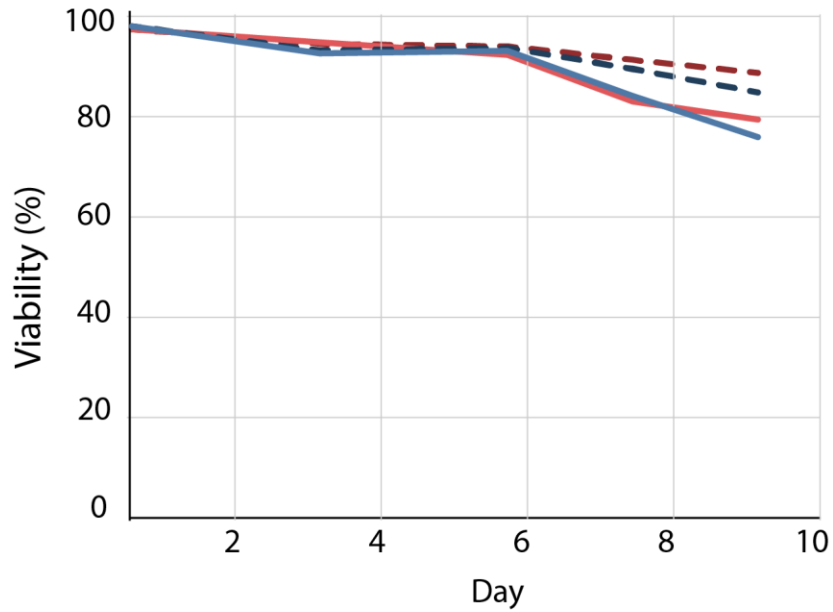


**Day 10**



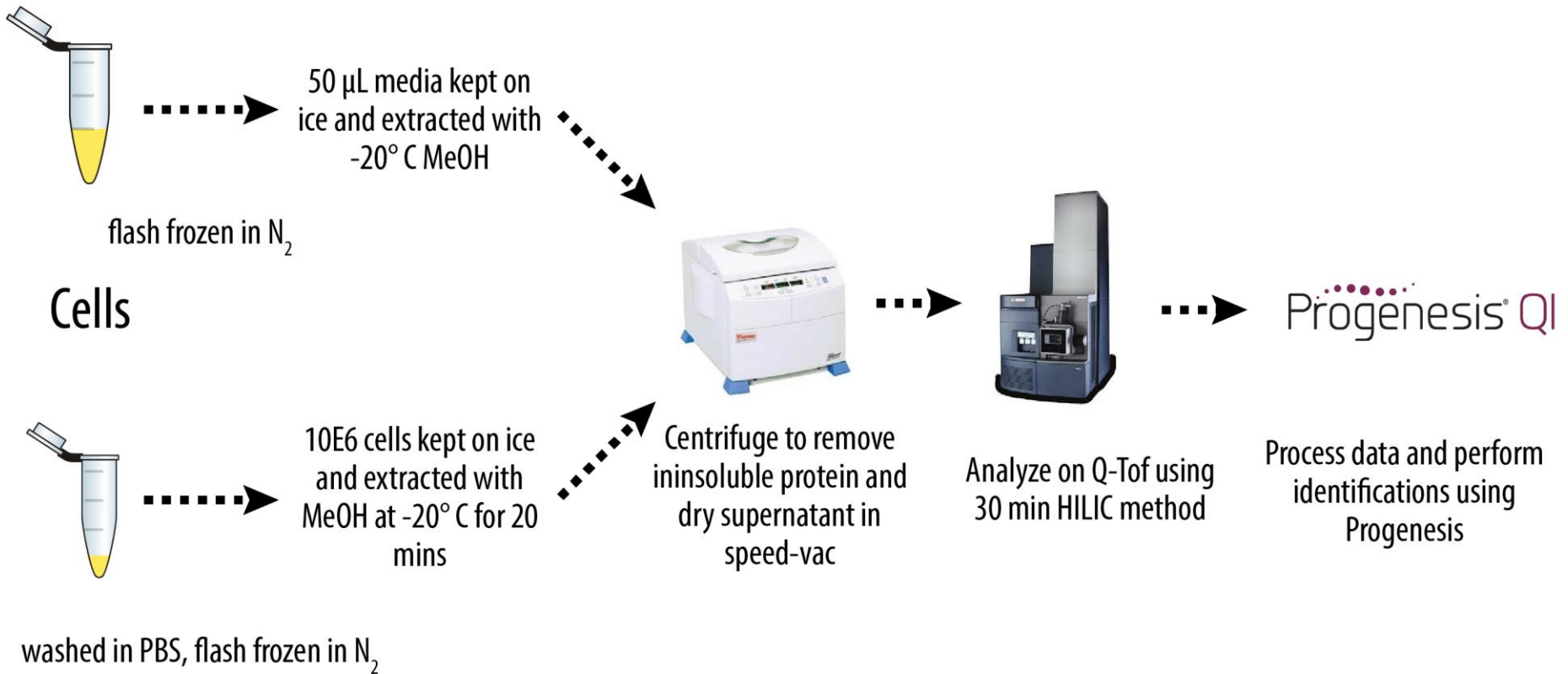
# Cell culture performance

Less viability and slightly faster growth without doxycycline



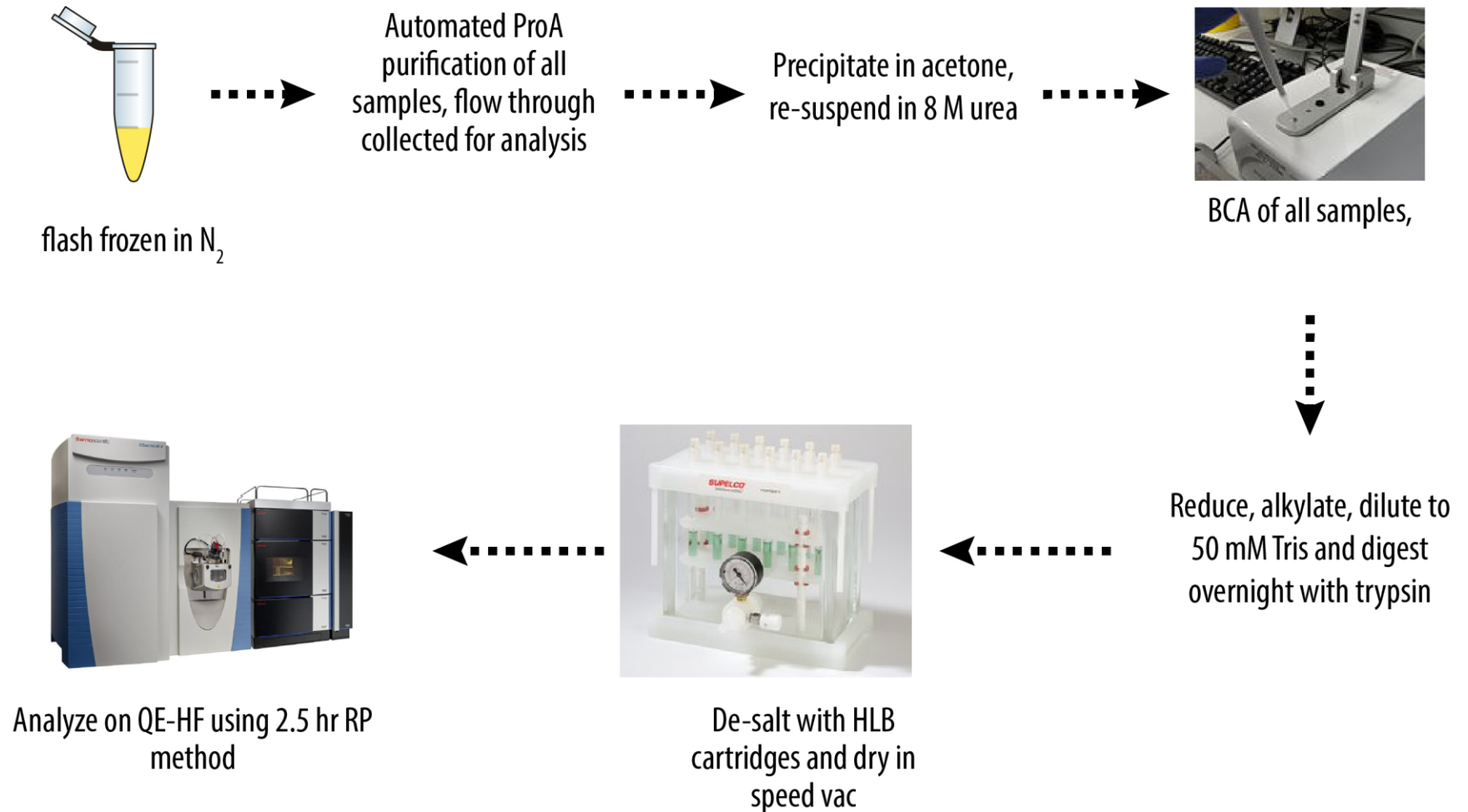
- Clone A
- Clone A + Dox
- Clone B
- Clone B + Dox

### Media



# Proteomics Sample Prep

## Media





# Proteomics Sample Prep

## Cell Pellets

### Cells



1E6 cells re-suspended in 8 M Urea/7 M GnHCl, DNA dispersed using probe sonicator, centrifuge and transfer supernatant



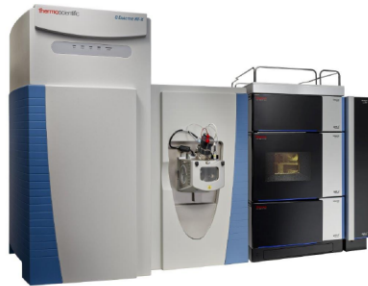
Reduce and alkylate samples, precipitate protein in cold EtOH



Re-suspend dried cell pellets in 8 M Urea, dilute with 50 mM Tris, digest with trypsin overnight



De-salt with HLB cartridges and dry in speed vac



Analyze on QE-HF using 2.5 hr RP method

Cells washed in PBS, flash frozen in N<sub>2</sub>

BCA of all samples, aliquot 100 µg

## Agenda

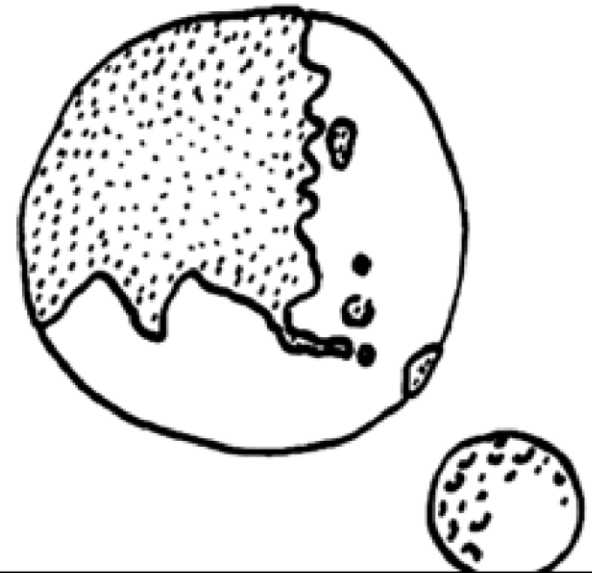
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Just - Evotec Biologics - Why Omics?

Inducible Cell line and Experimental Design

**Results**

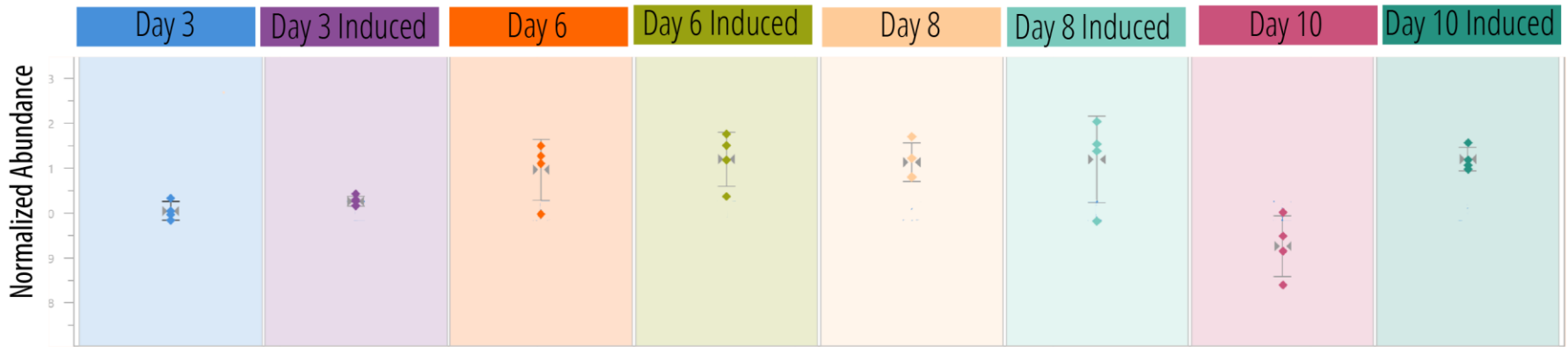
Conclusion



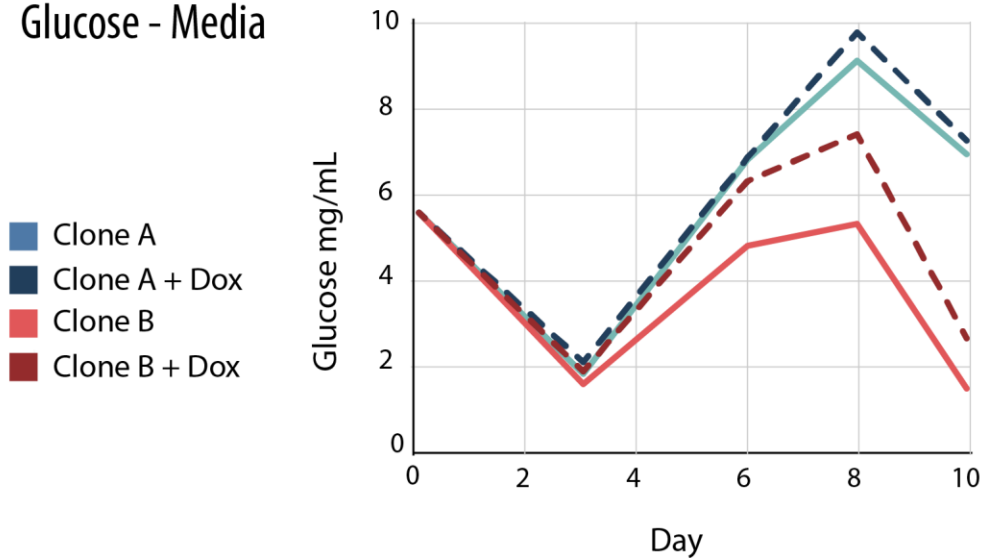
# Glucose

Doxycycline appears to decrease overall consumption of glucose

## Glucose - Media



## Glucose - Media

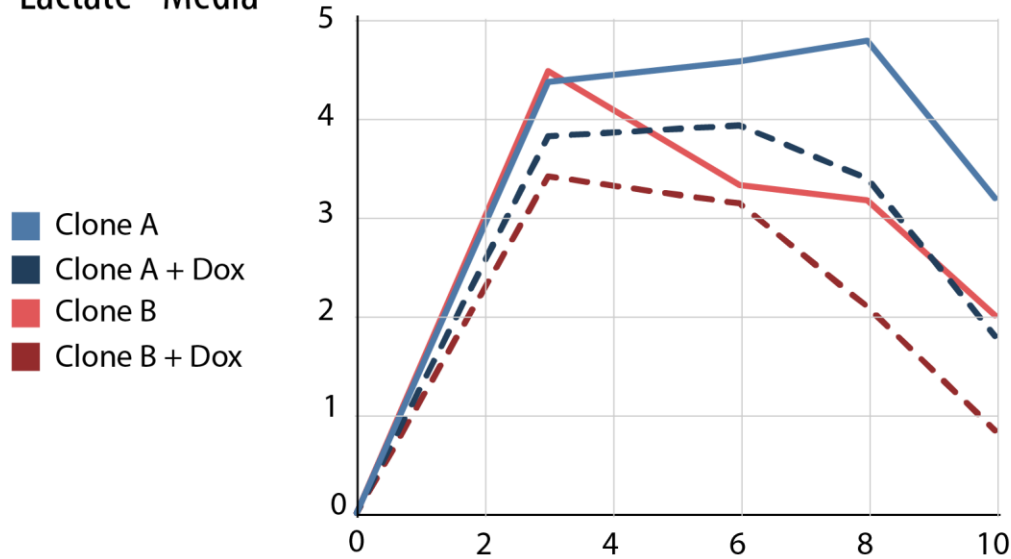


- Cells treated with doxycycline typically grow slower consuming less glucose and take longer to reach carrying capacity
- Hypothesize that this is due to increased energy being put toward antibody production

# Lactate

Shift to less lactate production, more lactate consumption

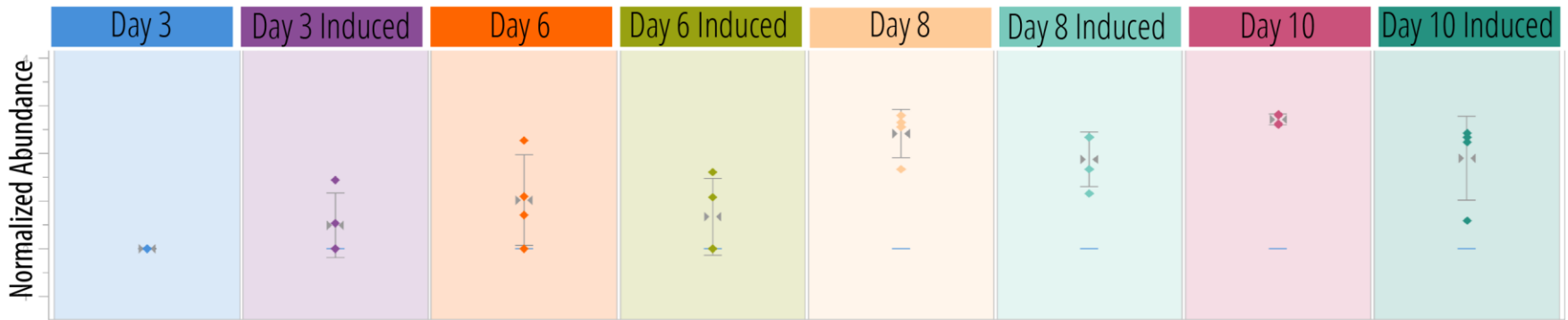
## Lactate - Media



- Lower lactate could also maybe explain growth/viability differences

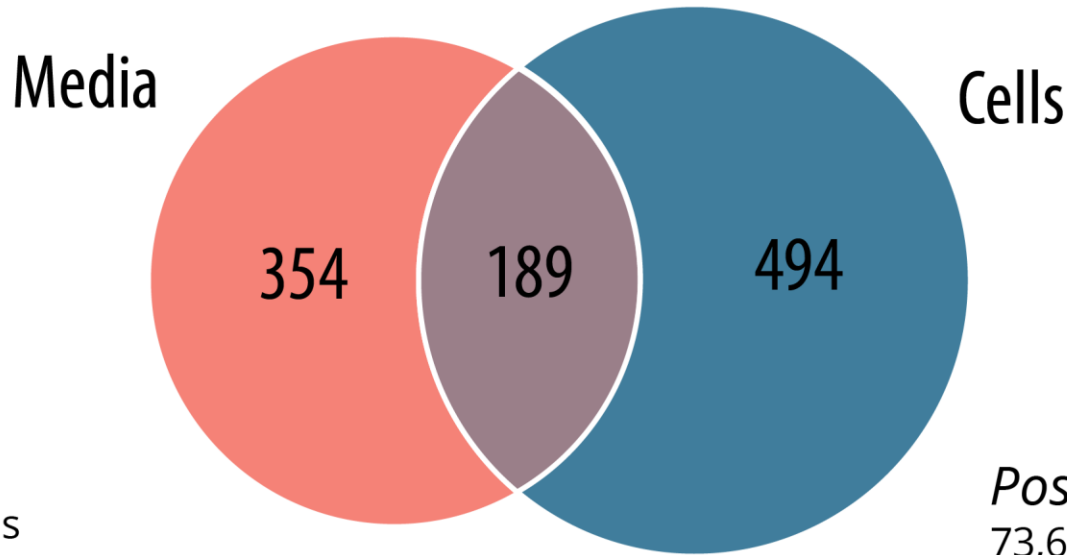
- This shift could also be explained by differences in GS expression, possibly through a push in the TCA-aKG-Glutamate cycle

## Lactate - Cells



# Over 200 metabolites identified from each sample set

Metabolomics results



## *Positive*

27,317 features

9,662 putative identifications

309 identified features

## *Negative*

13,144 features

3,658 putative identifications

279 identified features

## *Positive*

73,648 features

22,707 putative identifications

469 identified features

## *Negative*

9,096 features

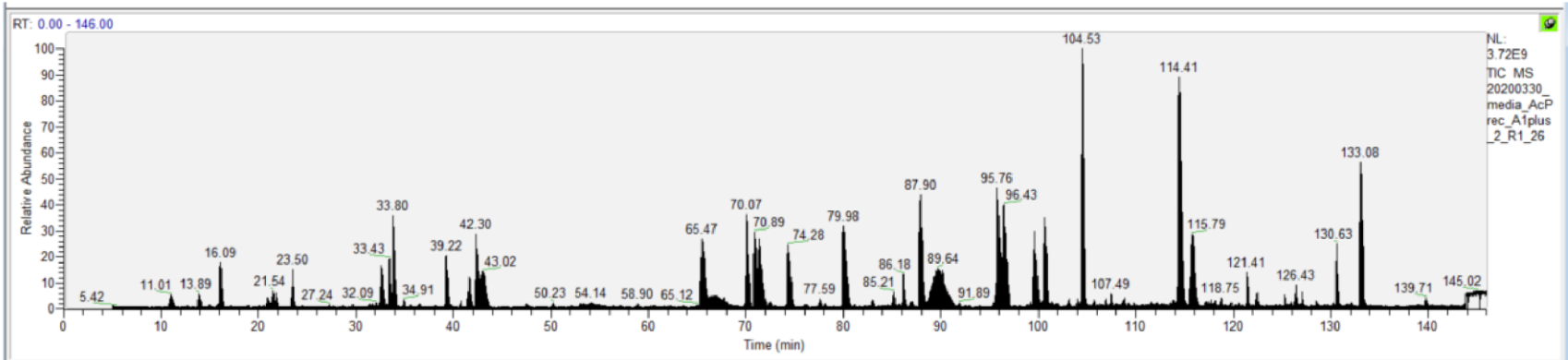
2,730 putative identifications

343 identified features

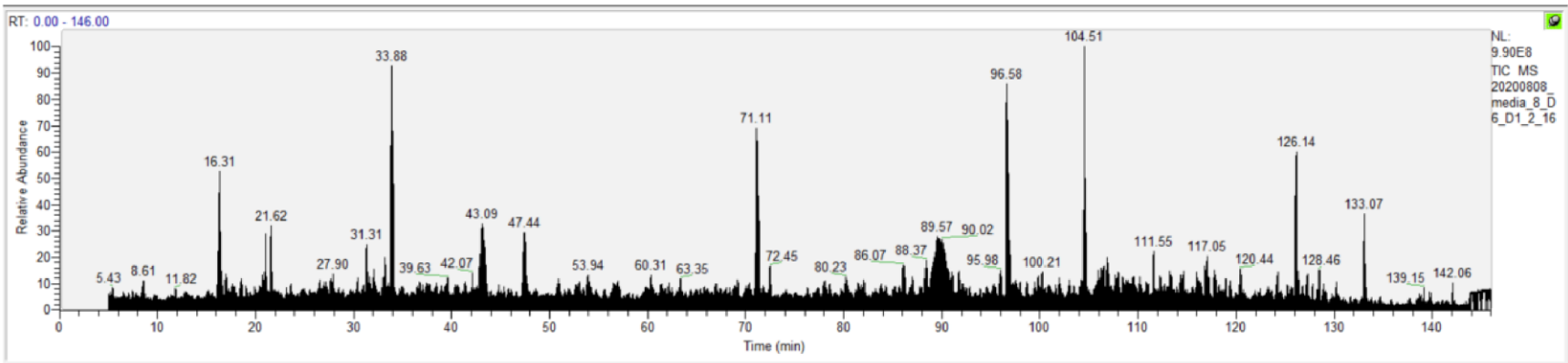
# Antibody removal with ProA improved media analysis

More low abundant proteins are visible in flow through

TIC - Media before ProA filtration



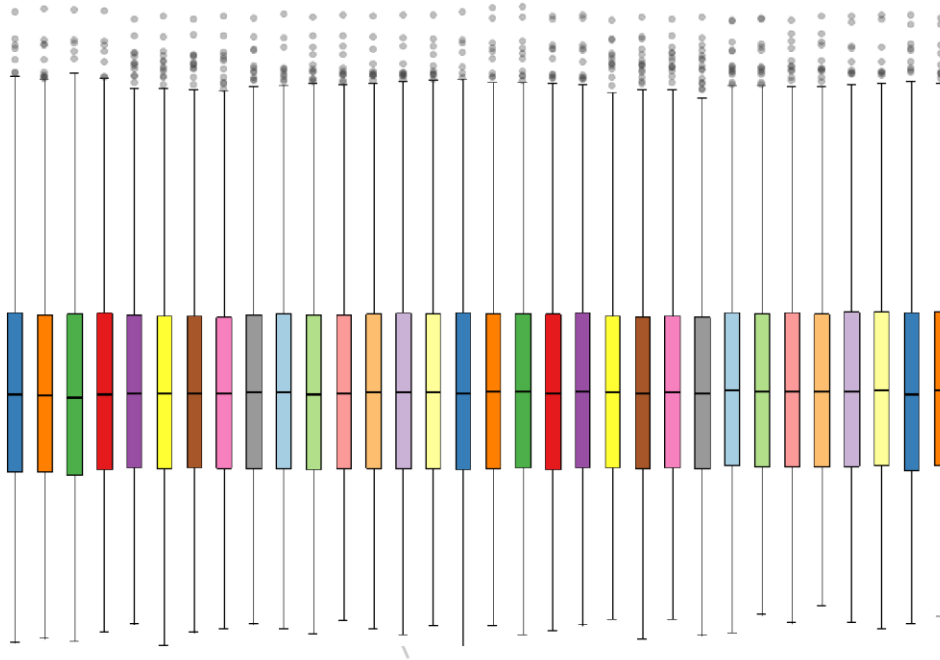
TIC - Media after ProA filtration



All samples were normalized to equivalent abundances

3,953 proteins identified with FDR < 1%

1,679 proteins identified with FDR < 1%

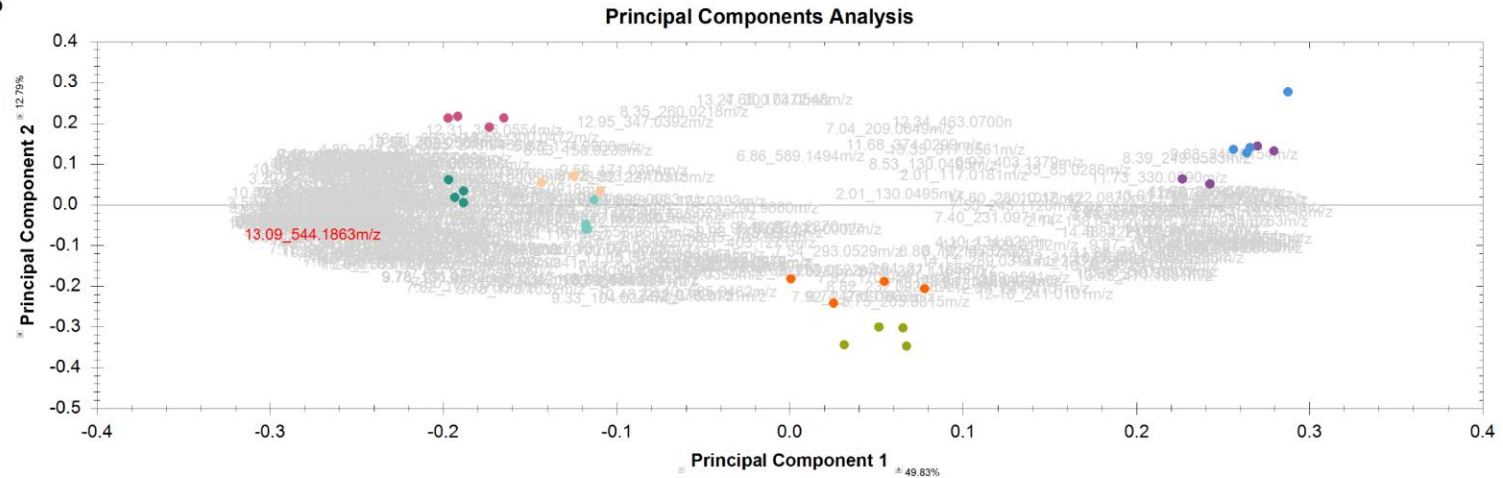


Raw data searched against database of mouse proteins using Proteome Discoverer

# Cells and media show separation by time in PCA

Cells show better separation by condition in metabolomics

## Cells



Day 3

Day 3 Induced

Day 6

Day 6 Induced

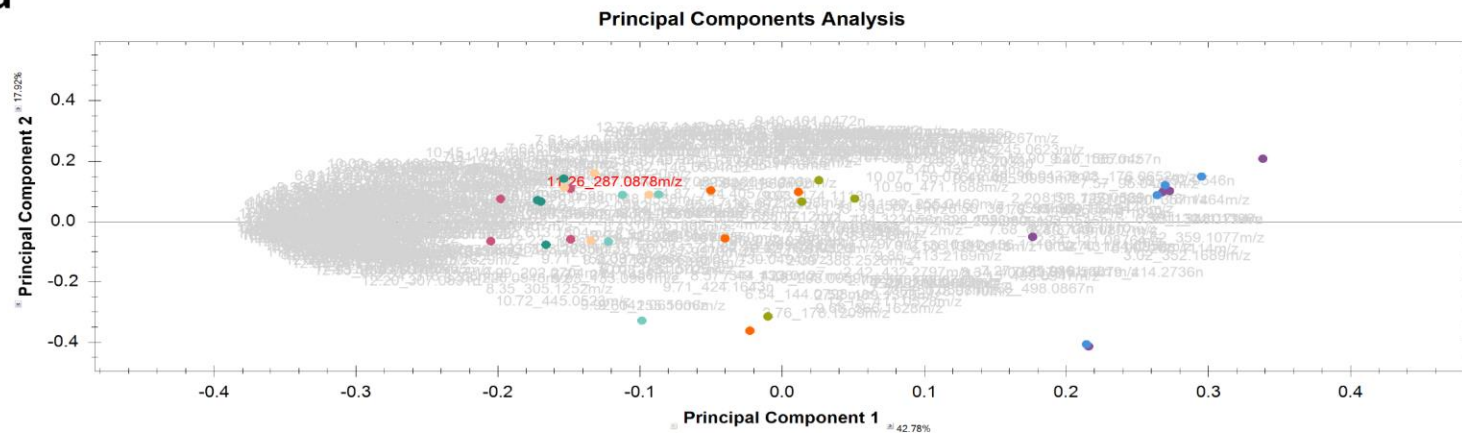
Day 8

Day 8 Induced

Day 10

Day 10 Induced

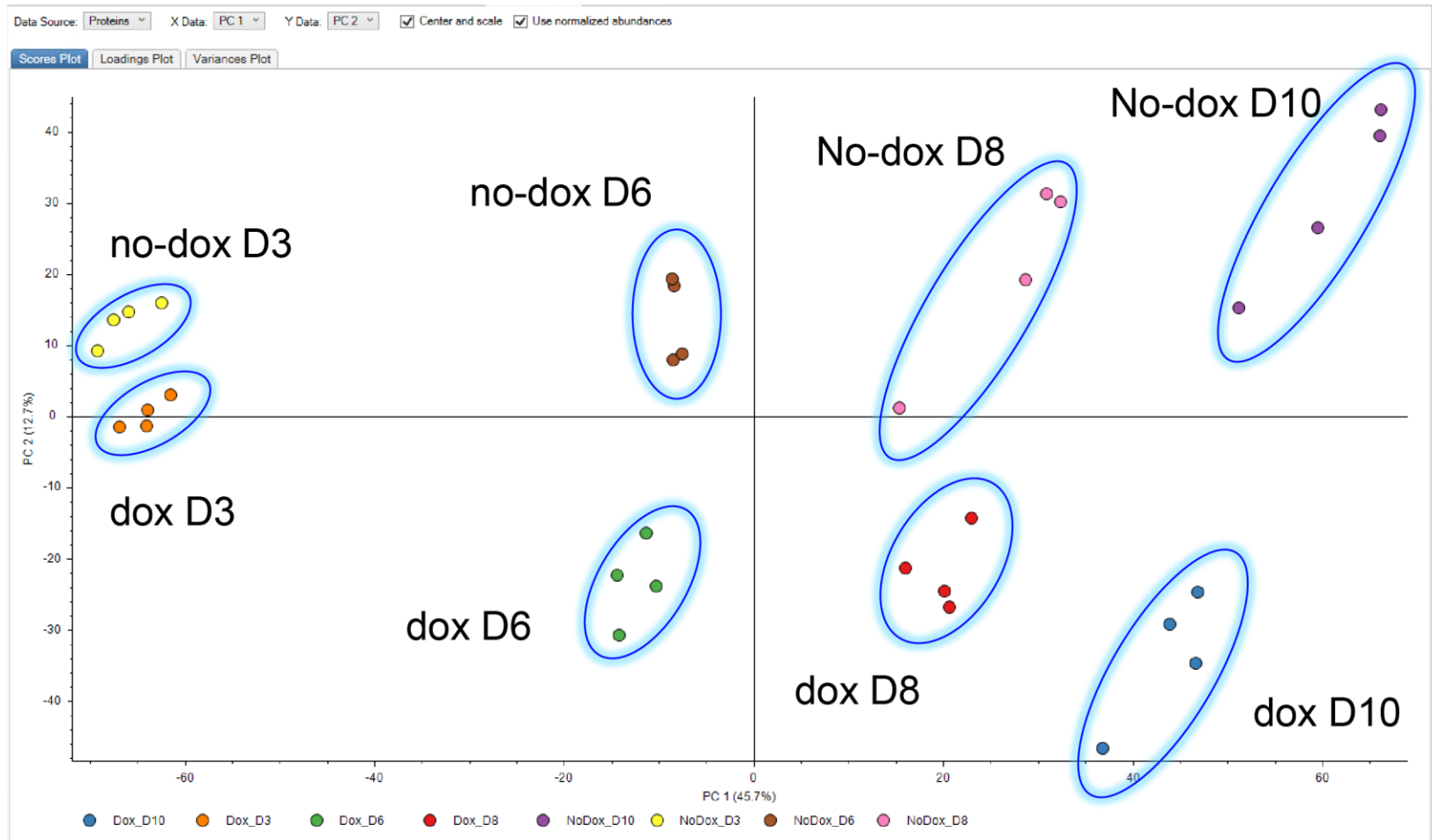
## Media





# Distinctive grouping by category seen in PCA analysis

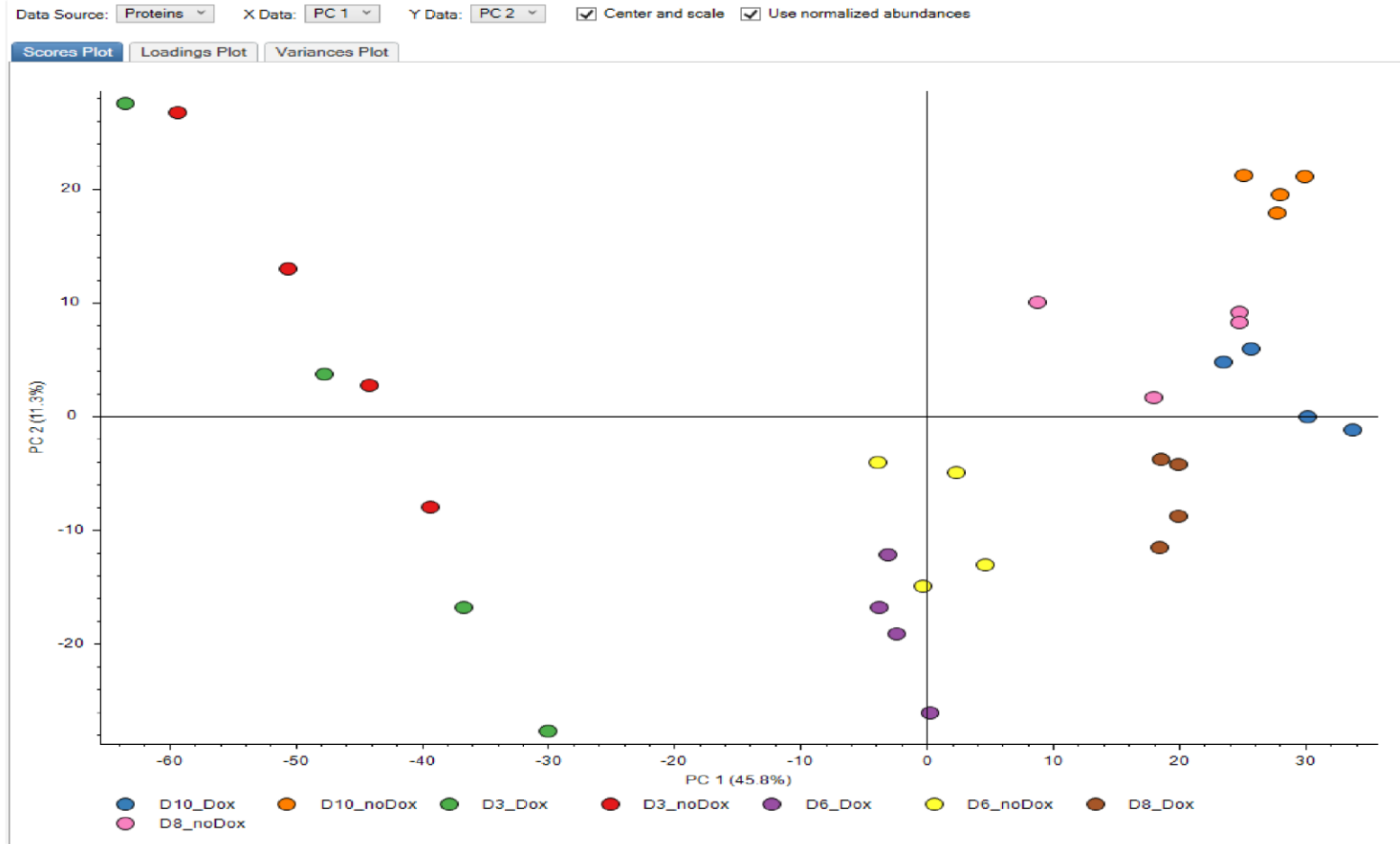
Proteomics Results - Cells



Day 3 Day 3 Induced Day 6 Day 6 Induced Day 8 Day 8 Induced Day 10 Day 10 Induced

# Media shows separation by time in PCA analysis

## Proteomics Results - Meida

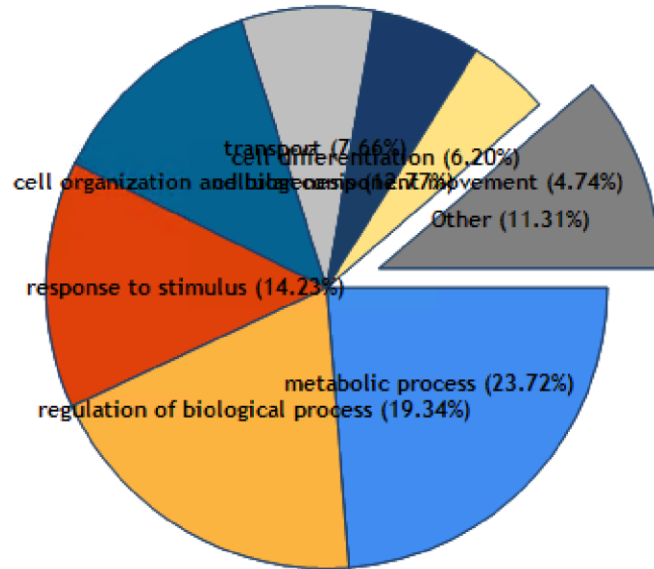


Day 3 Day 3 Induced Day 6 Day 6 Induced Day 8 Day 8 Induced Day 10 Day 10 Induced

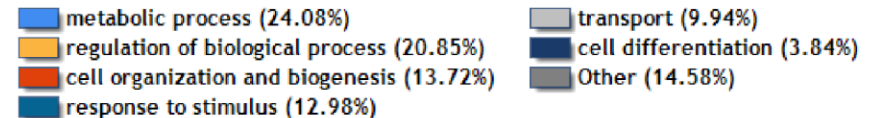
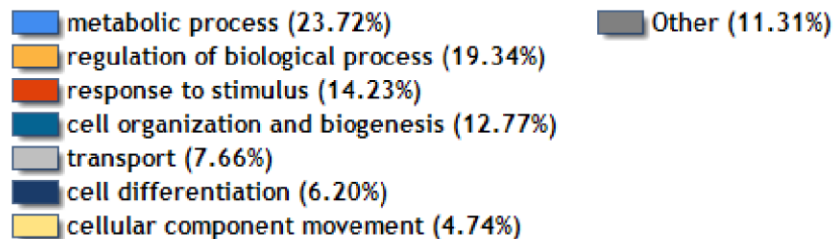
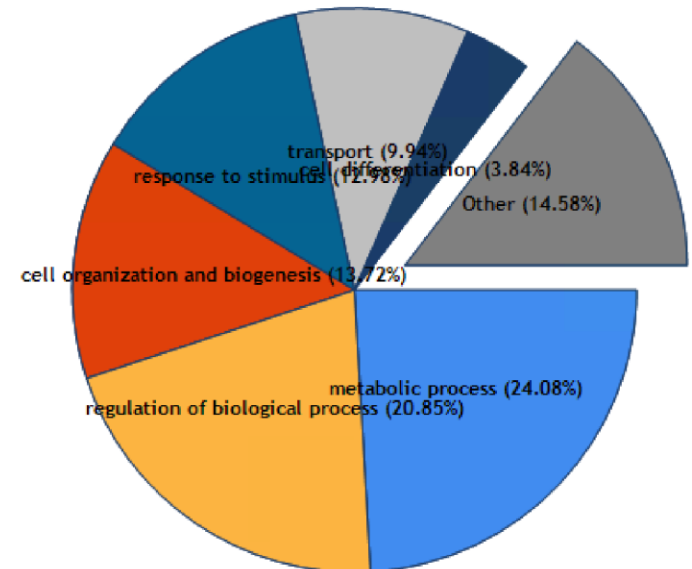
# Fewer proteins associated with transport, more with differentiation in untreated cells

GO enrichment of KEGG pathways

All proteins



Up-regulated proteins without doxycycline

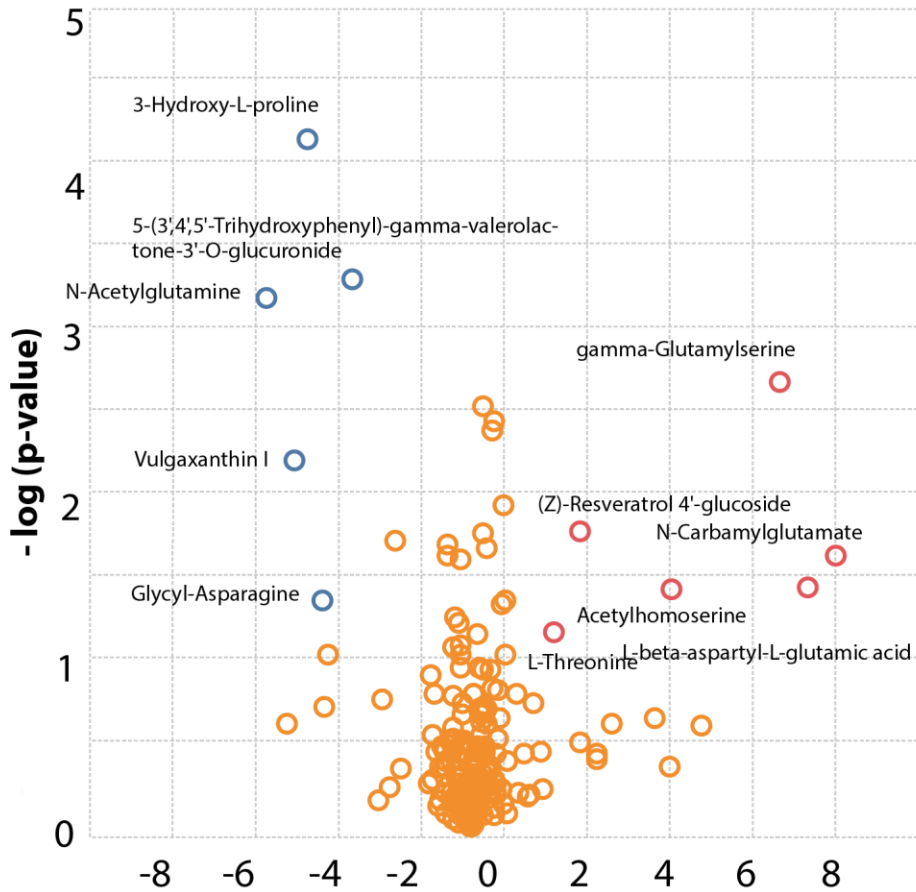


FDR ≤ 1%, >2 peptides

# Induced and uninduced samples similar at day 3

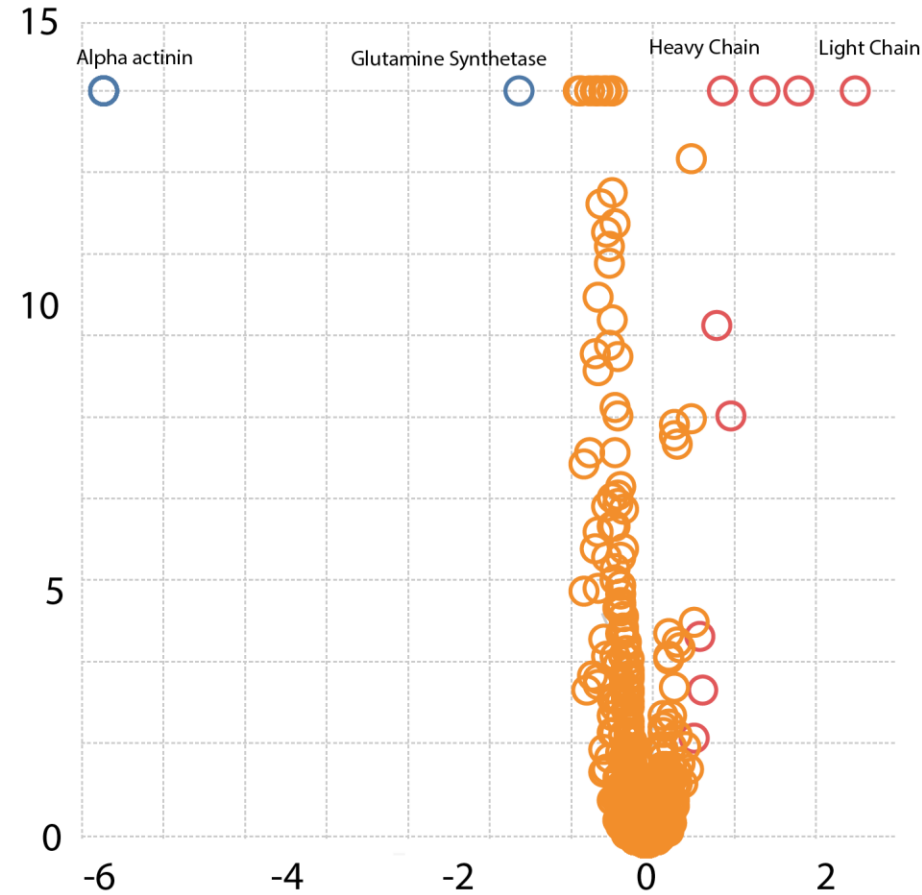
Metabolomics and Proteomics results: Day 3 Cells

## Metabolites



Log<sub>2</sub> Fold Change Induced - Uninduced

## Proteins

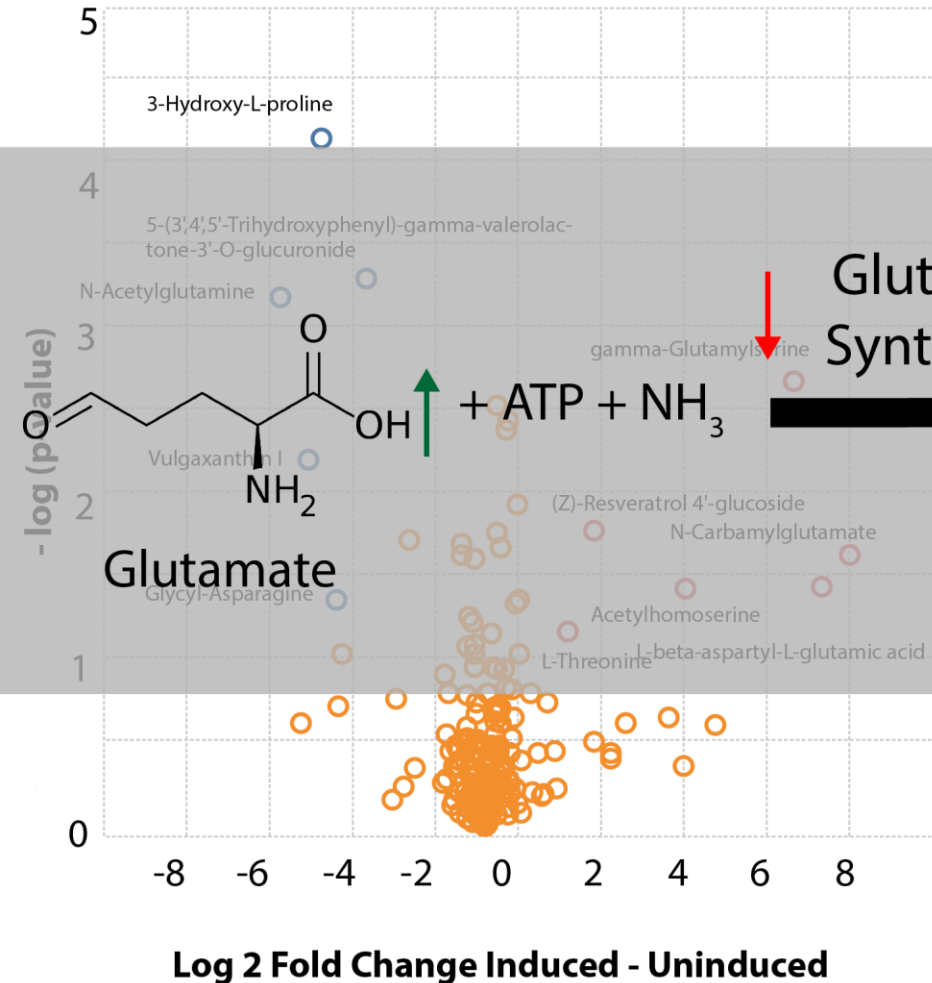


Log<sub>2</sub> Fold Change Induced - Uninduced

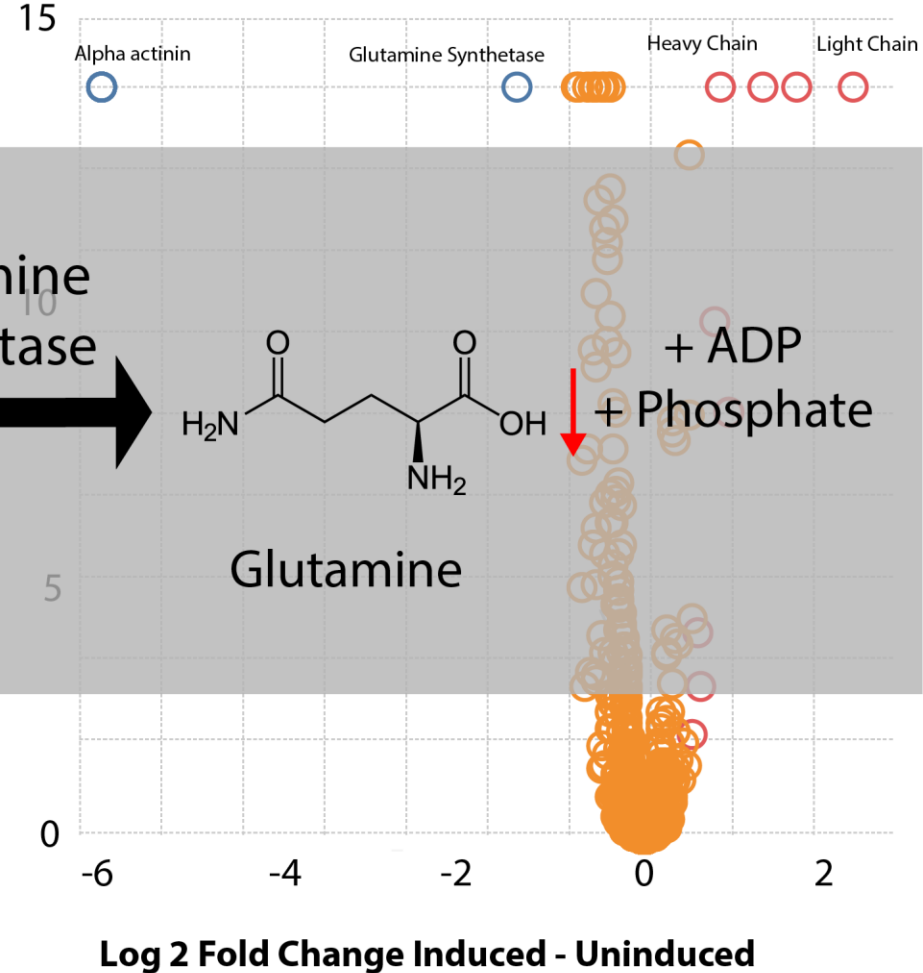
# Induced and uninduced samples similar at day 3

Metabolomics and Proteomics results: Day 3 Cells

## Metabolites



## Proteins



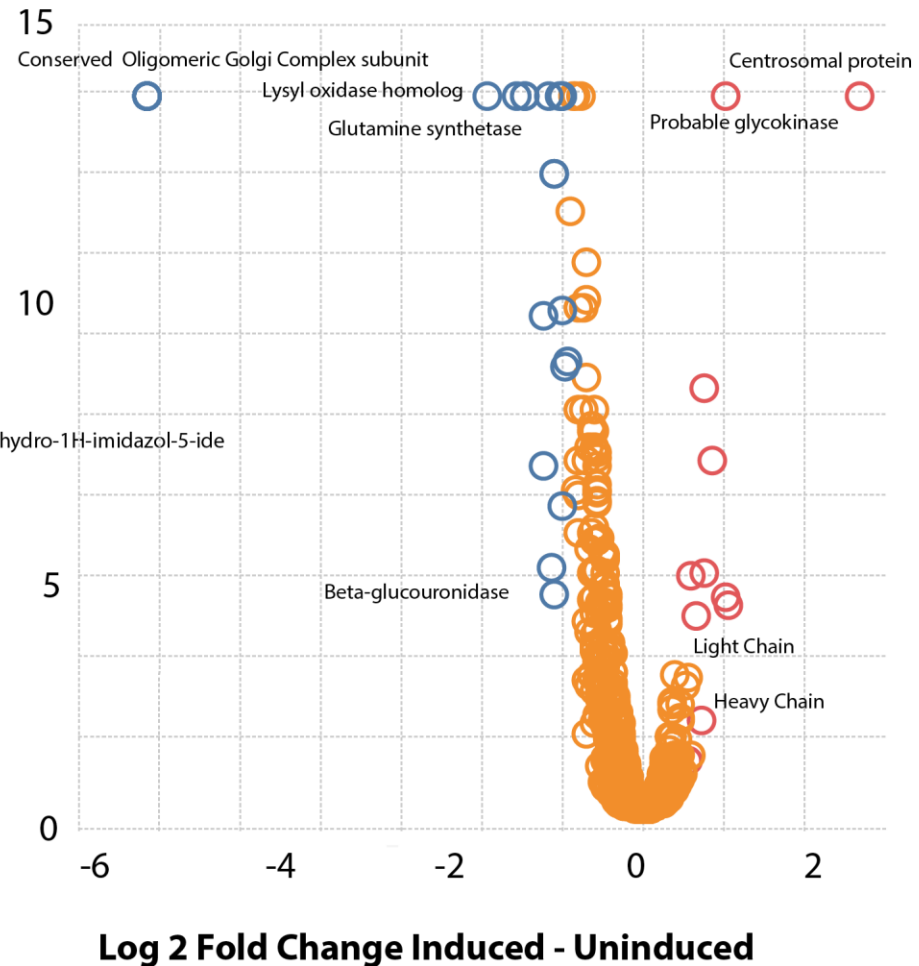
# Decreases seen in Glutamine and NAD

Metabolomics and Proteomics results: Day 6 Cells

## Metabolites



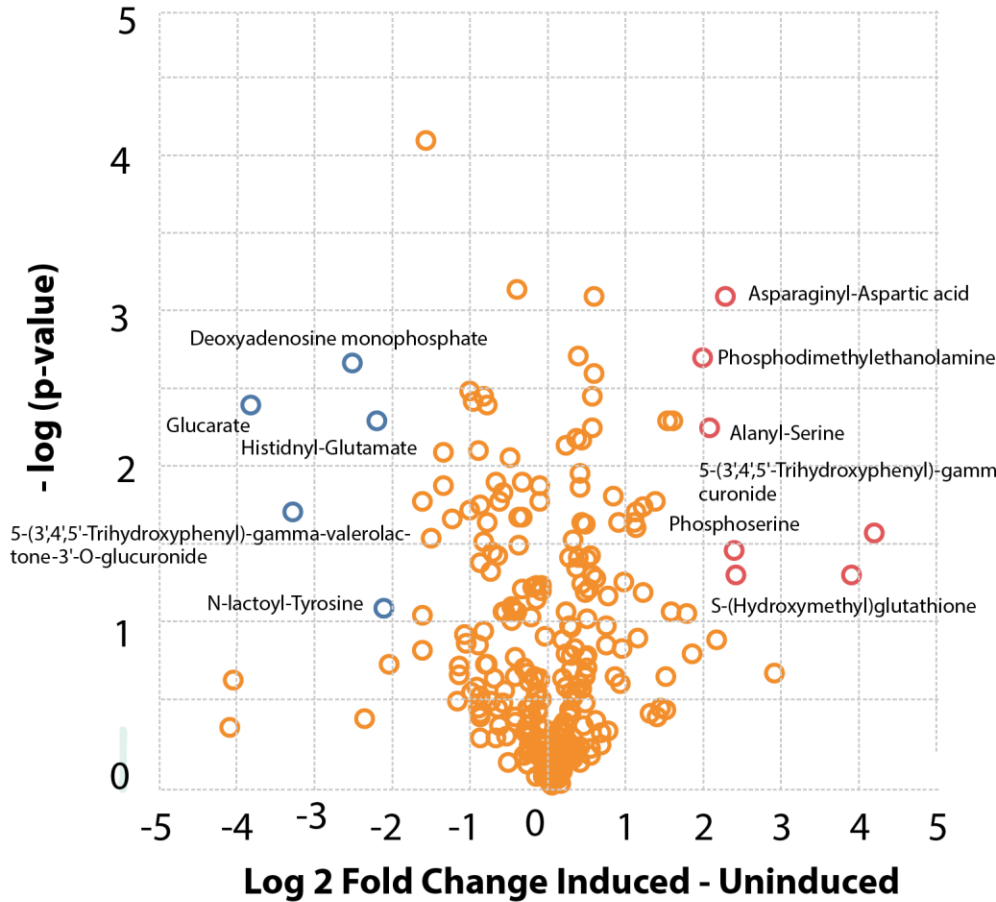
## Proteins



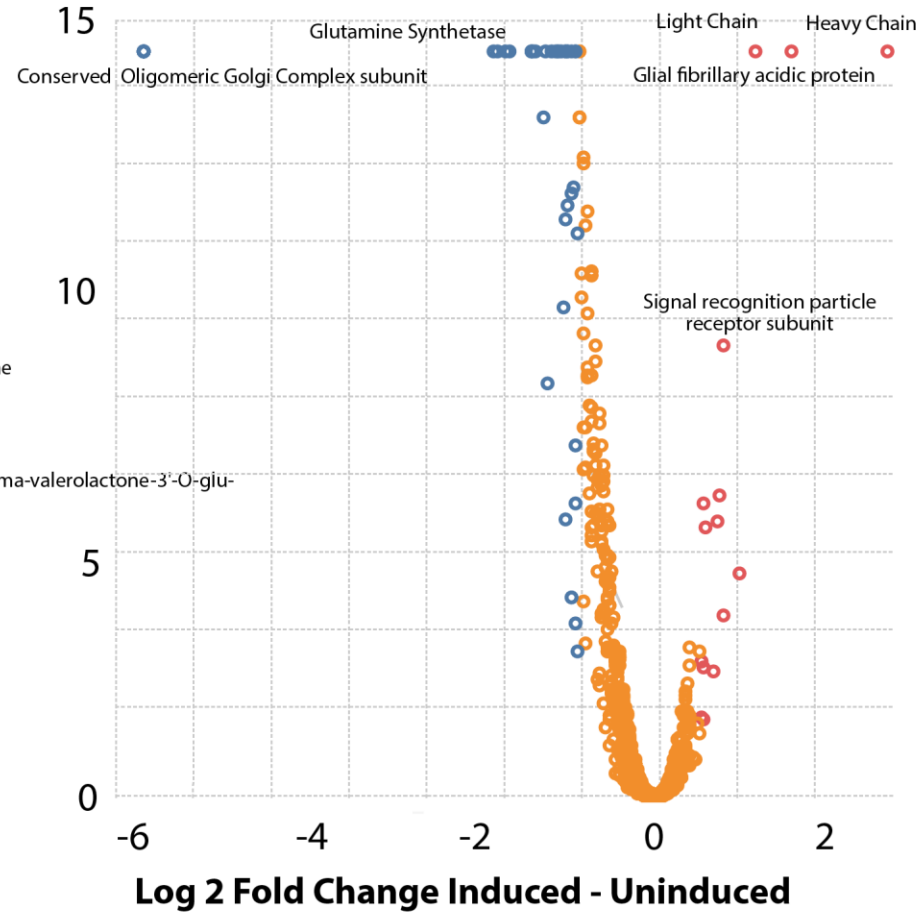
# Increasing number of proteins down-regulated

Metabolomics and Proteomics results: Day 8 Cells

## Metabolites



## Proteins

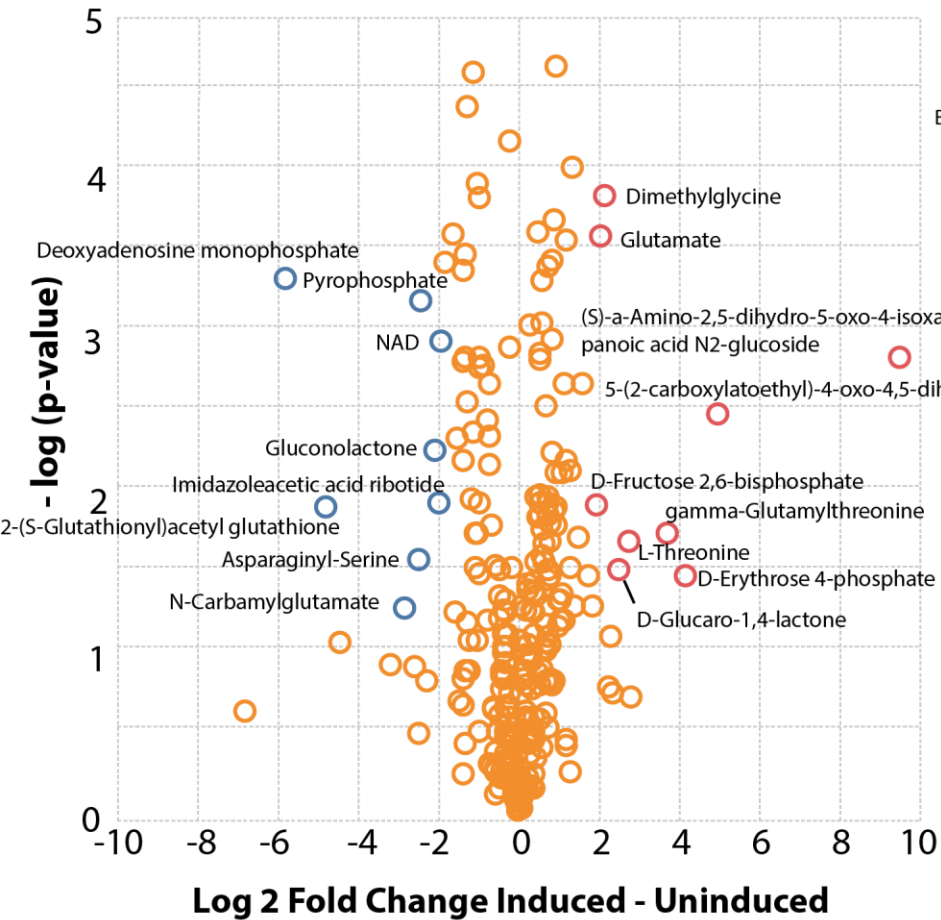




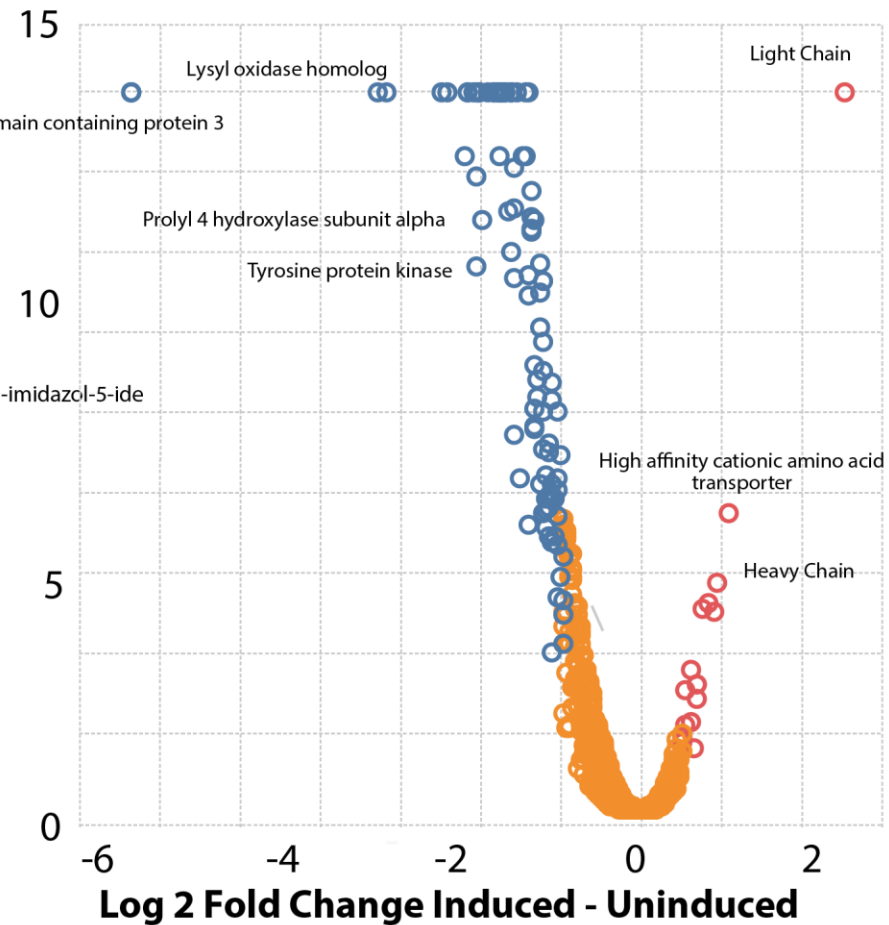
# Large number of proteins down-regulated by day 10

Metabolomics and Proteomics results: Day 10 Cells

## Metabolites



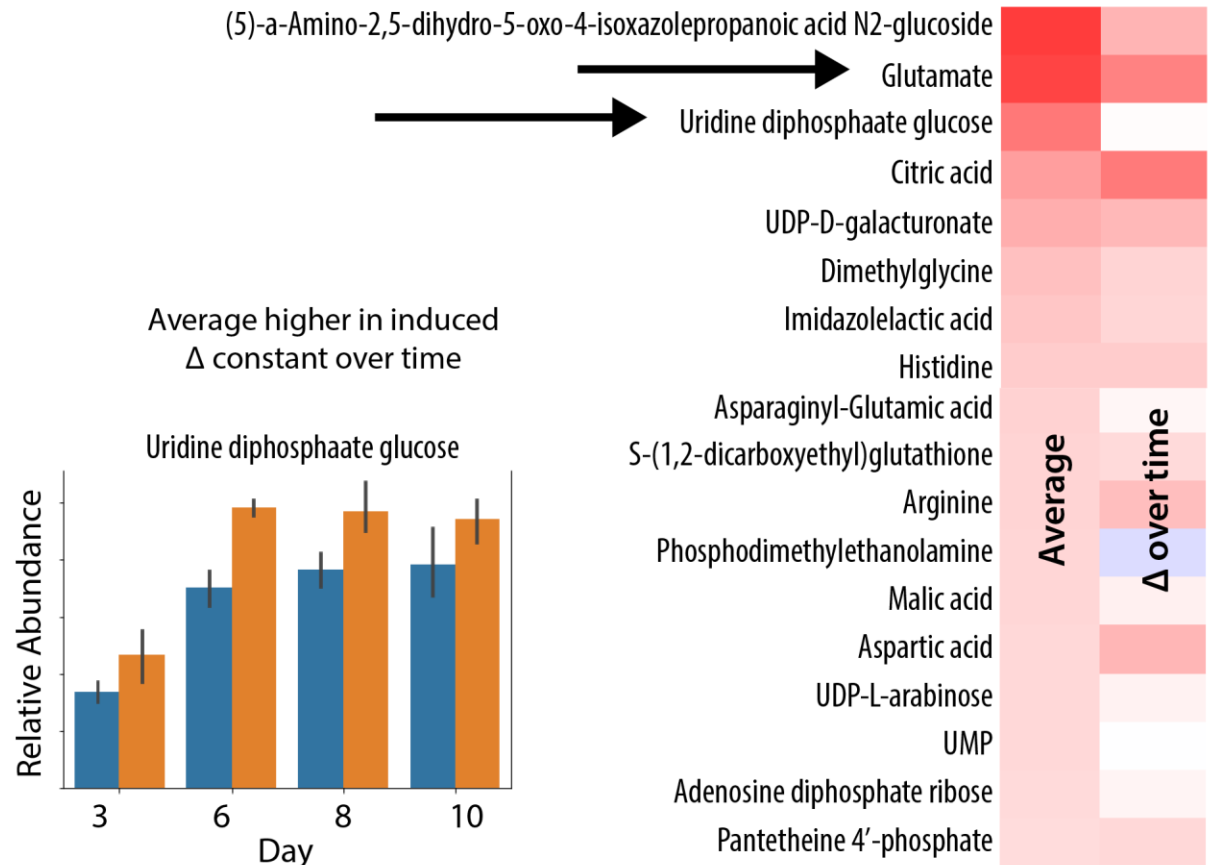
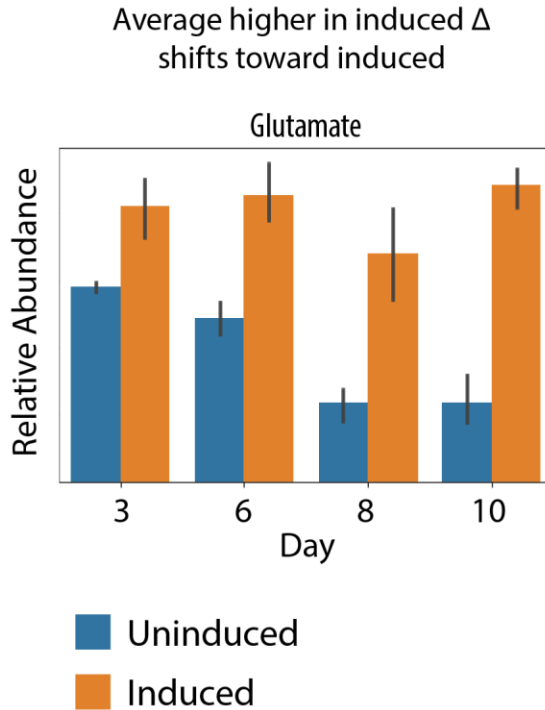
## Proteins





# Metabolomics - Cells: Effect of Induction

Species increased by induction



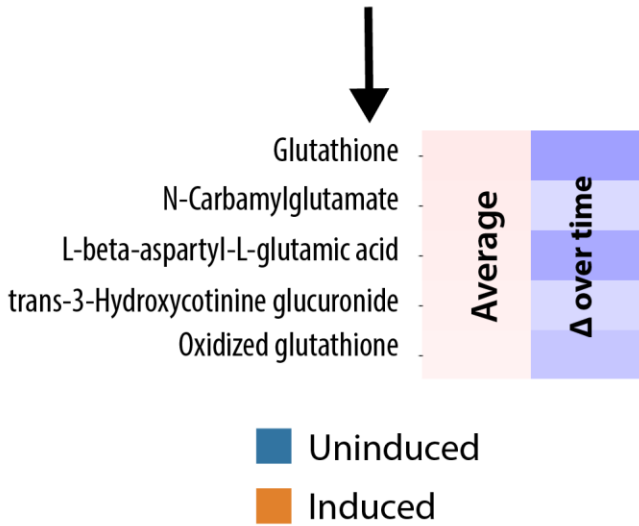
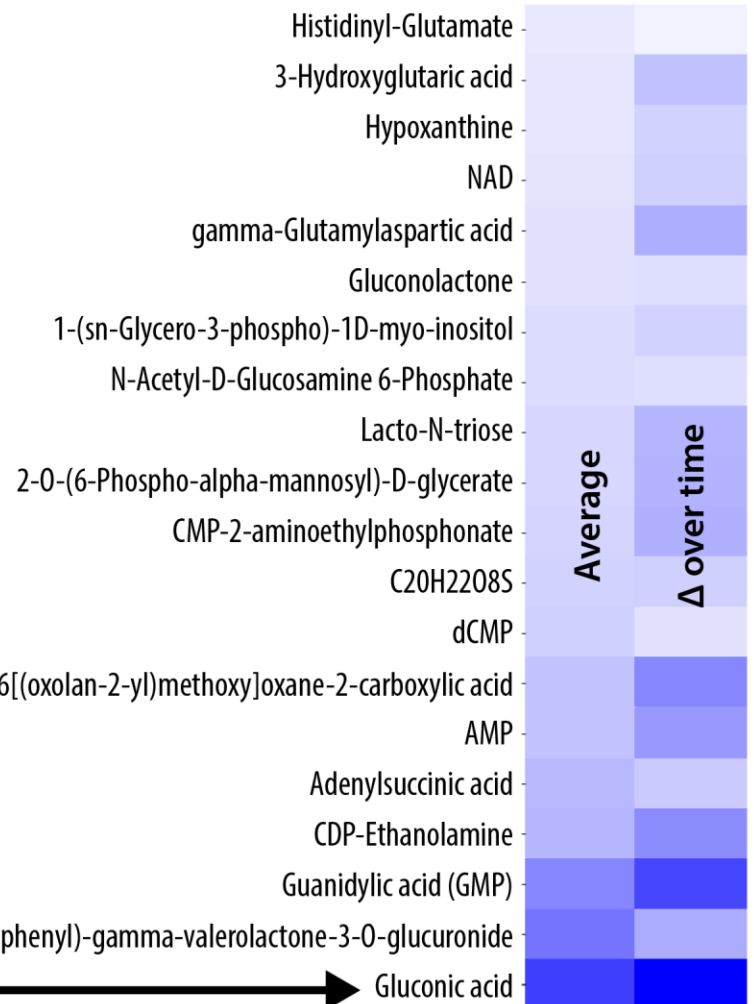
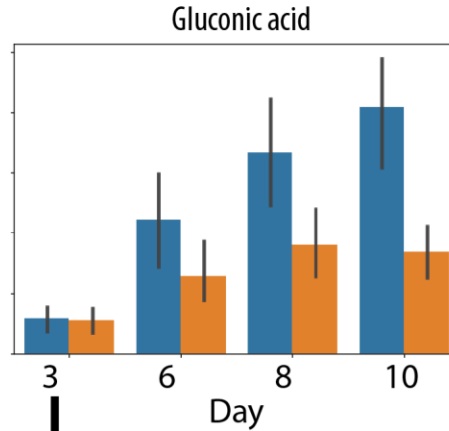
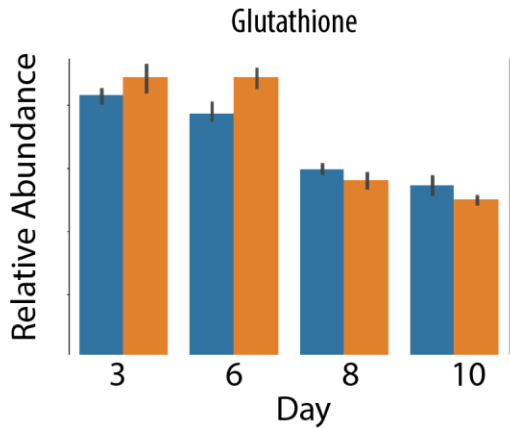
ASCA - ANOVA Simultaneous Component Analysis

Smilde et al, "ANOVA-simultaneous component analysis (ASCA): a new tool for analyzing designed metabolomics data", Bioinformatics, 2005.

Zwanenburg et al, "ANOVA-principal component analysis and ANOVA-simultaneous component analysis: a comparison". J. Chemometrics, 2011

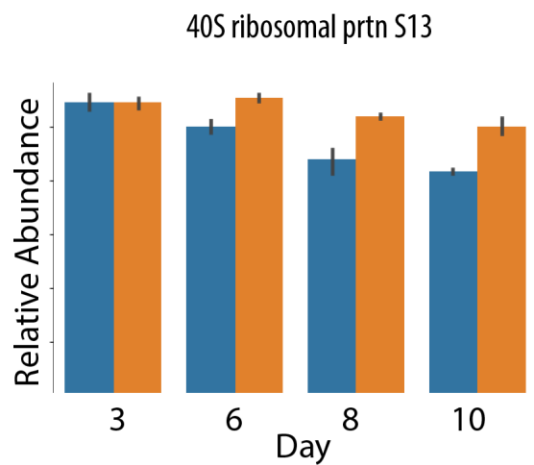
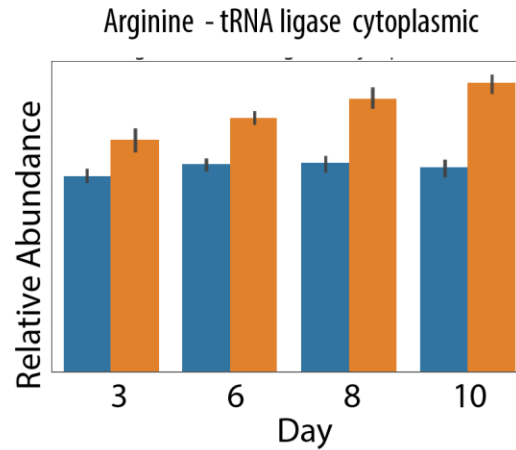
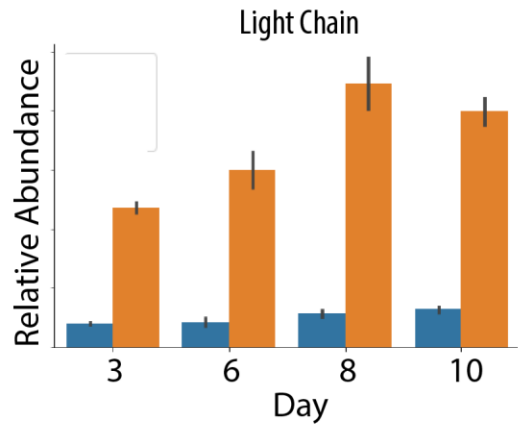
# Metabolomics - Cells: Effect of Induction

Species decreased by induction



# Proteins - Cells: Effect of Induction

Species increased by induction



■ Uninduced  
■ Induced

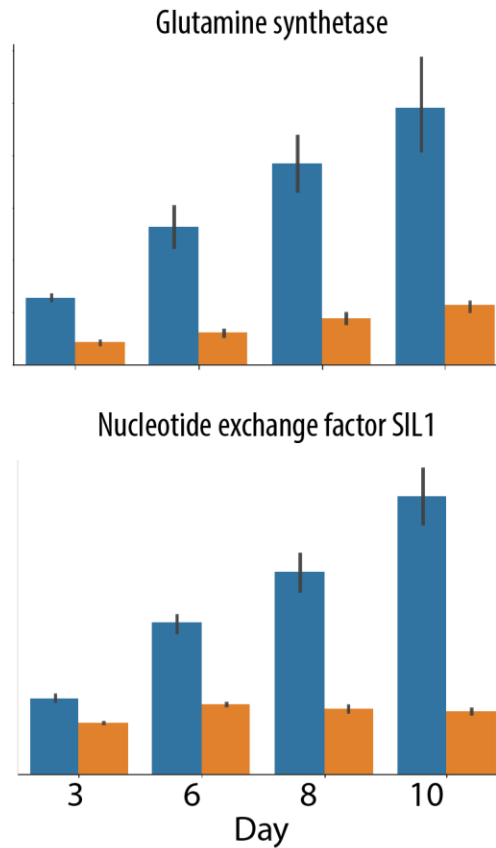
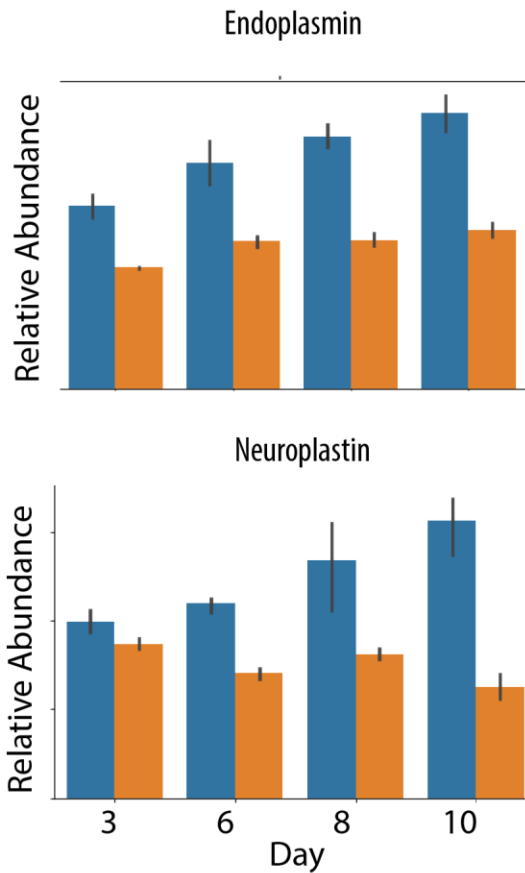
- Light Chain
- Eukaryotic translation initiation factor 4 gamma 2
- Calpain 1 catalytic subunit
- Cofilin-2
- 40S ribosomal prtn S24
- PDZ and LIM domain prtn 7
- 40S ribosomal prtn S13
- Heavy Chain
- Prtn ADP-ribosylarginine hydrolase
- Fibronectin type III domain containing prtn 3B
- Four and a half LIM domains prtn 1
- Arginine - tRNA ligase cytoplasmic
- Eukaryotic translation initiation factor 3 subunit H
- Elongation factor Tu, mitochondrial
- Drebrin
- 40S ribosomal prtn SA



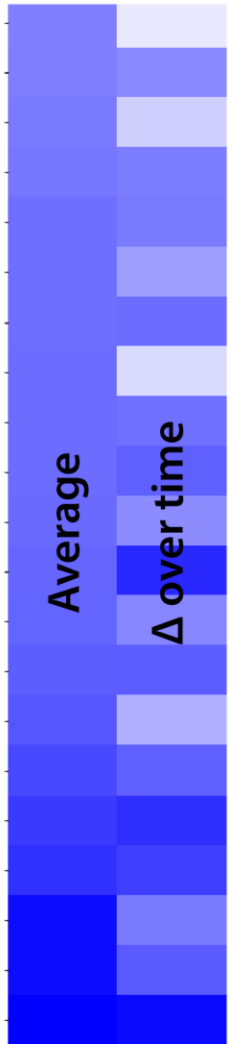
# Proteins - Cells: Effect of Induction

Species decreased by induction

■ Uninduced  
■ Induced



- Laminin subunit gamma-1
- GDP-fucose prtn O-fucosyltransferase 2
- Endoplasmin
- ER degradation-enhancing alpha-mannosidase-like prtn 3
- DNAj homolog subfamily C member 10
- Glutamine synthetase
- Prolyl 4-hydroxylase subunit alpha-1
- Twisted gastrulation prtn homolog 1
- TPR region domain containing prtn
- TPR region domain containing prtn
- Tubulointerstitial nephritis antigen-like
- GPI transamidase component PIG-T
- Selenium binding prtn 2
- Chitinase domain containing prtn 1
- Collagen alpha-1 (XII) chain
- Neuronal pentraxin receptor
- Neuroplastin
- Prtn OS-9
- Nucleotide exchange factor SIL1
- Prtn disulfide isomerase Creld2
- Laminin subunit beta 1
- Beta-hexosaminidase subunit alpha



## Agenda

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Just - Evotec Biologics - Why Omics?

Inducible Cell line and Experimental Design

Results

**Conclusion**



- Omics can provide a snapshot of cell physiology at different conditions
  - An inducible cell line exhibits slightly slower growth and decreased glucose consumption while producing protein
  - Over 3,900 protein and 650 metabolites were identified in cells
  - Over 1,679 proteins and 500 metabolites were identified in media
  - Significant shifts are seen in protein expression and metabolism are seen during protein production
  - Glutamine synthetase downregulated as part of induction system, corresponding metabolites affected
  - Ribosomes up-regulated in induced state, predicted to be due to high demand for protein production
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### **Just-Evotec Biologics**

Yuko Ogata

Gabe Stancu

Jeremy Shaver

Jeff Meyer

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