

Predictive Modelling of Stress-Related Behaviour Through Gene Expression Following Prenatal Glucocorticoid Exposure

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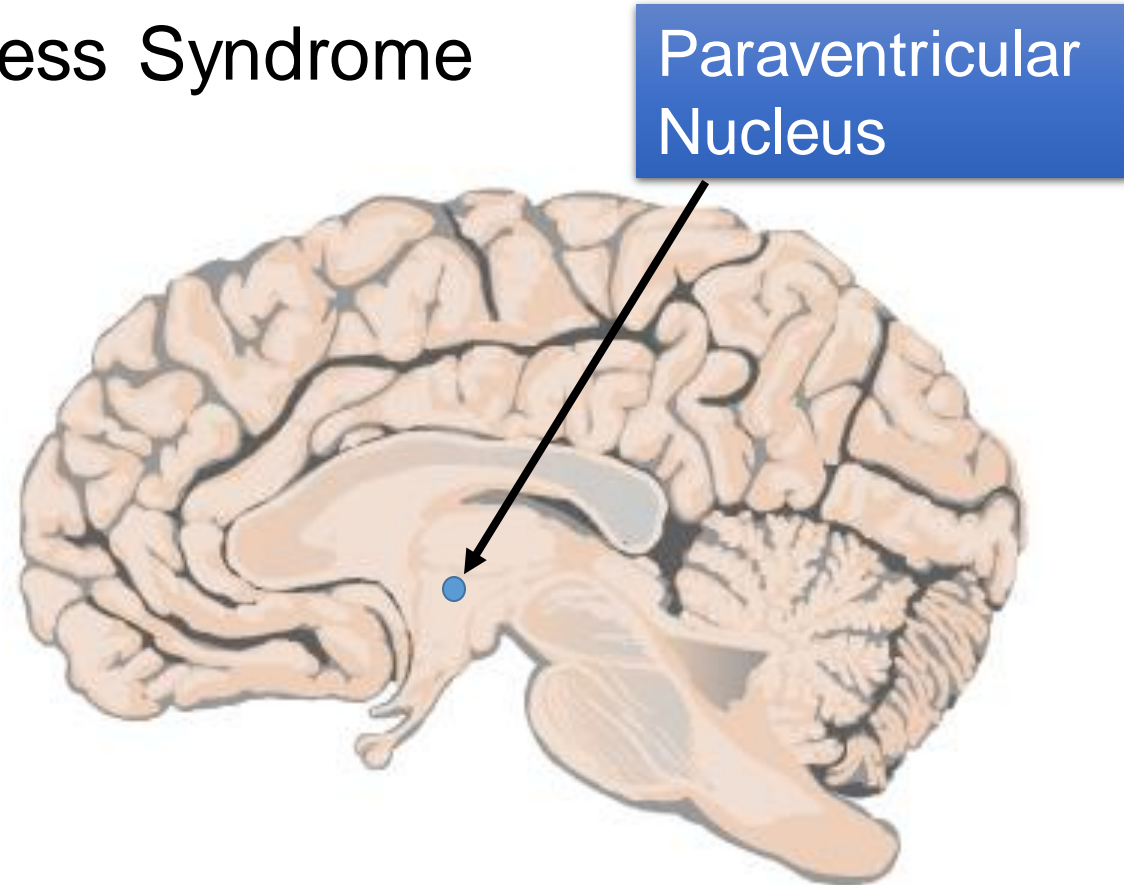
Glucocorticoids and Early Life Environment



- The early life environment has long term influences on future health
- Perinatal exposure to high levels of glucocorticoids has been linked to the development of:
 - heart disease
 - diabetes mellitus
 - depression and anxiety disorders

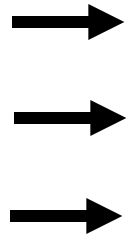
Synthetic Glucocorticoids (sGC)

- Administered to women at risk for pre-term labor (~10% of pregnant women)
- Reduces incidence of Respiratory Distress Syndrome
- Hypothalamic Paraventricular Nucleus (PVN) plays a central role in regulating stress response and behaviour
- Highly affected by antenatal synthetic glucocorticoid exposure



Experimental Method and Model

F₀: Treatment of Pregnant Guinea Pig with Betamethasone (1mg/kg) or saline



gd40-41
gd50-51
gd60-61

CONTROL

BETA

Males

Females

Males

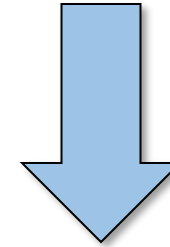
Females

Behavioural Tests:

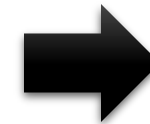
Startle Response (PND 23)

Open Field (PND 24)

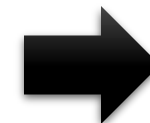
Post-Natal Day 40, PVN RNA was extracted from female offspring



RNA- Sequencing



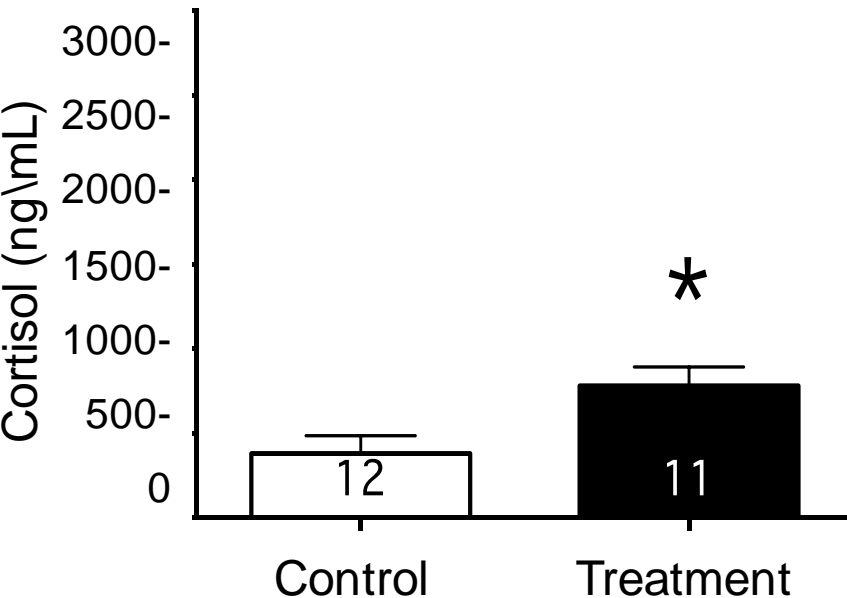
Tophat2 Alignment



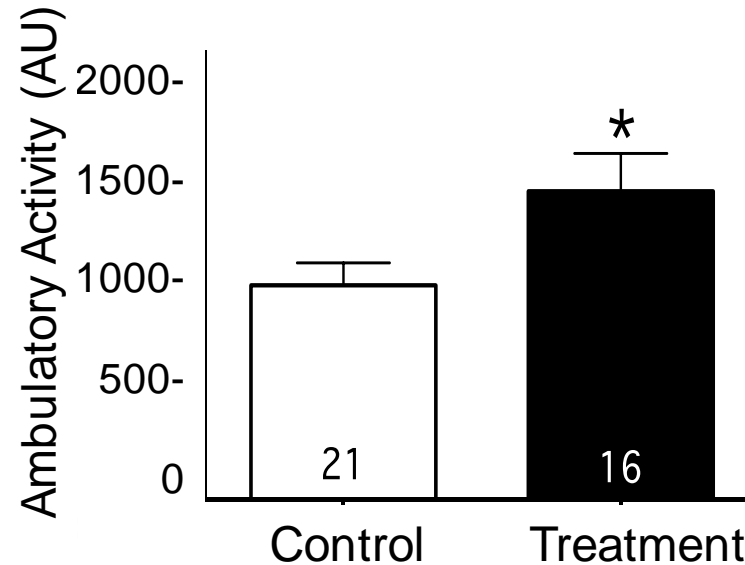
Differential Expression Analysis

Antenatal sGC Results in Significant Changes in Behaviour

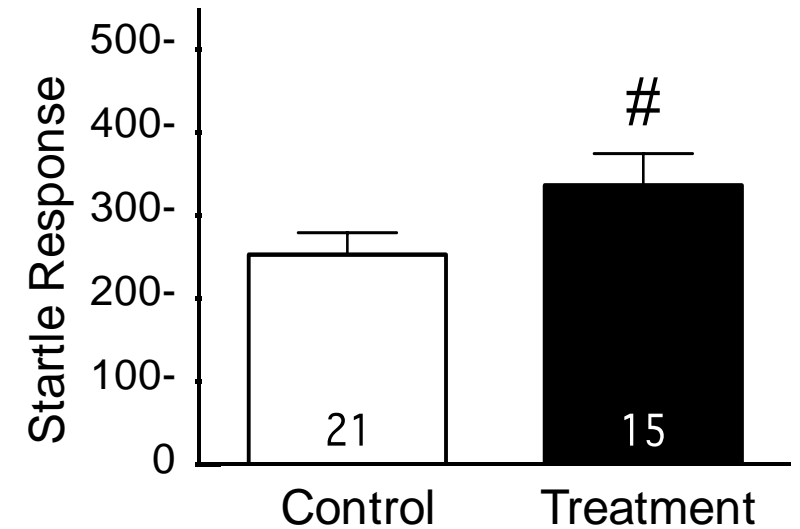
HPA Response to Open Field Stress



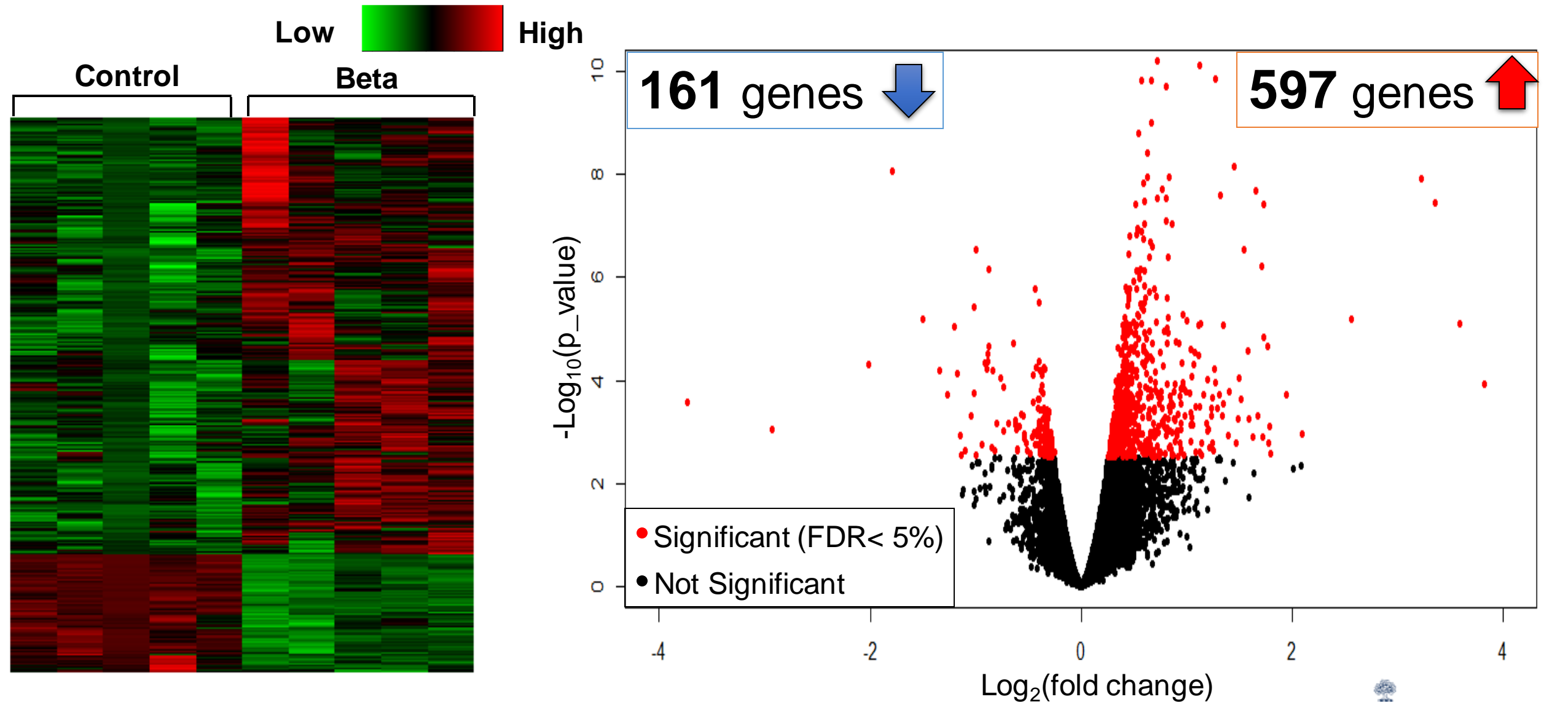
Total Locomotor Activity in the Open Field



Acoustic Startle Response



Antenatal sGC Results in Significant Changes in Gene Expression in PVN



Objective

To determine the relationship between gene expression in the PVN and behaviour

Hypothesis

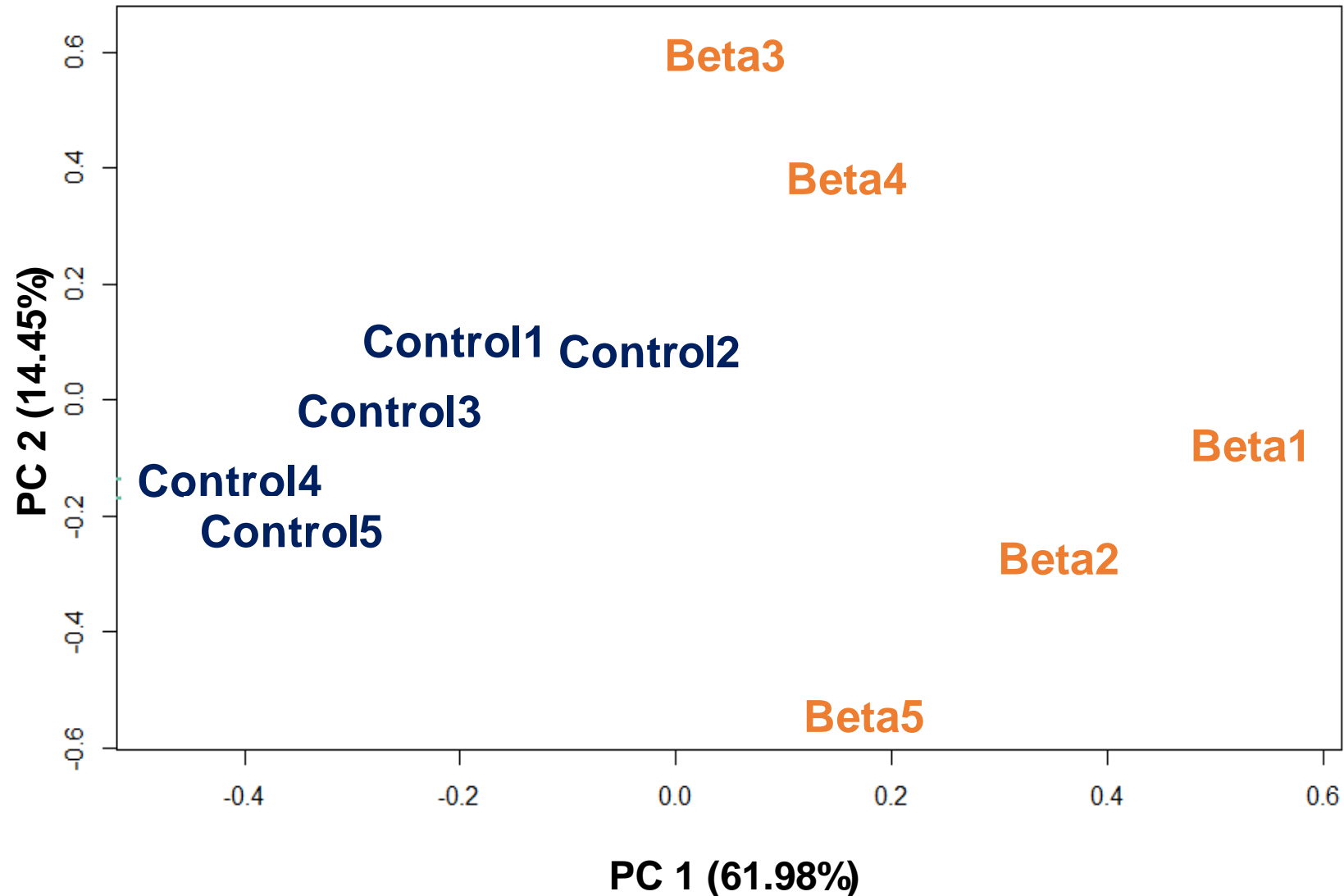
Transcriptional programming of the PVN by sGC is correlated to behavioural outcomes and can be used in predictive modelling

Methods

- Principal Component Analysis
- Multivariate Linear Regression

Principal Component Analysis (PCA)

- PCA is useful to describe multi-dimensional data.
- The expression of each gene = a new dimension
- We can reduce the dimensions of the data to the first two Principal Components that describe the majority of variation within the dataset.

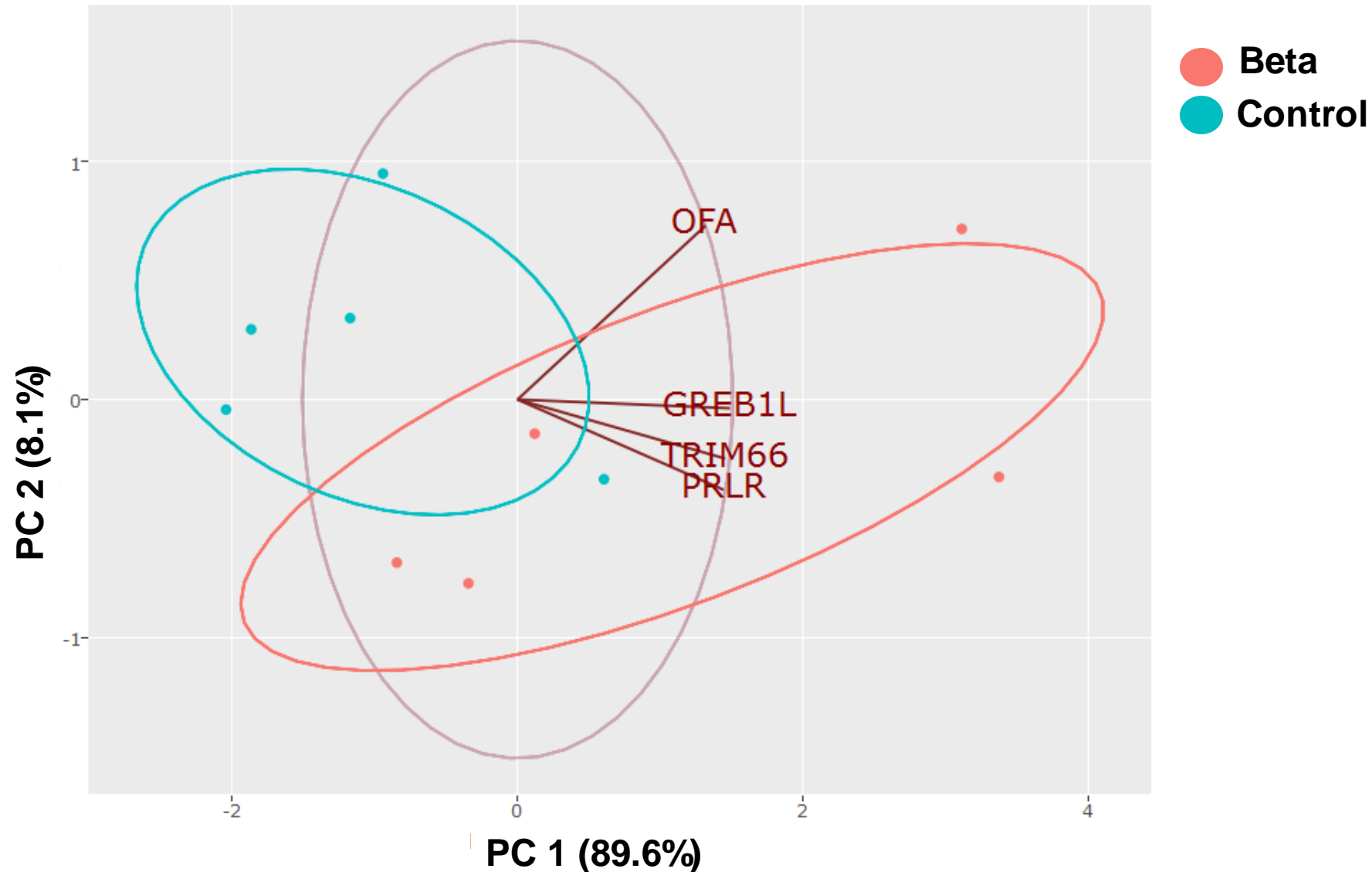


Circle of Correlations and PCA:

Determine the relationship between gene expression and behaviour



PCA of Gene Expression and Behaviour

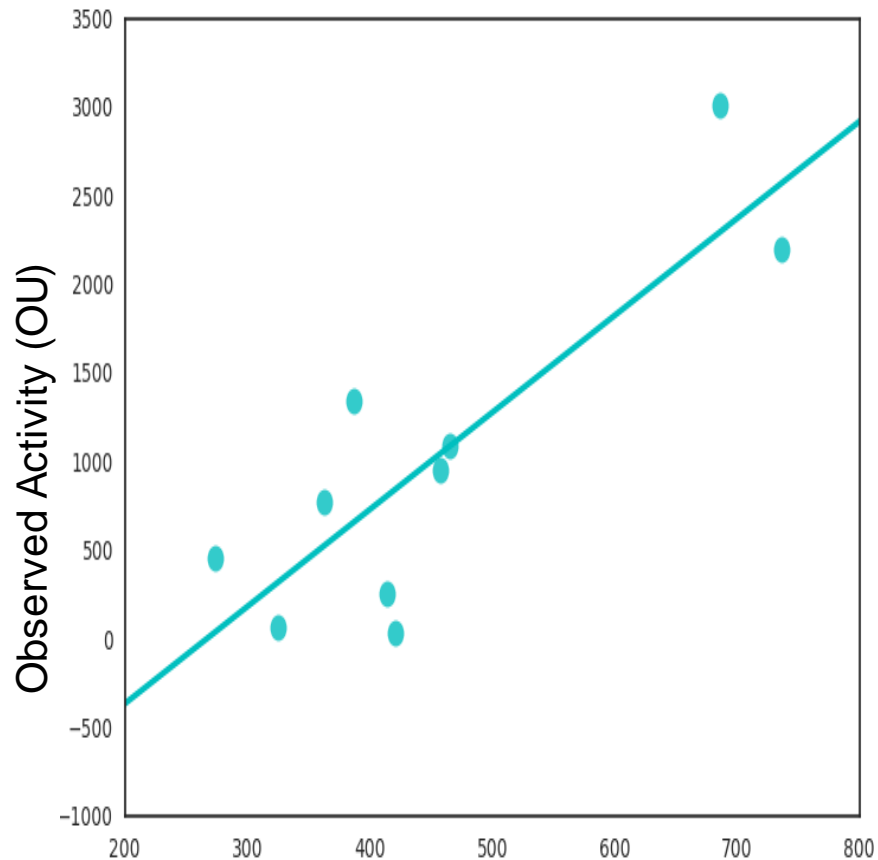


- PCA suggests that there may exist a relationship between the expression of GREB1L, TRIM66, PRLR and Open Field Activity
- Relationship is tested with Linear Regression

Linear Regression

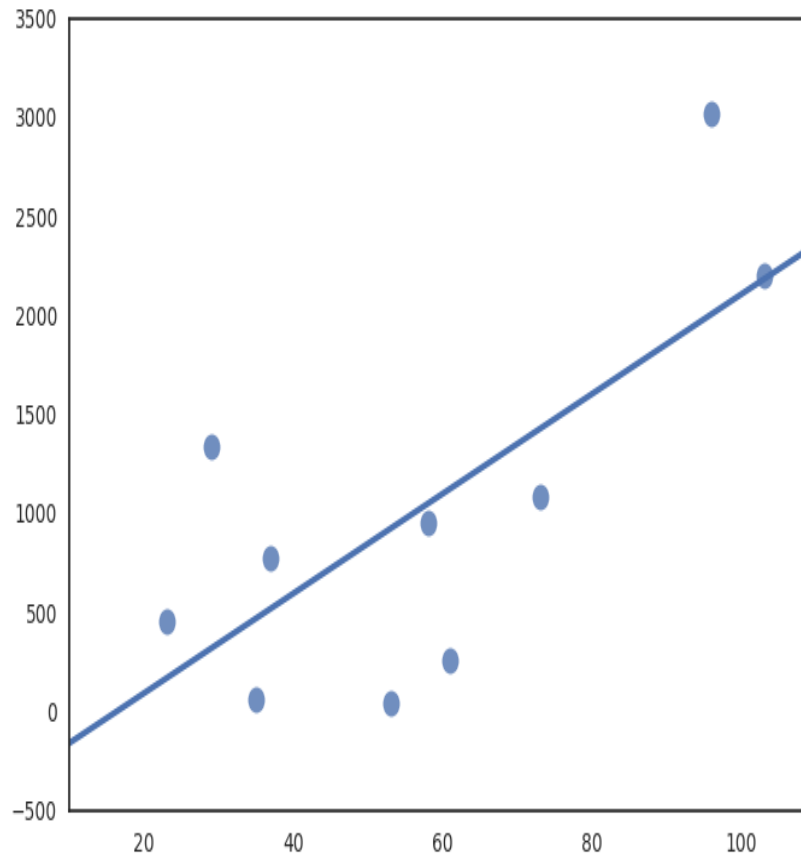
GREB1L

$R^2 = 0.71$, $p = 0.002$



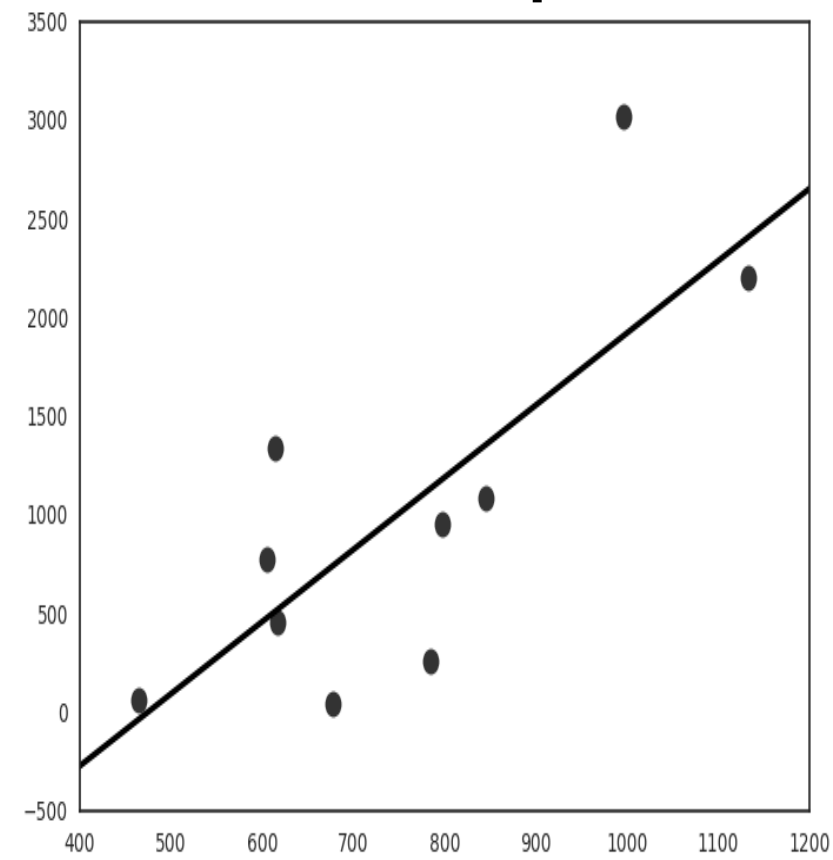
PRLR

$R^2 = 0.51$, $p = 0.019$



TRIM66

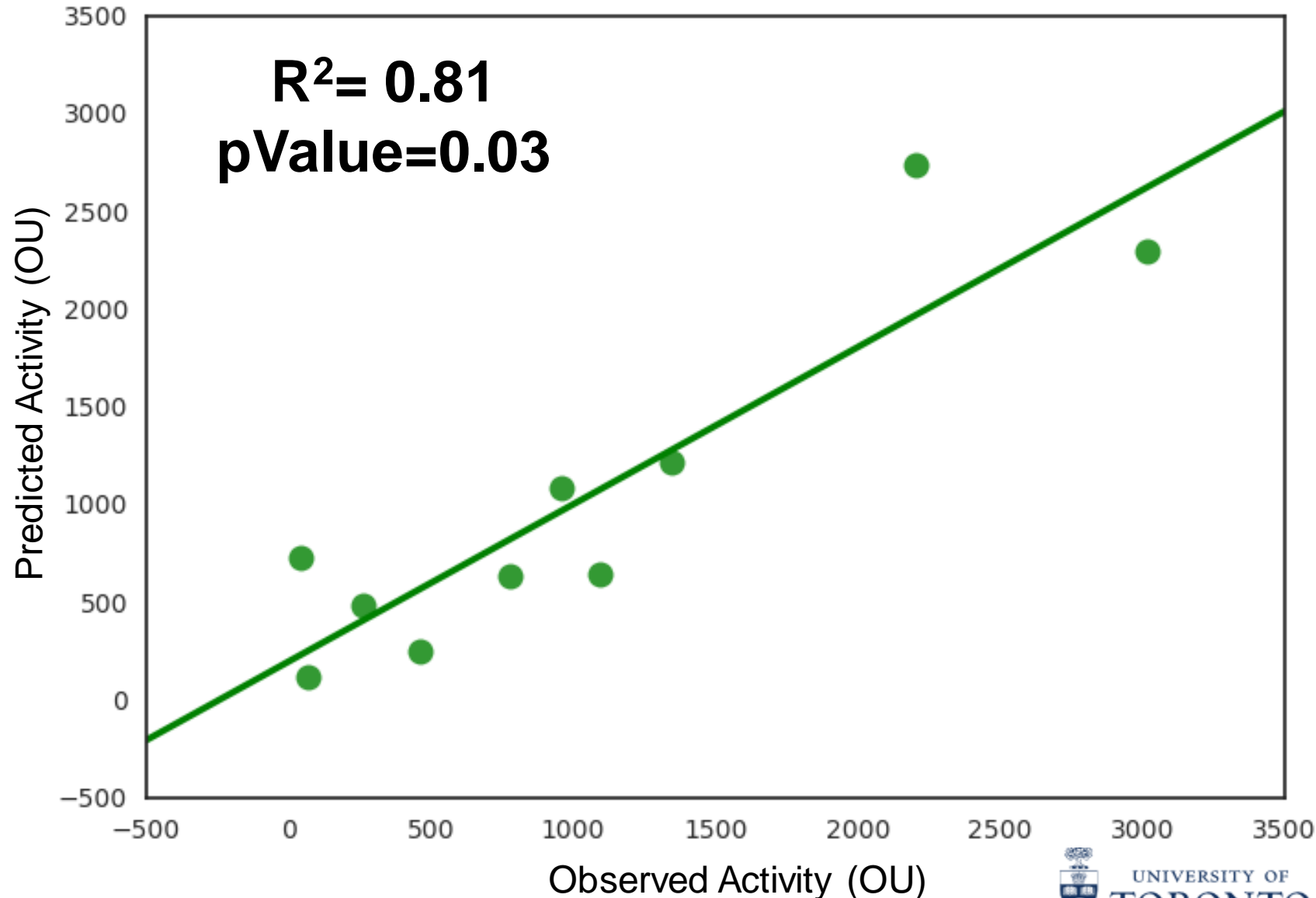
$R^2 = 0.58$, $p = 0.01$



Normalized Gene Expression (Counts)

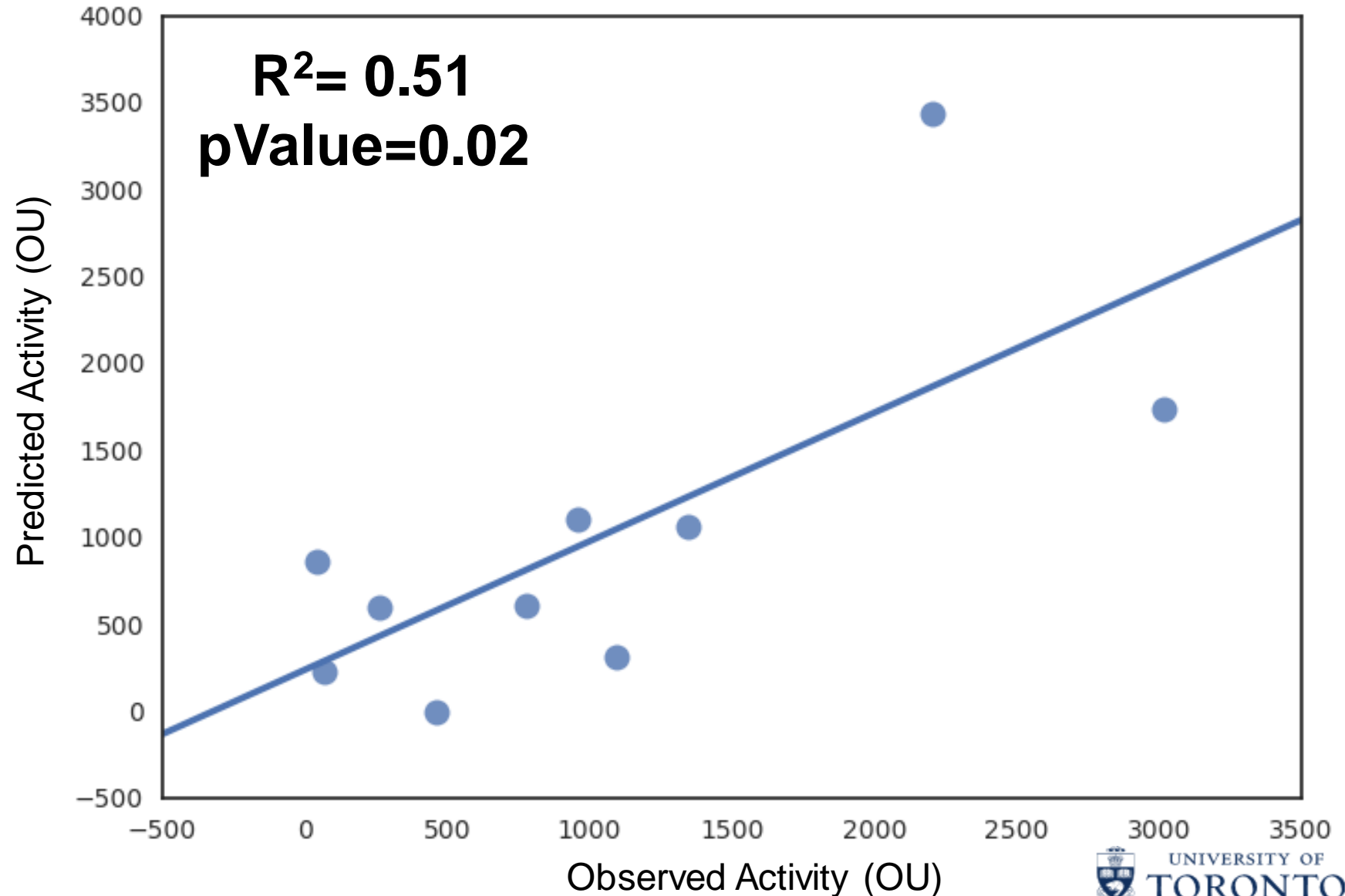
Multivariate Linear Regression Results

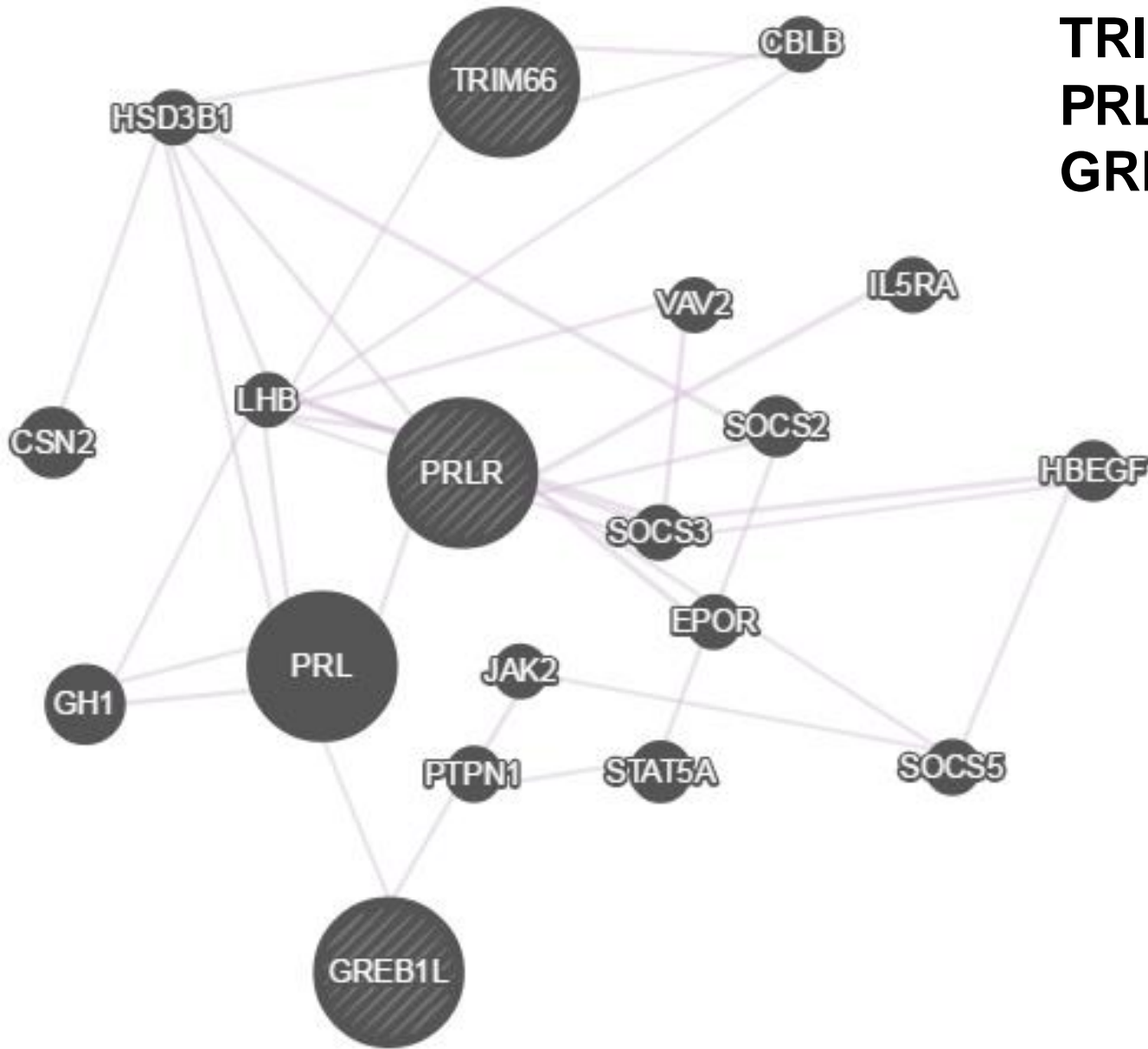
- Use the expression of 3 genes to model the relationship between gene expression and Open Field Activity
- Determine accuracy of model by plotting predicted value as a function of observed values and measuring R^2



Multivariate Linear Regression Cross Validation

- **Leave One Out**
Technique: remove one sample of your dataset to use as 'test' dataset. Repeat for all samples
- Obtain focused genes for further investigation





TRIM66: Transcription Regulation
PRLR: Prolactin Receptor
GREB1L: Estrogen Receptor Signaling

- These genes are co-expressed

Conclusions and Significance

- PCA and linear regression analyses suggest that a relationship exists between the expression of *Greb1l*, *Prlr* and *Trim66* and behaviour in the Open Field
- Additional samples are required to fully validate the model
- Insight into the potential mechanisms of antenatal sGC and how these molecular events may relate to behavior
- Proof-of-Principal for the use of gene expression profiles in disease prediction, detection, and prevention
 - Targeted therapeutic intervention

Acknowledgements

Matthews' lab

- Dr. Stephen Matthews
- Dr. Enrrico Bloise
- Mohsen Javam
- Alisa Kostaki
- Vasilis Moisiadis
- Guinevere Imperio
- Stephanie Baello
- Aya Sasaki
- Tam Lye
- Elizabeth Eng
- Maria Sqapi
- Alex Mouratidis
- Andrada Naghi
- Susmita Sarkar
- Chi-Lan Tran
- Loughlin Kelly



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