Transcriptomic Analysis of the Age-related Response to Endurance Exercise Training

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Aims of Project

- Extension of published project investigating age-related response to exercise training[1]
- Age-related decrease in aerobic capacity and muscle mass have been linked to declines in mitochondrial function[2].
- Therapies which maintain or improve mitochondrial function could potentially combat conditions such as sarcopenia[3].
- Most effective method of promoting mitochondrial biogenesis in skeletal muscle is exercise - biology not fully understood.
- Our aim is to examine the transcriptional response of skeletal muscle to exercise in different age-groups.

Key Questions

By measuring gene expression, we can study the transcriptomic response to exercise and ask the following:

- Is the initial response to exercise different to the response to prolonged exercise training?
- How does the transcriptional response to exercise vary between age groups?

The Data

12 male individuals:

- 6 young (~20 years old)
- 6 old (~74 years old)

Three time-points

- Before exercise (baseline)
- Two hours after a single bout of training (2h)
- After 12 weeks of aerobic exercise training (training)

Skeletal muscle samples from each individual were collected at each time-point and subjected to RNA-sequencing. Count data for ~22000 genes were converted to Fragments per Kilobase of transcript per Million mapped reads(FPKM)

Methods

- Untargeted differential expression analysis
- Targeted analysis of biomarkers of mitochondrial biogenesis, content, function and dynamics
- Gene ontology analysis to find up-regulated/down-regulated functions

Principal Component Analysis

Untargeted Differential Expression Analysis

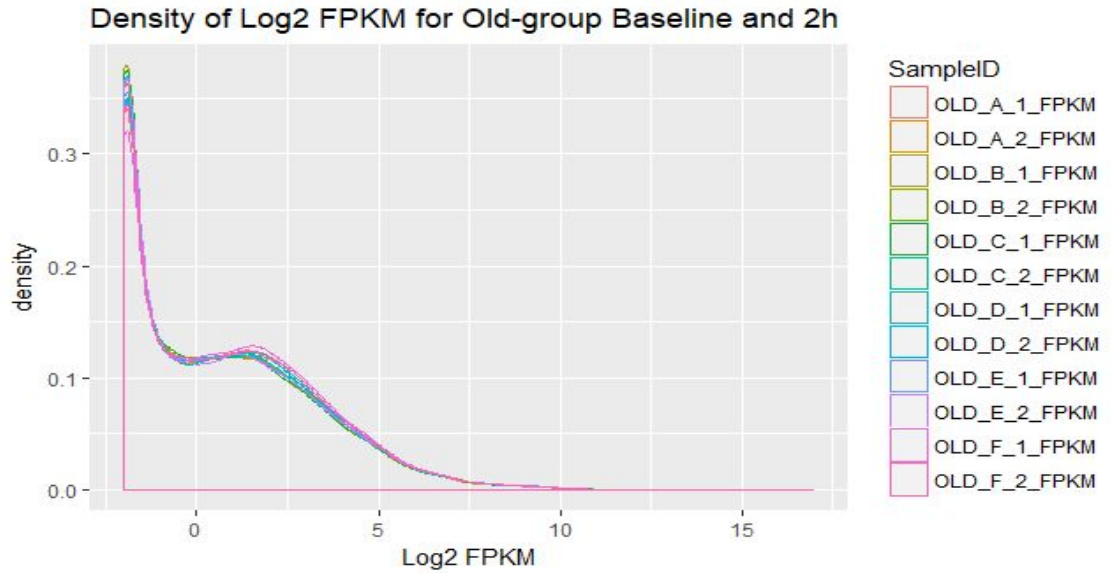
NOISeq

- NOISeq[4] is a bioconductor package in R
- Non-parametric differential expression analysis
- Filtering of genes with low expression across all samples
- Finds probability of differential expression by using log2-ratio of two conditions and value of difference between conditions and compares against noise distribution
- DEGs have probability of differential expression > 0.8 (i.e. 4x more likely to be differentially expressed than not)

Differential Expression Analysis in NOISeq

- Four Analyses:
  - Old-group baseline vs Old-group 2h
  - Old-group baseline vs Old-group training
  - Young-group baseline vs Young-group 2h
  - Young-group baseline vs Young-group training
- Identification of DEGs (probability > 0.8)
- Identification of DEGs shared between analyses
- Assessment of direction of fold change for DEGs

Filtering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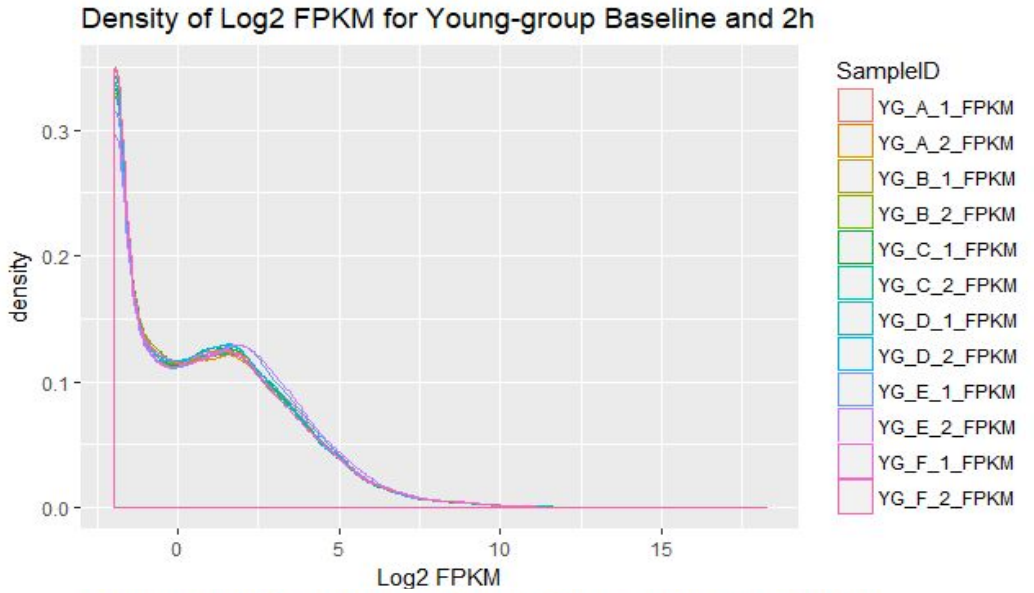
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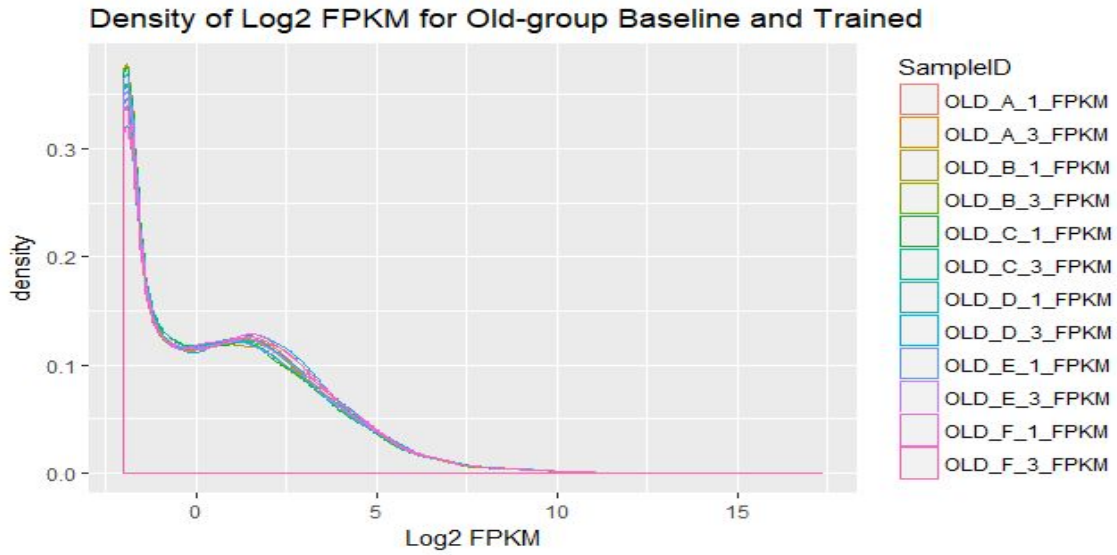
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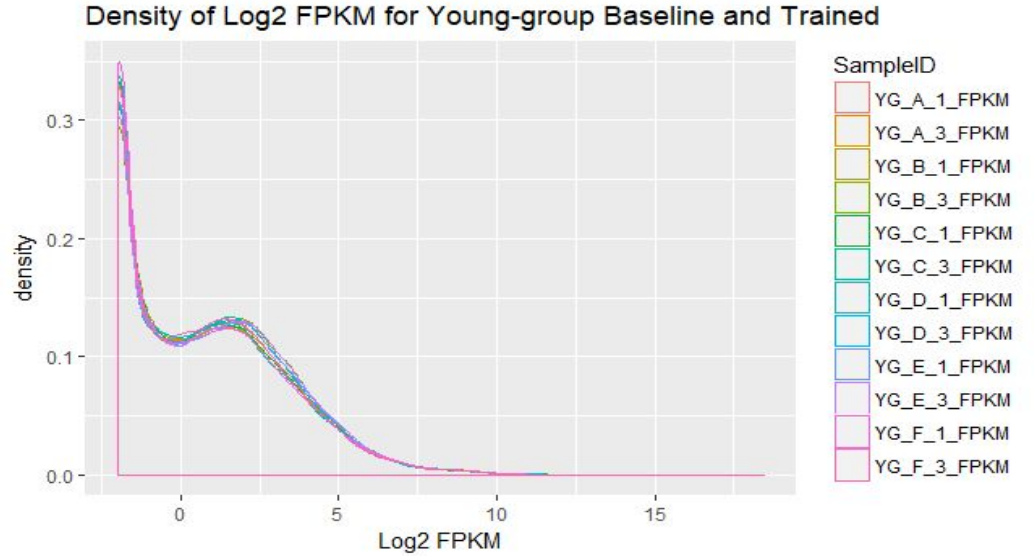
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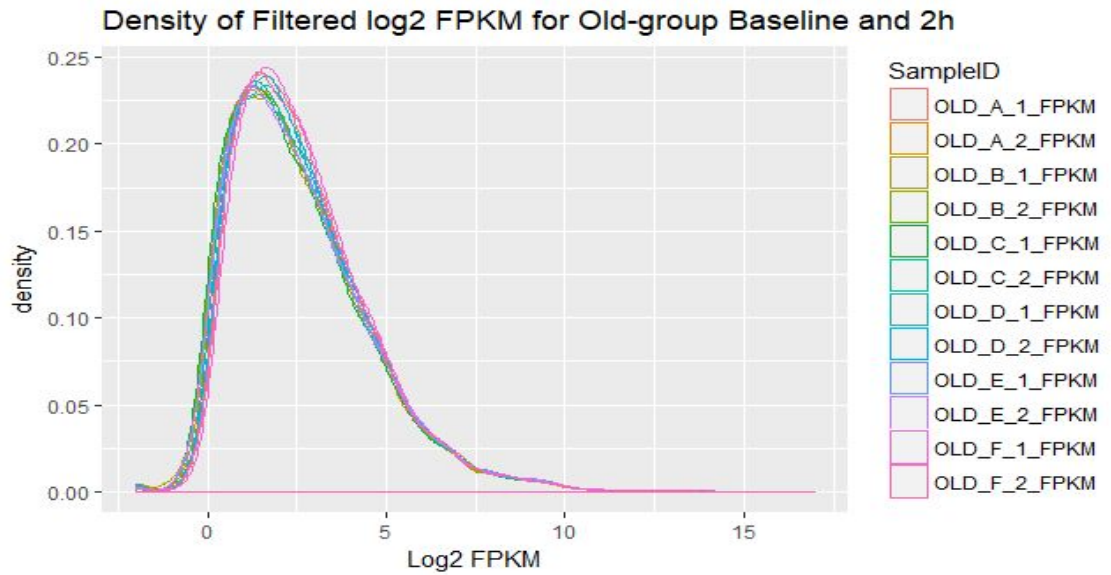
Filtering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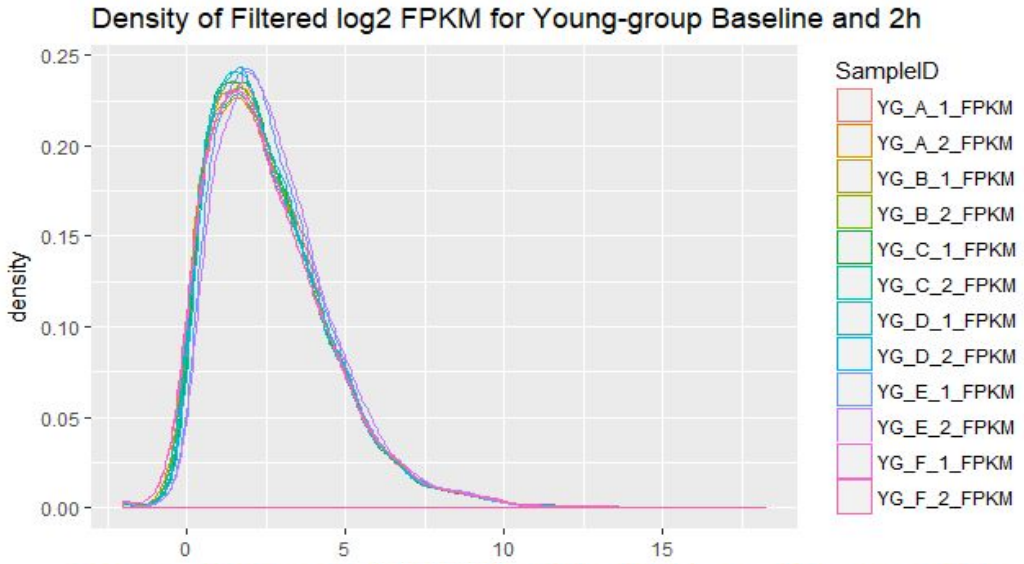
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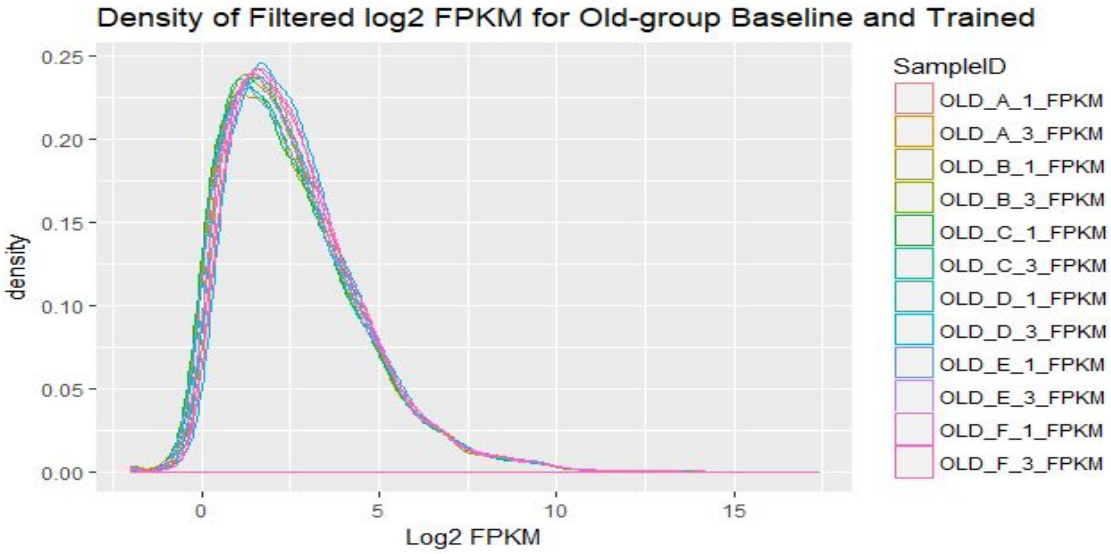
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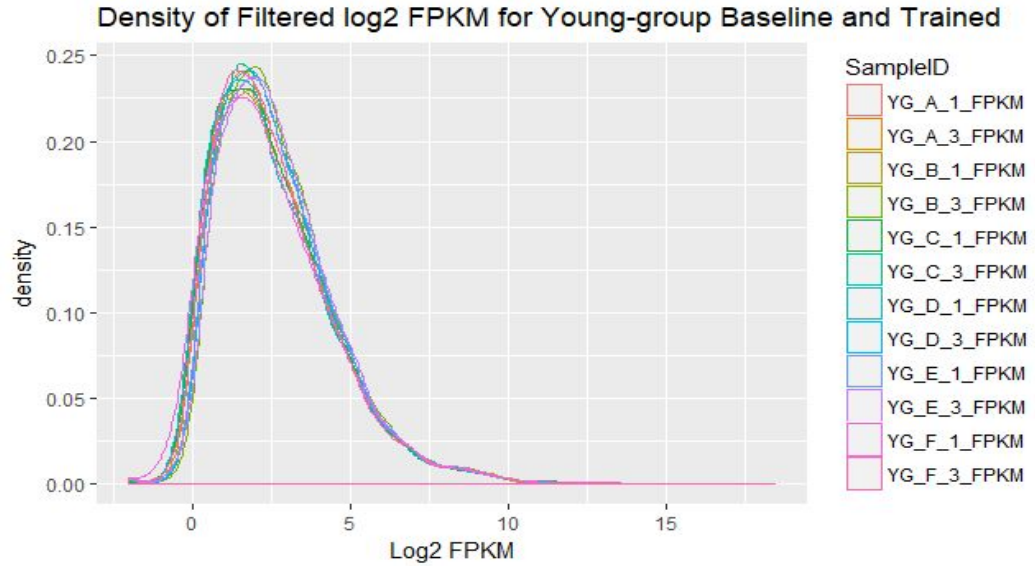
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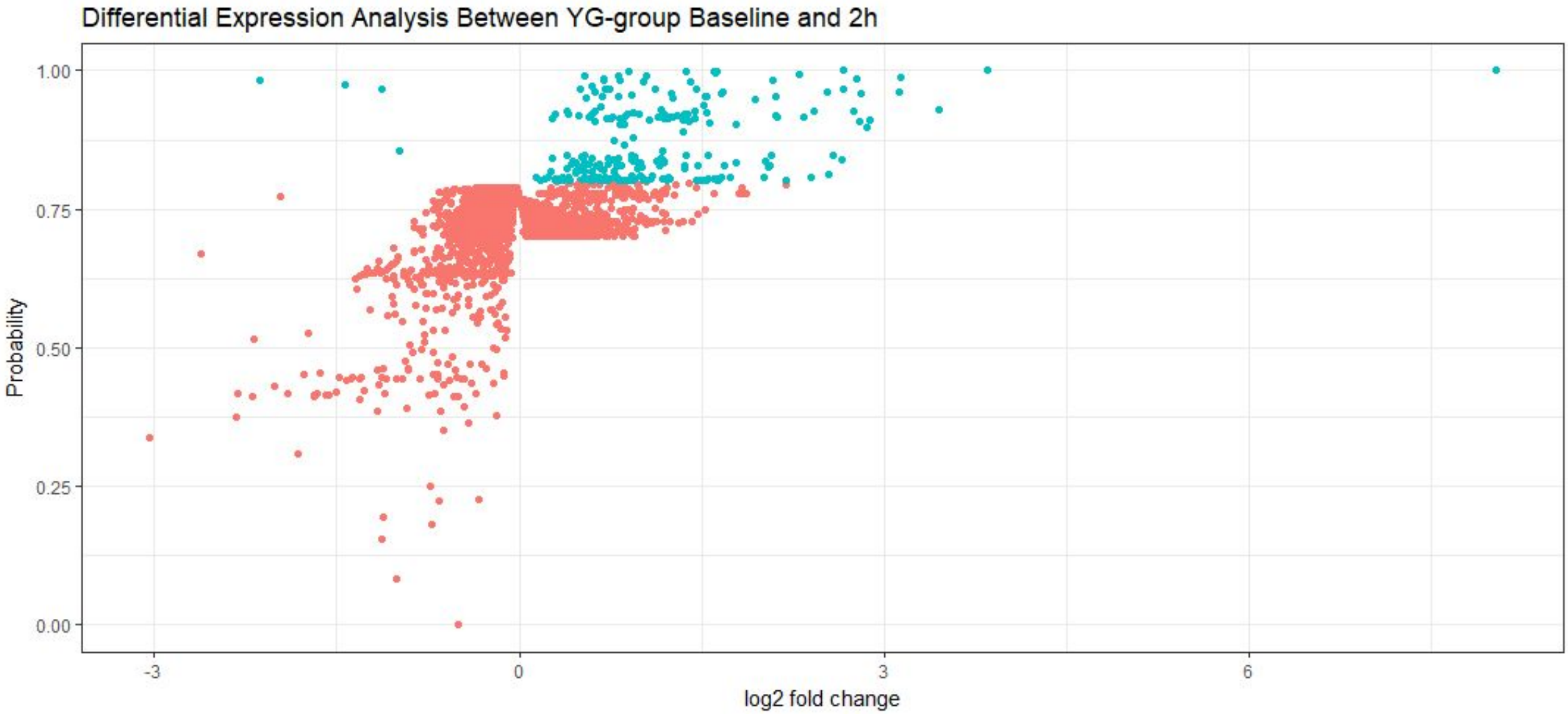
Differential Expression Analysis Between YG-group Baseline and 2h
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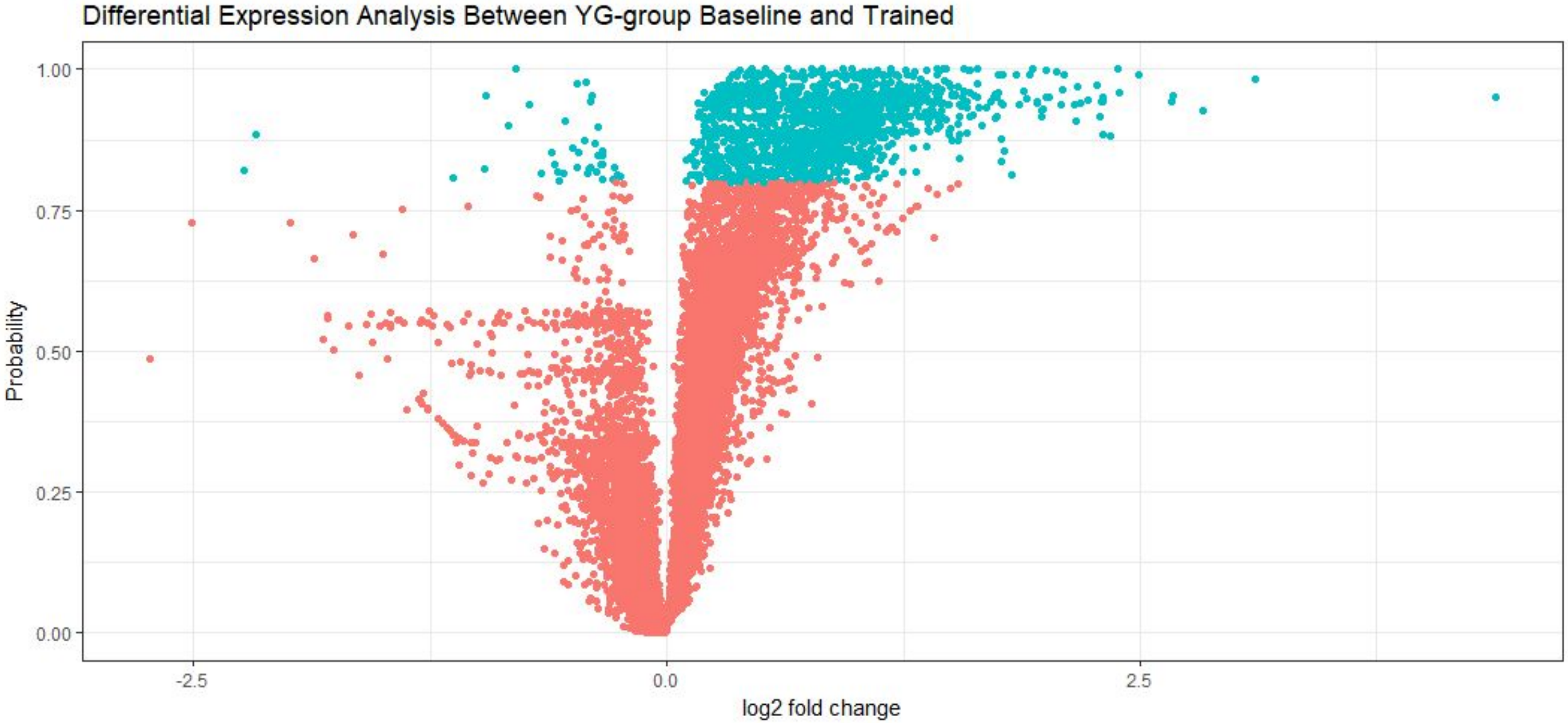
0.25
0.00
-3
0
3
6
log2 fold change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0.25
0.00
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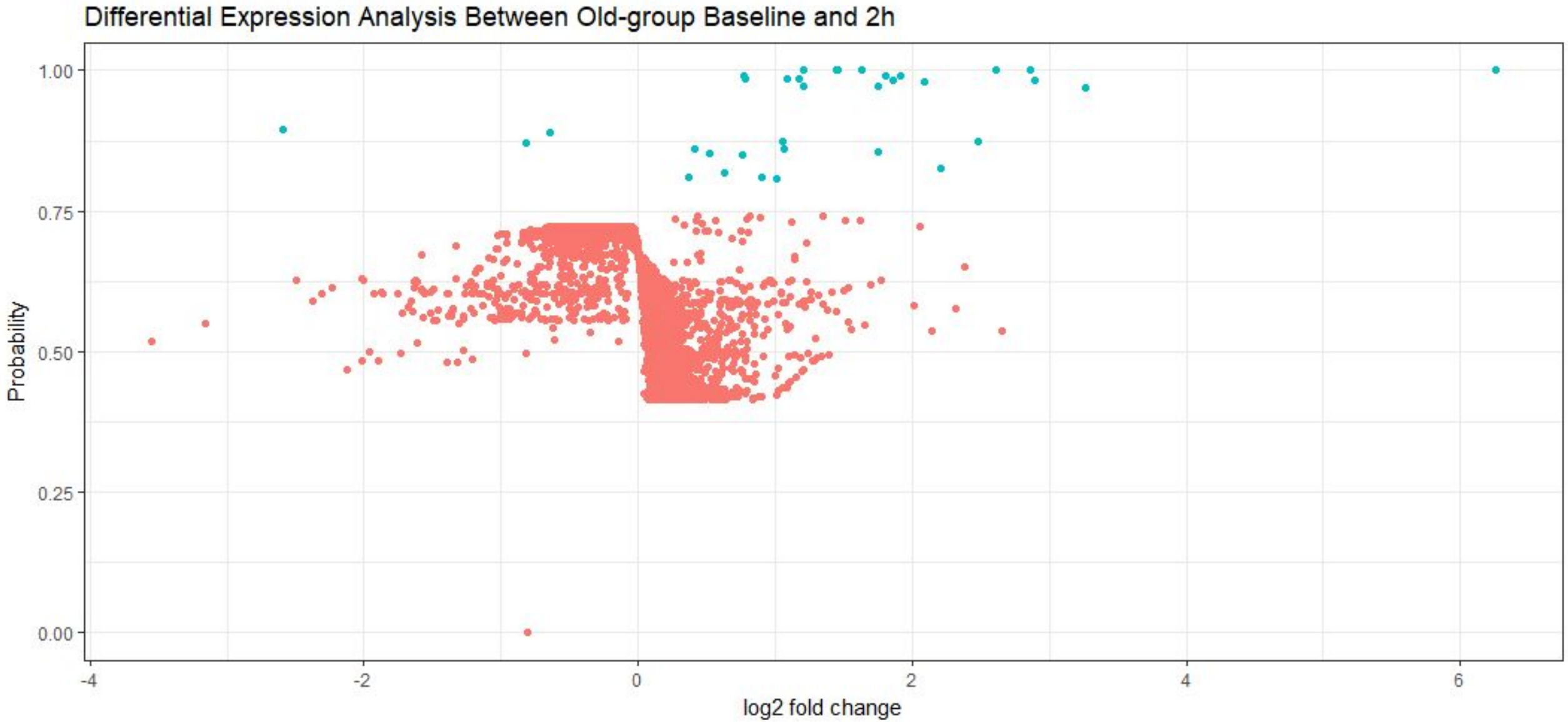
2.5
log2 fold change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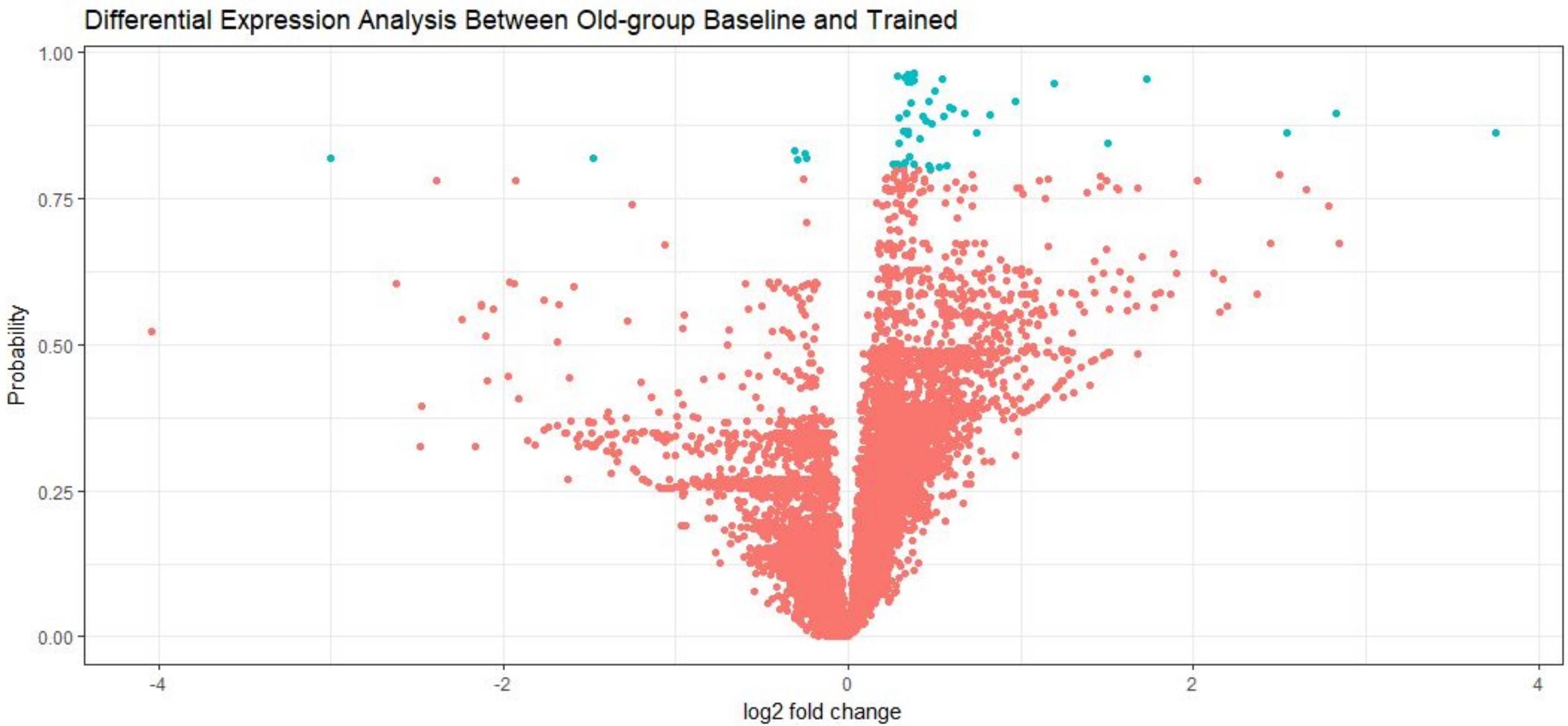
-2
0
2
4
6
log2 fold change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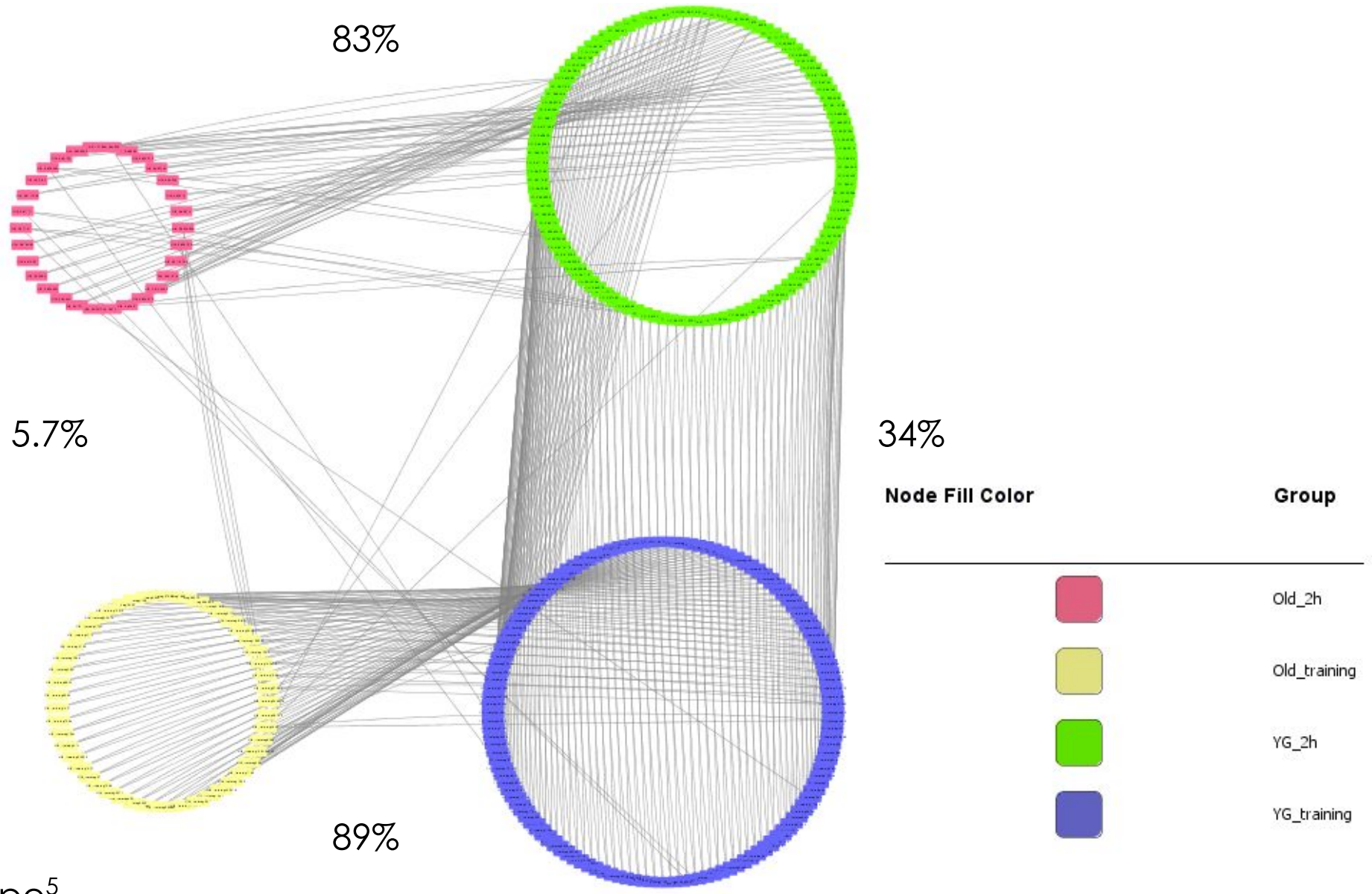
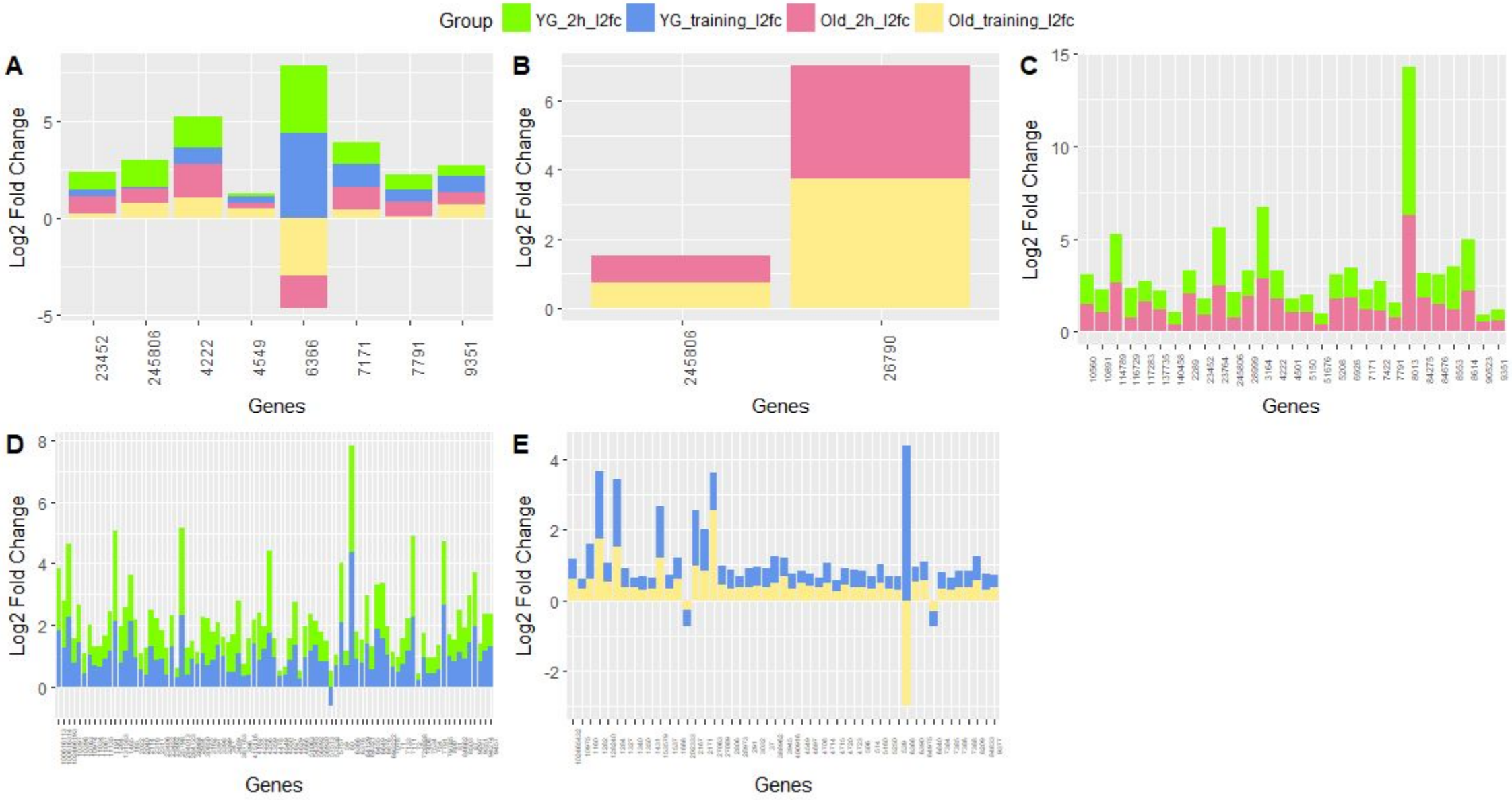
1.00
0.75
0.50
0.25
0.00
Probability
-4
-2
0
2
4
log2 fold change

DEGs network



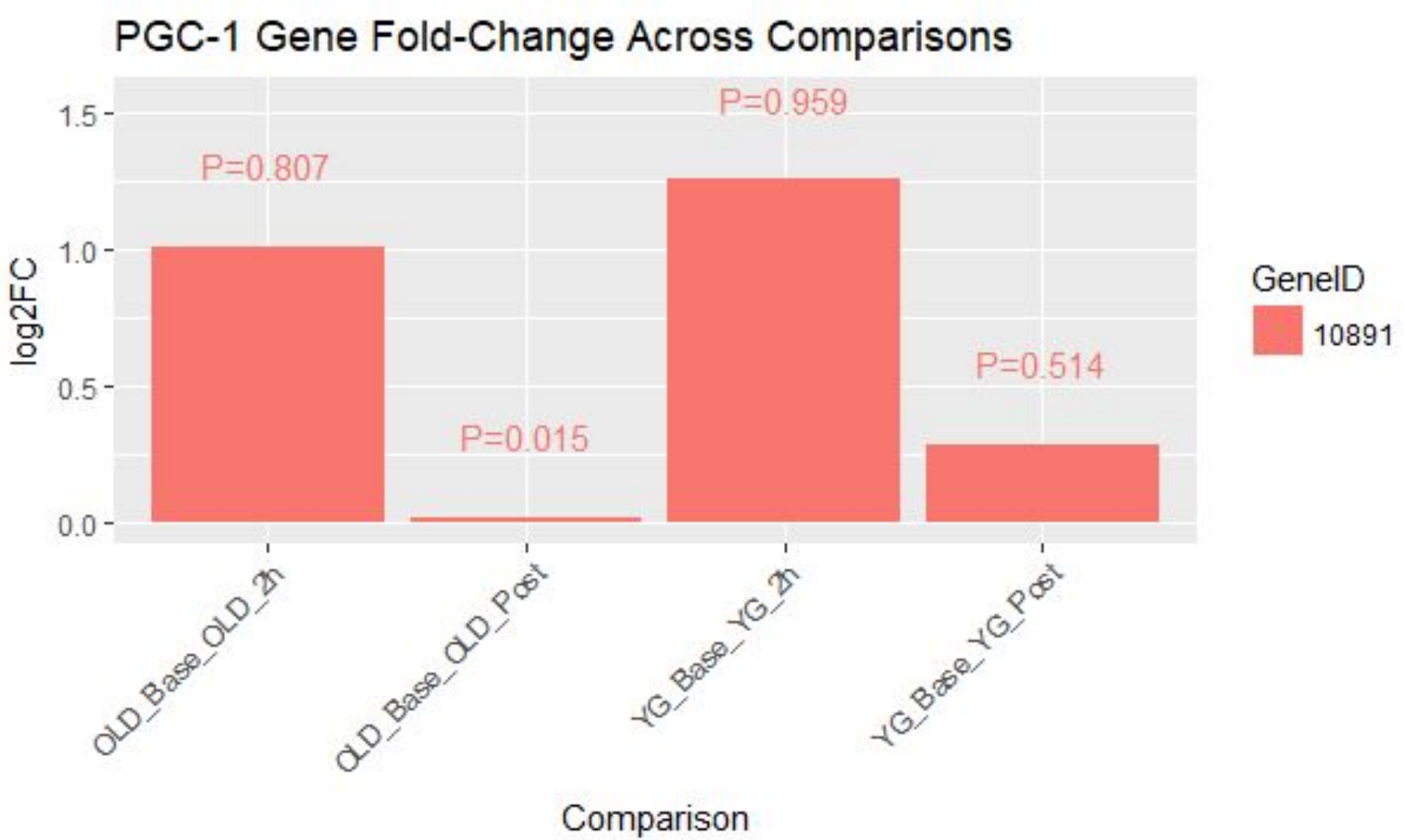
Created in Cytoscape[5]

Log2 fold change of shared DEGs

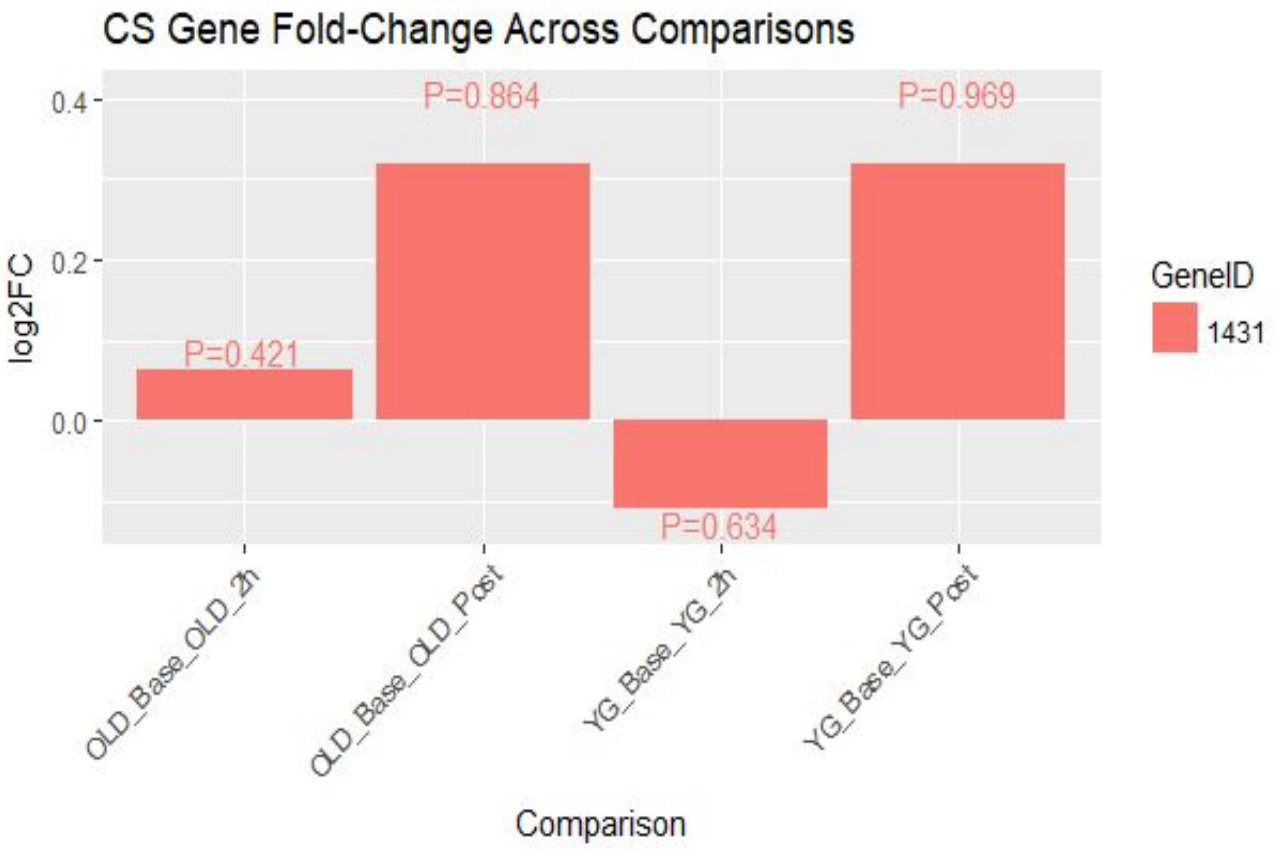


Targeted Analysis of Biomarkers for Mitochondrial Biogenesis, Content, Function, and Dynamics

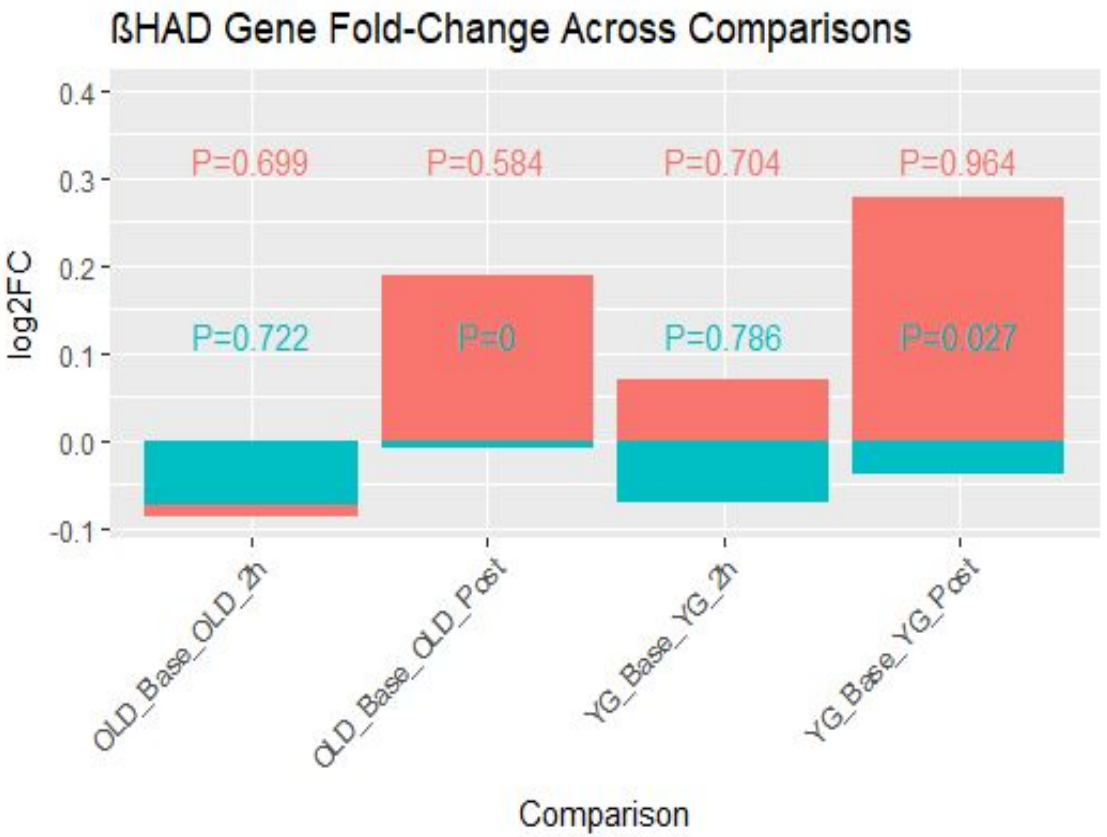
Mitochondrial Biogenesis

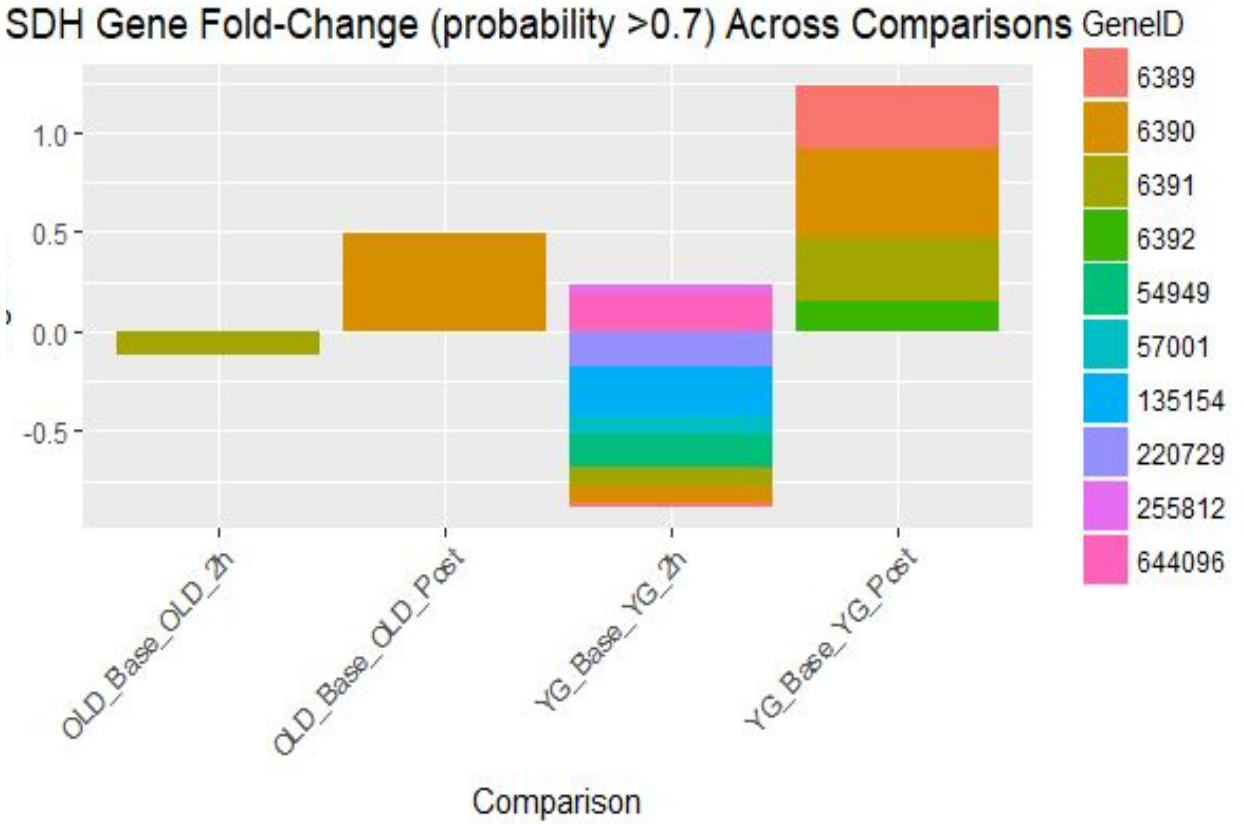


Markers of Mitochondrial Content

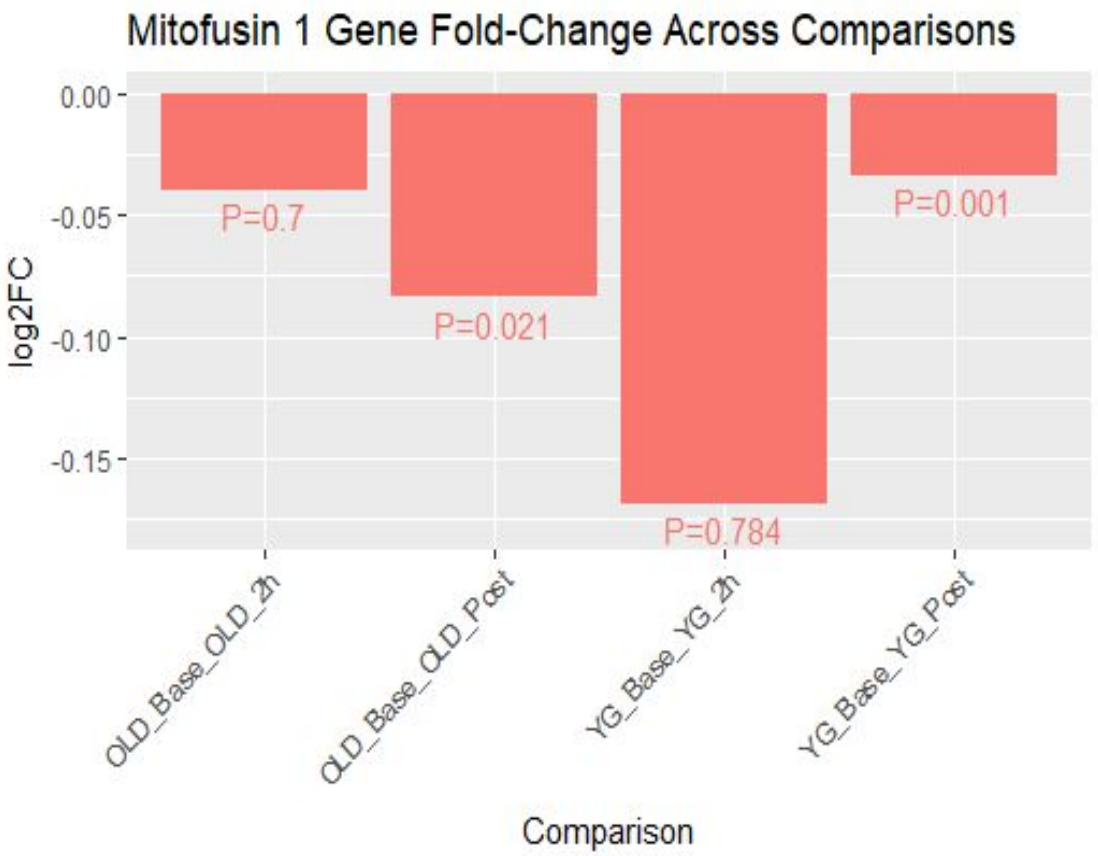


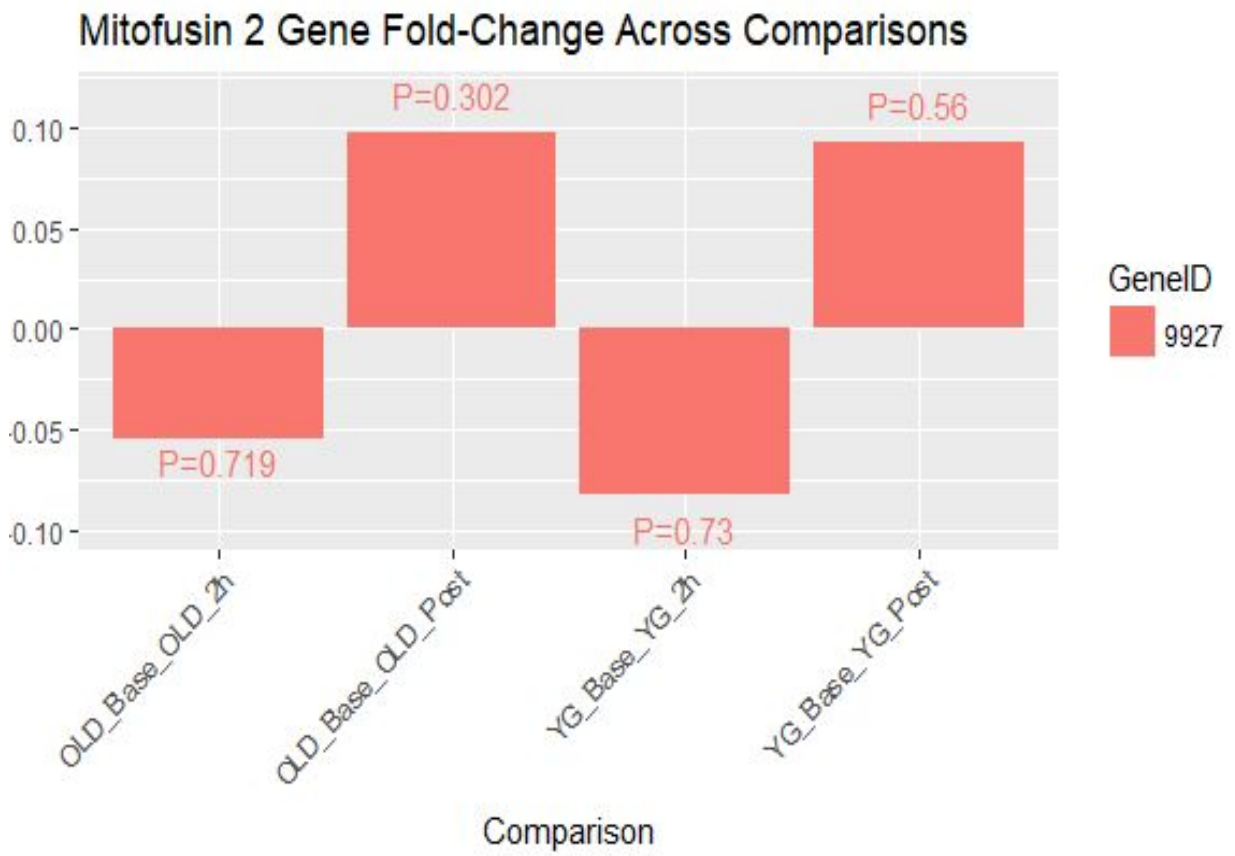
Markers of Mitochondrial Function



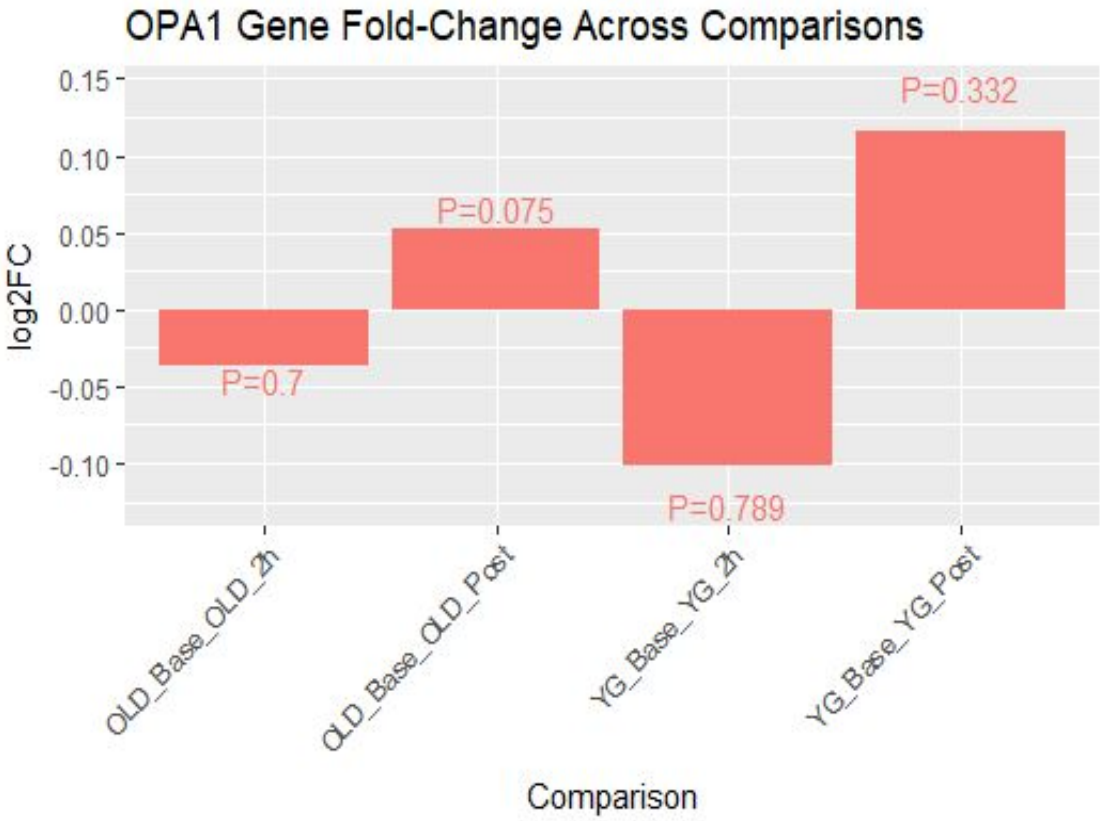


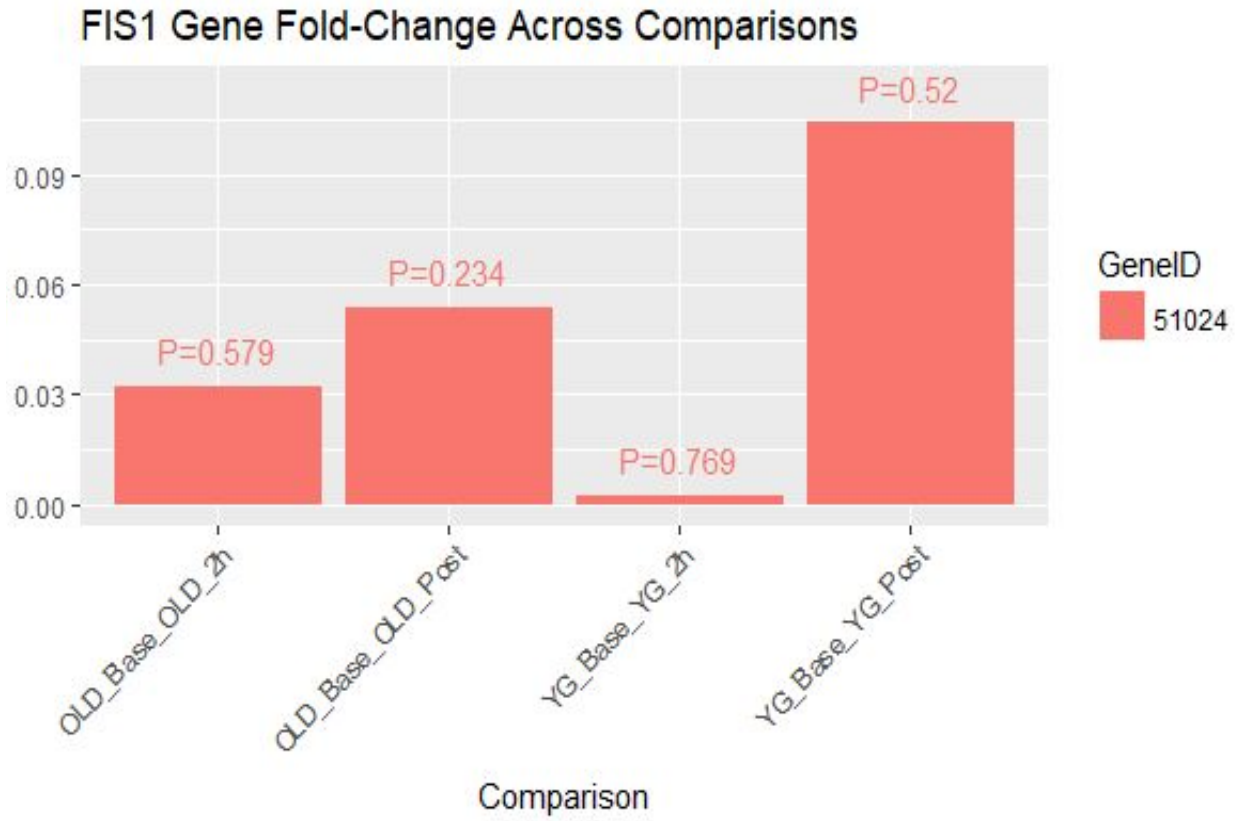
Markers of Mitochondrial Dynamics





Markers of Mitochondrial Dynamics





Young Group

Two hour:

- Increased mitochondrial biogenesis (PGC upregulation)
- Weak evidence of decreased mitochondrial content (COXIV downregulation)
- Weak evidence of decreased mitofusion (MFN1, OPA1 downregulation)

Trained:

- Increased mitochondrial content (CS and COXIV upregulation)
- Increased mitochondrial function (βHAD, SDH upregulation)

Old Group

Two hour:

- Increased mitochondrial biogenesis (PGC upregulation)
- Weak evidence of decreased mitochondrial content (COXIV downregulation)

Trained::

- Increased mitochondrial content (CS and COXIV upregulation)
- Increased mitochondrial function (SDH upregulation)

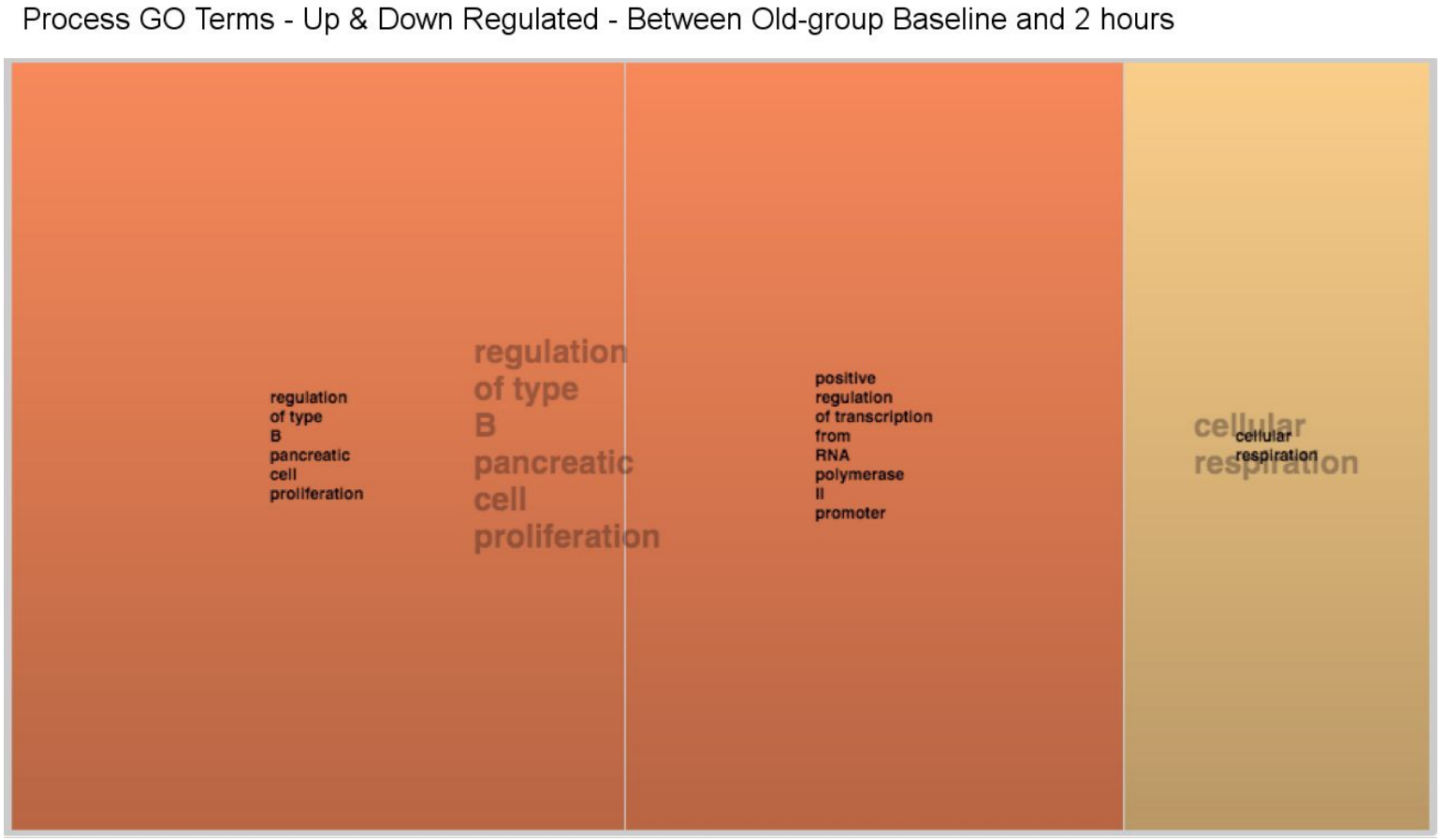
Gene Ontology Analysis

Introduction and Processing

- Each dataset produced by NOISeq was annotated to give non-unique GO Terms with p-values for the Gene's differential expression
  - Three GO Term Categories separated - Component, Function and Process
- Each GO Term was compiled into a contingency table
- Fisher's Exact Test was applied to get a p-value for each GO Term
- Adjusted using False Discovery Rate
- Compiled into a summary table and filtered to give only significant findings
- Process repeated for only Up and Down Regulated Genes
- Gives 36 datasets - REVIGO[6] - Tree Maps
- Run through baseline vs 2 hour then vs Trained

Baseline vs 2 hours

Process - Old UP & Down

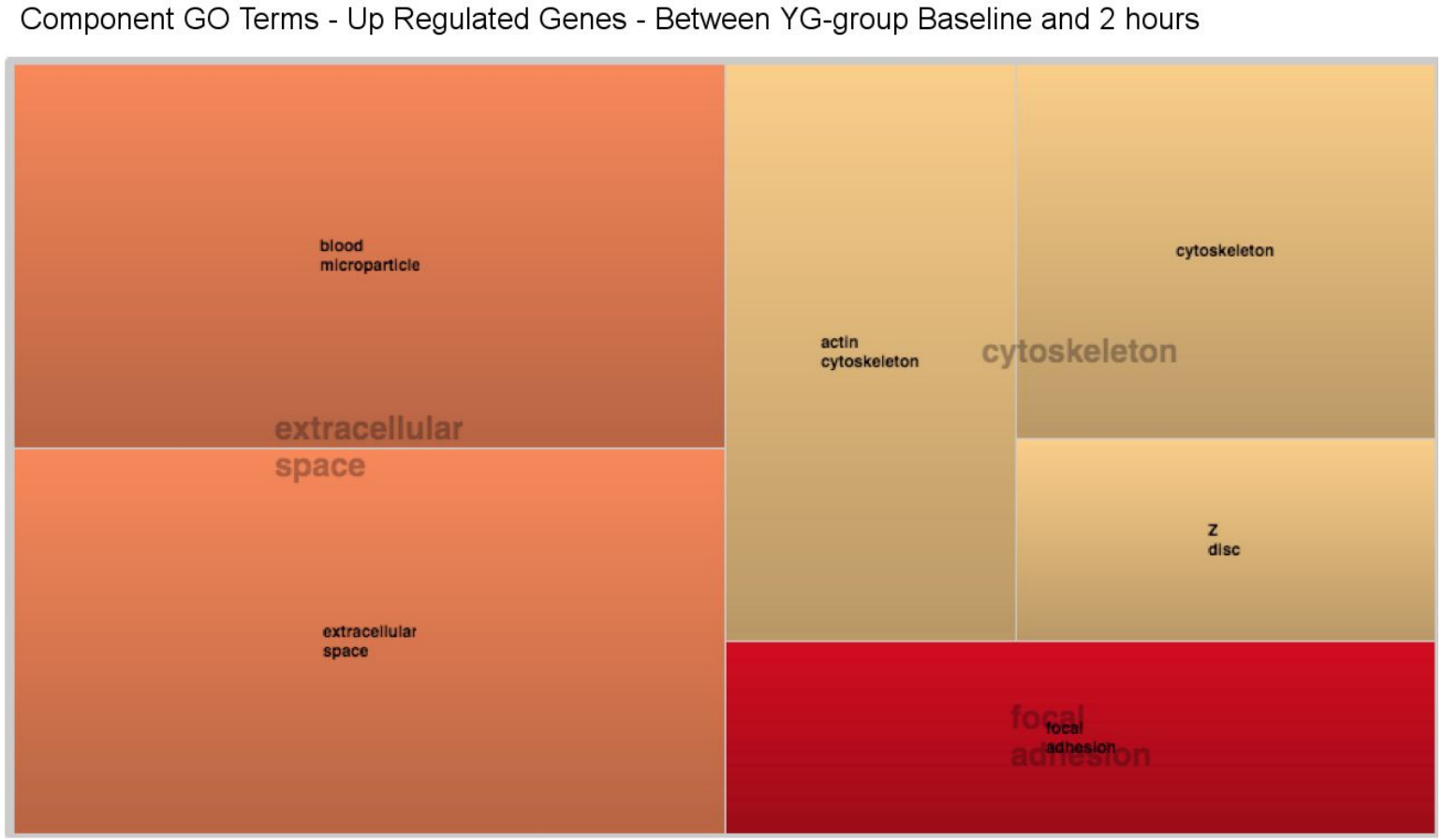


Pancreatic related gene and cellular respiration

Process - Young UP

Reparation and damage control

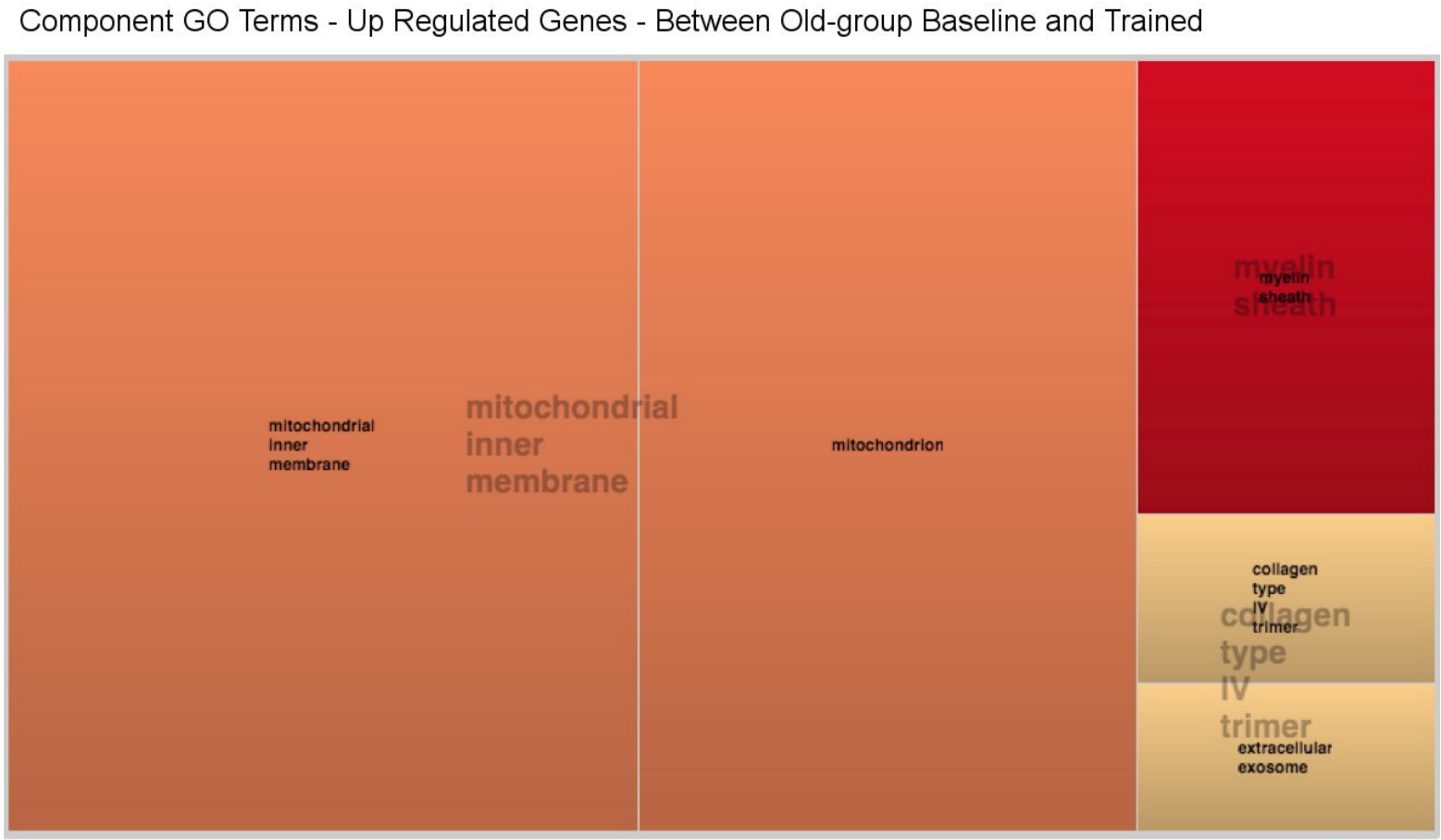
Component - Young UP



More varied, repairs and to extracellular space

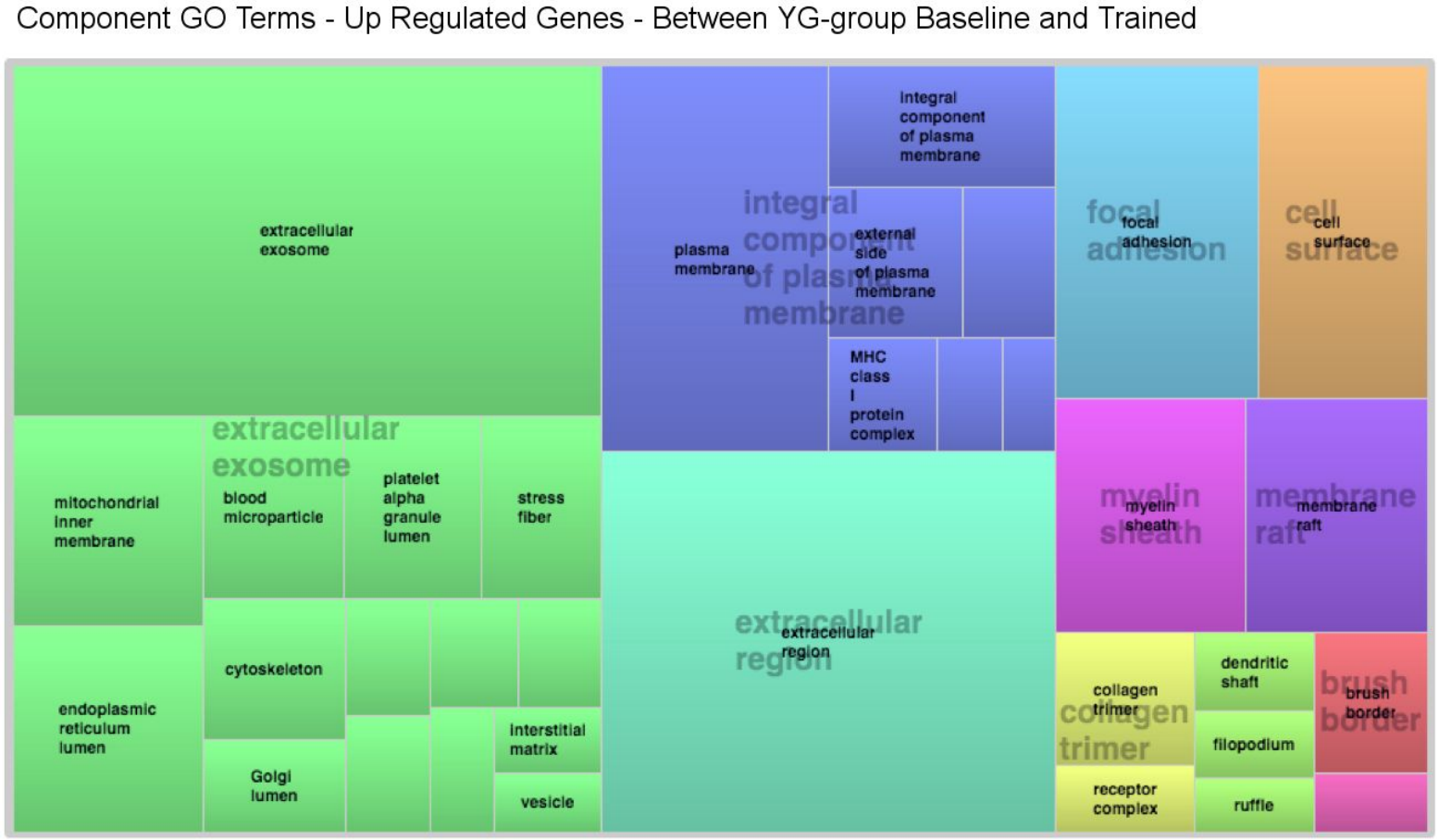
Baseline vs trained

Component - Old UP



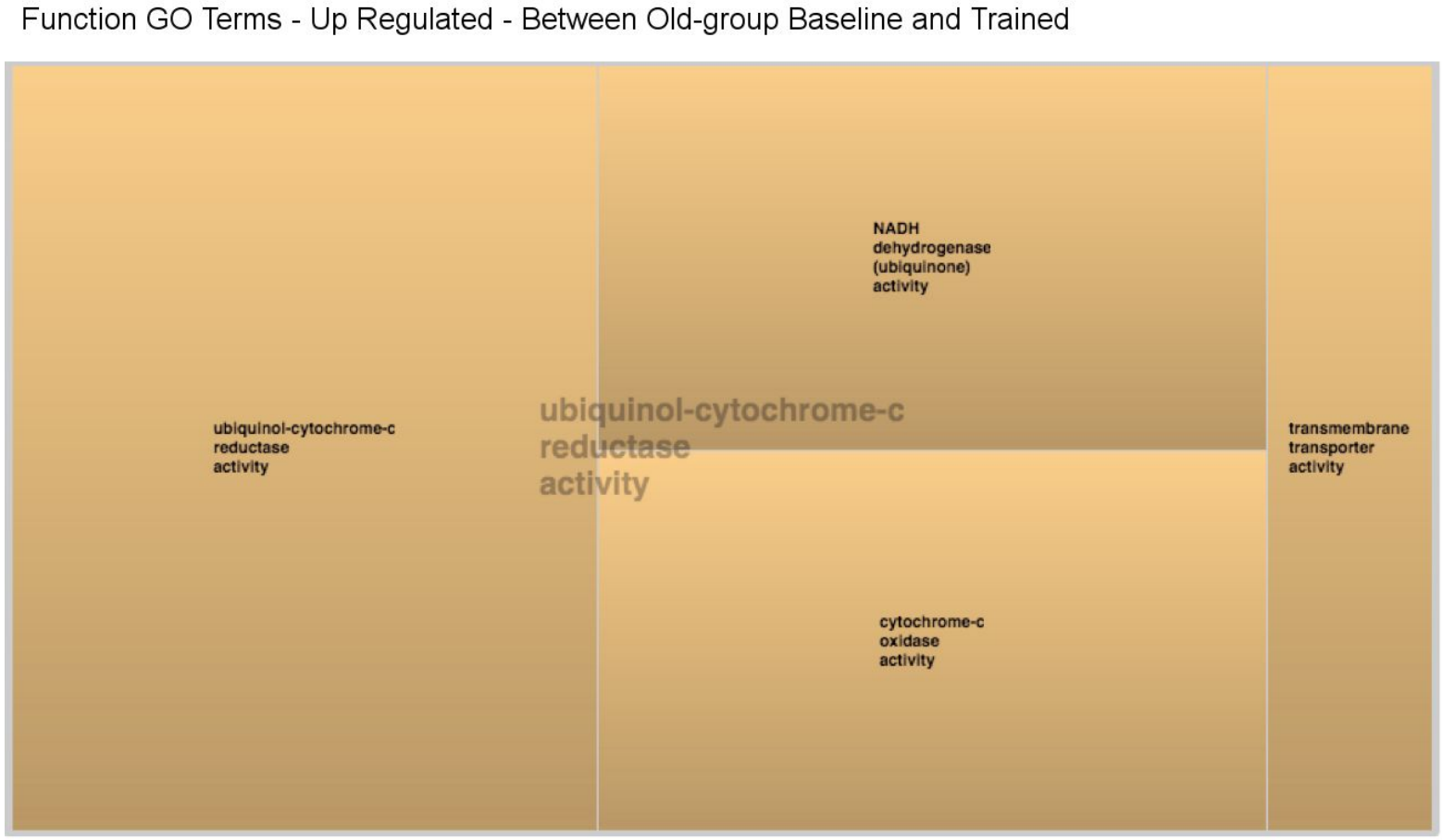
Focus largely on mitochondria

Component - Young UP



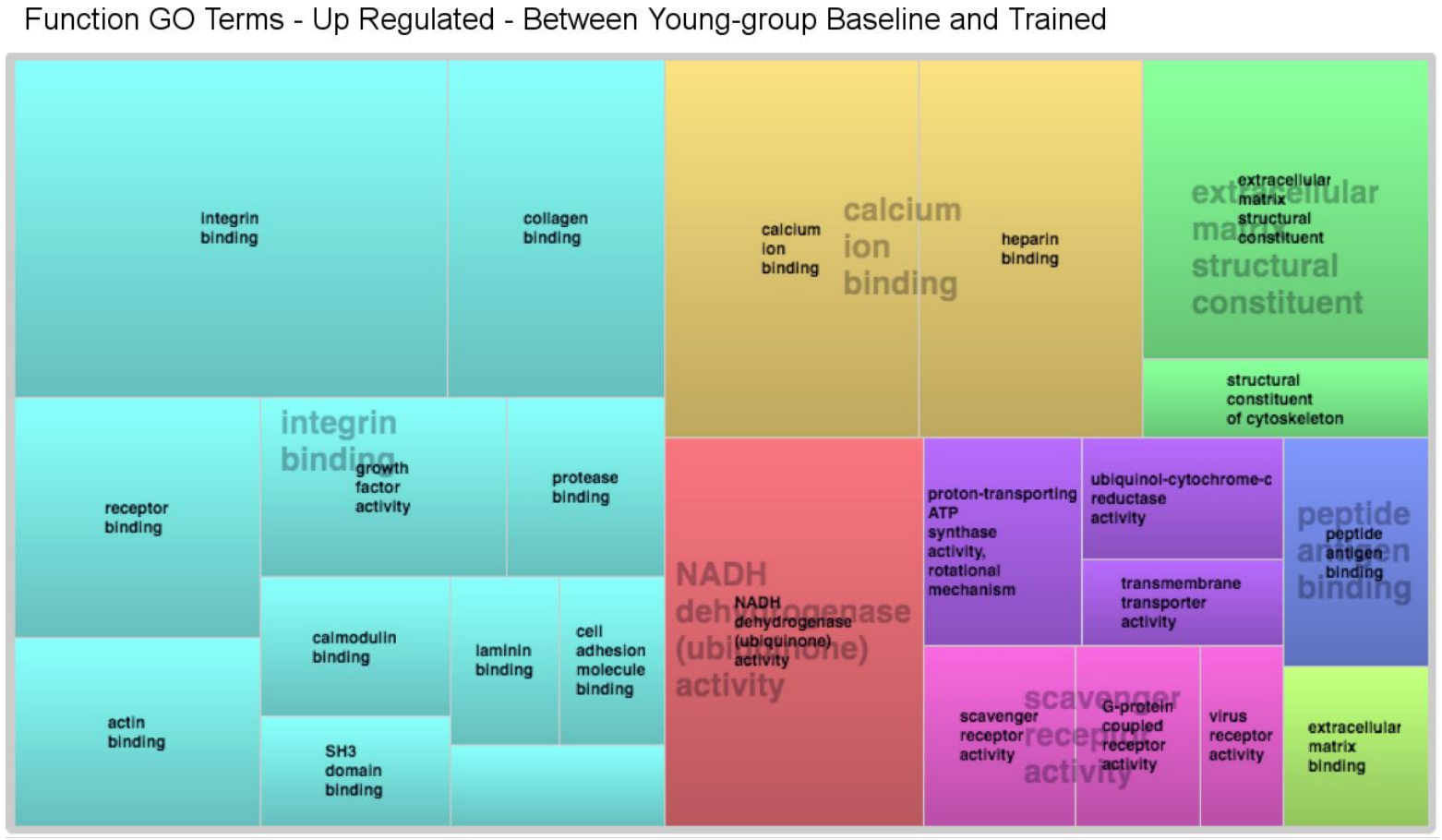
Much more varied response, significant component extracellular

Function - Old UP



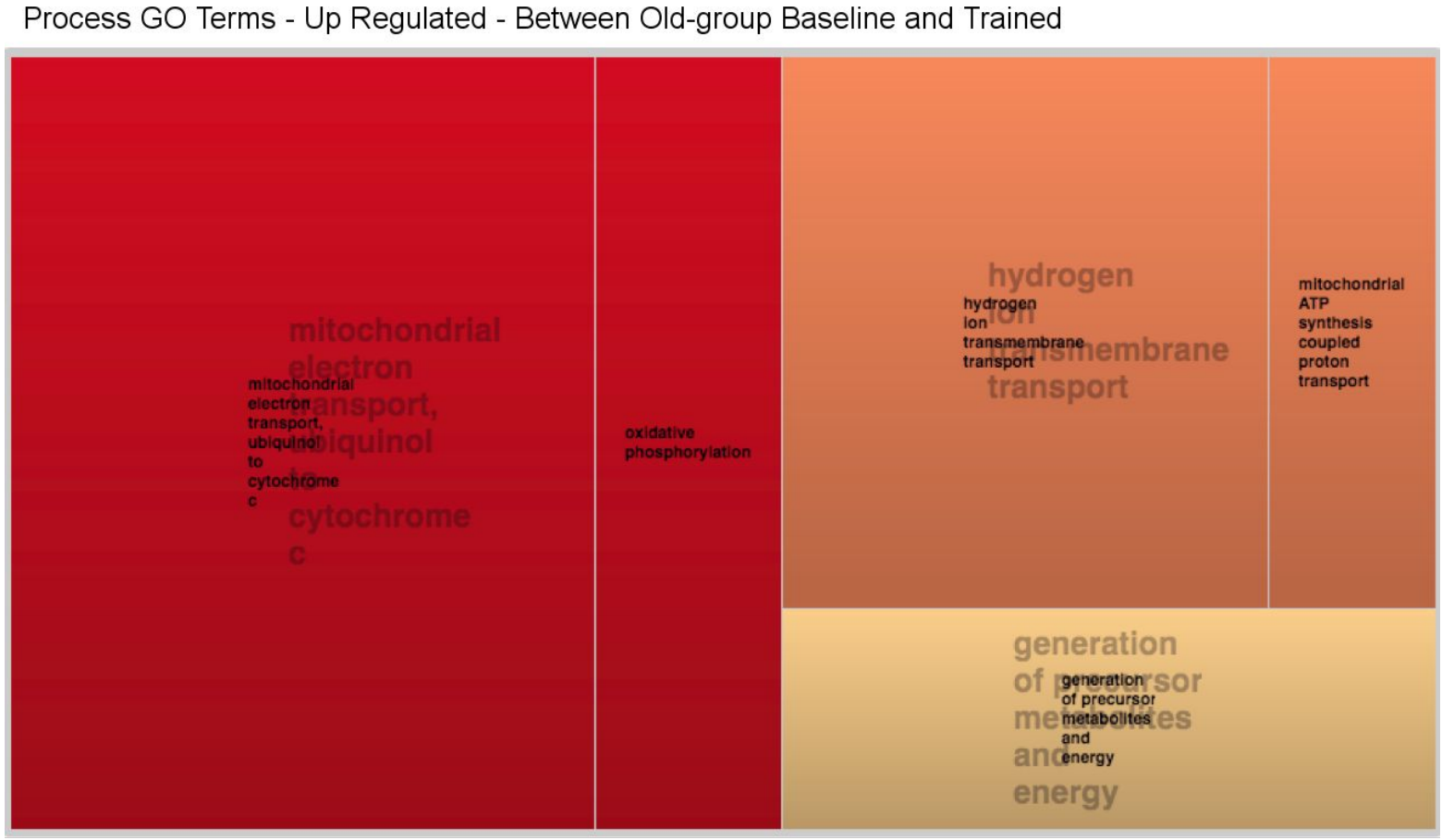
All involved in mitochondrial functions

Function - Young UP



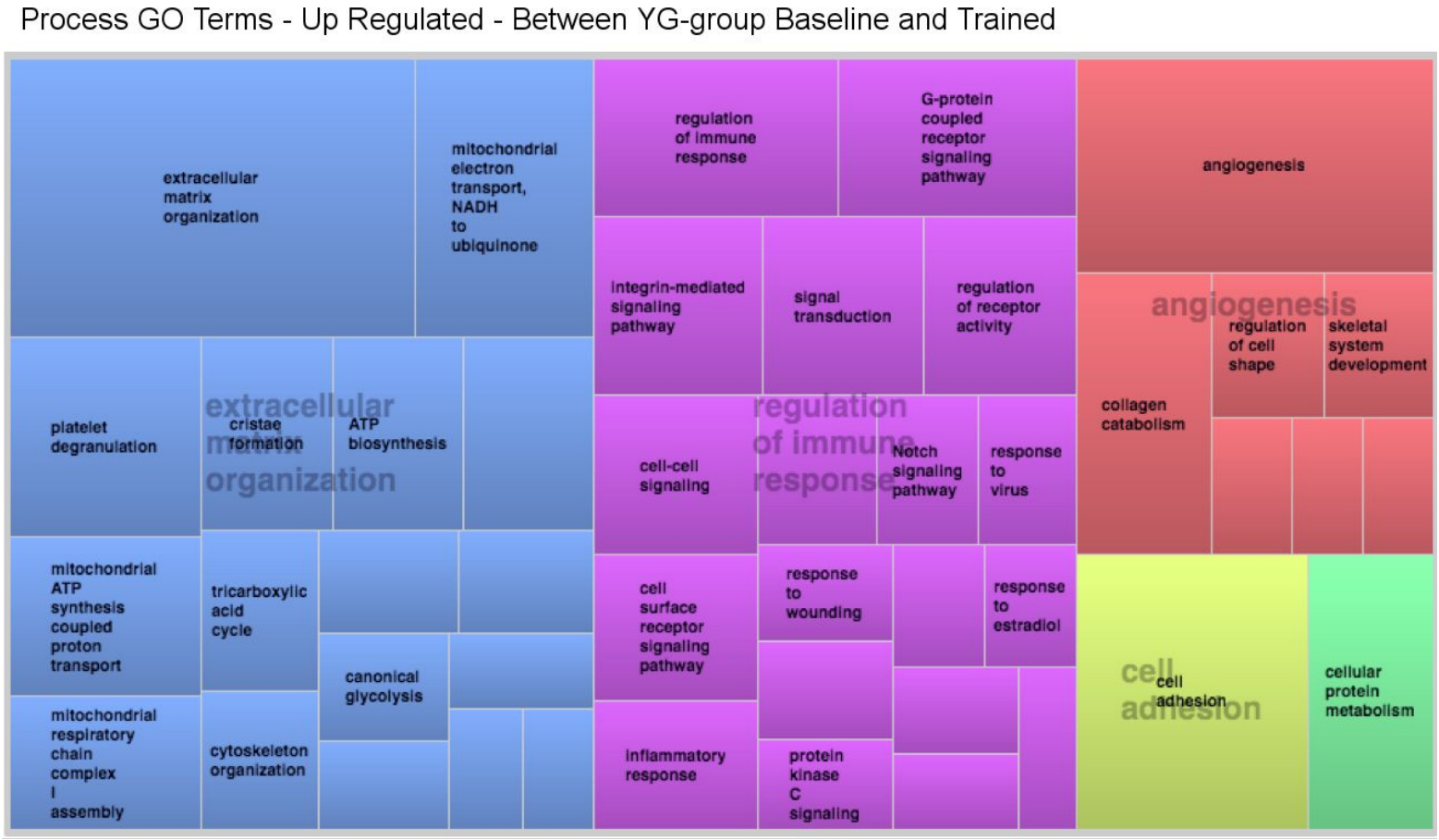
Mitochondrial functions as well as muscle building e.g. integrin

Process - Old UP



Mitochondrial processes and generation of precursors

Process - Young UP

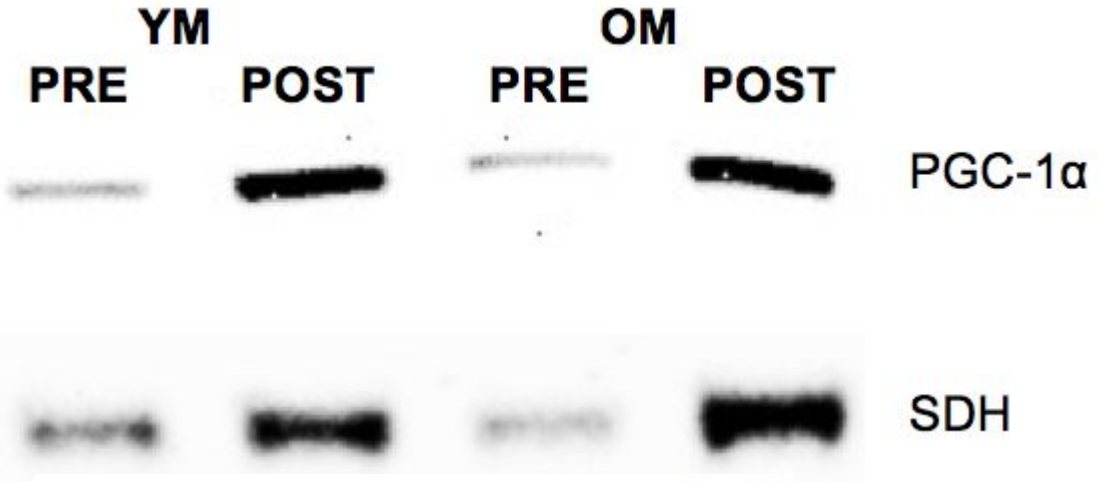


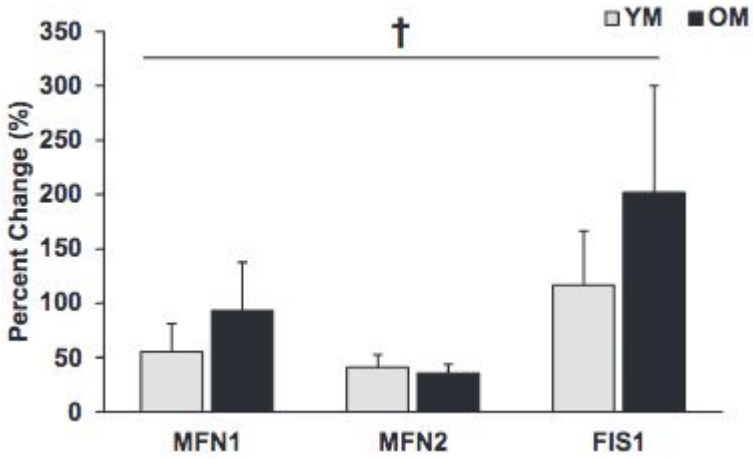
Much more diversified and includes growth processes

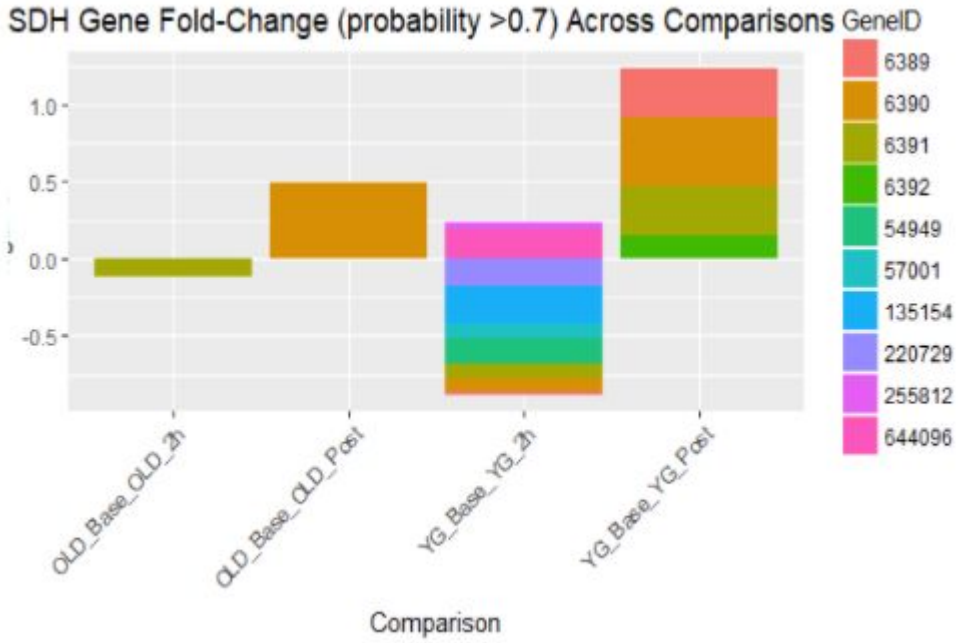
Conclusions

Our study vs previous study

- The power of Bioinformatics (previous study from 2014)







How Does the transcriptional response to exercise vary between 2h and trained groups?

- 5.7-34% of DEGs in 2h samples were shared with trained samples indicating most of response is different
- 2h
  - Significant upregulation (Probability > 0.8) of markers of mitobiogenesis and weak evidence of downregulation (Probability > 0.6) of markers of mitochondrial content
- Trained
  - Significant upregulation (Probability > 0.8) of markers of mitochondrial content and mitochondrial function (Probability >0.9)

How Does the transcriptional response to exercise vary between age groups?

- 83-89% of DEGs in old-groups were shared with the young-group of same time-point - indicates very similar response between age-groups, but young response was much more varied
- Before training, weak evidence of downregulation (Probability >0.7) for markers of mito-fusion was seen in the young group, but not in the old group
- After training, significant upregulation (Probability > 0.9) in SDH a marker of of mitochondrial function in both groups, with the young group showing upregulation in an additional marker βHAD (Probability > 0.95)

On Sarcopenia

Future direction

- More samples to allow predictive modelling and network analysis and to increase statistical power
- Additional data types such as proteomics, aerobic capacity and muscle size to link expression changes to
- KEGG annotations for pathway analysis

References

1. Konopka AR, Suer MK, Wolff CA, Harber MP. Markers of human skeletal muscle mitochondrial biogenesis and quality control: Effects of age and aerobic exercise training. J Gerontol A Biol Sci Med Sci. 2014; 69: 371–378.
2. Johnson ML, Robinson MM, Nair SK. Skeletal muscle aging and the mitochondrion. Trends Endocrinol. Metab. 2013; 24: 247–256.
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4. Tarazona S, Furio-Tari P, Turra D, Di Pietro A, Nueda MJ, Ferrer A, Conesa A. Data quality aware analysis of differential expression in RNA-seq with NOISeq R/Bioc package. Nucleic Acids Res. 2015; 43(21): e140.
5. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13(11): 2498 - 2504.
6. Supek F, Bošnjak M, Škunca N, Šmuc T. REVIGO summarizes and visualizes long lists of Gene Ontology terms. PLoS ONE. 2011; 6(7): e21800 .

Data and Code

https://github.com/annegarrett/Group_Project