



Transcriptomic Analysis of Early-Stage Developing Retinal Ganglion Cells Induced from Human Stem Cells

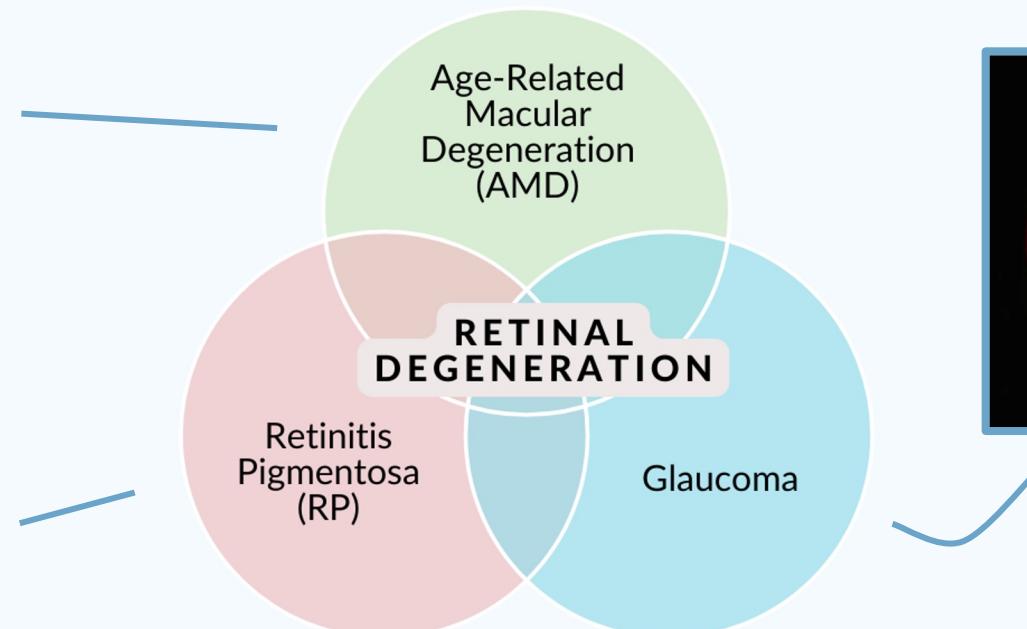
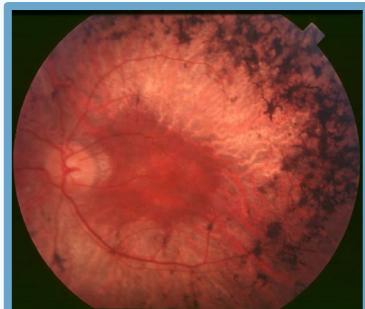
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36th Annual URC Conference (Spring 2023)

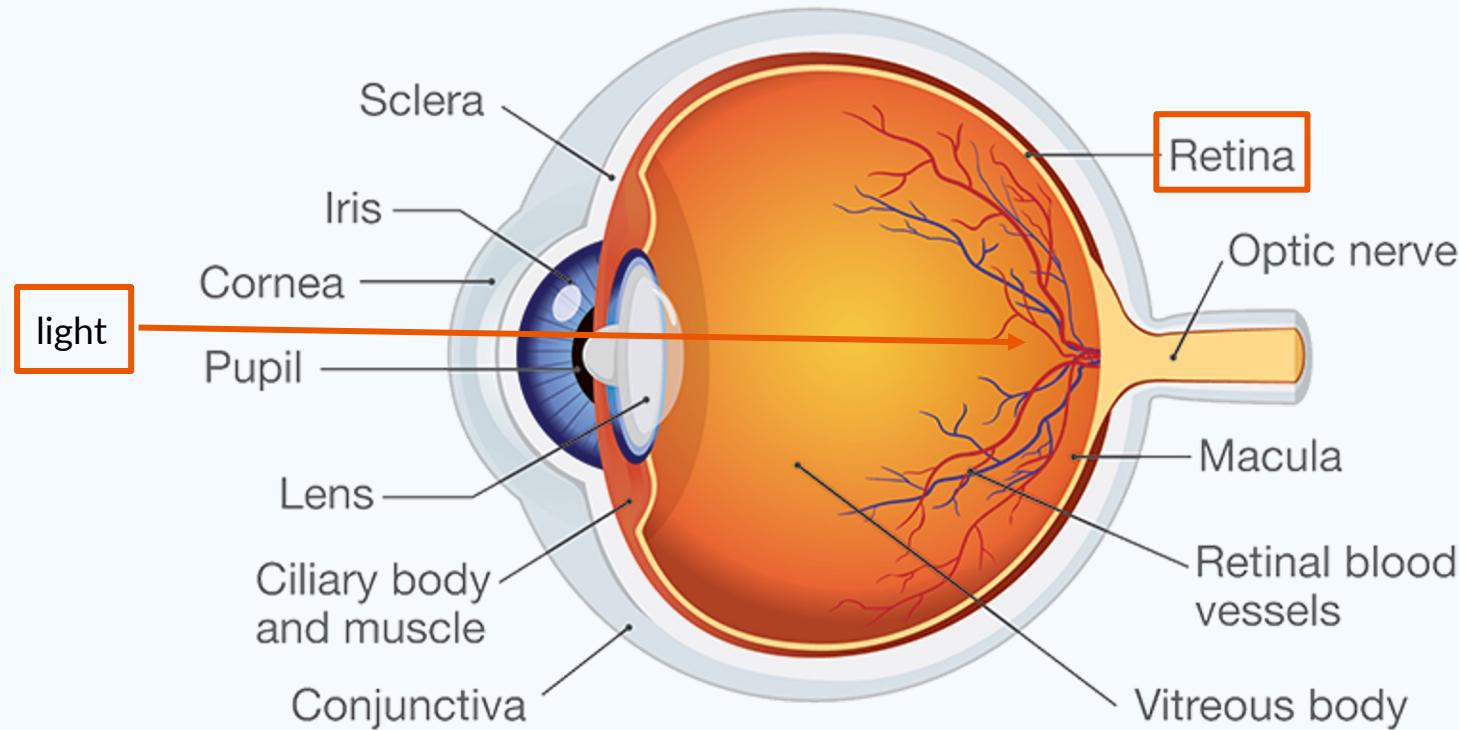
285 MILLION

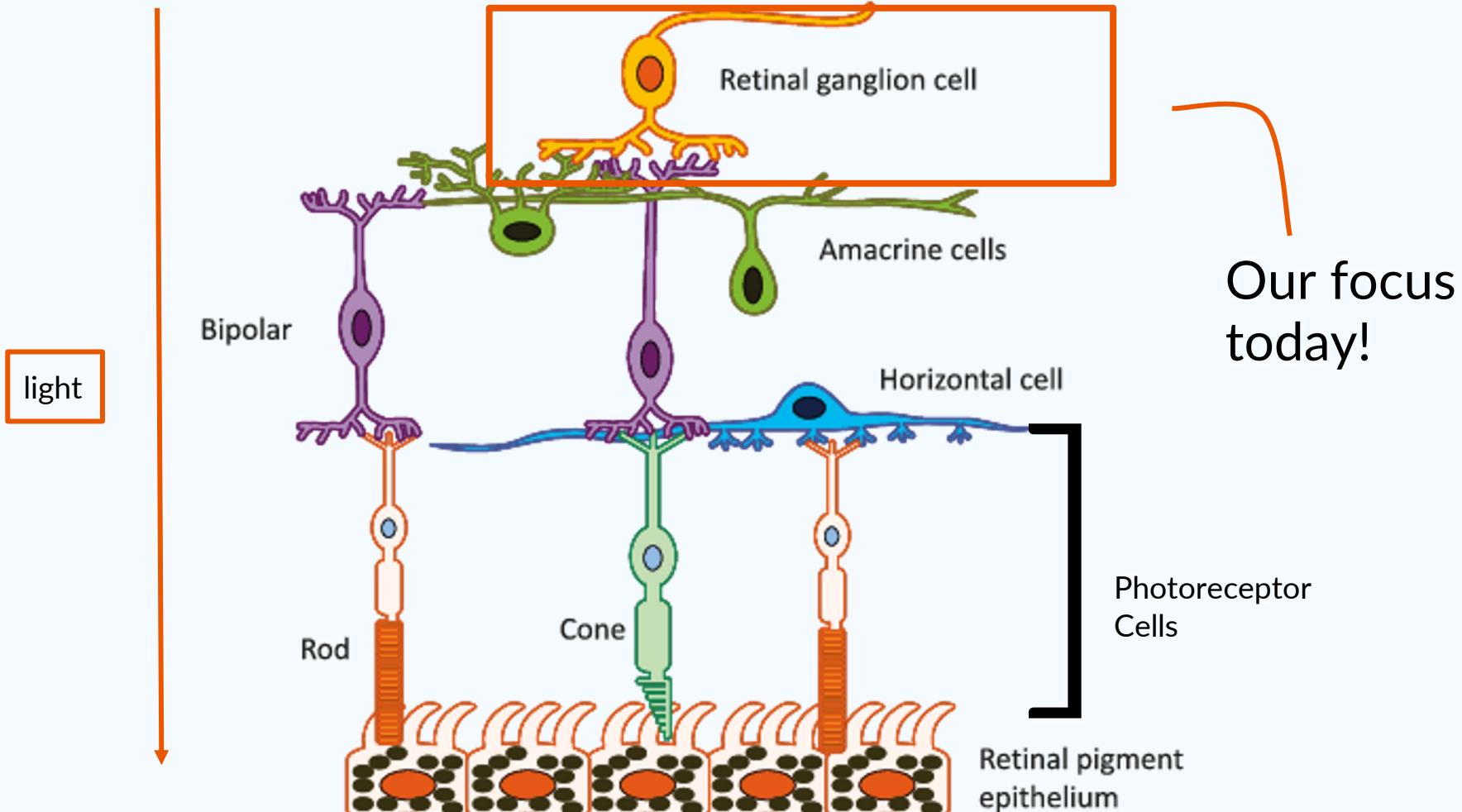
AFFECTED WORLDWIDE (2022)



The Retina & Retinal Ganglion Cells (RGCs)

Human Eye Anatomy

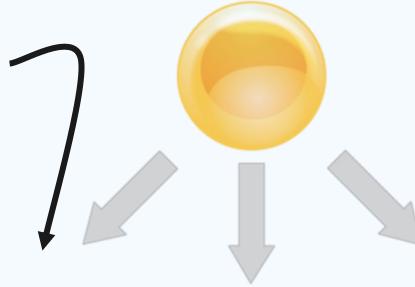




Pluripotent Stem Cells (PSCs) & 'Reprogramming'

IN VIVO:
“points of no return”

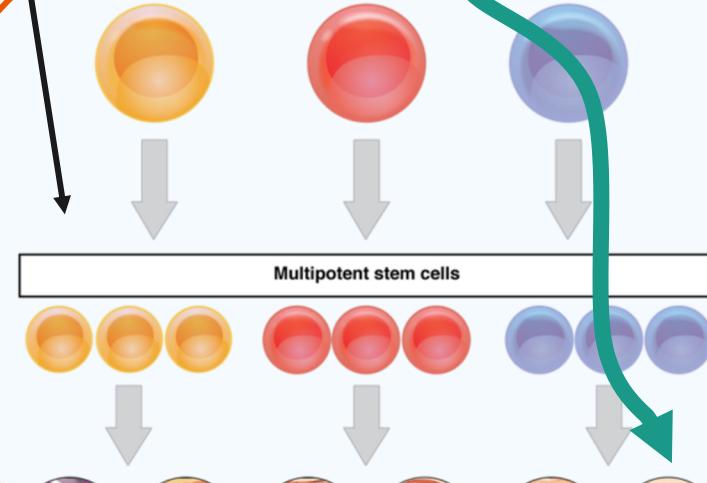
Totipotent embryonic stem cell



IN VITRO:
“induced”
PSCs using
TFs

Pluripotent embryonic stem cells

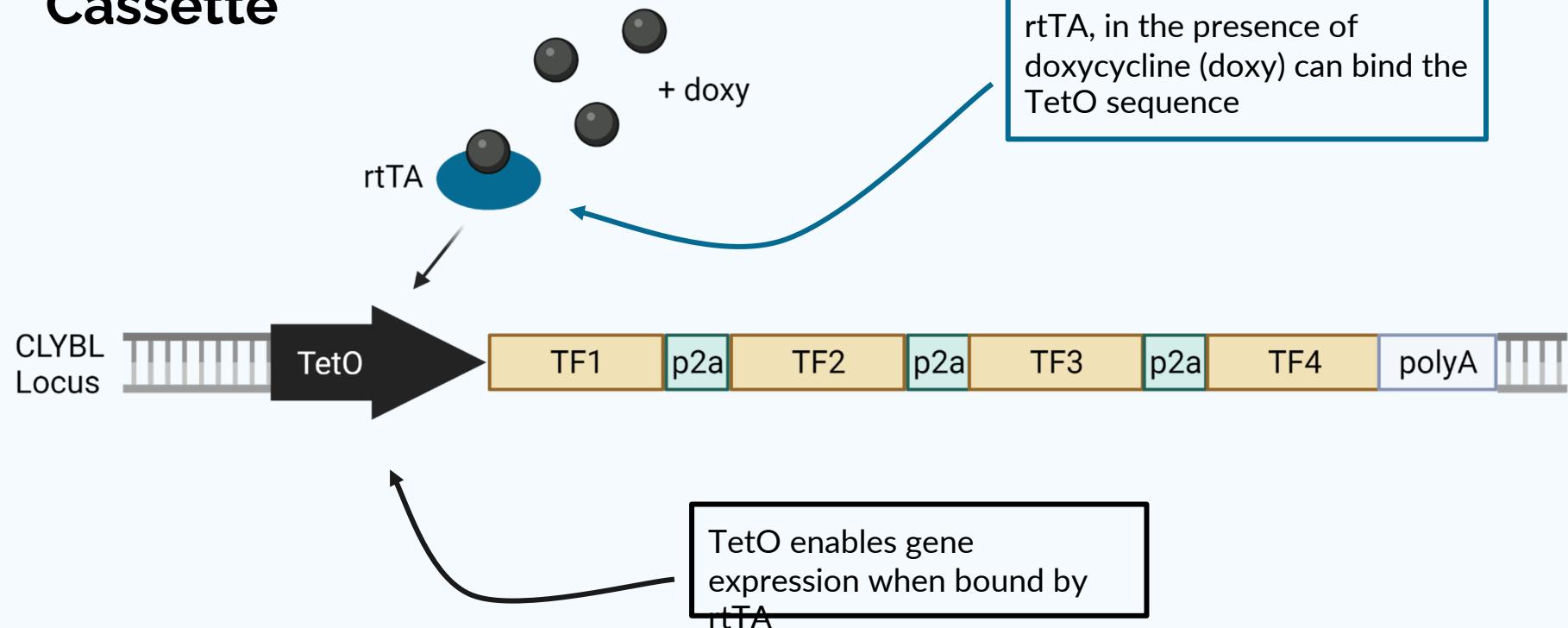
Endoderm line Mesoderm line Ectoderm line



We want to
take iPSCs and
create
“induced”
RGCs

Previously, Wahlin Lab has shown that we can make induced Retinal Ganglion Cells (RGC-iNs) using a specific tetra-transcription factor cassette.

The Doxycycline Inducible Cassette



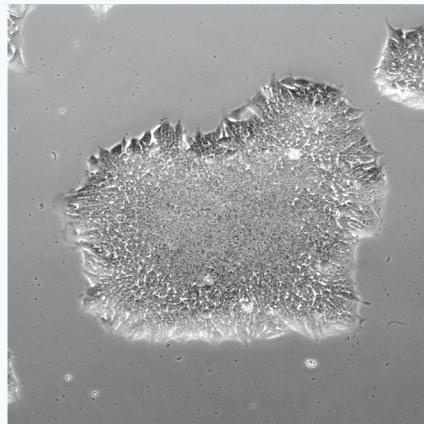
Differentiation of iPSCs into induced RGCs (RGC-iNs)



Kevin Mazo



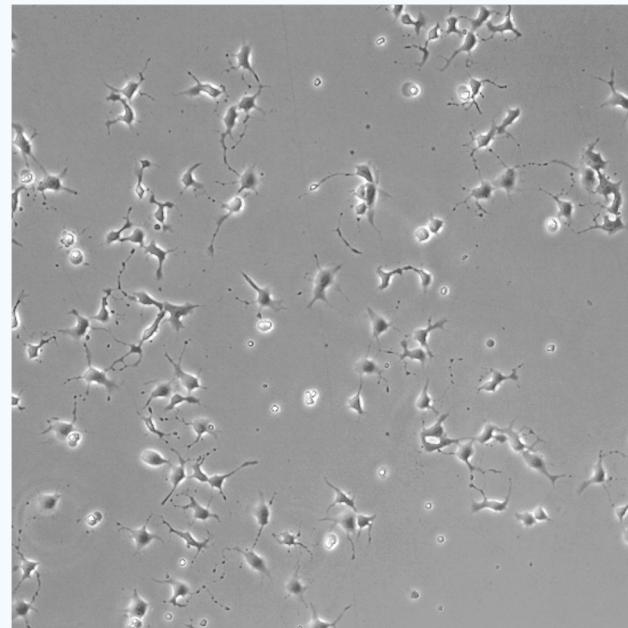
Devansh Agarwal



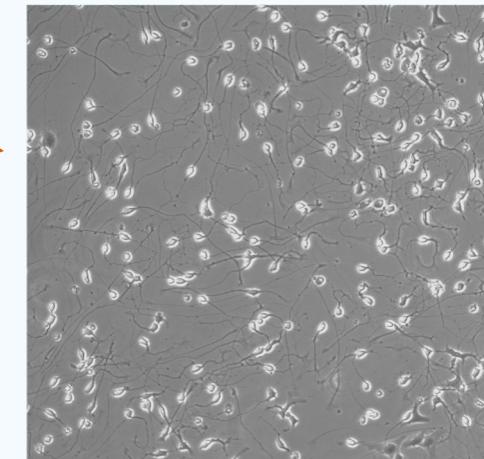
24HR
post-
doxy



1D post-doxy



5D post-
doxy



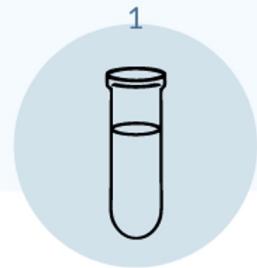
Hypothesis

There are important transcriptional events taking place at early timepoints in RGC-iN development which can guide future efforts in regeneration.

Bioinformatics Analysis

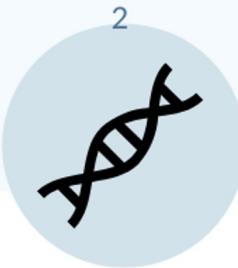
Sequencing & Data Visualization

Summary of Transcriptomic Analysis



RNA Extraction

Take cells with the NAIP2 expression cassette (experimental group) and cells without NAIP2 expression cassette (CTL) and extract RNA.



RNAseq

Send RNA samples out to perform sequencing, obtain reads.



Galaxy Workflow

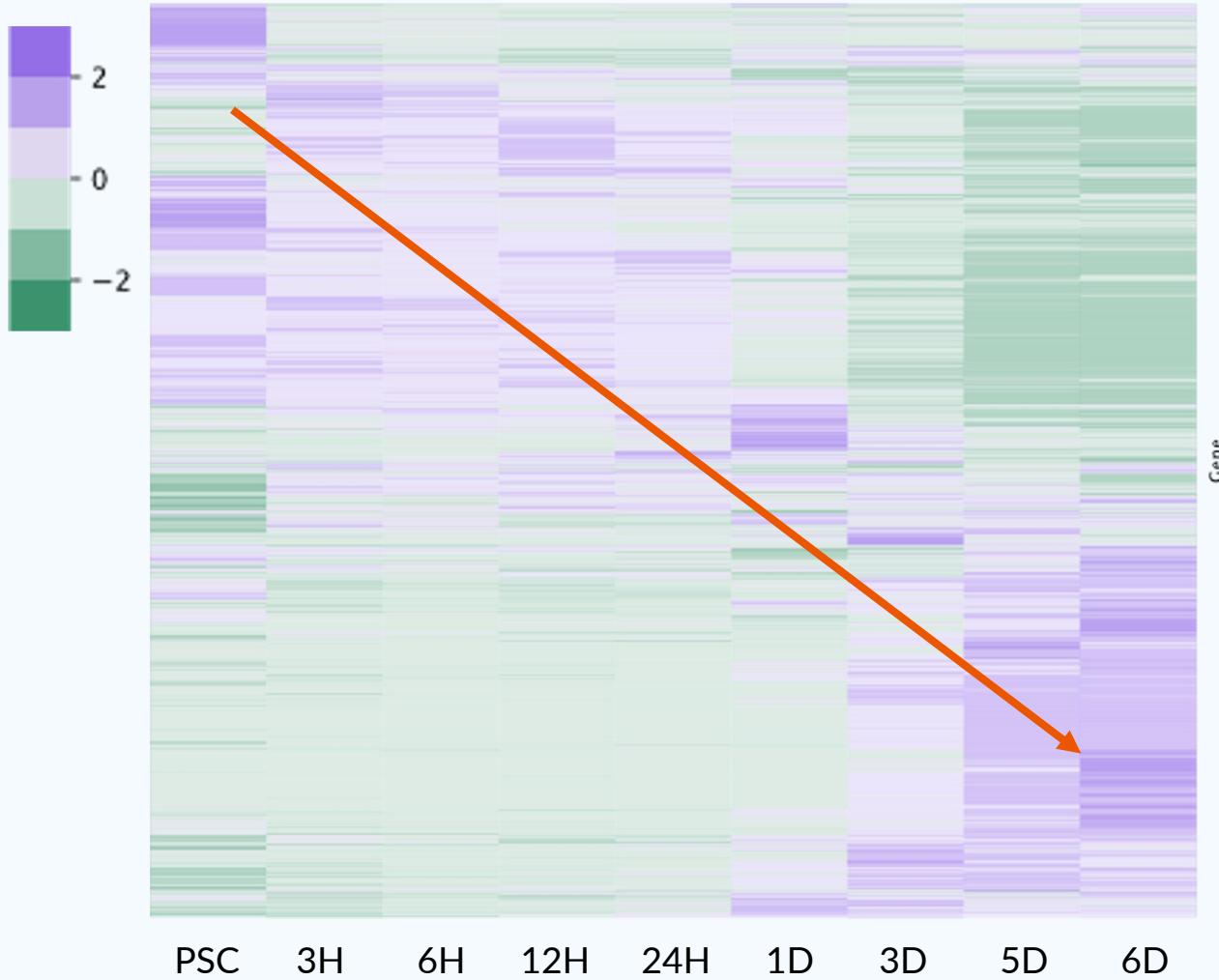
Process raw data from RNAseq so that we can interpret our results. Additionally, perform quality control checks on the data.



Data Visualization & Analysis

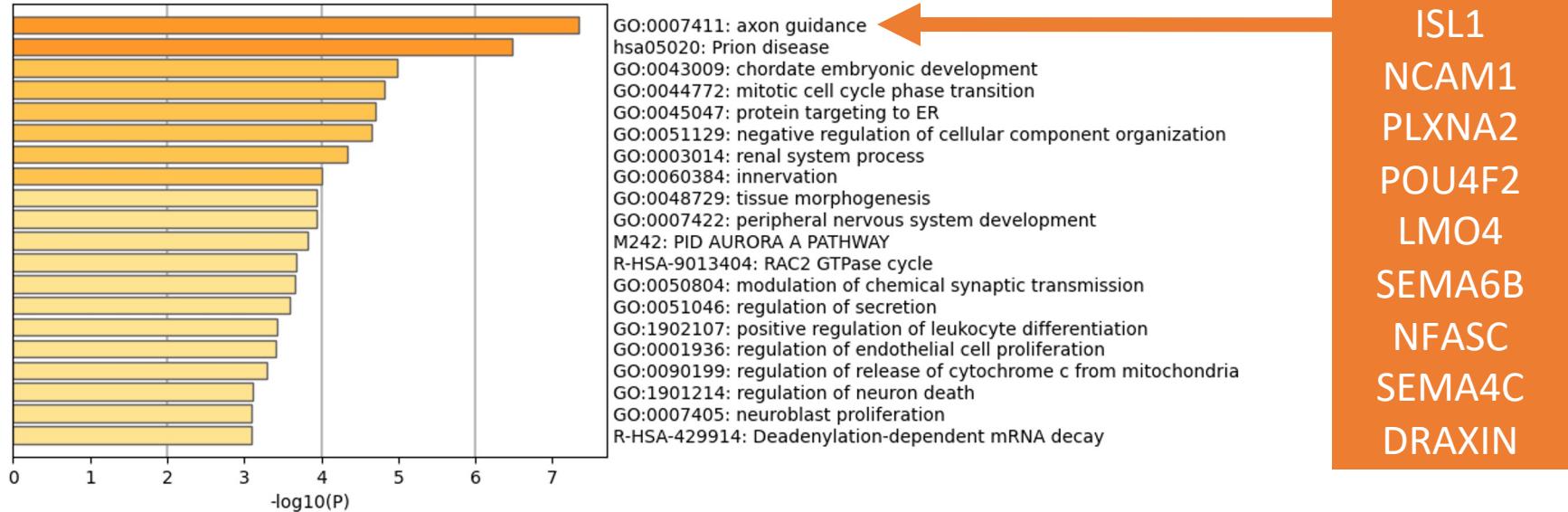
Using expression values outputted by the Galaxy workflow (specifically DESeq2), plot data with various methods to show trends and show an RGC-like expression profile in our experimental group.

Temporal Patterning of Gene Expression Levels



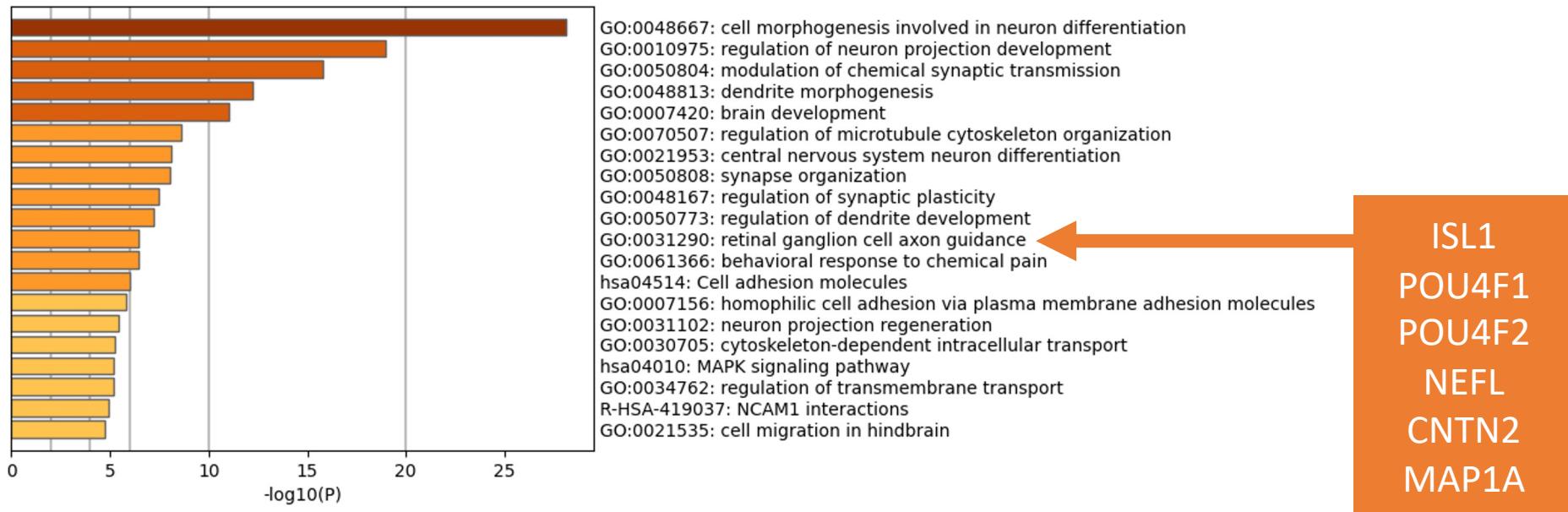
We can see a clear trend indicating a dramatic shift in transcription

Neuronal genes and pathways appear in about 24 hours!



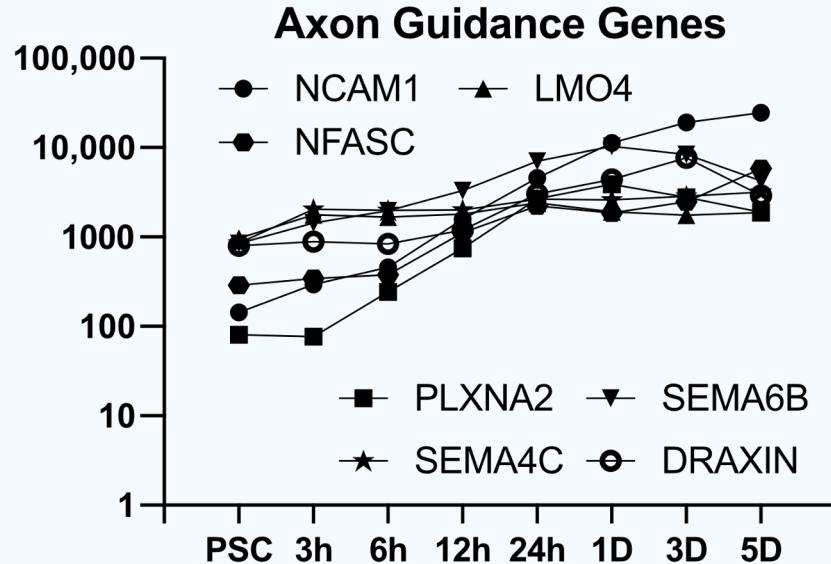
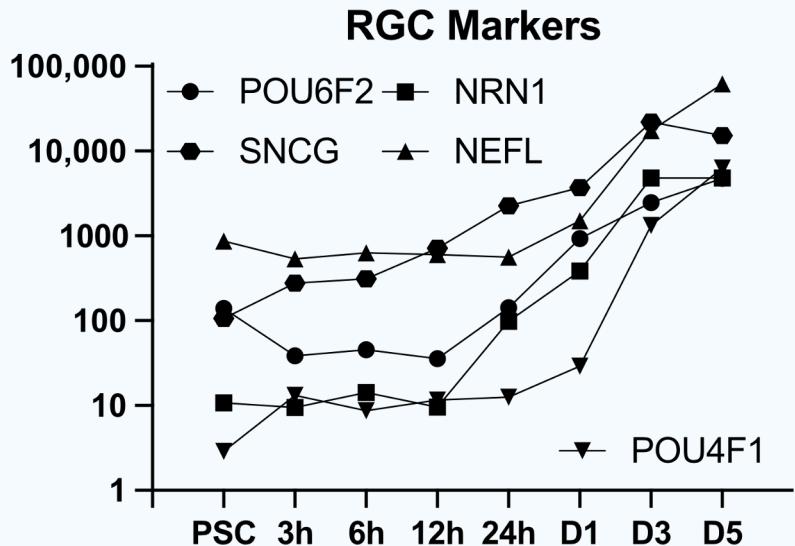
Metascape Pathway Analysis from Top Differentially Expressed Genes in PSCs and 24h pre-treated PSCs

By Day 5, almost all the enriched pathways reflect a neuronal fate, and we begin to see RGC-specific pathways as well



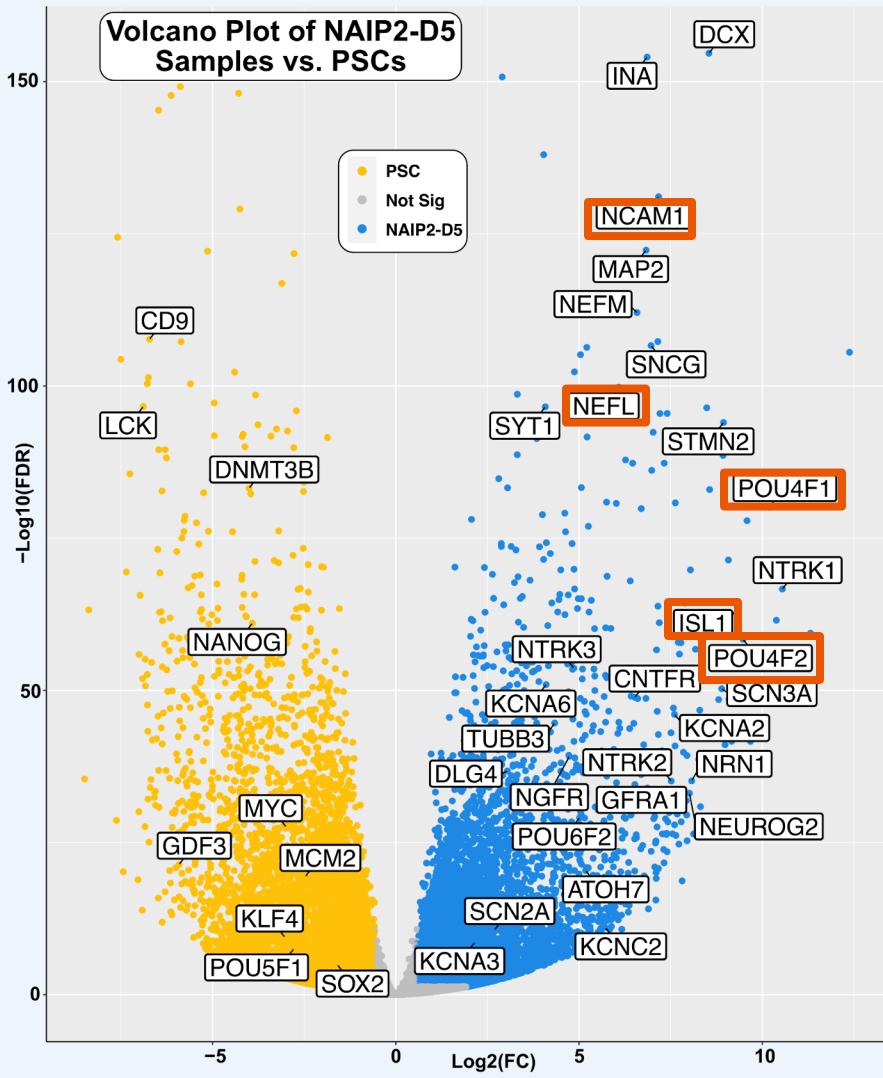
Metascape Pathway Analysis from Top Differentially Expressed Genes in PSCs and D5 pre-treated PSCs

Patterns of Specific Genes of Interest



We can analyze specific genes of interests using charts like line graphs and volcano plots (next slide).

Volcano Plot of NAIP2-D5 Samples vs. PSCs



Exploring Differentially Expressed Genes Between PSCs and NAIP2-D5 Cells

Here we have labeled some genes that we know to be important, but volcano plots are also good tools for de-novo analysis.

Future Directions

- Using results from the bioinformatic analysis, we can potentially refine our induction protocols
 - Investigating key processes from pathway analysis
 - Investigating the ways in which key transcription factors/genes function
- Single cell analysis of RGC-iNs
- Photoreceptor Cells (PRs) are another big area of cell death leading to retinal disorders
 - induced PRs

Special Thanks!



Wahlin Lab

Dr. Karl J. Wahlin
Devansh Agarwal
Kevin Mazo
Ali Dragozova



Undergraduate Research Conference

Prof. Claire Meaders
Dr David Artis
Bev Fruto
URC Team