DIVING INTO ALZHEIMER'S DISEASE

with transcriptome analysis of a zebrafish model

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THE UNIVERSITY of ADELAIDE

What does the Alzheimer's disease brain look like?

Higher resolution

e.g. Transcriptome measurements using RNA-seq [3-6],
Proteomic measurements using mass spectrometry

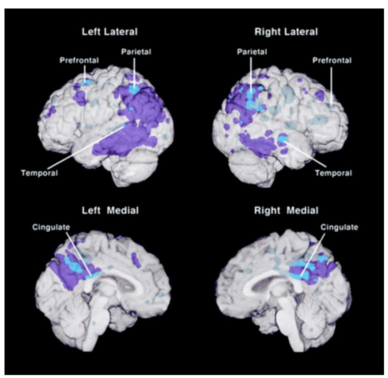
To investigate early events in Alzheimer's disease, we need a study here.

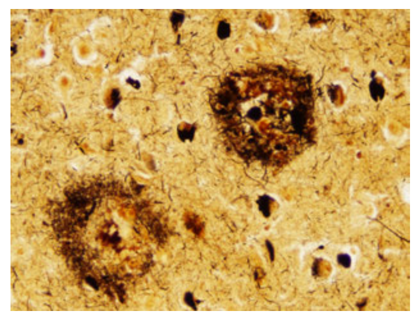
Upregulated by AD	#	PValue
cell adhesion	10	8.05E-04
response to wounding	7	1.17E-03
adherens j		
cell death		
platelet al		
iron ion bi		
protein oli		
myeloid ce		

Downregulated by AD	#	PValue
precursor metabolites & energy	18	7.47E-10
organelle envelope	18	2.35E-05
microtubule-based process	11	4.66E-05
ribonucleotide binding	29	9.92E-04
synaptic vesicle membrane	4	9.95E-04
synapse part	8	5.60E-03
CNS neuron development	3	2.87E-02
actin cytoskeleton	7	2.97E-02
mitochondrial matrix	6	4.77E-02

Young adult brain

Post-mortem elderly brain





Lower resolution

e.g. brain imaging, autopsies, cerebrospinal fluid Aβ measurements[1, 2]

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Likely confounding from overexpressing multiple mutant human genes

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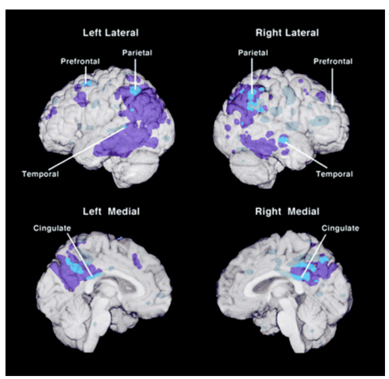

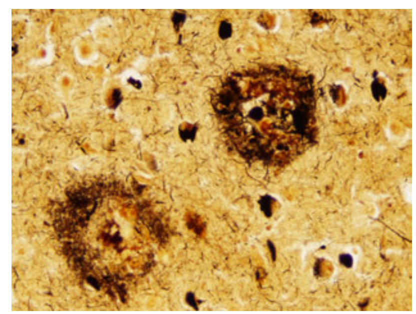

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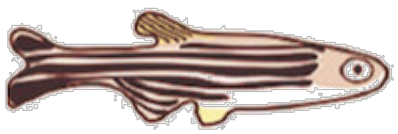
RNA-seq on zebrafish with a single, endogenous Alzheimer's disease mutation.



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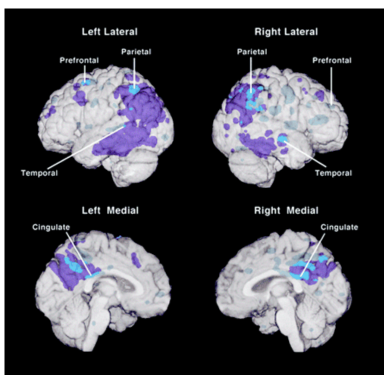

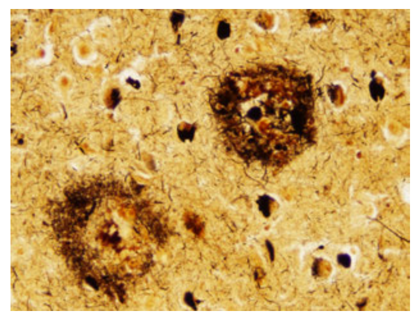

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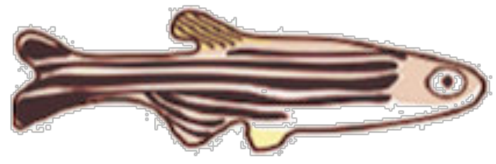
A zebrafish model of Alzheimer's disease



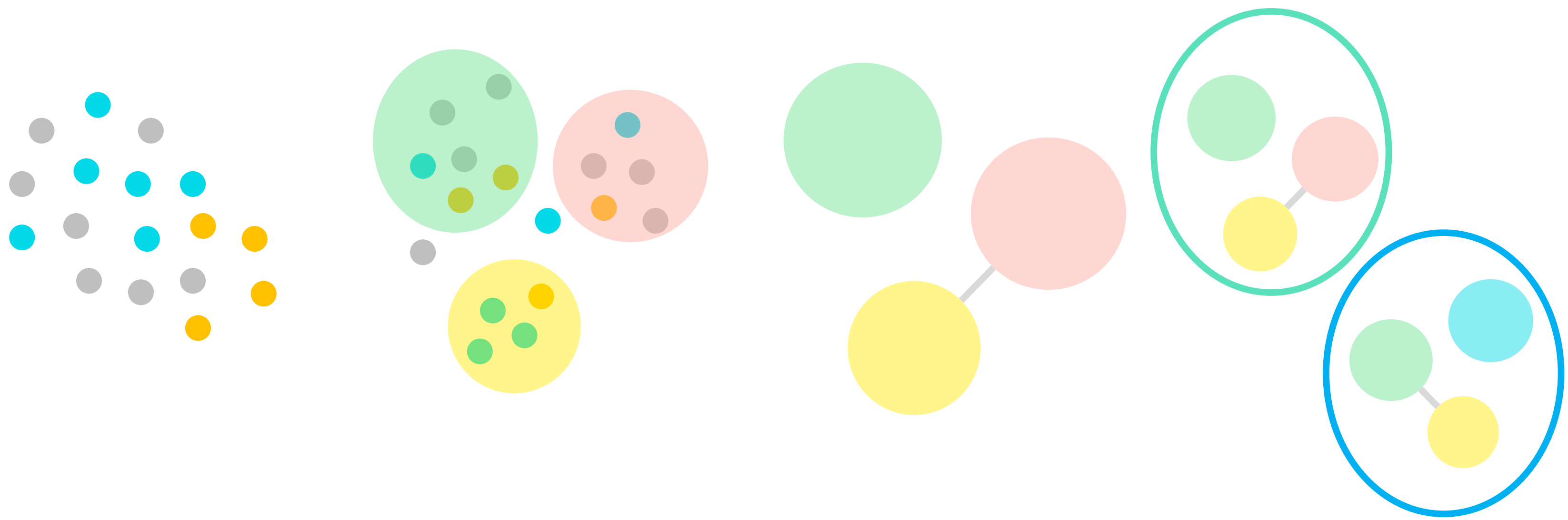
Mutant *psen1* genotype: K97Gfs/+

- K97Gfs is equivalent to a known familial Alzheimer's disease mutation[7-9].
- Heterozygous mutation, so that the mutant protein product is expressed at physiologically relevant levels.
- All mutant and wildtype zebrafish are siblings raised in the same tank. At 6 months and 24 months, brains removed for total RNA sequencing.

7. Sato N *et al. Journal of Neurochemistry* **1999**;72:2498-505.
8. Newman M *et al. Hum Mol Genet* **2014**;23: 602-17, doi:10.1093/hmg/ddt448.
9. Moussavi Nik SH *et al. Hum Mol Genet* **2015**, doi:10.1093/hmg/ddv110.


RNA-seq
Analysis

Can *psen1* mutant zebrafish model the gene expression changes underlying Alzheimer's disease?



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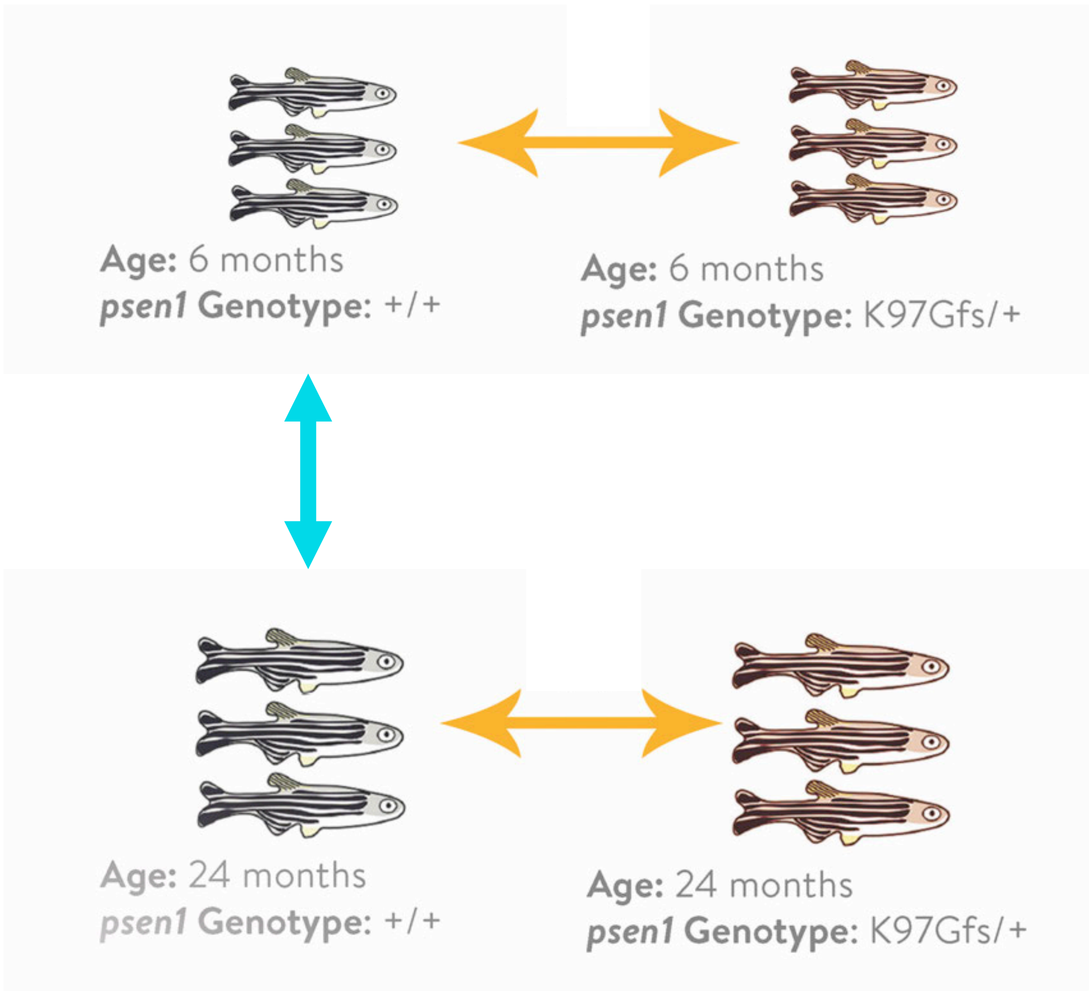
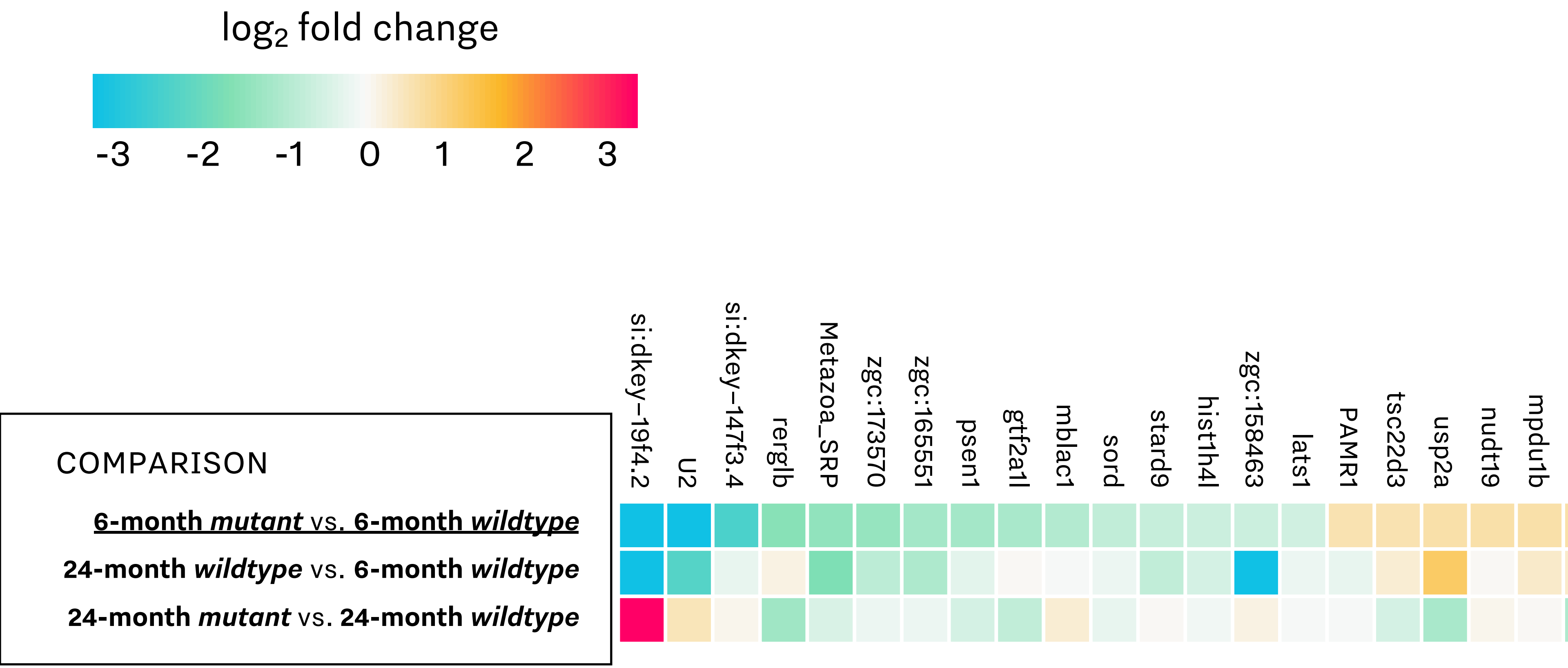
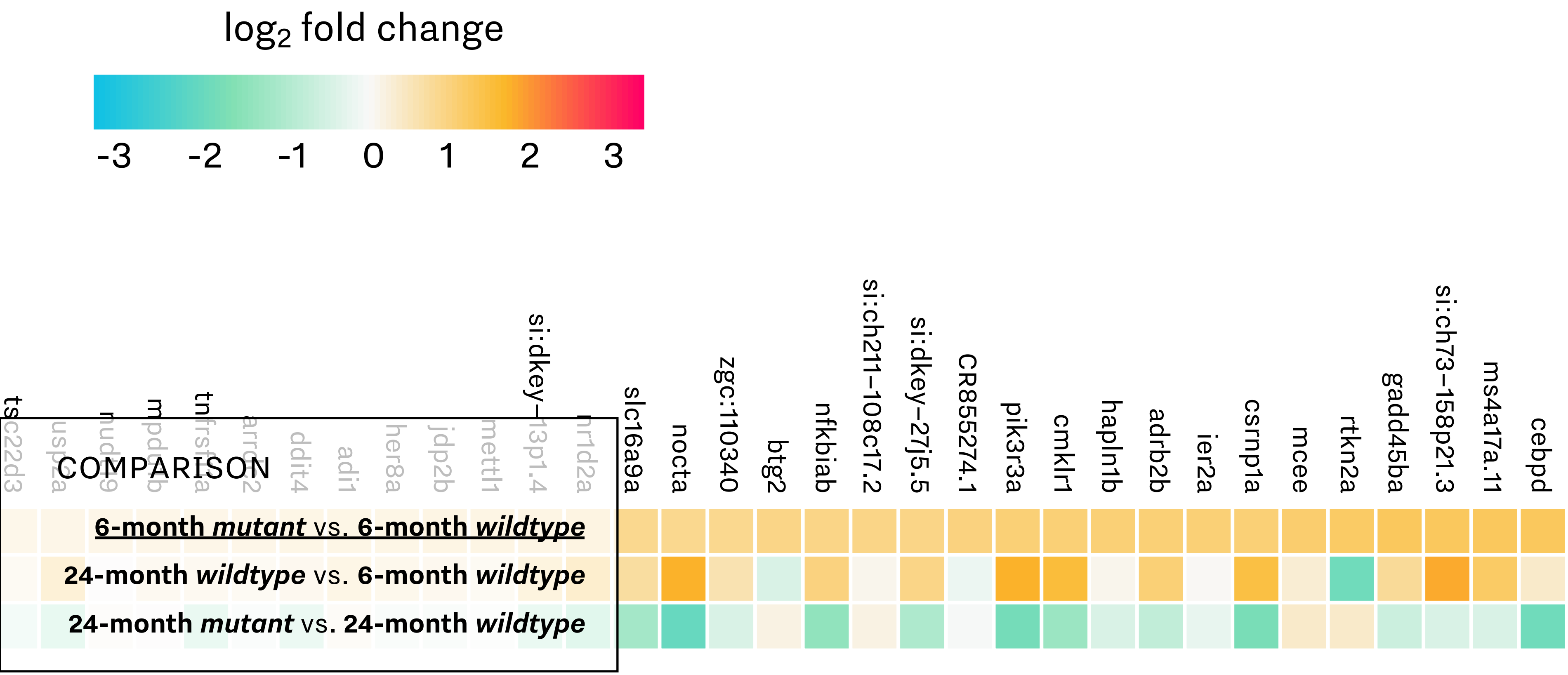
- Differential gene expression analysis using moderated *t*-tests (*limma*).





1. Gene expression changes in 6-month-old mutant brains imply **premature aging**.

2. In 24-month-old mutant brains, gene expression is **inverted**.



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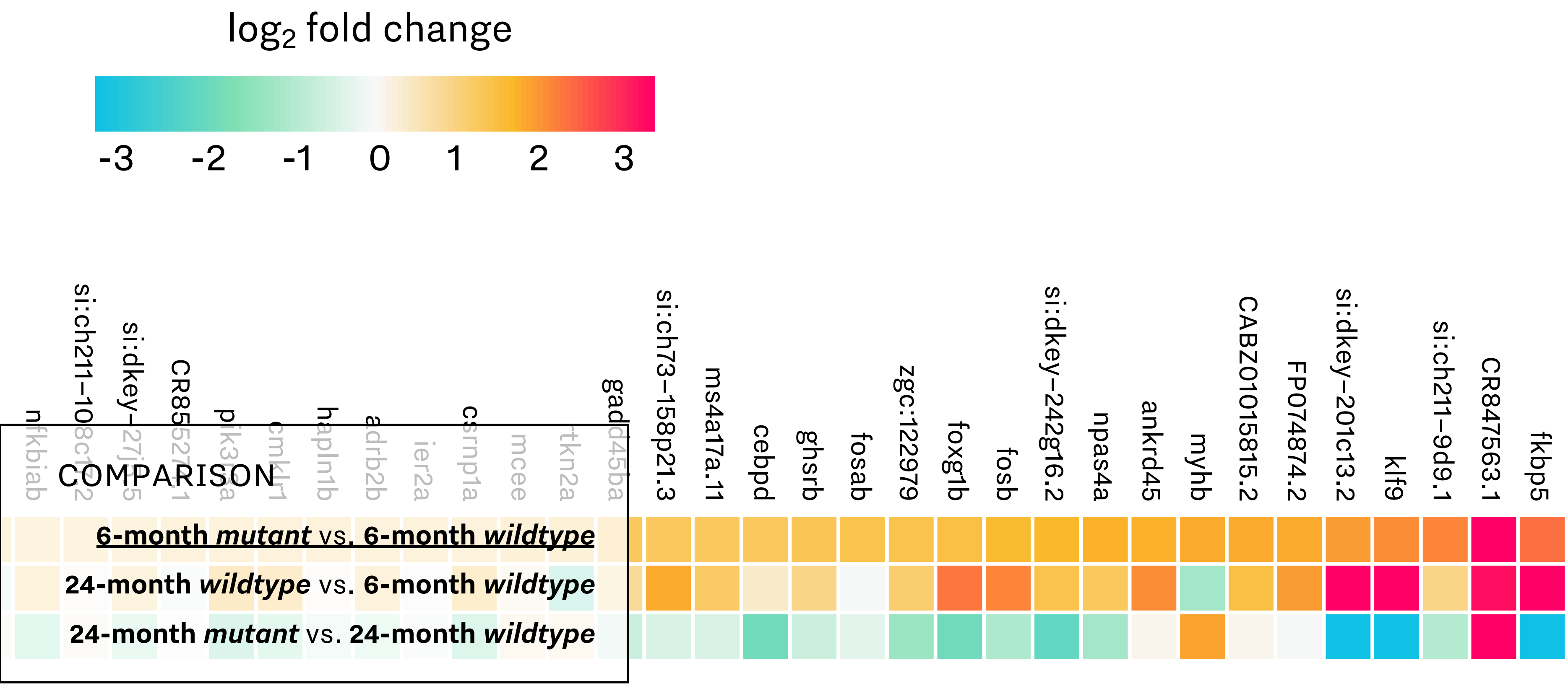

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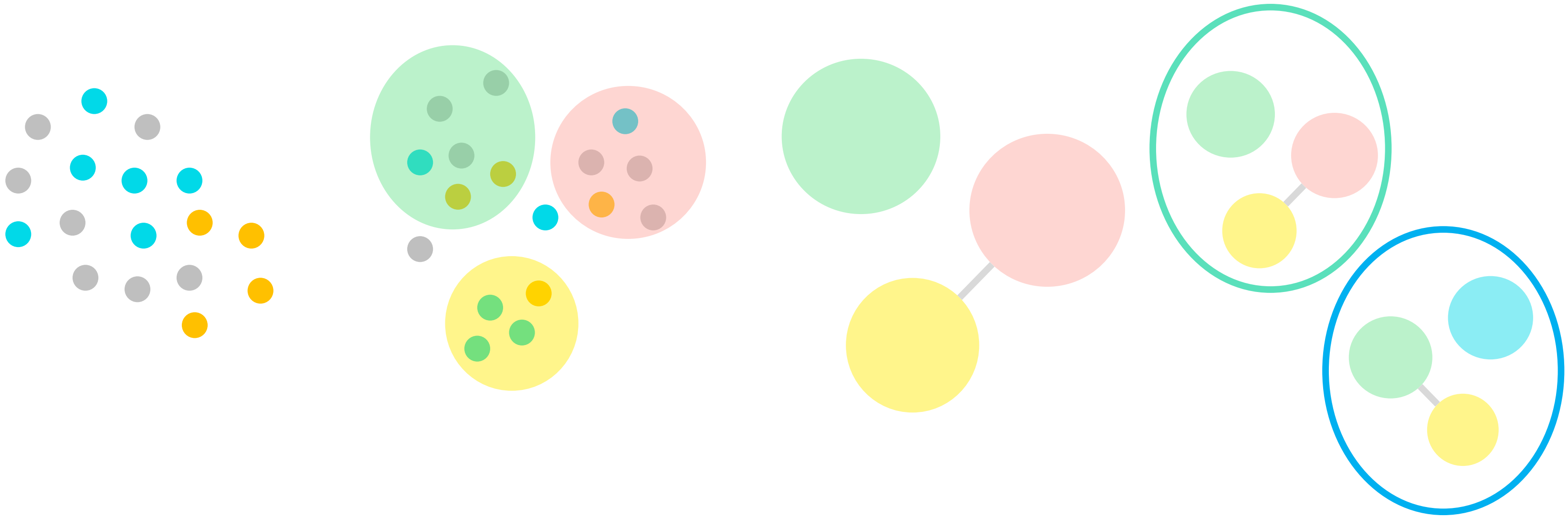

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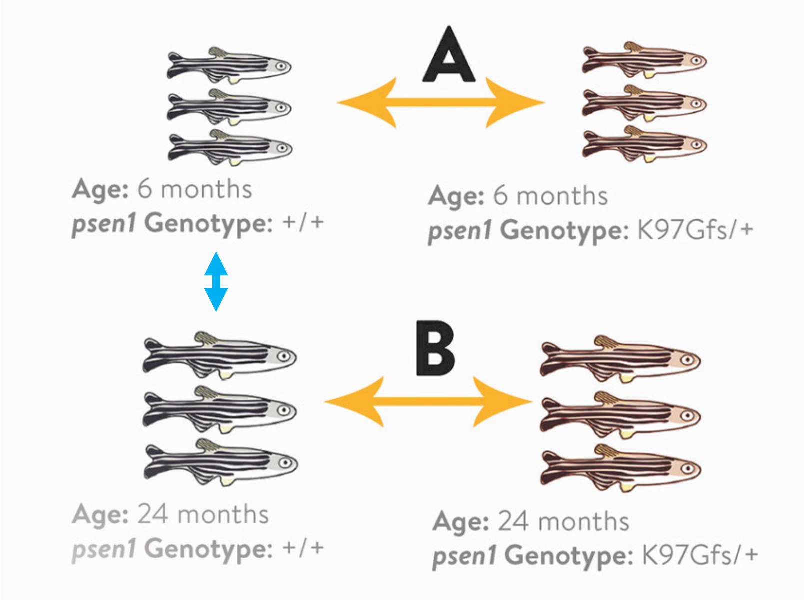
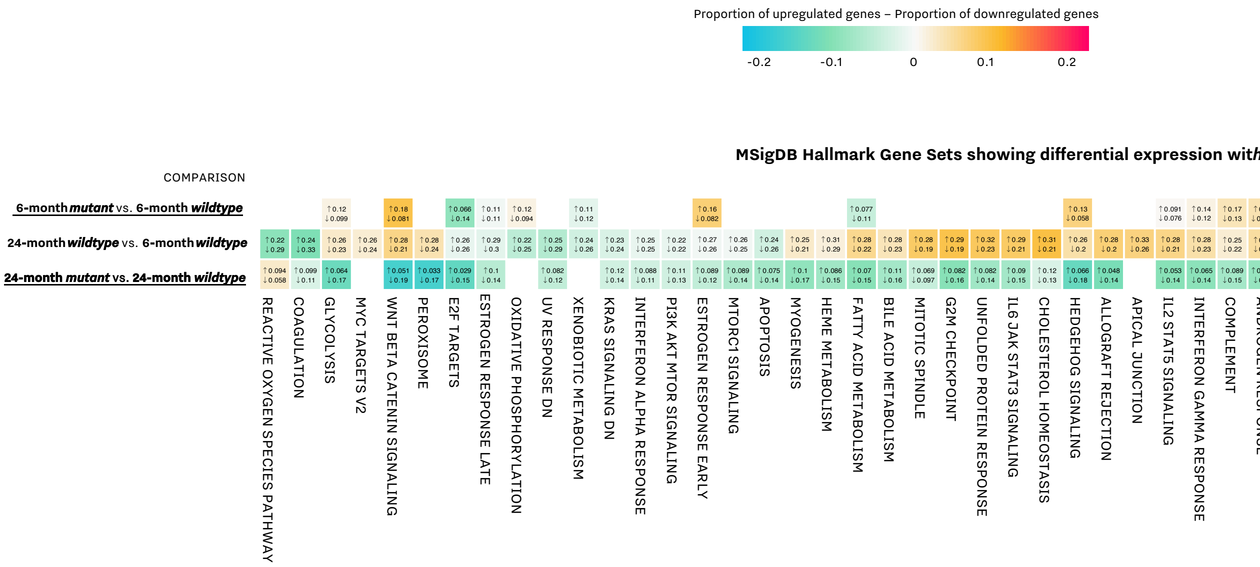
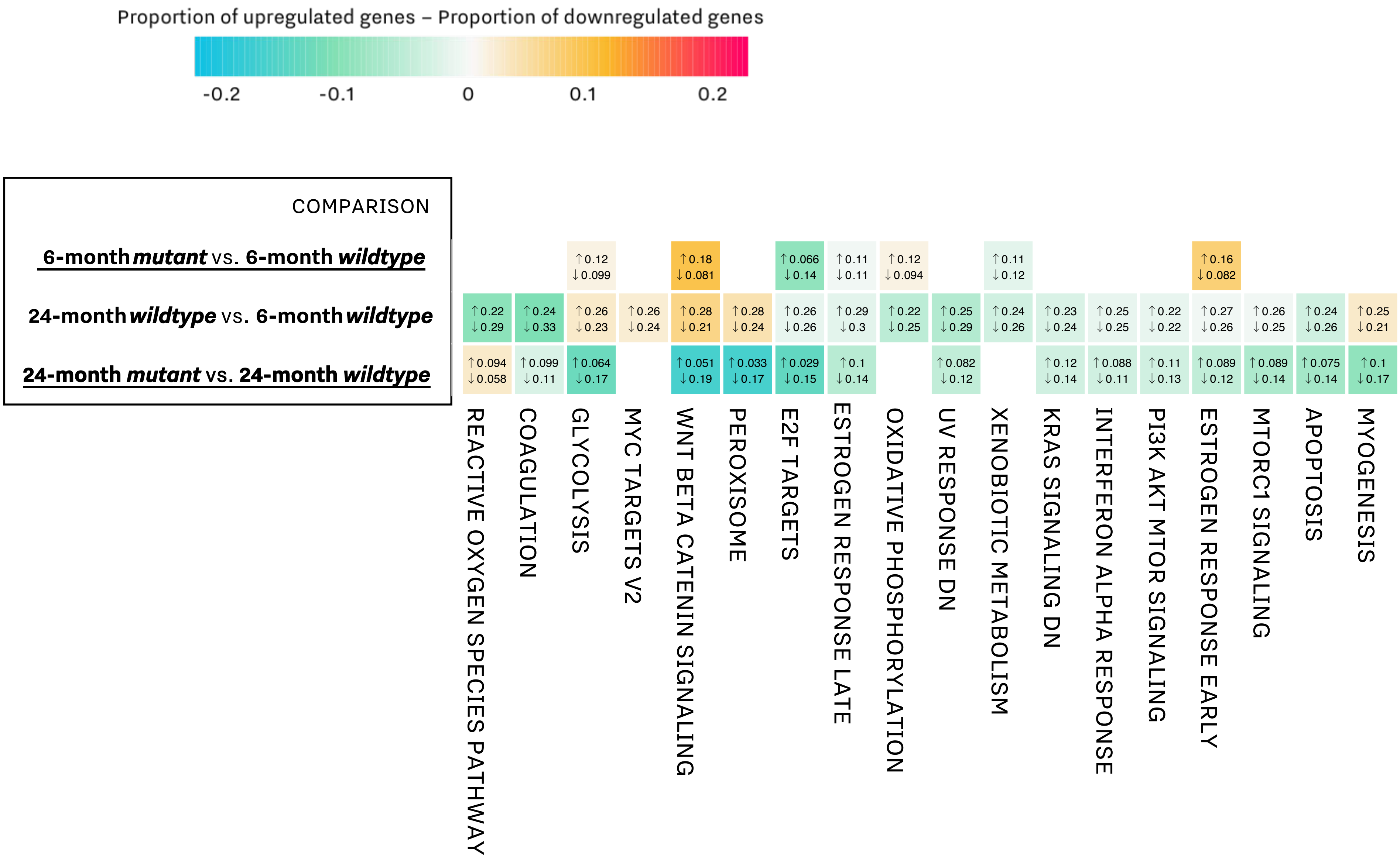
Which biological processes are altered in *psen1* mutants?

- A **gene set** is a group of genes involved in a biological process.
- Hallmark gene sets from MSigDB aggregated from multiple studies[10].
- Gene set testing (using *FRY* and *ROAST*) to identify differentially expressed gene sets for each comparison[11].





10. Liberzon et al. *Cell Systems*. 2015 Dec;6(23):417-25
11. Giner & Smyth. *F1000Research*. 2016 5(2605). DOI: 10.7490/f1000research.1113351.1


Proportion of upregulated genes – Proportion of downregulated genes
-0.2
-0.1
0
0.1
0.2
COMPARISON
6-month ***mutant*** vs. 6-month ***wildtype***
24-month ***wildtype*** vs. 6-month ***wildtype***
24-month ***mutant*** vs. 24-month ***wildtype***
↑0.12 ↓0.099
↑0.18 ↓0.081
↑0.066 ↓0.14
↑0.11 ↓0.11
↑0.12 ↓0.094
↑0.11 ↓0.12
↑0.16 ↓0.082
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REACTIVE OXYGEN SPECIES PATHWAY
COAGULATION
GLYCOLYSIS
MYC TARGETS V2
WNT BETA CATENIN SIGNALING
PEROXISOME
E2F TARGETS
ESTROGEN RESPONSE LATE
OXIDATIVE PHOSPHORYLATION
UV RESPONSE DN
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KRAS SIGNALING DN
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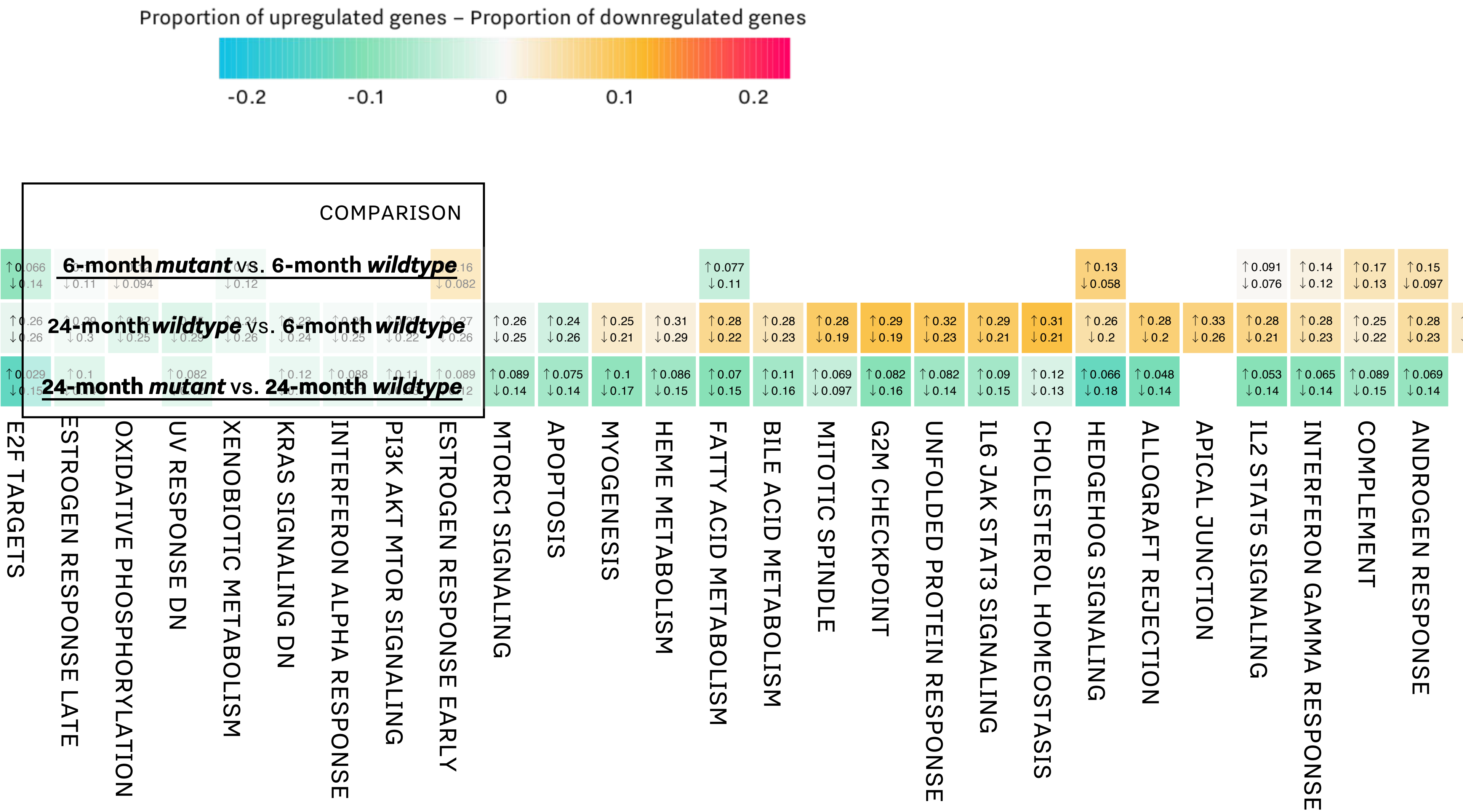
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G2M CHECKPOINT
UNFOLDED PROTEIN RESPONSE
IL6 JAK STAT3 SIGNALING
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HEDGEHOG SIGNALING
ALLOGRAFT REJECTION
APICAL JUNCTION
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INTERFERON GAMMA RESPONSE
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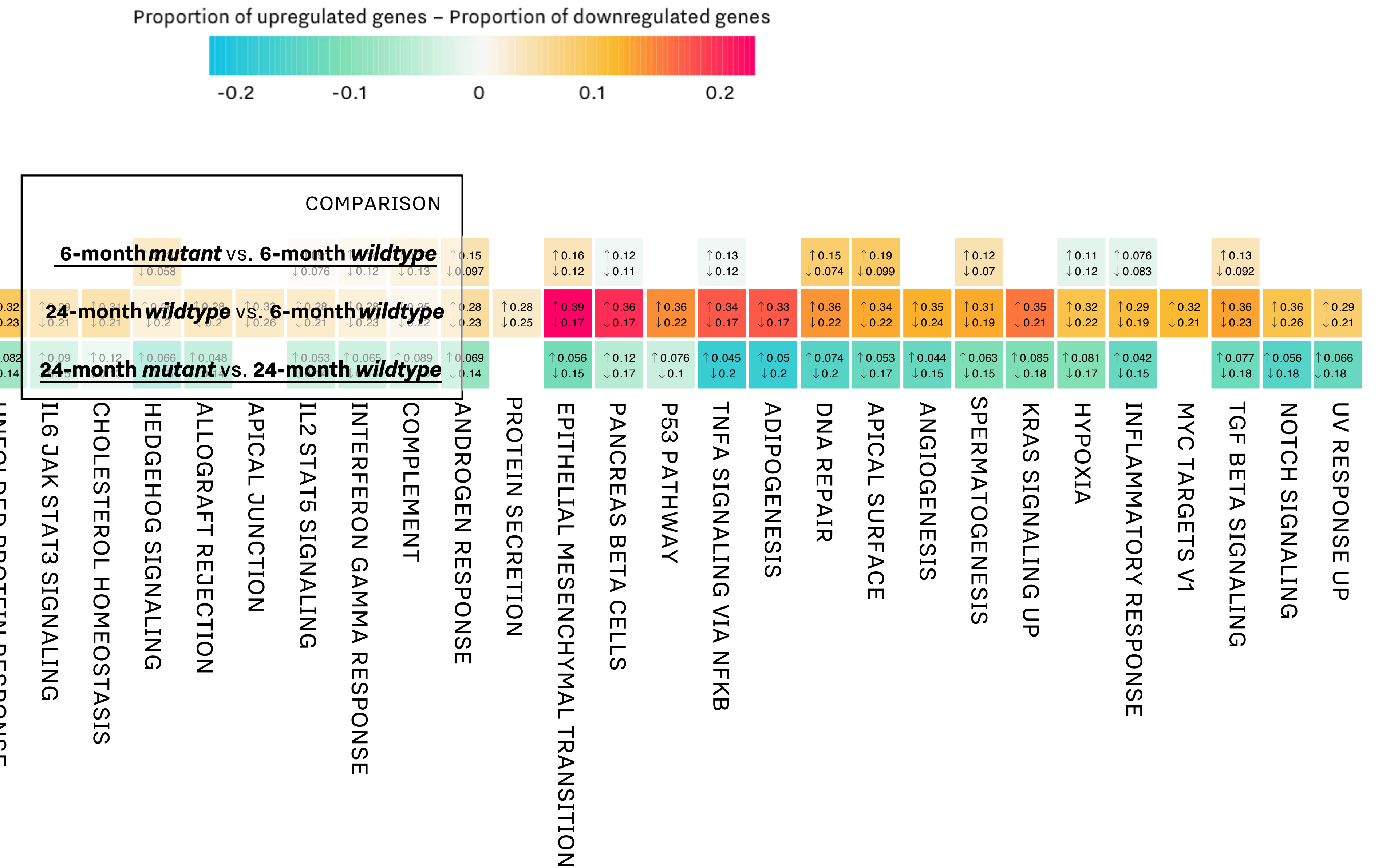

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P53 PATHWAY
TNFA SIGNALING VIA NFKB
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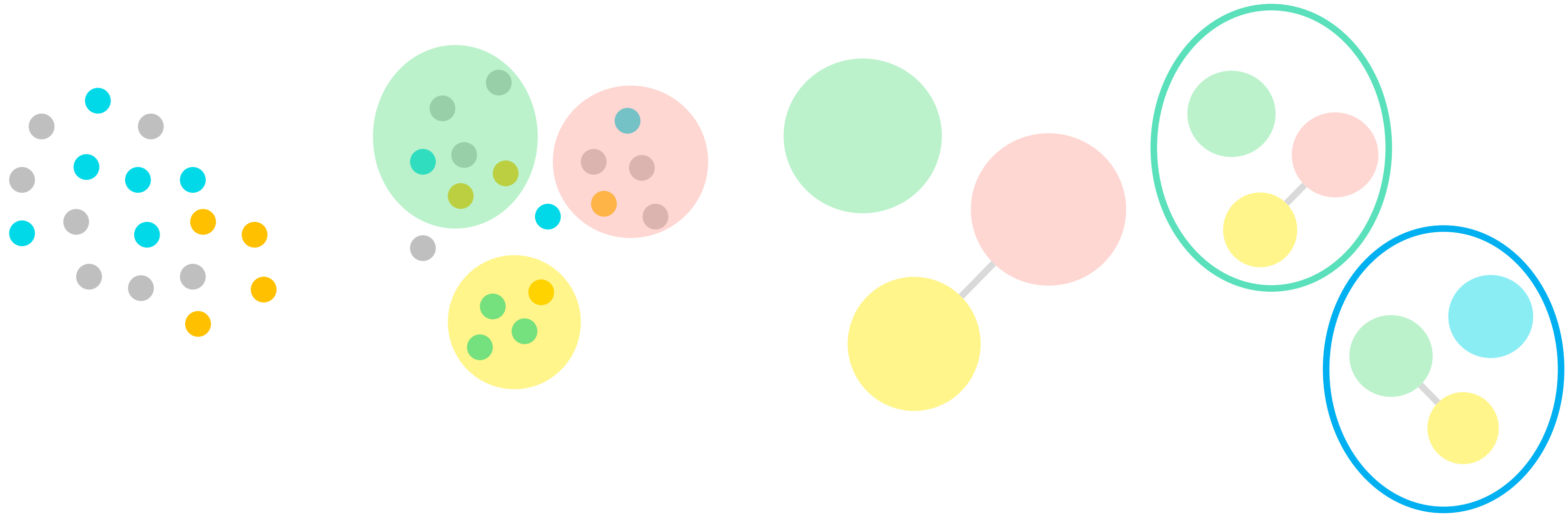
APICAL SURFACE
ANGIOGENESIS
SPERMATOGENESIS
KRAS SIGNALING UP
HYPOXIA
INFLAMMATORY RESPONSE
MYC TARGETS V1
TGF BETA SIGNALING
NOTCH SIGNALING
UV RESPONSE UP

Can *psen1* mutant zebrafish model the gene expression changes underlying Alzheimer's disease?



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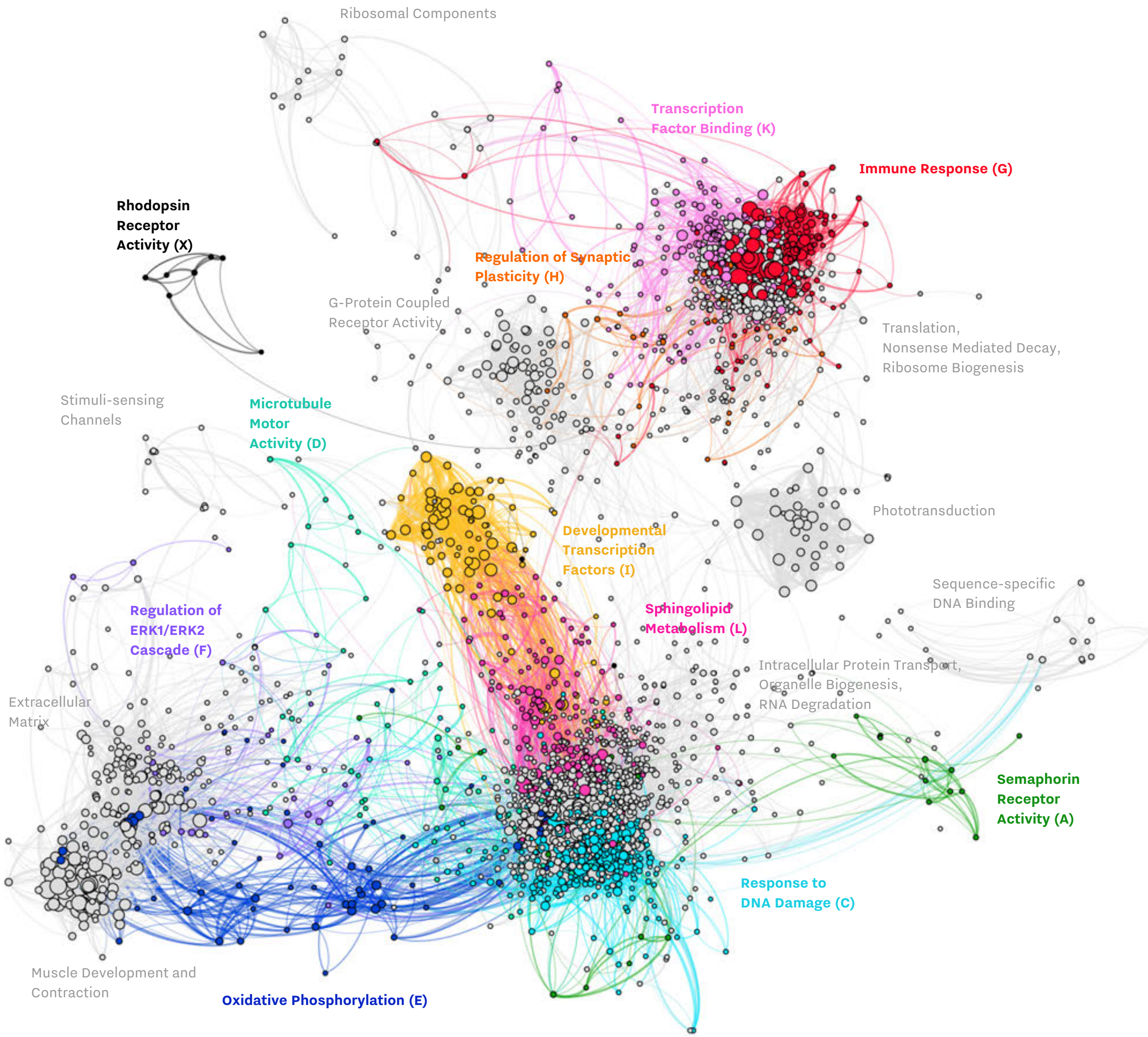
How are biological processes connected in the zebrafish brain transcriptome?

- Weighted gene co-expression network analysis[12] allows us to determine “modules” or groups or genes with correlated expression.
- Most modules show functional enrichment.
- Many modules are altered in *psen1* mutants (using module-trait correlation).



Visualisation produced by plotting adjacency matrix (representing the gene co-expression network) in Gephi.

12. Zhang & Horvath. *Statistical Applications in Genetics and Molecular Biology*. 2005. 4(1).

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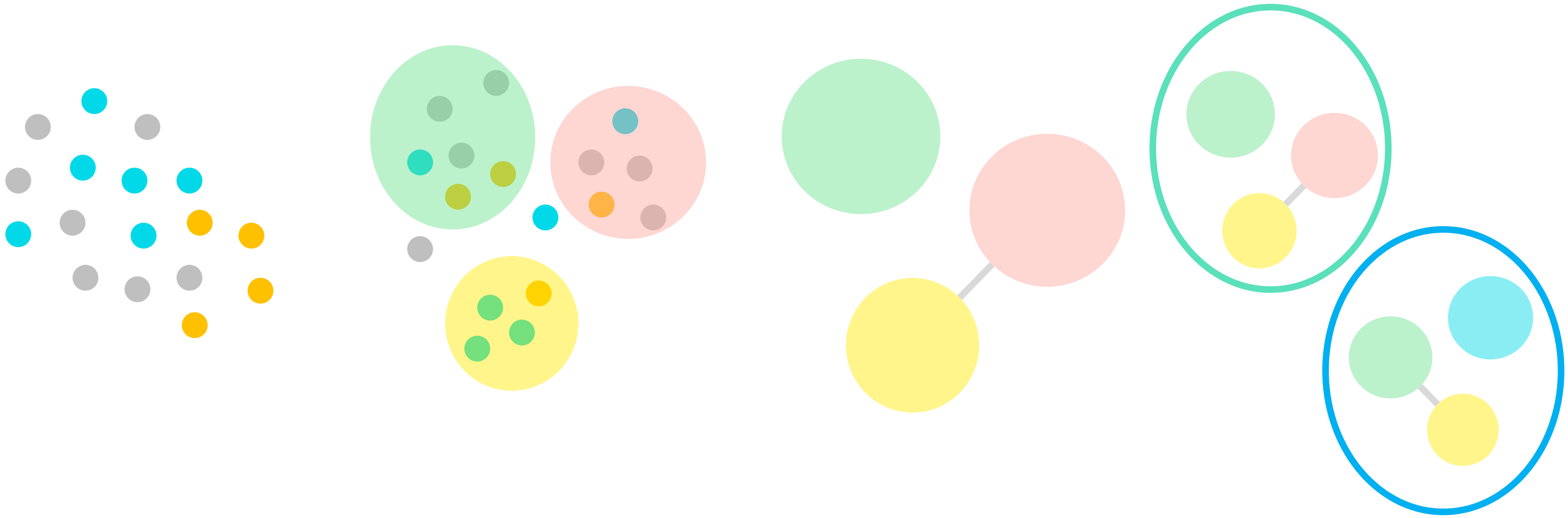

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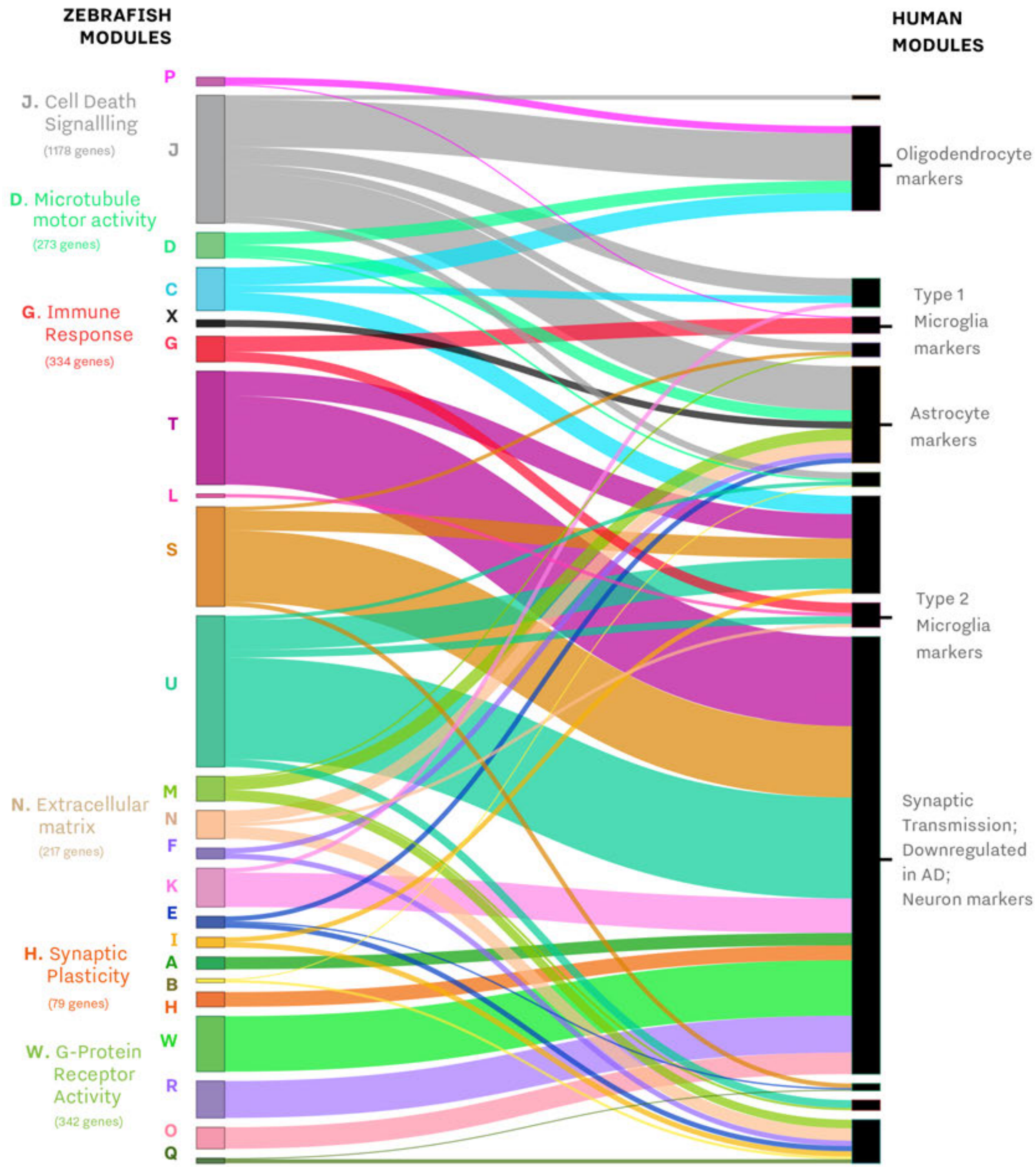
- Constructed gene co-expression network for a publically-available human Alzheimer's disease microarray-based dataset[13], with homologous human genes and *collapseRows* function[14].
- Used module preservation statistics[15] to determine whether module properties and genes are preserved between zebrafish and human brain.



13. Antonell et al. *Neurobiol Aging.* 2013 Jul; 34(7) 1772-1778.
14. Miller et al. *BMC Bioinformatics.* 2011. 12(322).
15. Langfelder et al. *PLOS Computational Biology.* 2011. 7(1):e1001057.

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Acknowledgements

SUPERVISORS

Stephen Pederson

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