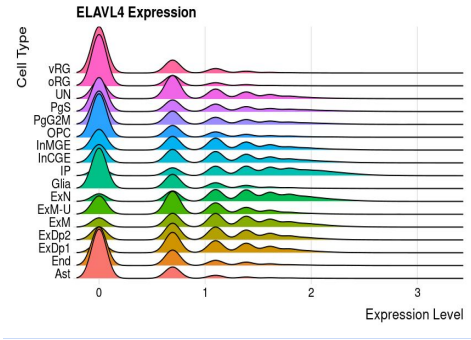
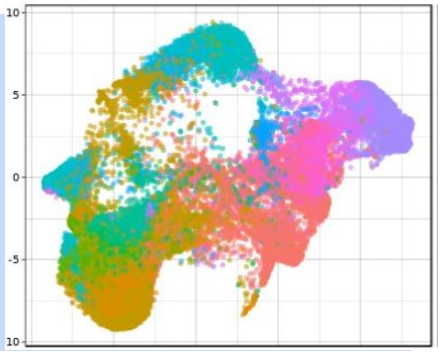


IDEA AlzApp / D2ARC
Spring / Summer 2022



NSCI

The Neural Stem Cell Institute, Rensselaer NY

Sally Temple (Director)

Nathan Boles (PI)

Tom Kiehl (PI)

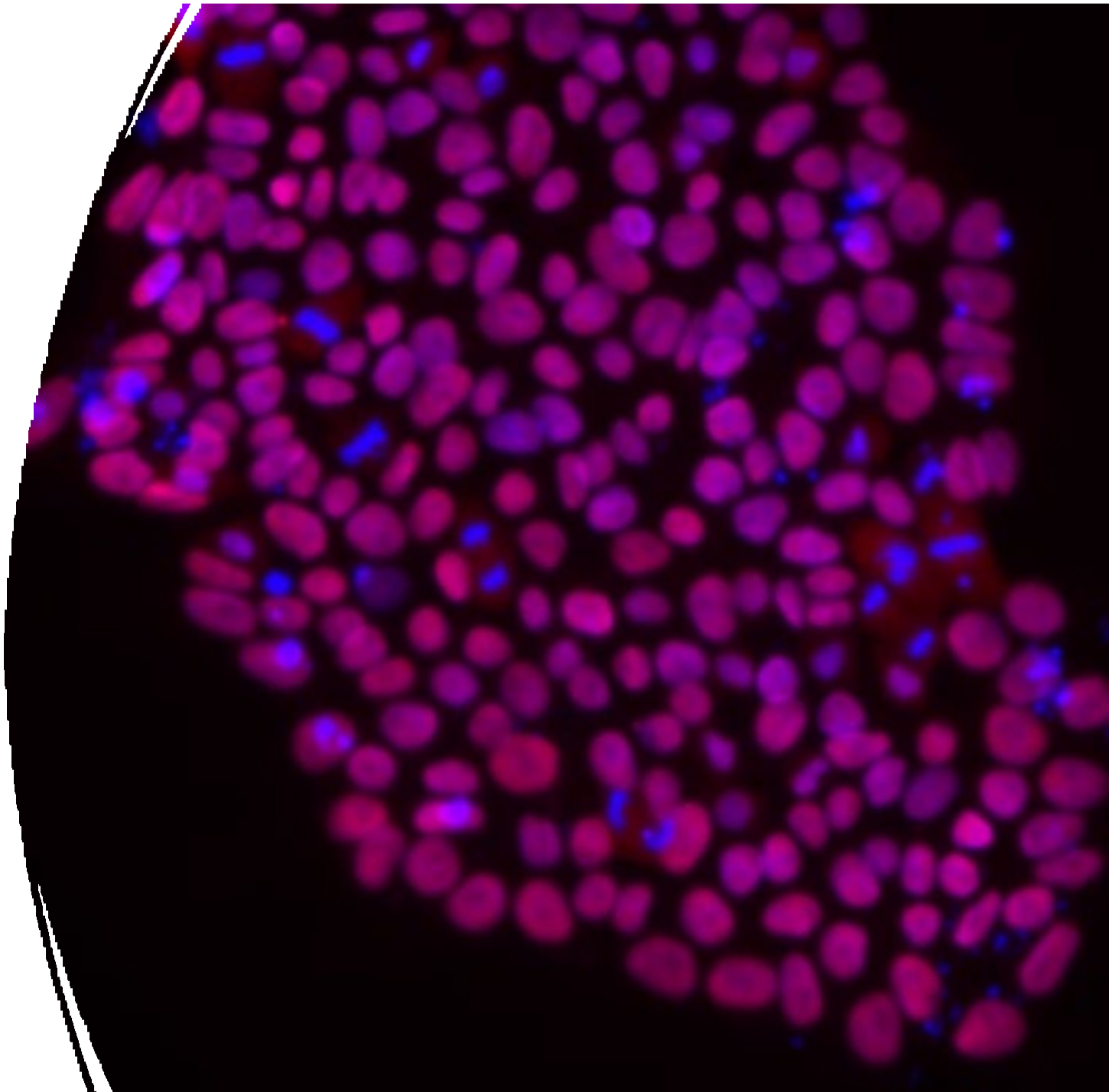
IDEA

Rensselaer **Institute for Data Exploration and Applications**

Kristin Bennett (Associate Director)
John S. Erickson (Director of Research Ops)
Keith Fraser (PI, Biological Sciences)

Rachael White
(UG, Computational Biology)
Haowen He
(UG, Mathematics)

Using Stem Cell Technology to Combat Age-Related Neurodegenerative Disease



Frontotemporal Dementia

Decades of healthy life before diagnosis

KEY QUESTION: What changes occur in tauopathy brains prior to clinical symptoms??

Clinical Symptoms Diagnosis

The Dataset: scRNAseq data from Bowles et al., 2021

Cell

Article

ELAVL4, splicing, and glutamatergic dysfunction precede neuron loss in *MAPT* mutation cerebral organoids

Kat Bowles Alison Goate

Mount Sinai

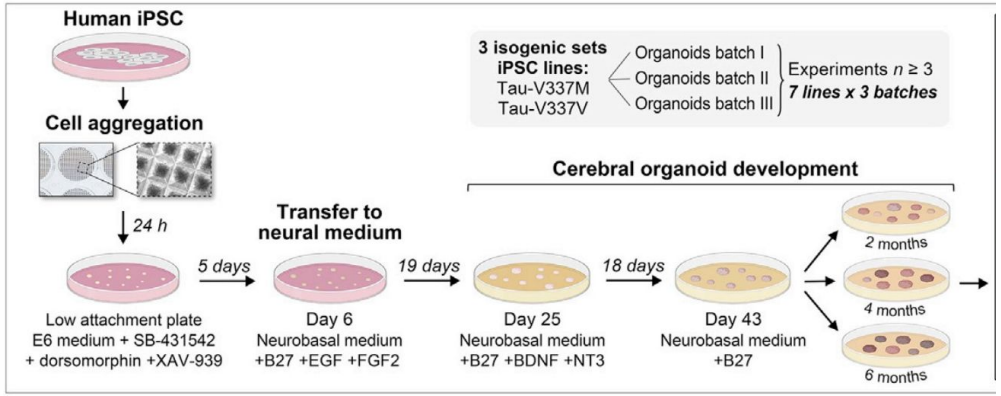
NSCI NEURAL STEM CELL INSTITUTE

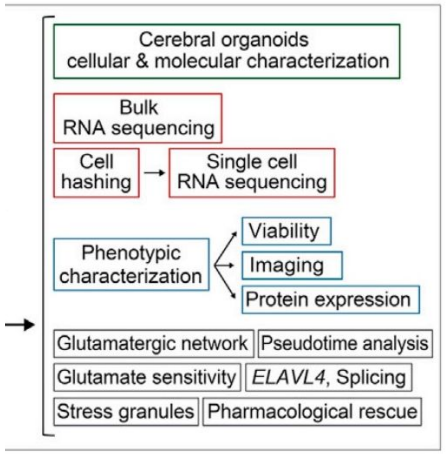
NEW YORK GENOME CENTER

Tau Consortium

Authors

Kathryn R. Bowles, M. Catarina Silva, Kristen Whitney, ..., Justin K. Ichida, Alison M. Goate, Sally Temple





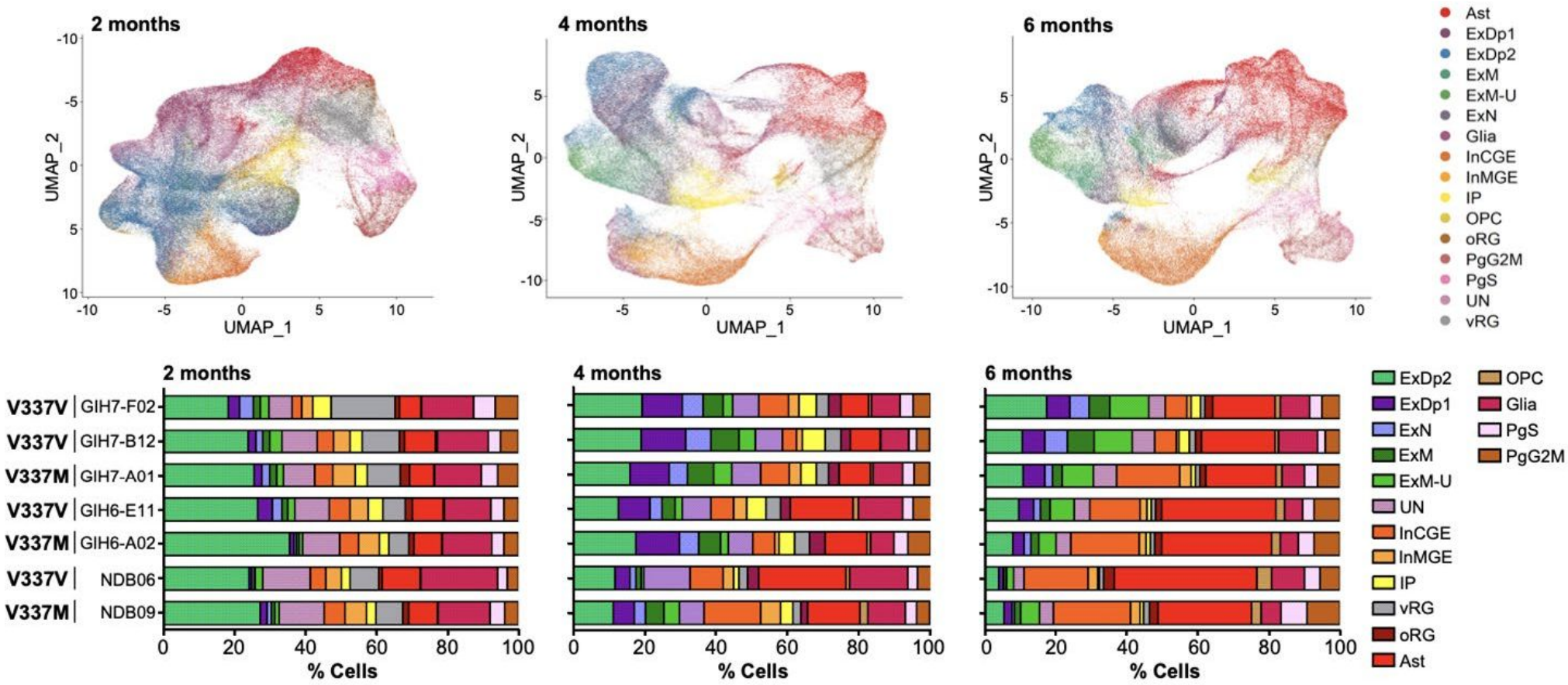
- Studied the impact of a key gene mutation (MAPT) giving rise to tauopathy in human brain, using iPSC-based organoid models
- Selected *V337M MAPT* as model mutation (100% penetrant, behavioral variant FTD)
- organoids from 3 original stem cell donors with CRISPR-corrected isogenic equivalents (controls)
- 370,000 single cells subjected to single-cell RNAseq on 10X

Findings from single cell RNA-seq analysis:

Organoids contain 16 major neuronal/glial cell types

Kat Bowles Alison Goate

Mount Sinai



- Mostly cortical phenotypes
- Mature over time, increased neuron differentiation

Conclusions

- Can we model a disease of aging with brain organoids which by their nature are ‘immature? ✔
- Can we use organoids to study disease progression? ✔
- How do different brain cell types respond to disease conditions? ✔
- Are these models valuable for a) disease phenotyping and b) testing candidate therapeutics? ✔

Data Summary	Questions we want to ask
7 iPSC lines differentiated into brain organoids 3 V337M MAPT mutation 3 isogenic controls	What is the impact on gene expression of having a MAPT mutation?
3 timepoints – 2,4,6 months	What is the impact at timepoints – 2,4,6 months
3 replicates	Look for consistency across lines and replicates
Bulk-RNA seq from each sample – the genes that are expressed in each sample	What are differentially expressed genes expressed by all the cells over time?
370,000 single cell RNA-seq – the genes that are expressed by about 5000 cells per sample	What are the differentially expressed genes expressed by individual cell types, neurons astrocytes and subtypes, over time?
	Which cells express my favorite gene? Is it expressed differently between mutant and control? With time?

Objective: Making this dataset accessible for the biologist

Single cell data – one of fastest growing areas of biology

This dataset has great value, but currently not easy to use:

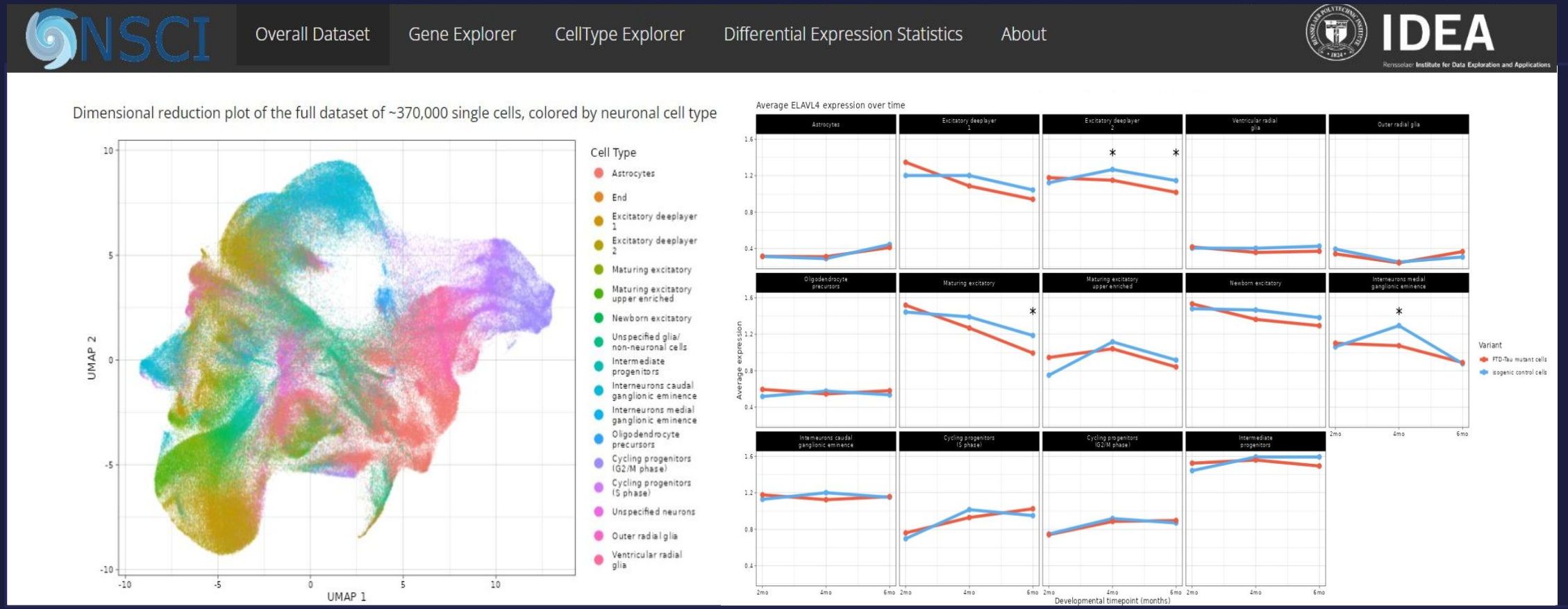
- Dataset is huge – takes significant computing power even to load
- Requires specialized knowledge to ask questions

OUR GOAL:

Make this dataset accessible to the biology researcher and general user *irrespective* of computational abilities, maximizing value for research into tauopathies such as Alzheimer's Disease

Solution: FTD MINDER

An Open-Source Web Application for scRNAseq Data Exploration



Neural Stem Cell Institute
Sally Temple, Nathan Boles, Thomas Kiehl

Institute for Data Exploration and Applications at Rensselaer Polytechnic
Kristin Bennett, John S. Erickson, Rachael White, Haowen He

Questions we want to ask:

- **How do the expression patterns of my favorite gene differ between FTD-Tau mutant and control cells, and over time?**
- **How is my favorite gene expressed (or not expressed) in different neuronal cell types?**

Which genes are differentially-expressed by individual cell types, neurons astrocytes and subtypes?

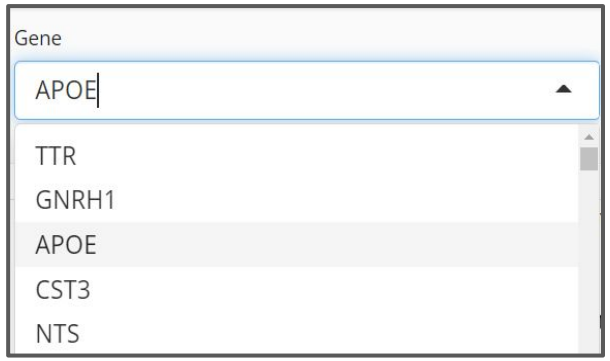
Which genes are differentially-expressed in FTD-Tau mutant cells relative to controls, and how do these expression patterns change over time?

How do the different neuronal celltypes communicate with each other in mutant cells versus controls? What cell-cell communication networks are represented, and how do these networks change over time?

Gene Explorer View *analyzes expression trends of individual genes of interest*

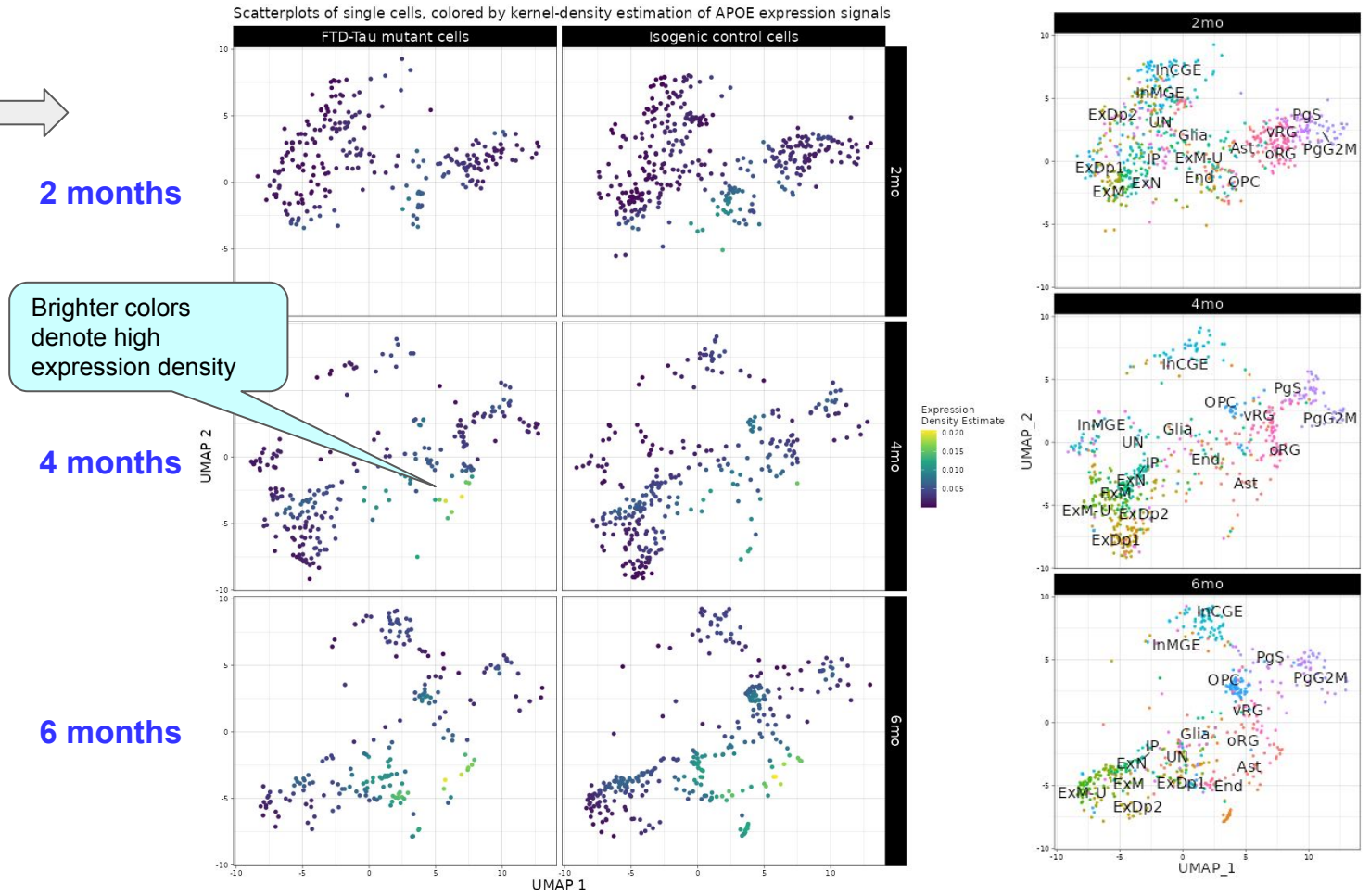
Overall Dataset | Gene Explorer | CellType Explorer | Differential Expression Statistics

Gene selection:



- **What are the expression trends of my gene of interest…**
  - **As presenting in FTD-Tau-affected organoid cells compared to isogenic controls?**
  - **Over time?**
- **Right, dimensional reduction (UMAP) scatterplots of individual cells, split by time + variant, colored by (a) normalized expression and (b) cell type**

FTD-Tau cells **isogenic controls** **all cells, for reference**

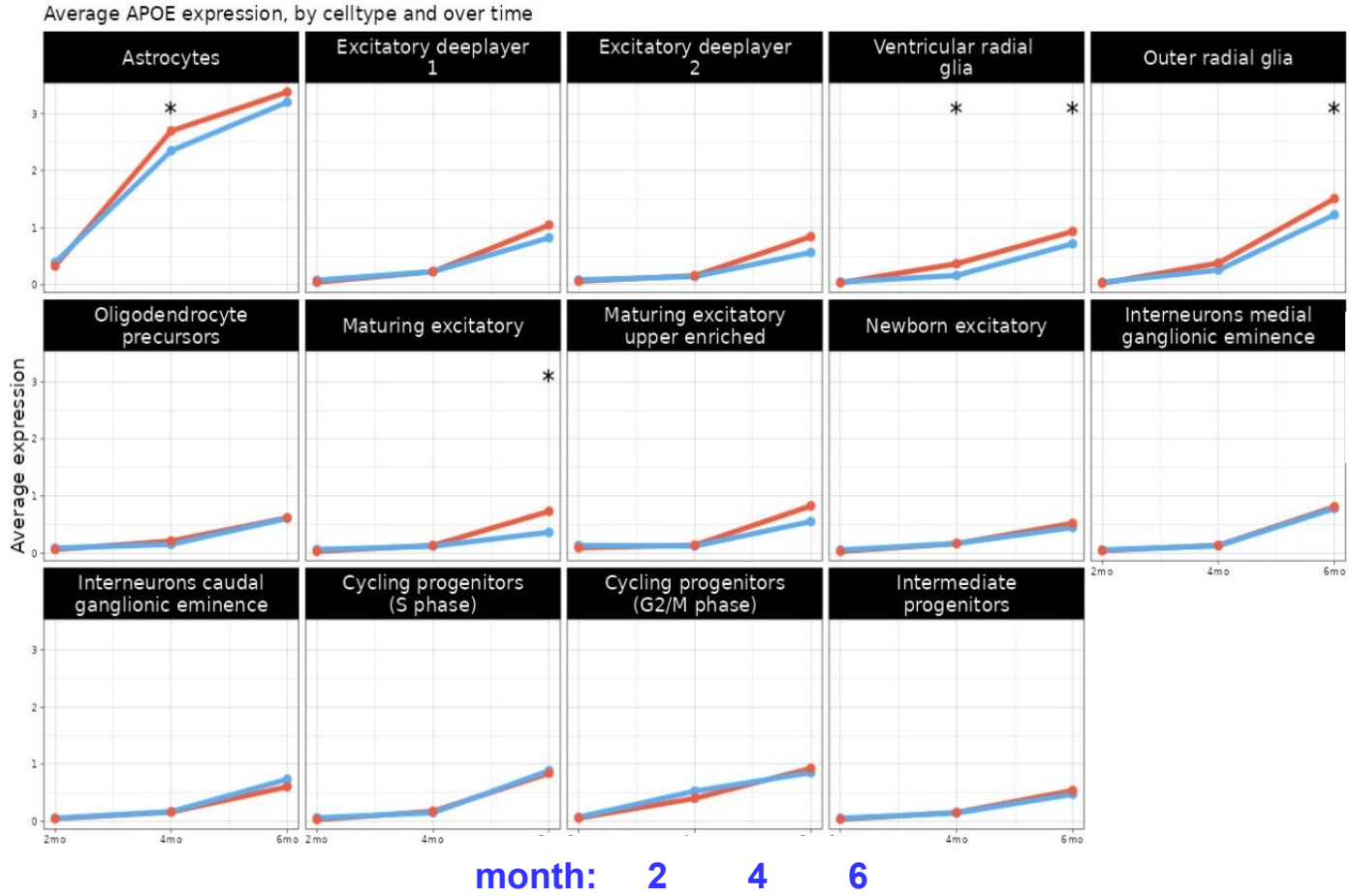


Gene Explorer View *tracks single-gene expression trends across neuronal cell types*

Overall Dataset | Gene Explorer | CellType Explorer | Differential Expression Statistics

- *What are the expression profiles of **my gene of interest** in each represented **cell type**, over **time**?*

Gene selection:



FTD-Tau cells
Isogenic controls

month: 2 4 6

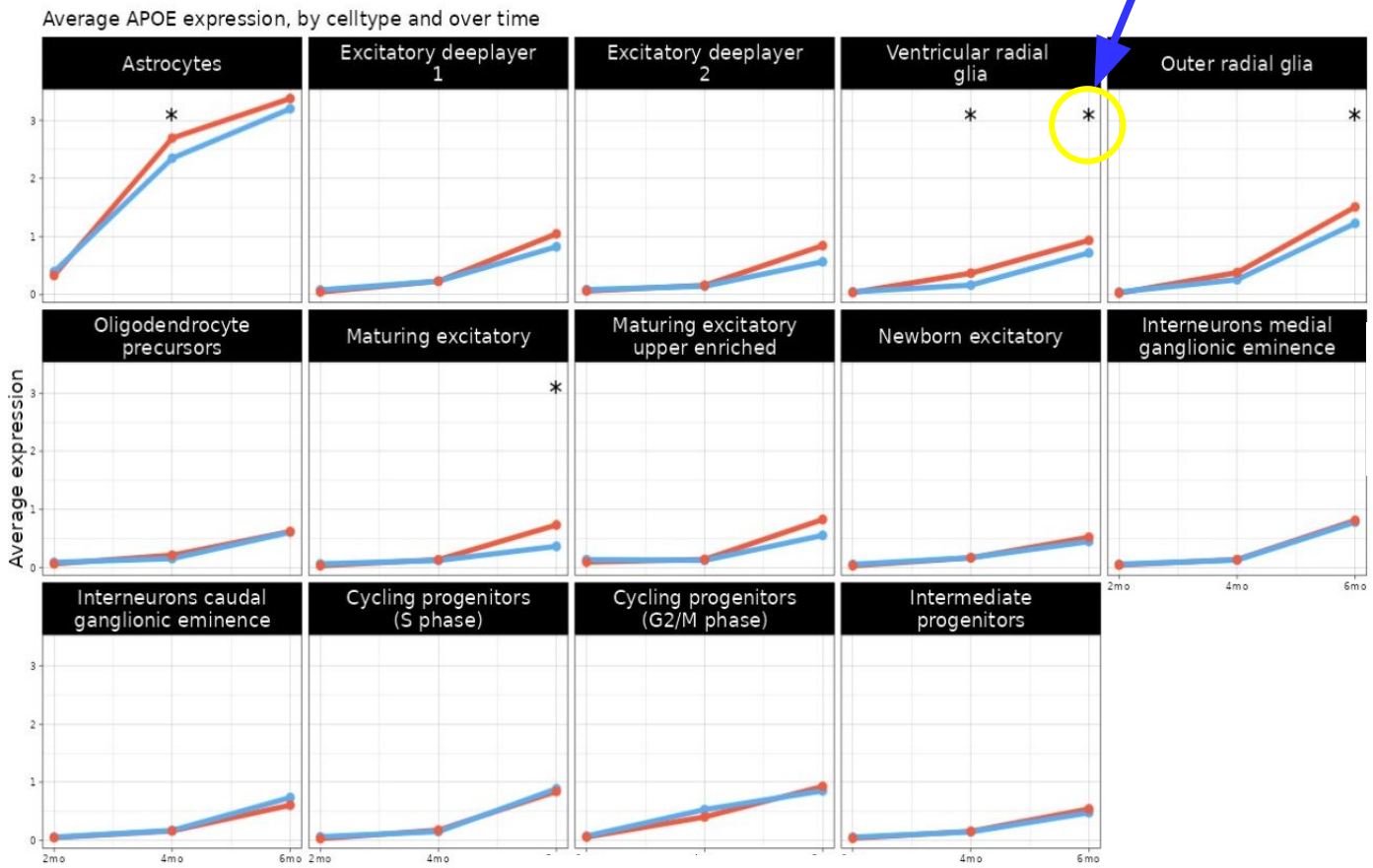
Gene Explorer View tracks single-gene expression trends across neuronal cell types

Overall Dataset | Gene Explorer | CellType Explorer | Differential Expression Statistics

- *What are the expression profiles of **my gene of interest** in each represented **cell type**, over **time**?*

Gene selection:

Differential expression significance annotations



FTD-Tau cells
Isogenic controls

month: 2 4 6

Questions we want to ask:

How do the expression patterns of my favorite gene differ between FTD-Tau mutant and control cells, and over time?
How is my favorite gene expressed (or not expressed) in different neuronal cell types?
Which genes are significantly differentially-expressed by individual cell types, neurons and glial subtypes? (i.e. what are marker genes?)
How do the different neuronal cell types communicate with each other in mutant cells versus controls? What cell-cell communication networks are represented, and how do these networks change over time?
Which genes are significantly differentially-expressed in FTD-Tau mutant cells relative to controls, and how do these expression patterns change over time?

CellType Explorer View *summarizes biological profiles of individual neuronal and glial cell types*

Overall Dataset | Gene Explorer | CellType Explorer | Differential Expression Statistics

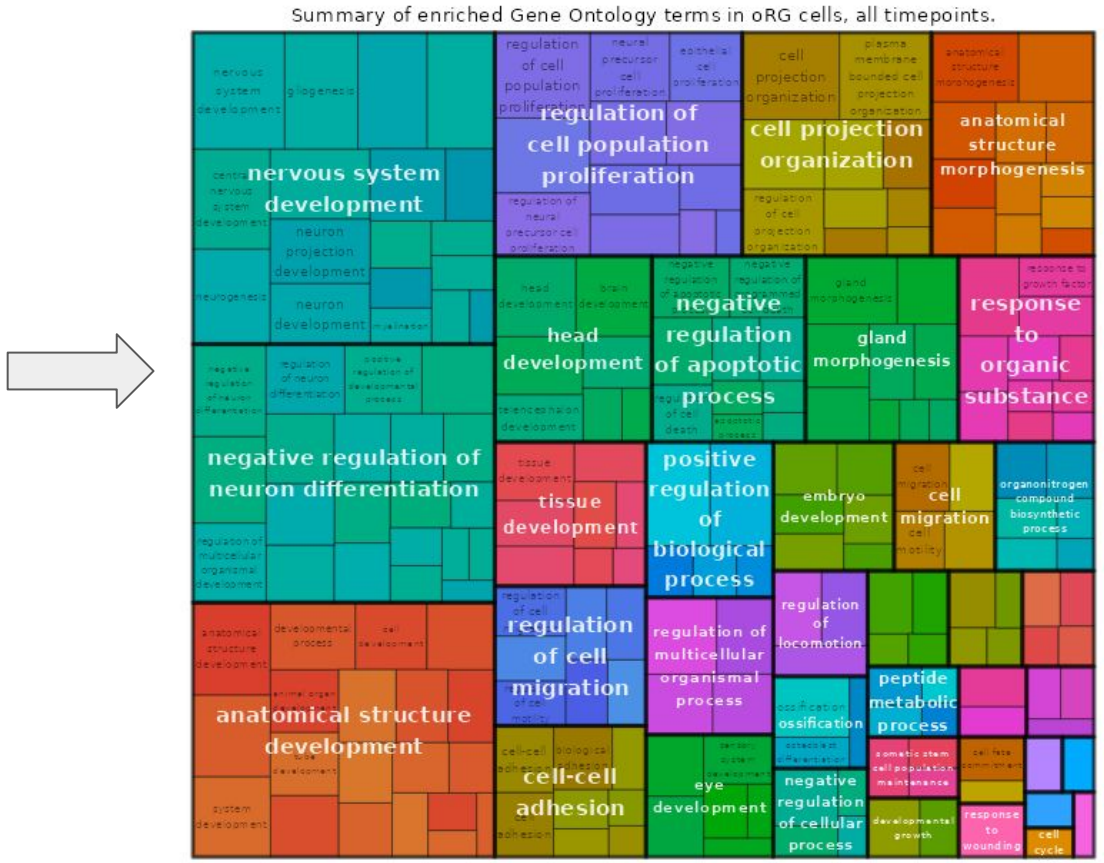
Select a cell type:

Outer Radial Glia

Show 10 entries Search:

	Gene	avg_log2FC	p_val
1	SFRP1	2.27266133506659	0
2	FABP7	2.1272139842273	0
3	PTN	2.12629128864291	0
4	HOPX	2.11482795601417	0
5	SLC1A3	1.89743286227067	0
6	VIM	1.70143866600855	0
7	PTPRZ1	1.56649366015413	0
8	PEA15	1.45272840942231	0
9	DBI	1.34818794484693	0
10	AC018730.1	1.34288796285723	0

Showing 1 to 10 of 100 entries Previous 1 2 3 4 5 ... 10 Next



Questions we want to ask:

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Which genes are differentially-expressed in FTD-Tau mutant cells relative to controls, and how do these expression patterns change over time?

CellType Explorer View *highlights cell-cell communication networks in each cell type- for mutant vs. control cells, and at specific timepoints*

Overall Dataset | Gene Explorer | CellType Explorer | Differential Expression Statistics

Jin, S., Guerrero-Juarez, C.F., Zhang, L. et al. Inference and analysis of cell-cell communication using CellChat. Nat Commun 12, 1088 (2021).

Select a cell type:

Astrocytes

Select a developmental timepoint:

6 months

Wildtype/mutant:

FTD-Tau-affected organoid cells

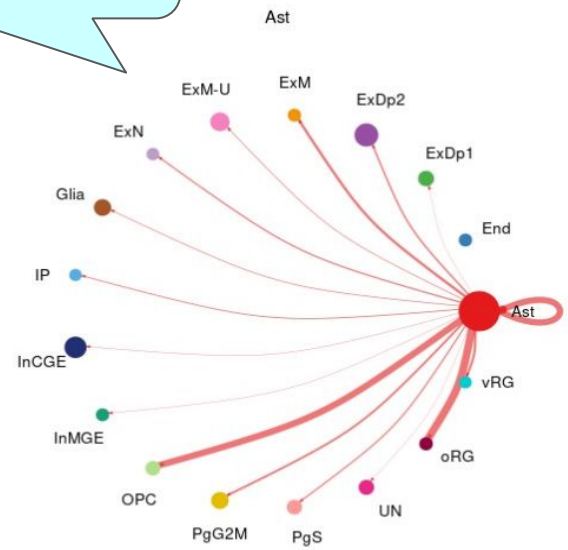
Show 10 entries Search:

	source	target	pathway	prob	pval
1	Ast	Ast	EGF	4.920947e-07	0.03
2	Ast	Ast	LAMININ	2.531450e-05	0.000833333333333333
3	Ast	Ast	NGL	6.397194e-06	0.01
4	Ast	Ast	NRXN	6.495521e-05	0.01
5	Ast	Ast	VISFATIN	2.460469e-06	0.02
6	Ast	ExDp2	CDH	1.083722e-04	0.01
7	Ast	ExDp2	EPHA	1.377863e-05	0.003
8	Ast	ExDp2	EPHB	3.469225e-05	0.00625
9	Ast	ExDp2	LAMININ	3.257901e-05	0.000833333333333333
10	Ast	ExDp2	NRXN	2.007610e-04	0.00166666666666667

Showing 1 to 10 of 75 entries Previous 1 2 3 4 5 ... 8 Next

Inter-cell-type interaction network found at the selected timepoint when selected celltype acts as ligand

Pathway enrichment statistics furnishing the network



The size of the circle of various colors in the periphery indicates the number of cells. The larger the circle, the more cells. The cells that emit arrows express ligands, and the cells that the arrows point to express receptors. The more ligand receptor pairs, the thicker the line.

CellType Explorer View *shows representation of signaling pathways of interest in the detected cell types*

Overall Dataset | Gene Explorer | CellType Explorer | Differential Expression Statistics

User selects biological pathway of interest

Select pathway to explore its communication network in the dataset

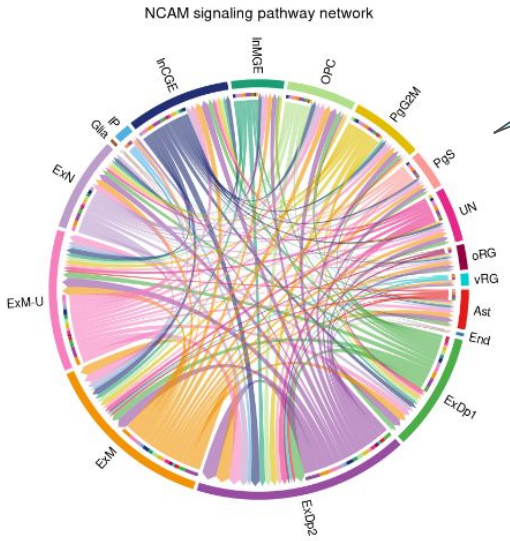
NCAM | Go

Left, cellular communication visualization outputs for different analytical tasks, showing the inferred intercellular communication network for selected signaling.

Right, analysis of the communication. Heatmap shows the relative importance of each cell group based on the computed four network centrality measures of selected signaling. Histogram shows the relative contribution of each ligand-receptor pair to the overall communication network of selected signaling pathway, which is the ratio of the total communication probability of the inferred network of each ligand-receptor pair to that of selected signaling pathway.

Hierarchy plot | Heatmap | Chord plot | Circle plot

Visualization of pathway representation across all neuronal cell types



Key signaling roles | Contribution of each L-R | Pathway annotation

Show 10 entries | Search:

	interaction_name	pathway_name	ligand	receptor	evidence	annotation
1834	NCAM1_FGFR1	NCAM	NCAM1	FGFR1	PMID: 12791257	Cell-Cell Contact
1835	NCAM1_NCAM1	NCAM	NCAM1	NCAM1	KEGG: hsa04514	Cell-Cell Contact
1836	NCAM1_NCAM2	NCAM	NCAM1	NCAM2	KEGG: hsa04514	Cell-Cell Contact
1837	NCAM1_L1CAM	NCAM	NCAM1	L1CAM	KEGG: hsa04514	Cell-Cell Contact
1838	NCAM2_L1CAM	NCAM	NCAM2	L1CAM	KEGG: hsa04514	Cell-Cell Contact

Showing 1 to 5 of 5 entries | Previous | 1 | Next

Corresponding statistics

Questions we want to ask:

How do the expression patterns of my favorite gene differ between FTD-Tau mutant and control cells, and over time?
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Which genes are significantly differentially-expressed in FTD-Tau mutant cells relative to controls, and how do these expression patterns change over time?

Differential Expression Statistics Browser

reports differential gene expression between mutant and control cells, for any experimental group of interest

Overall Dataset | Gene Explorer | CellType Explorer | Differential Expression Statistics

Developmental Timepoint (Month): All

CellType: All

Gene: All

P-Value Threshold (max 0.01): 0.01

Download selected data

User-specified experimental categories to test differential expression for

Results

Show 10 entries

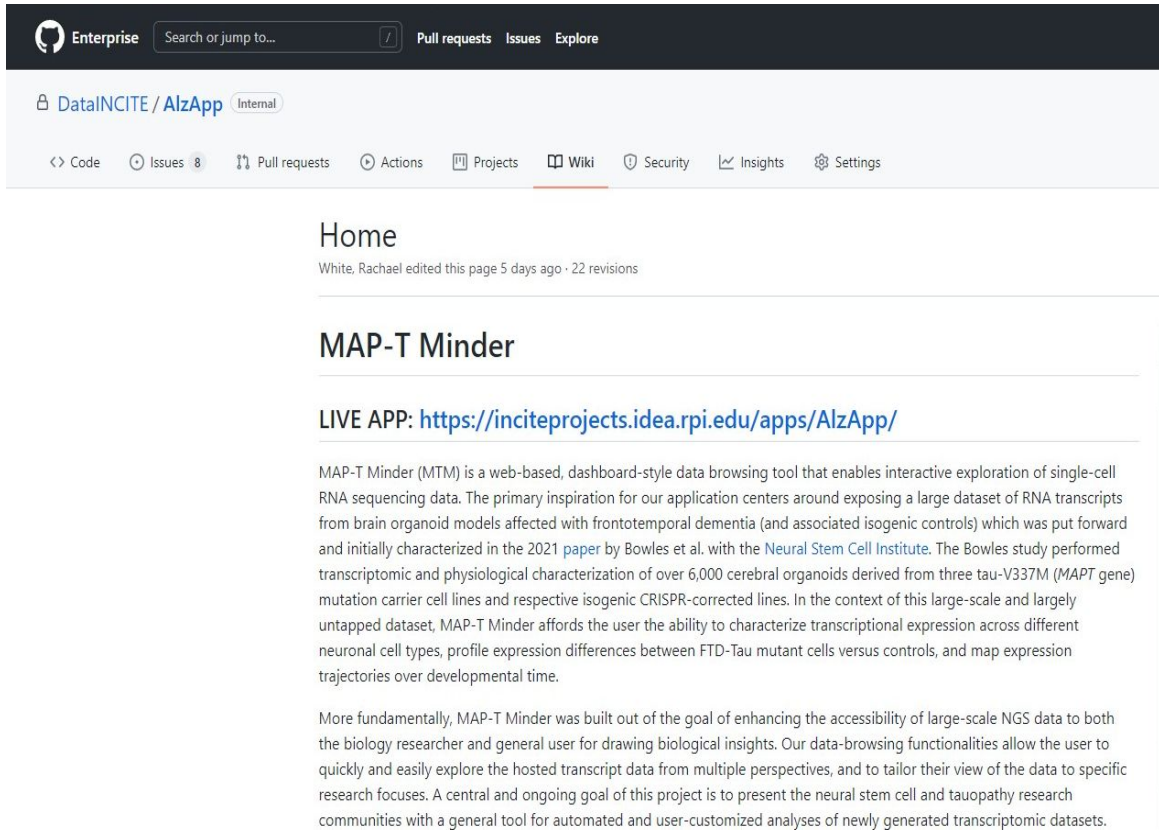
Search:

	Gene	D.E. P Value	Bonferroni-Corrected P Value	Average Log2 Fold Change in mutant from control for this celltype and timepoint	Percent of V337M cells expressing gene within this celltype and timepoint	Developmental timepoint	Celltype	D.E. status
1	CHCHD2	0.0	0.0	0.8277	63.10%	2	ExDp2	Significantly expressed in V337M
2	PGRMC1	0.0	0.0	0.5414	87.50%	2	ExDp2	Significantly expressed in V337M
3	CHCHD2	0.0	0.0	0.6498	41.50%	4	ExDp1	Significantly expressed in V337M
4	CHCHD2	0.0	0.0	0.8357	41.50%	4	ExDp2	Significantly expressed in V337M
5	RP11-701H24.2	0.0	0.0	-0.7017	56.20%	4	ExDp2	Significantly expressed in V337M
6	CHCHD2	1.6e-277	6.7e-273	0.6346	58.40%	6	Ast	Significantly expressed in V337M
7	UBE3A	1.4e-267	5.9e-263	0.3422	66.60%	4	ExDp2	Significantly expressed in V337M

Resources for Learning More and Visiting the App!

Source code and full documentation freely available at our production GitHub:

https://github.rpi.edu/DataINCITE/AlzApp/



Live application is hosted publically at:

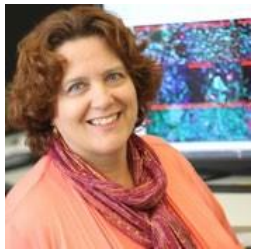
https://inciteprojects.idea.rpi.edu/alzapp/app/alzapp/

Acknowledgements

NSCI
NEURAL STEM CELL INSTITUTE


Taylor Bertucci


David Butler


Susan Goderie

Guo-Li Ming
Hongjun Song
Sergiu Pasca
Se-Jin Yoon

Tau Consortium

Steve Haggarty MGH
Alison Goate MSSM
Ken Kosik UCSB
Celeste Karch Wash U
Sally Temple NSCI
Justin Ichida USC
Martin Kampmann UCSF
John Crary MSSM
Khadijah Onanuga - PM

Tau Consortium

RAINWATER Charitable Foundation

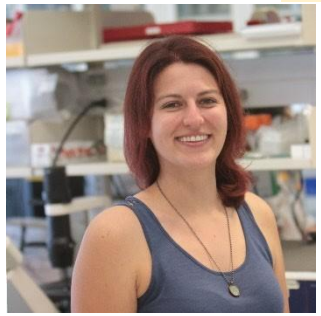
curePSP UNLOCKING THE SECRETS OF BRAIN DISEASE

NINDS NIA

AFTD The Association for Frontotemporal Degeneration FIND HELP·SHARE HOPE


Susan Borden

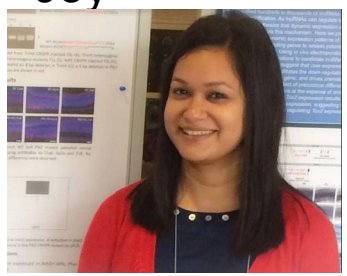

Shona Joy

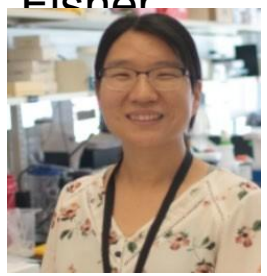

Liz Fisher


Jenny Wang

neuracell

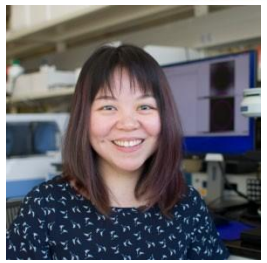

Nathan Boles


Rebecca Chowdhury


Xiuli Zhou


Anne Messer


Steve Lotz


Isabel Tian


Brian Unruh

Questions?

Potential Q & A

- **What programming framework is the app implemented with?**
  - MAPT Minder is an RShiny application developed in the R programming language. Source code is managed using Git and freely accessible in our production GitHub. We are deployed on a public, institutionally-curated and well-resourced production server.

- **What additional programming frameworks/analysis technologies does the app draw on for analyses?**
  - Major programming softwares / R frameworks used:
    - *Seurat; CellChat; G:Profiler; Revigo; MAST*
  - Refer to https://github.rpi.edu/DataINCITE/AlzApp/wiki#key-resources-employed for references

- **How do we handle performance restraints due to dataset size?**
  - The majority of the visualizations shown in the app draw on pre-calculated data (data calculated, subset, or otherwise drawn from the original dataset prior to app runtime). For the visualizations that must be rendered at runtime (namely the Gene Explorer gene expression feature plots based on selected genes), we draw on a downsampled version of the complete, normalized dataset containing a subset of ~10% of cells per celltype, which we have found to be approximately representative of the overall trends.

Potential Q & A

- **How many genes are available for querying?**
  - Genes now available for selection in dropdown are **all ~40,000** represented in the complete Bowles dataset
  - Performance is kept in check despite this number because we use backend, R-based search handling, rather than front-end Javascript-based data loaders as many apps do by default
- **How is the data normalized and scaled? Is the data displayed in the different plots normalized and scaled uniformly?**
  - We draw on the data as it was originally normalized and scaled for the Bowles paper, which used the SCTransform protocol regressing out specific common confounding sources of variation. The exceptions are our gene-specific expression trajectory line plots, which show gene expression averaged from raw read counts at each time point within each individual celltype and subsequently log-normalized; and our gene-specific violin plots, which also show expression distributions log normalized from raw read counts.
- **What server is hosting the live app?**
  - The app is currently hosted on a server institutionally-funded and maintained by the Rensselaer Polytechnic Institute Incite Projects Directive. We are currently expanding our server resources to avoid multi-user performance limitations .