

Multi-Omic Analysis of Induced Biotherapeutic Protein Production in CHO Cells Reveals Substantial Shifts in Energy Allocation

Gabriel Stancu, PhD – May 2021

PEGS Boston - Optimizing Protein Expression

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Talk outline...

Introduction and Experimental Design

Transcriptomics

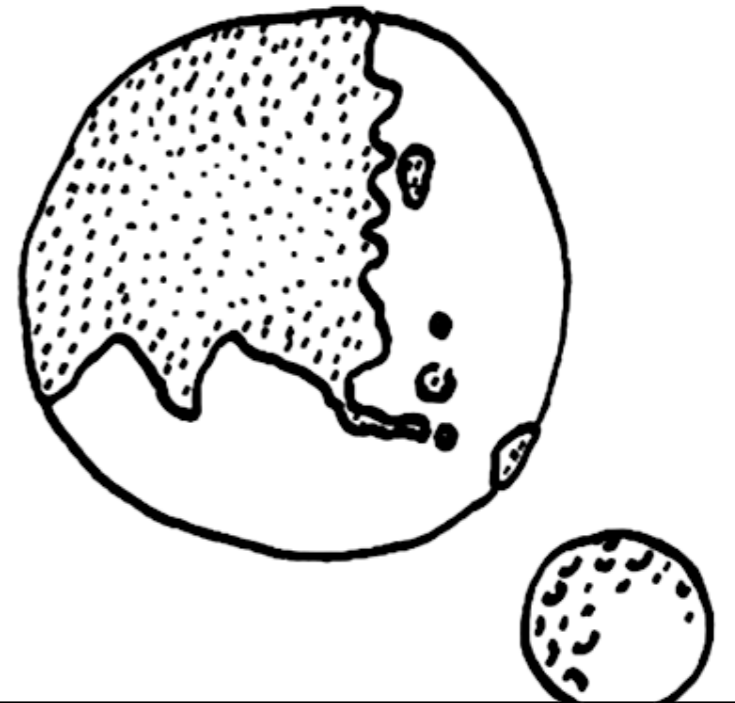
- Data Processing and Analysis

Proteomics – Metabolomics

- Multivariate 'omic analysis

Conclusion

- What does this tell us about CHO clone screenings?



Introduction and background

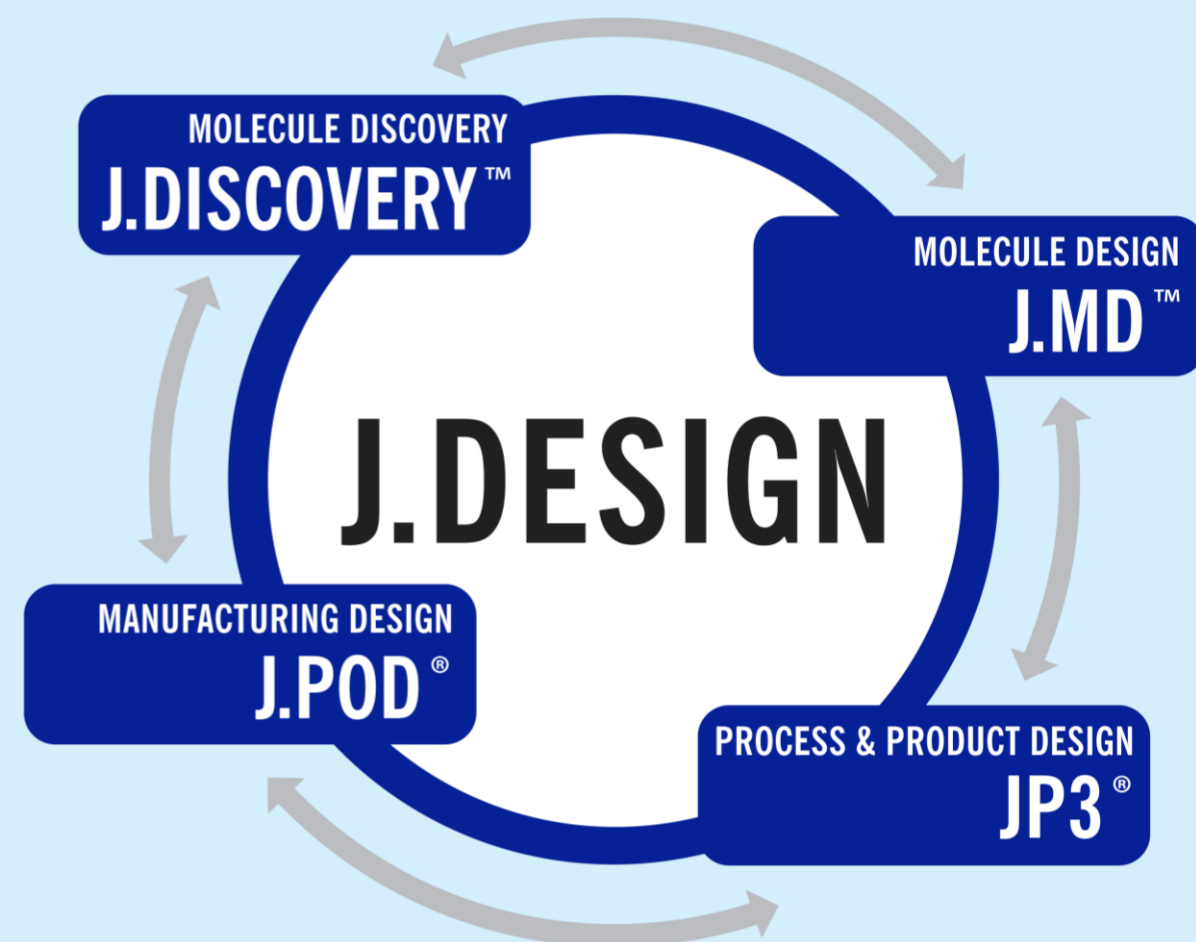
Just - Evotec Biologics

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- Company focused on biologics discovery, development and manufacturing optimization
- Main goal of easing and increasing access to high quality biotherapeutics

CHO Expression Systems

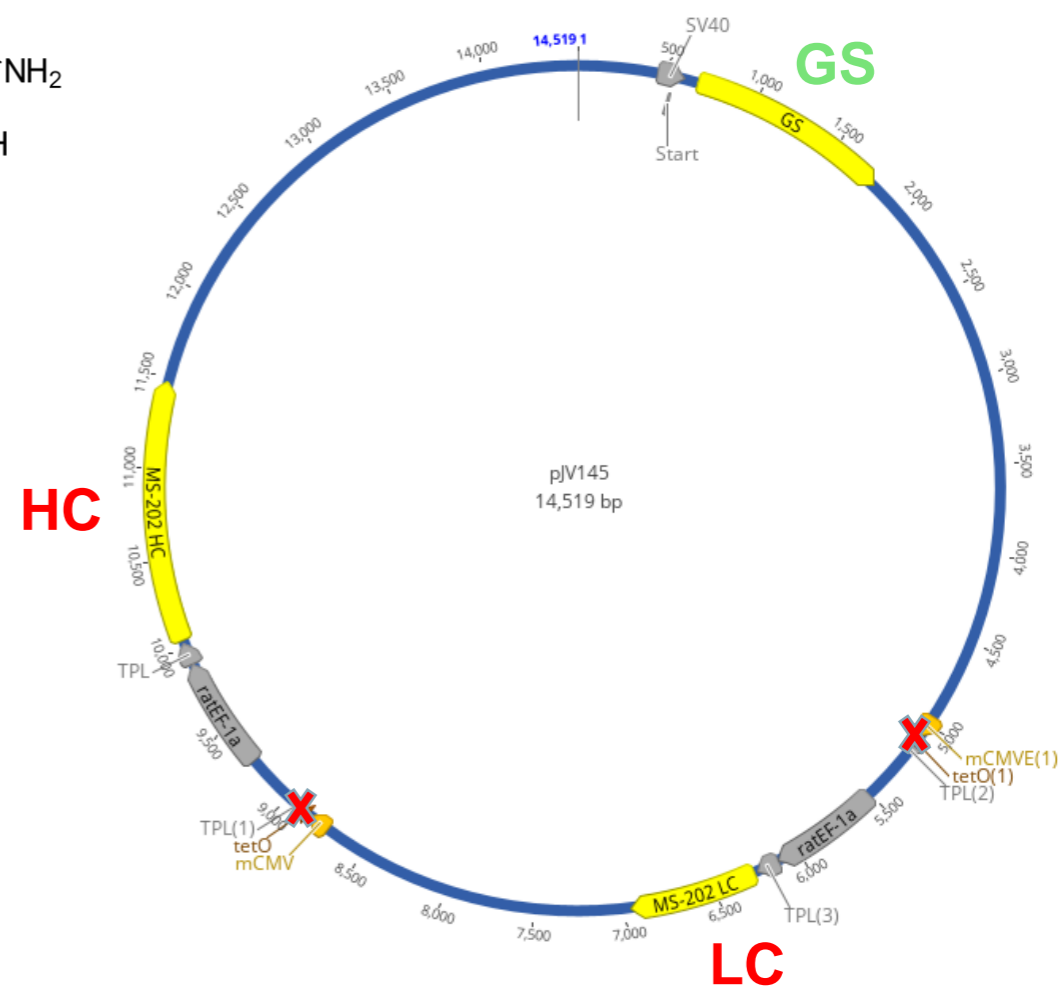
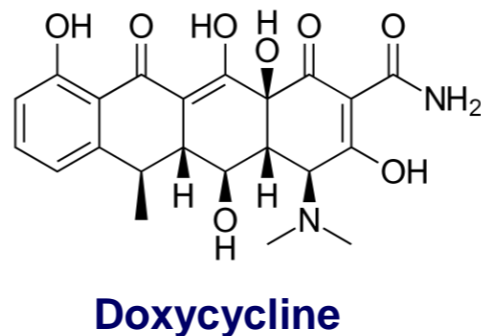
- Time and cost required to find high/efficient producing clones
- Long term stability of cells in bioreactors for manufacturing consistency



Experimental Design

Tet-Inducible System – Control vs. Treated

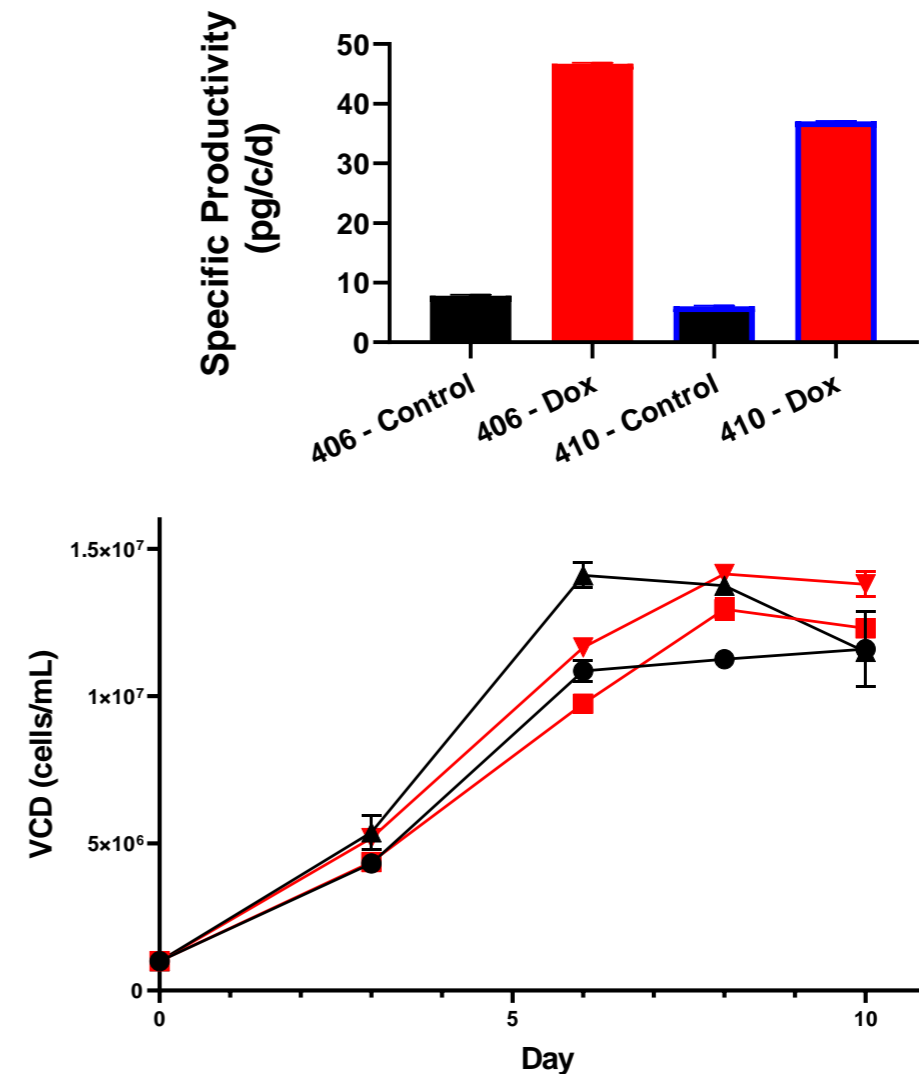
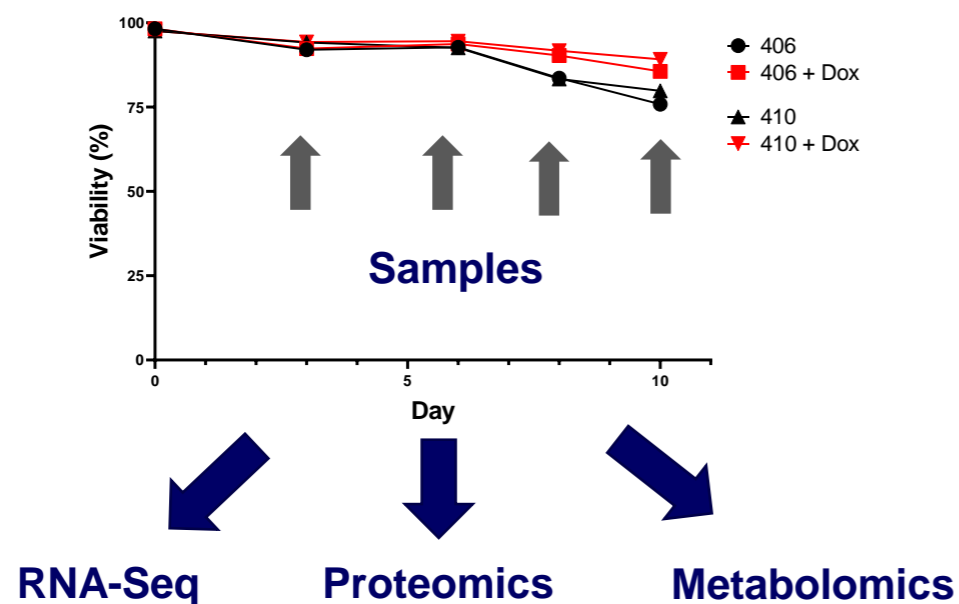
- 6F5 Horizon Cell Line - Tetracycline-controlled transcriptional activation
- Tet-On System doxycycline binding to rtTA promotes expression
- What are the driving forces and responses regulating high IgG1 expression ?



Fed-Batch Production – CHO Cells

Horizon Cell line – IgG1 expression

- Two different clones expressing IgG1 Antibody 1
- Fed-batch with standardized feeding on days 3, 6 and 8
- Cells and spent media collected after counting and before each feed



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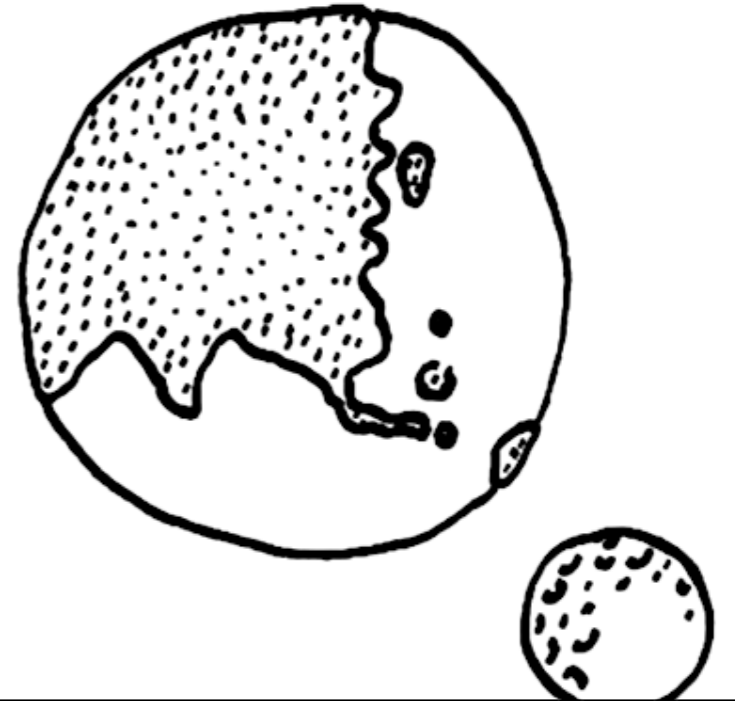
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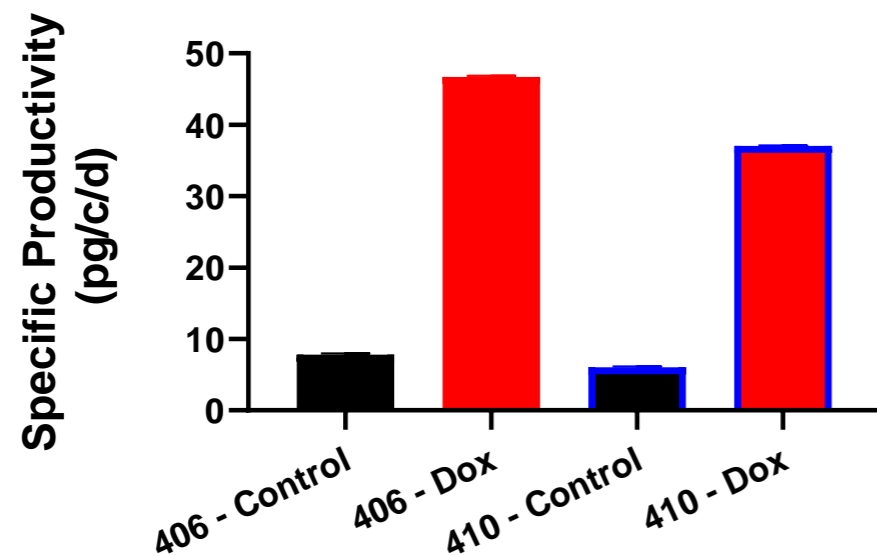
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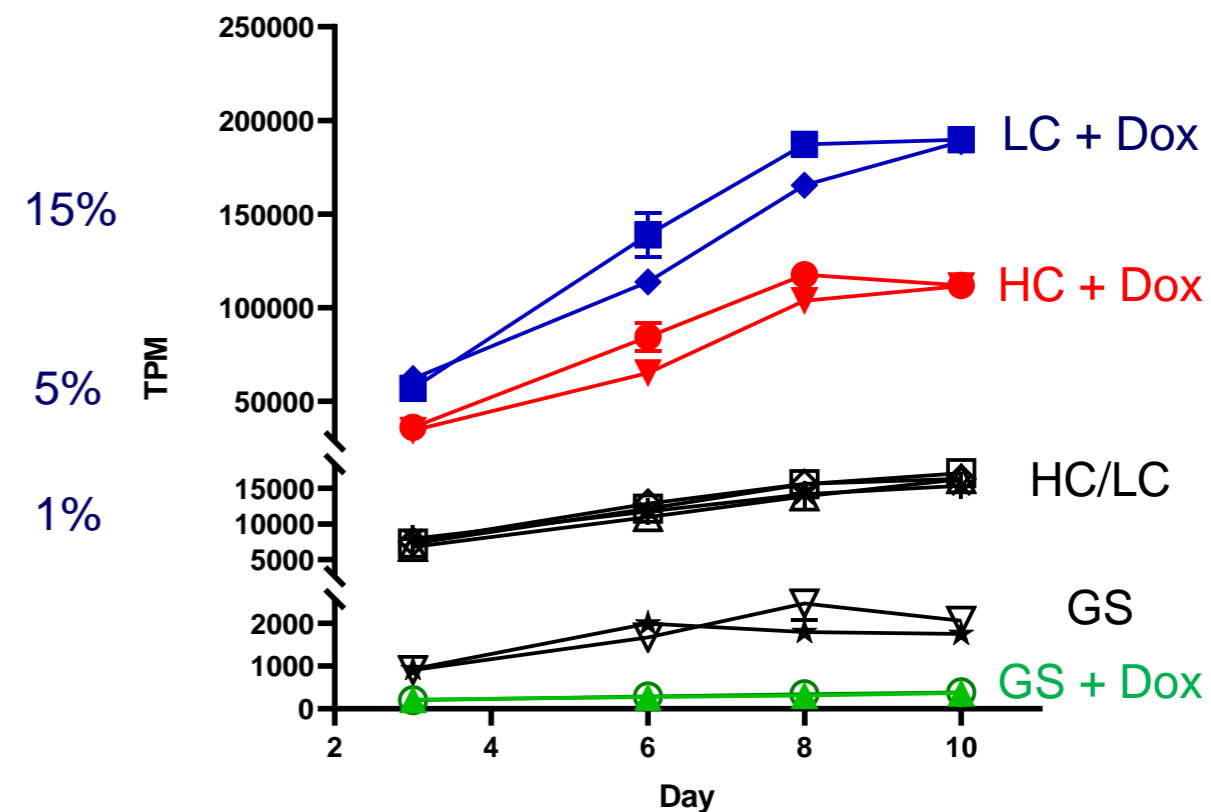
Transcriptome – Antibody Expression

Balance between Constitutive Genes and Antibody 1

- IgG1 HC and LC mRNA as an increasing percentage of the total transcriptome
- IgG1 mRNA trends with specific productivity of the cell line

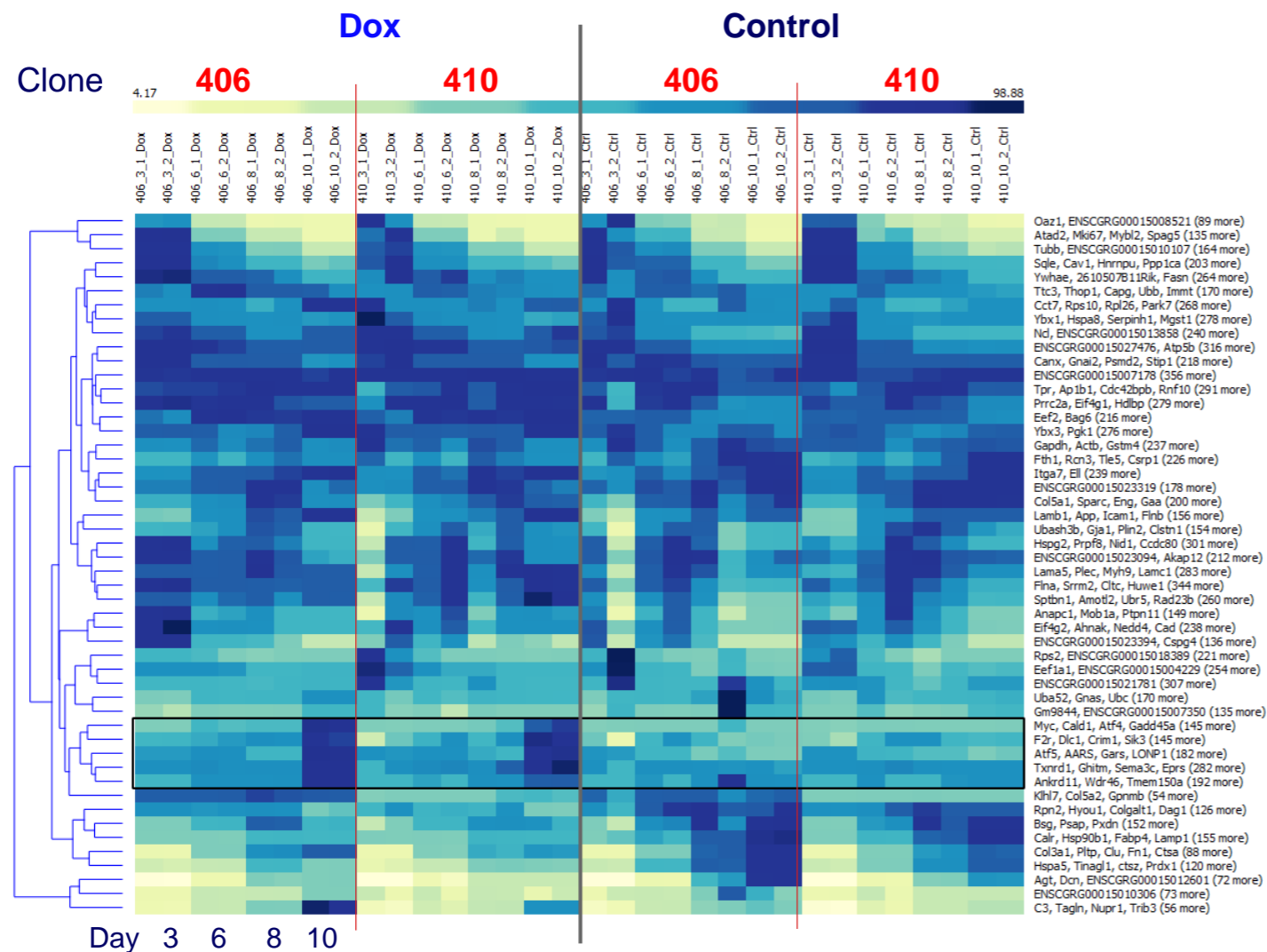


%Transcriptome

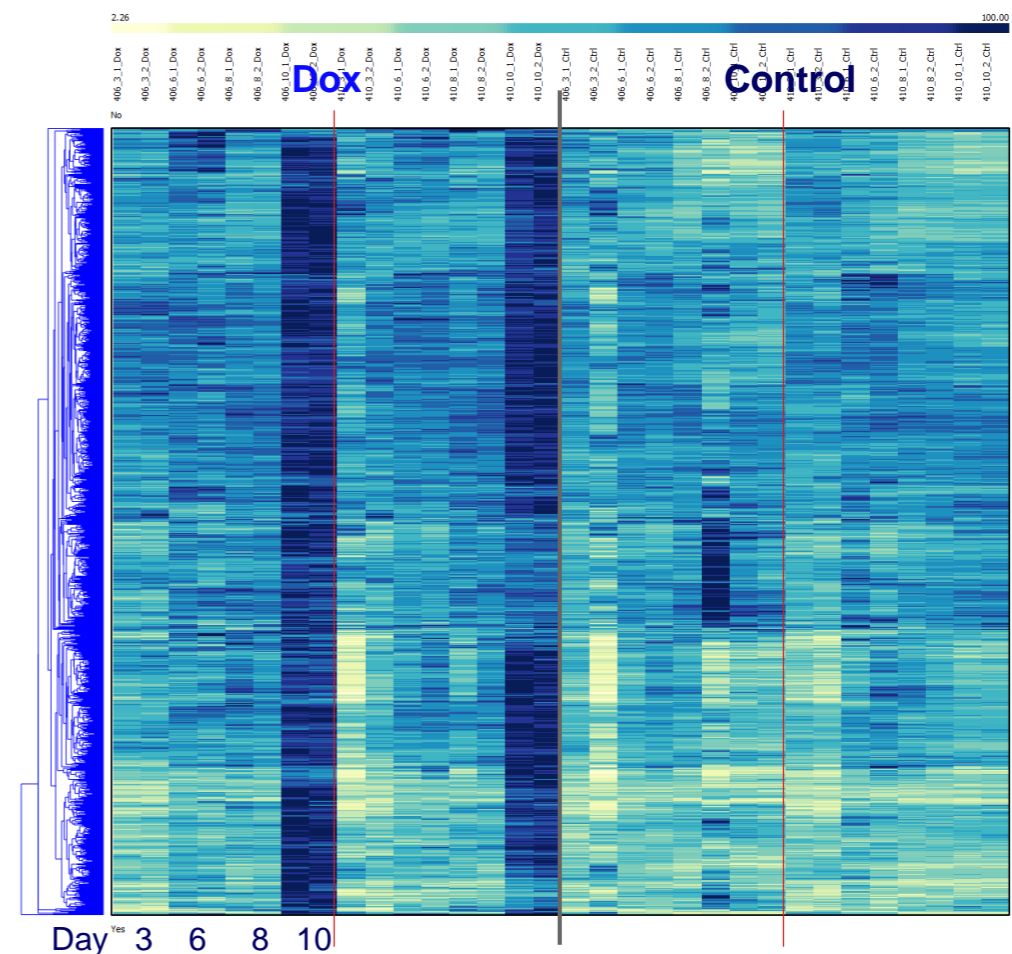


Transcriptomics Heat Map

k-Means clustering - percent of max normalization

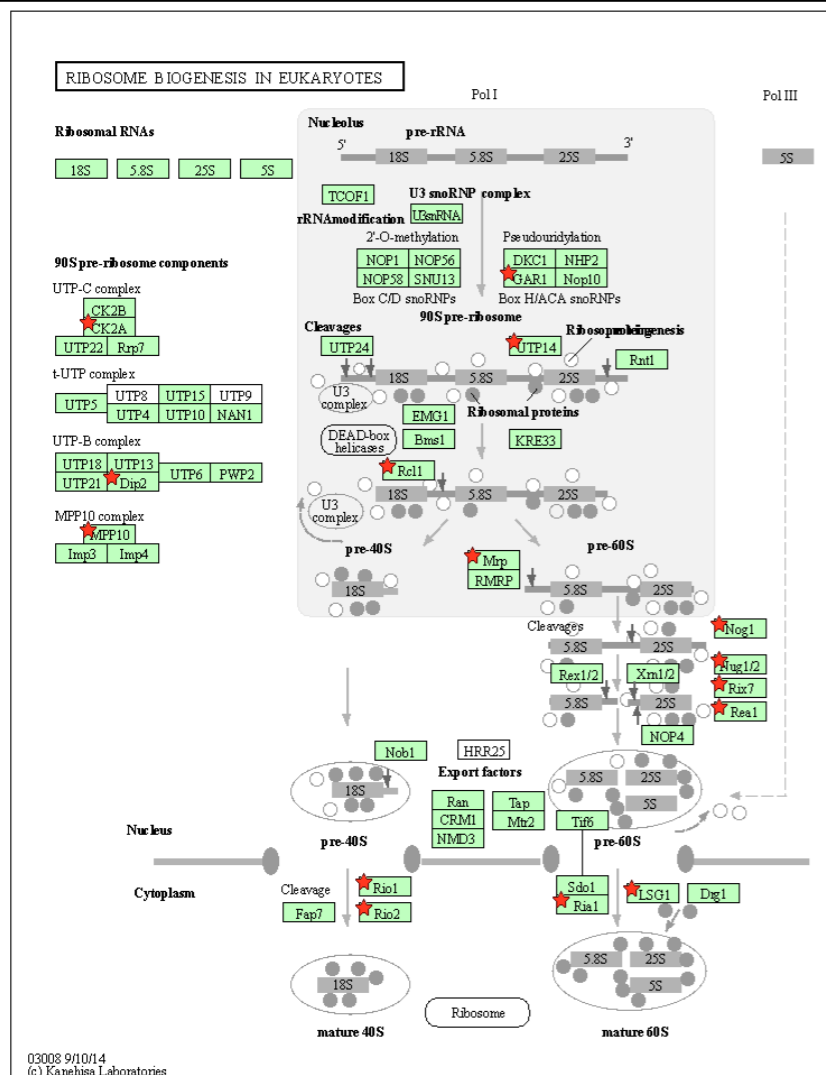


Vector Quantization – Nearest Mean
Grouping of Transcripts



Clustering Transcriptome - Gene Analysis

Database for Annotation, Visualization and Integrated Discovery (DAVID)



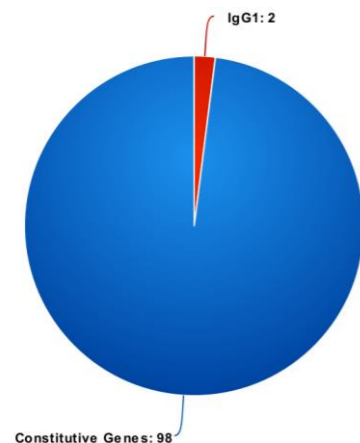
Annotation Cluster 5	Enrichment Score: 4.89	G	Count	P_Value	Benjamini
<input type="checkbox"/> KEGG_PATHWAY	Ribosome biogenesis in eukaryotes	RT	17	3.1E-8	3.6E-6
<input type="checkbox"/> GOTERM_BP_DIRECT	ribosome biogenesis	RT	13	2.1E-4	4.4E-2
<input type="checkbox"/> UP_KEYWORDS	Ribosome biogenesis	RT	10	3.3E-4	3.5E-3

- Transcriptomics analysis shows extensive changes that appear and develop over the life-course of the production
- Dox induction seem to activate Ribosome Biogenesis pathway to sustain high Antibody 1 expression
- These changes likely triggered by fundamental differences in energy allocation – as will be covered later in the presentation

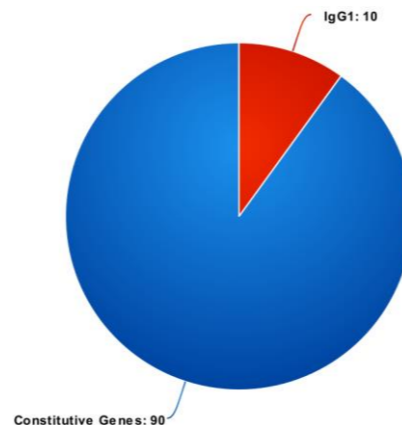
Transcriptome – Are CHO Cells Dynamic?

Balance between Constitutive Genes and Antibody 1

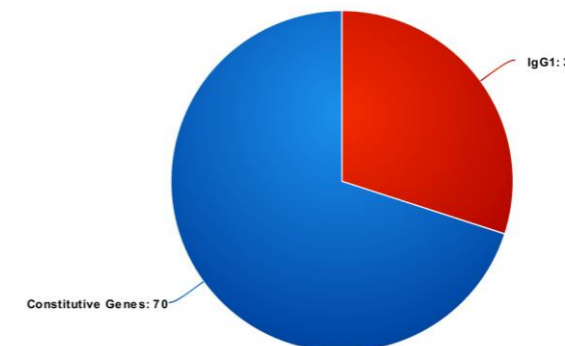
Control



Day 3

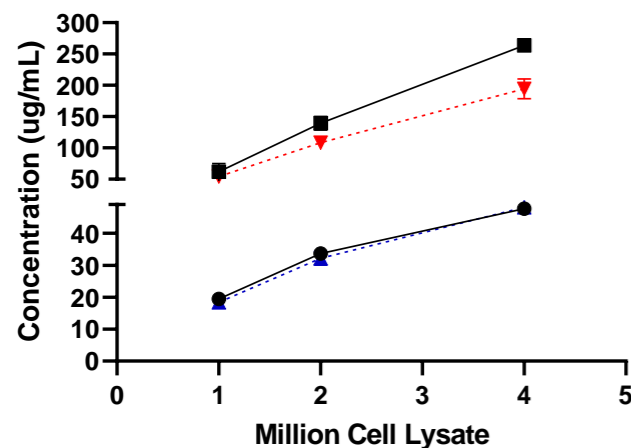


Dox

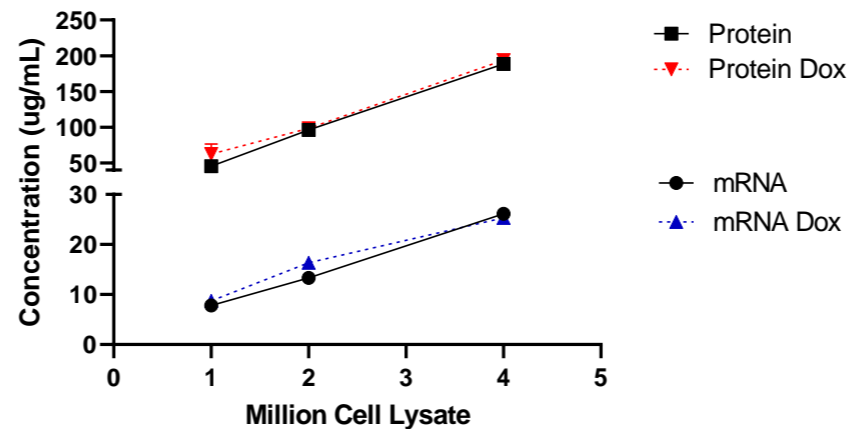


Apparent

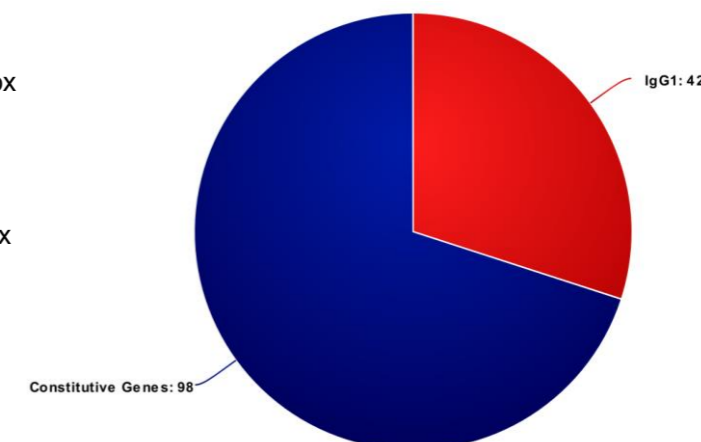
6F5 Antibody 1 Clone 406



6F5 Antibody 1 Clone 410



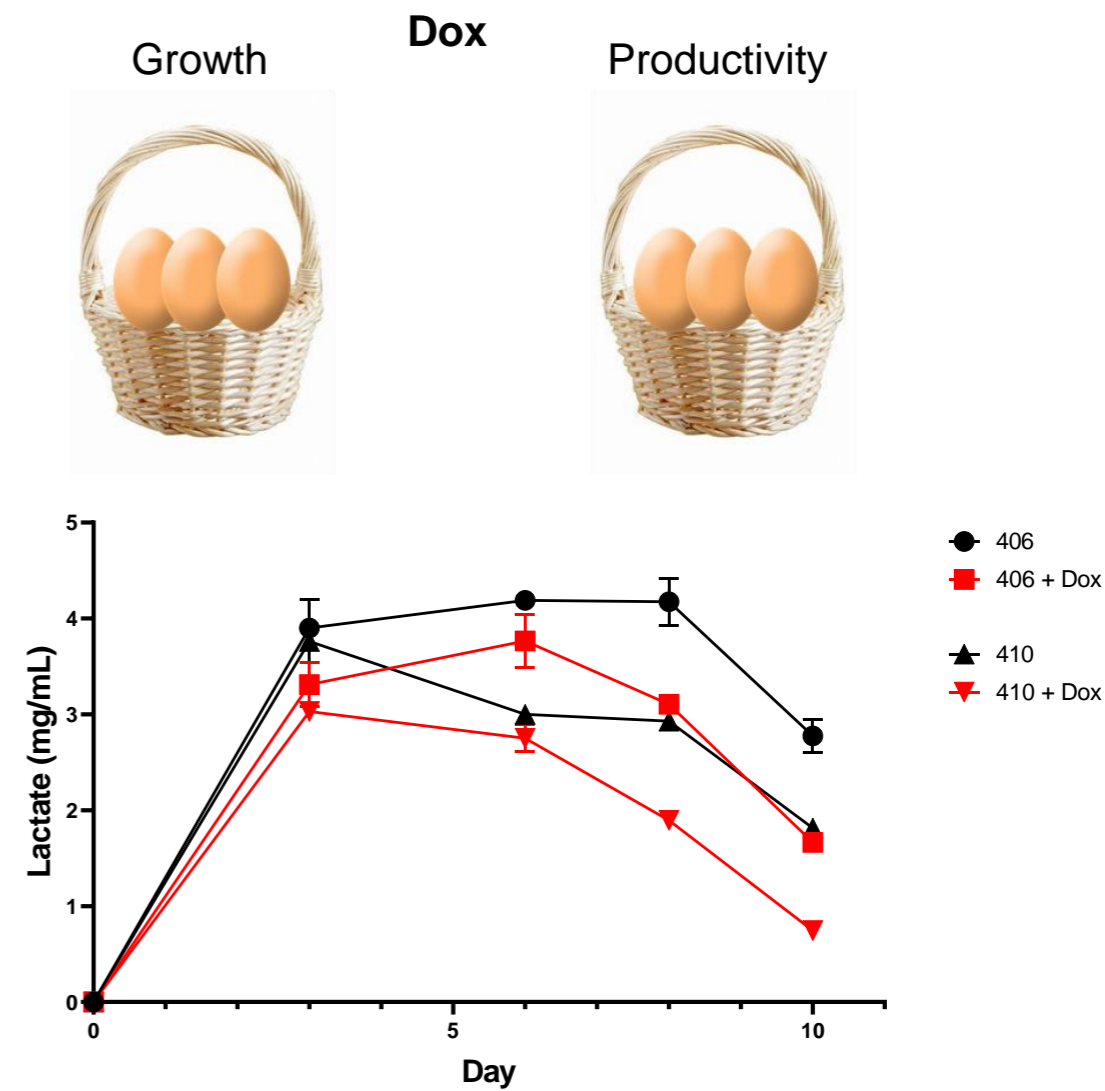
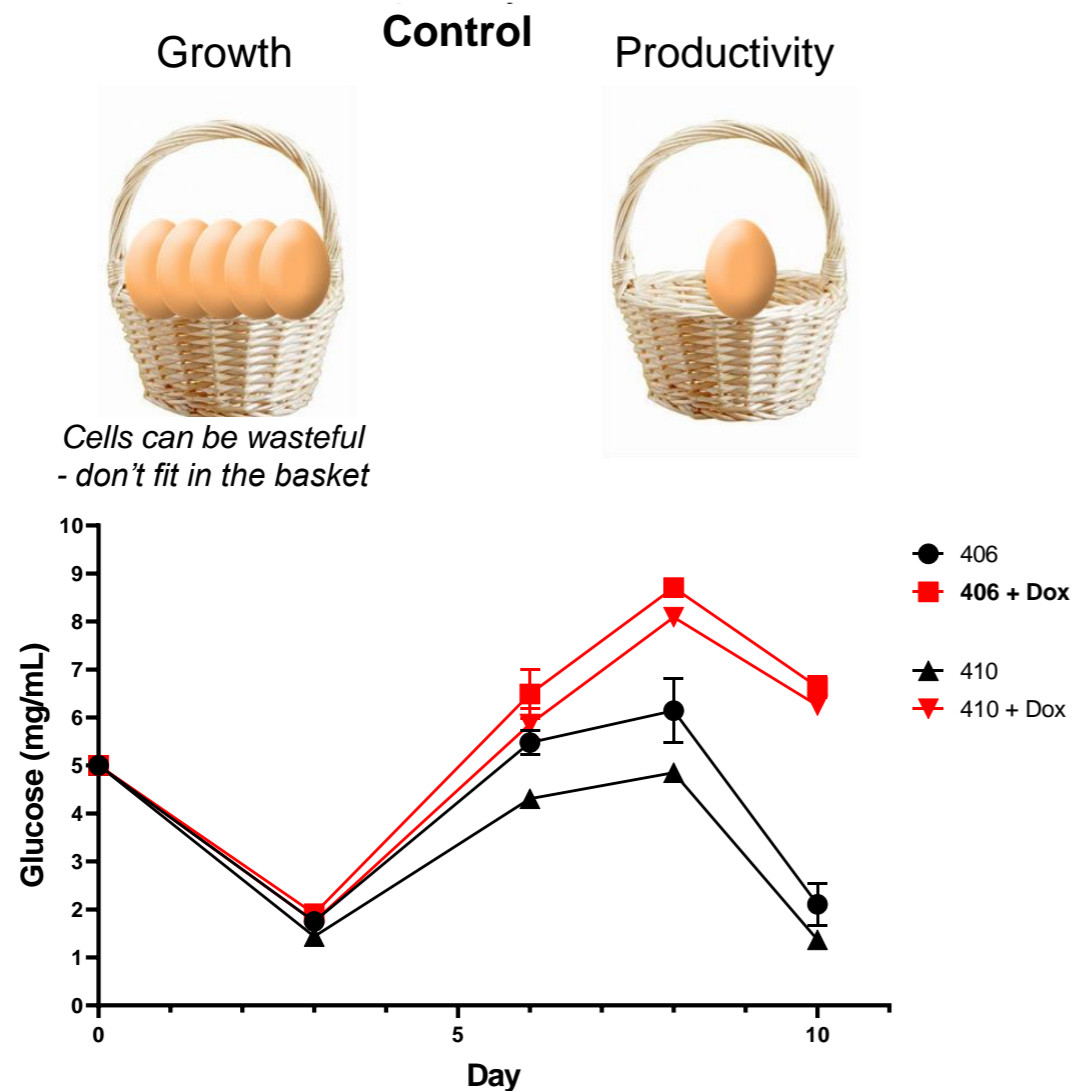
Day 10



Dynamic

Are CHO Cells Dynamic?

Indicators from Proteomic and Metabolomic Energy Allocation



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Transcriptomics

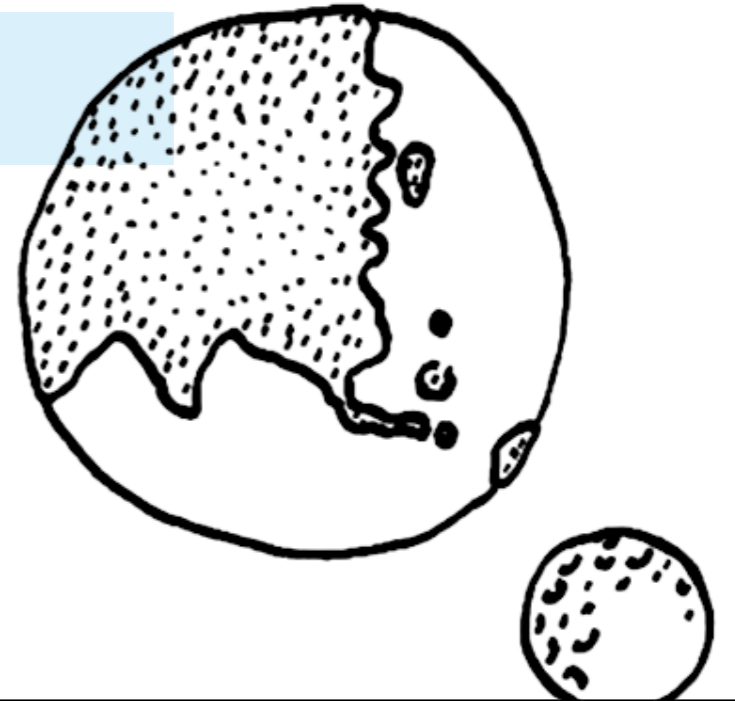
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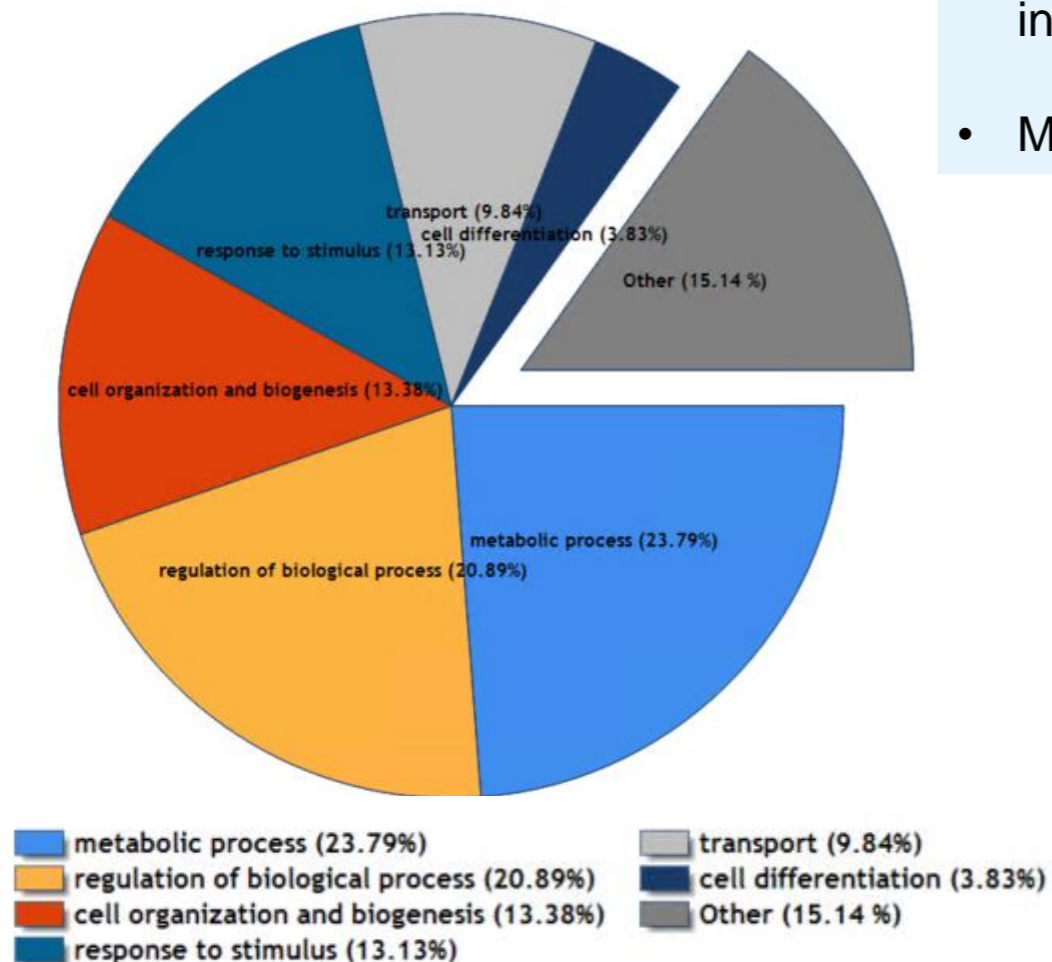
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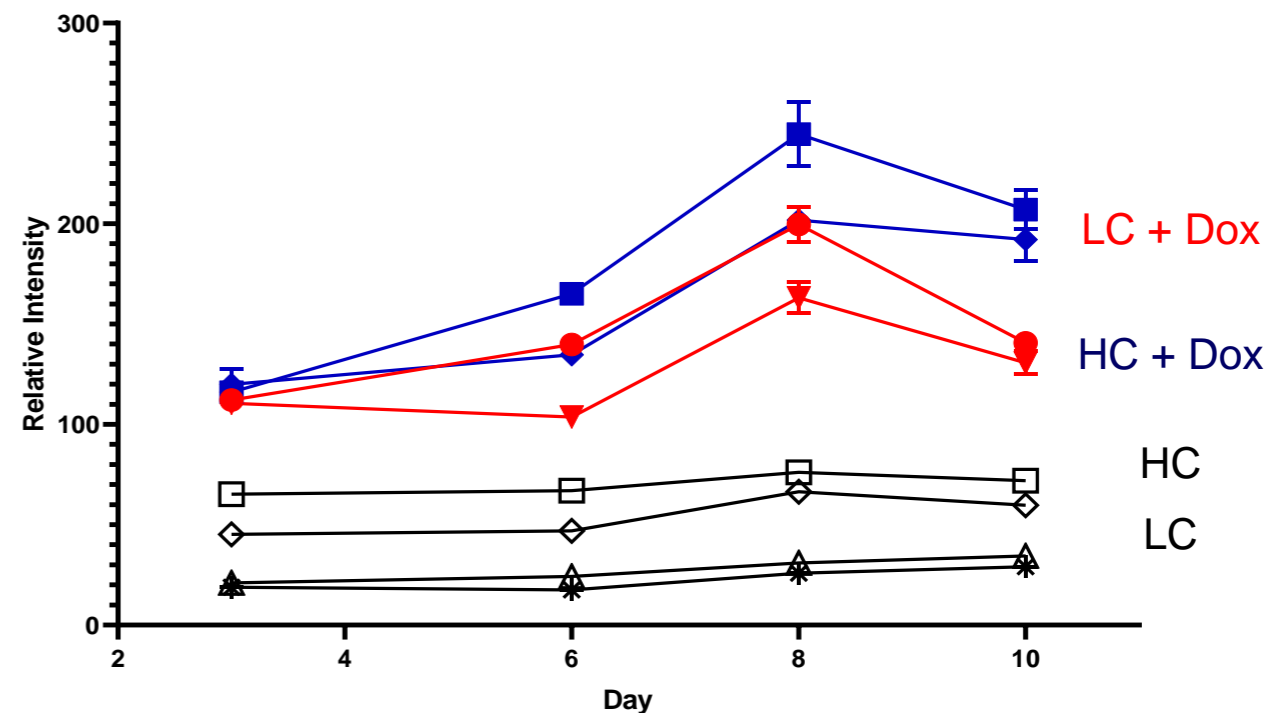
Proteomics: Pathway analysis

Cell normalization of mouse proteins excluding Antibody 1

Biological Process Categories >3%

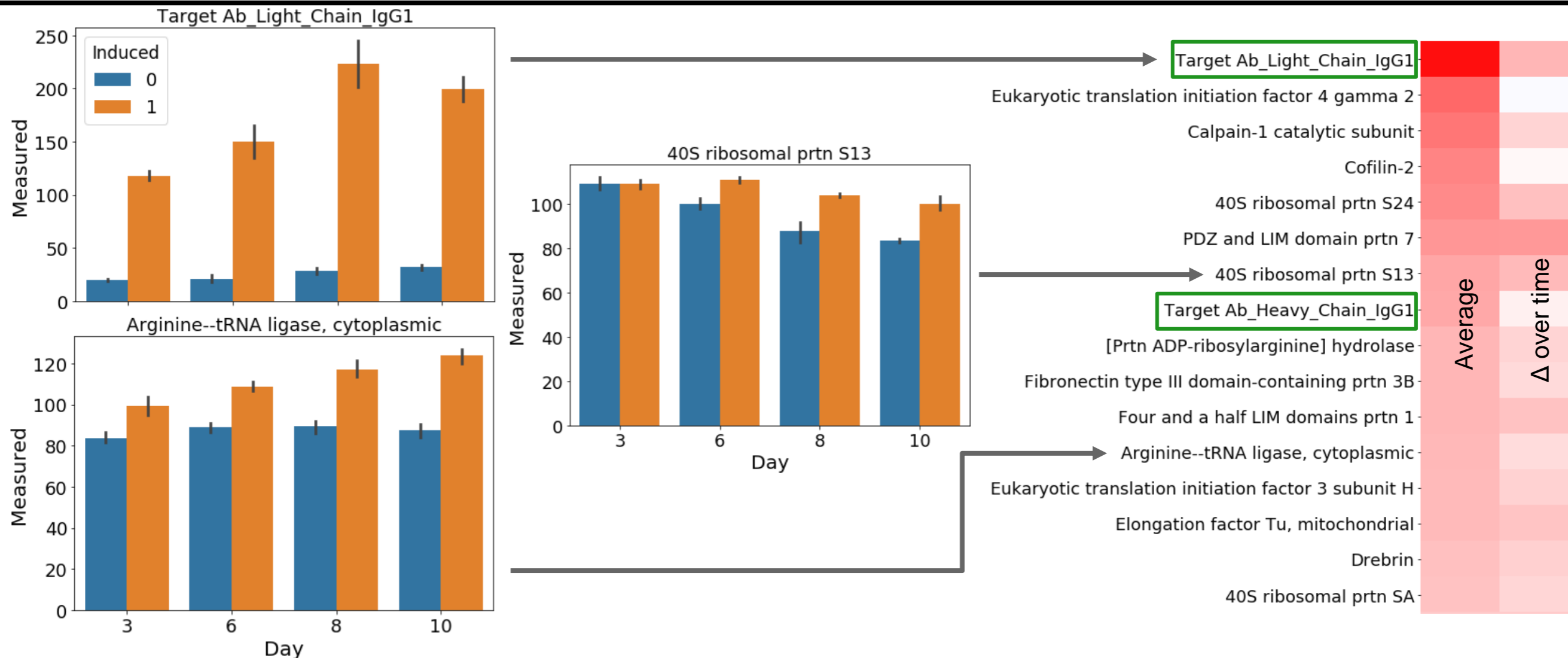


- Proteomic analysis of cellular pathways and bioprocesses relative to intracellular IgG1 expression
- Multivariate analysis can be used to track consistent changes over time



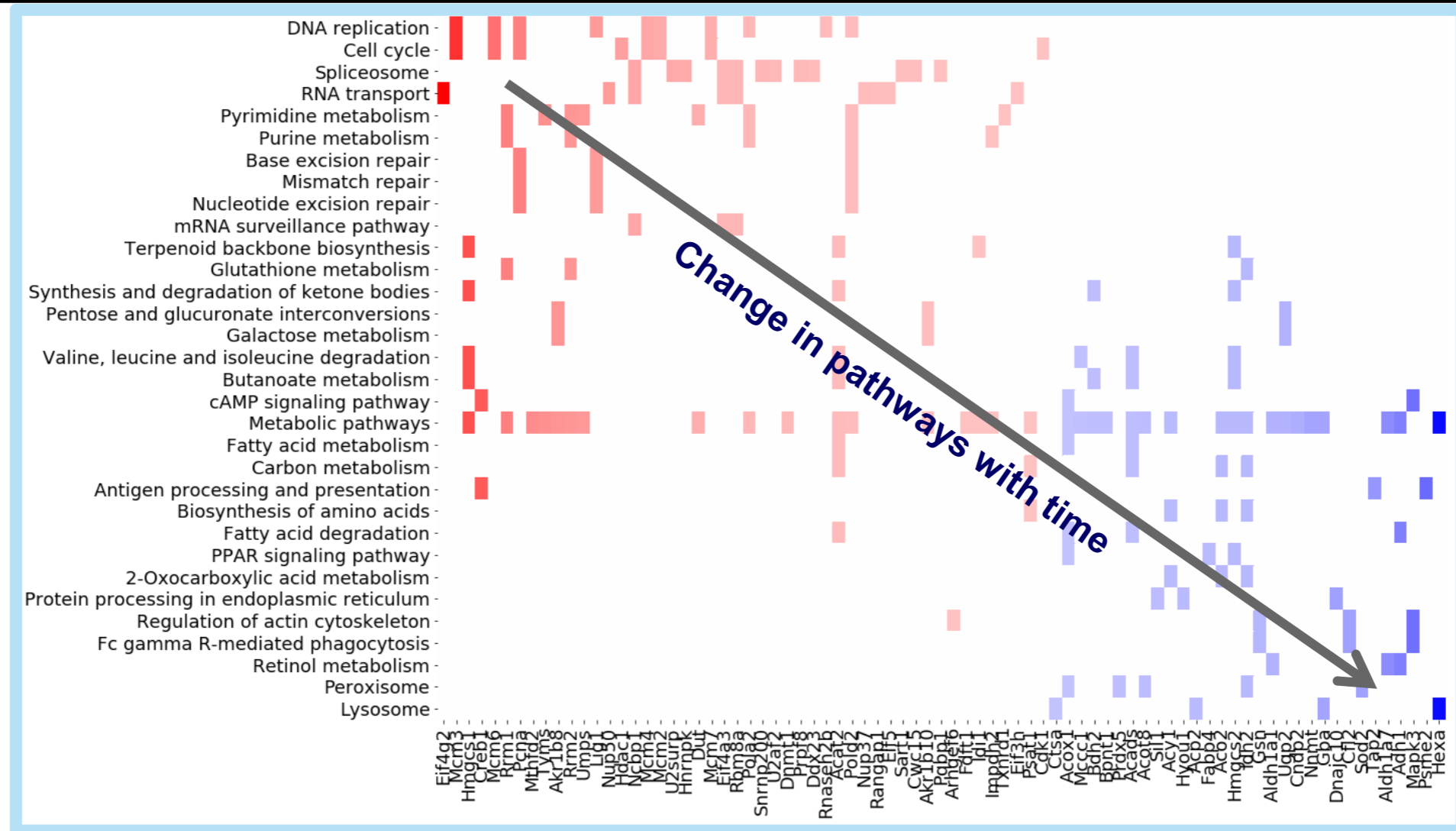
Proteomics: Effect of induction

Species that are increased by induction



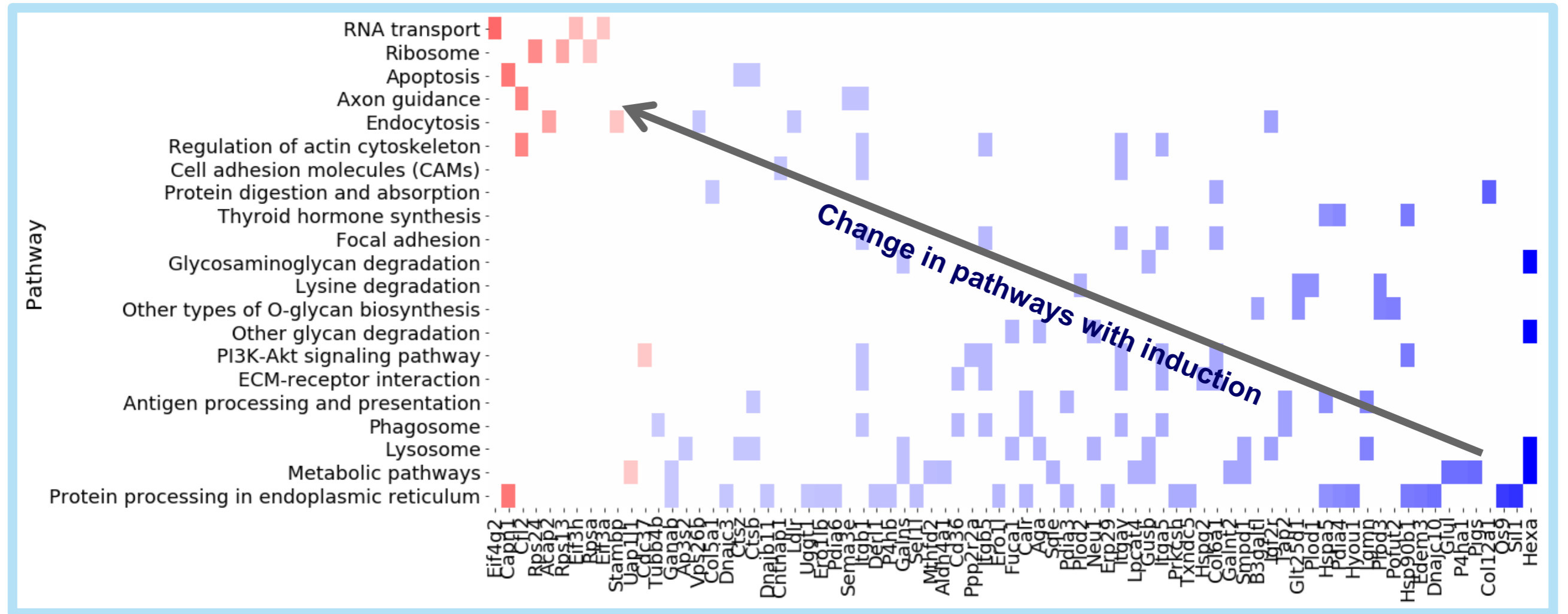
Pathways indicated by proteomics

Red-highlighted pathways increase with induction, blue decrease



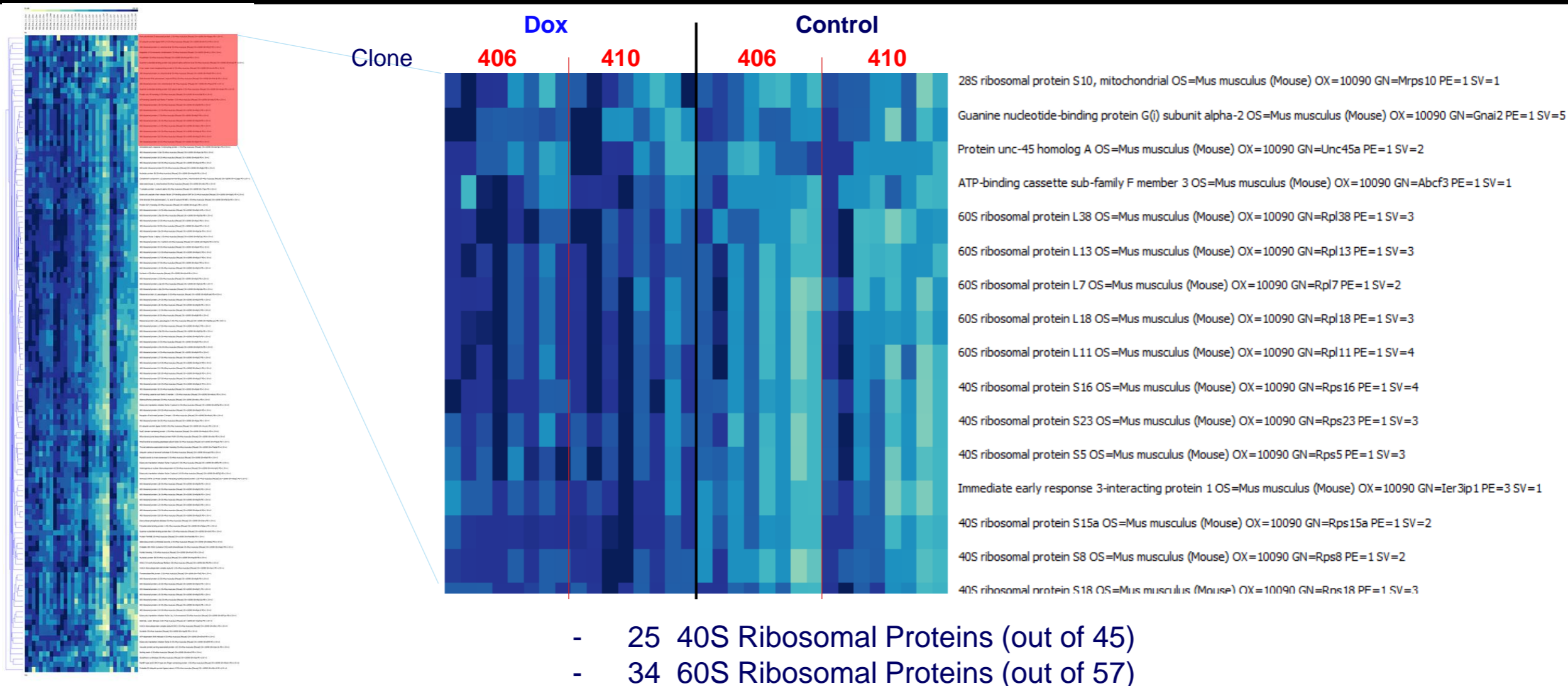
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Proteomics: Ribosomal Proteins

Clustering of 40S and 60S ribosomal proteins



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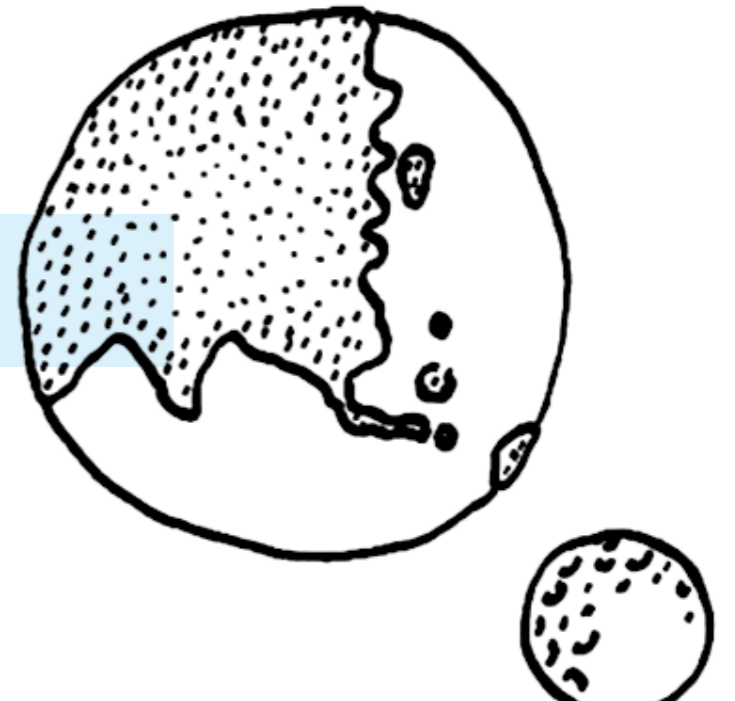
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Summary

Multi-Omic analysis of induced biotherapeutic protein production



- Changes in the transcriptome appear to alter the dynamics of cellular energy allocation
- CHO expression systems seem constrained by the biophysical characteristics of the hosts cells
- This study aims to better understand how we characterize clones on specific productivity and stability for better bioreactor performance
- Highlights the difficulty in further improvements to reduce costs and time for biologics development and manufacturing

Acknowledgements

Just - Evotec Biologics subject matter experts!

Analytical Sciences

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Data Sciences

- Jeremy Shaver, PhD

Cell Line Development

- Sherman Ku, PhD
- Jeff McGrew, PhD

QUESTIONS AND ANSWERS

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