Exploring the Impact of Valproic Acid on Neural Progenitor Cells through Cell Villages and Differential Gene Expression Analysis

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ABSTRACT

Valproic acid (VPA) is a commonly used mood stabilizer and antiepileptic drug. In utero exposure to VPA is associated with increased risk for neurodevelopmental disorders (NDDs). Neural progenitor cells (NPCs) are crucial in neurodevelopment and may be vulnerable to VPA. We sought to determine VPA's effect on NPCs using cell viallges, a method which pools together cell lines in a shared *in vitro* environment. Stem-cell-derived NPCs from 12 donors were combined in equal proportions, then separated into two villages, treated with VPA or water control. Single-cell RNA sequencing and the *Dropulation* algorithm were used to profile gene expression and cell donor identity. We performed differential gene expression analysis using Voom and *Dream* and identified 356 differentially expressed genes that changed in response to VPA. Continued research into the effects of VPA can transform our understanding of NDDs, leading to improved healthcare, early interventions, and better outcomes for individuals with challenging neurodevelopmental conditions.

INTRODUCTION

- The purpose of this experiment was to test the effects of valproic acid on neural progenitor cells
- Statistical anaylsis was used to determine differential gene expression
- VPA induces differentiation and inhibition of proliferation in NPCs

Stem cell-derived NGN2-accelerated progenitors (SNaPs) model early human brain development
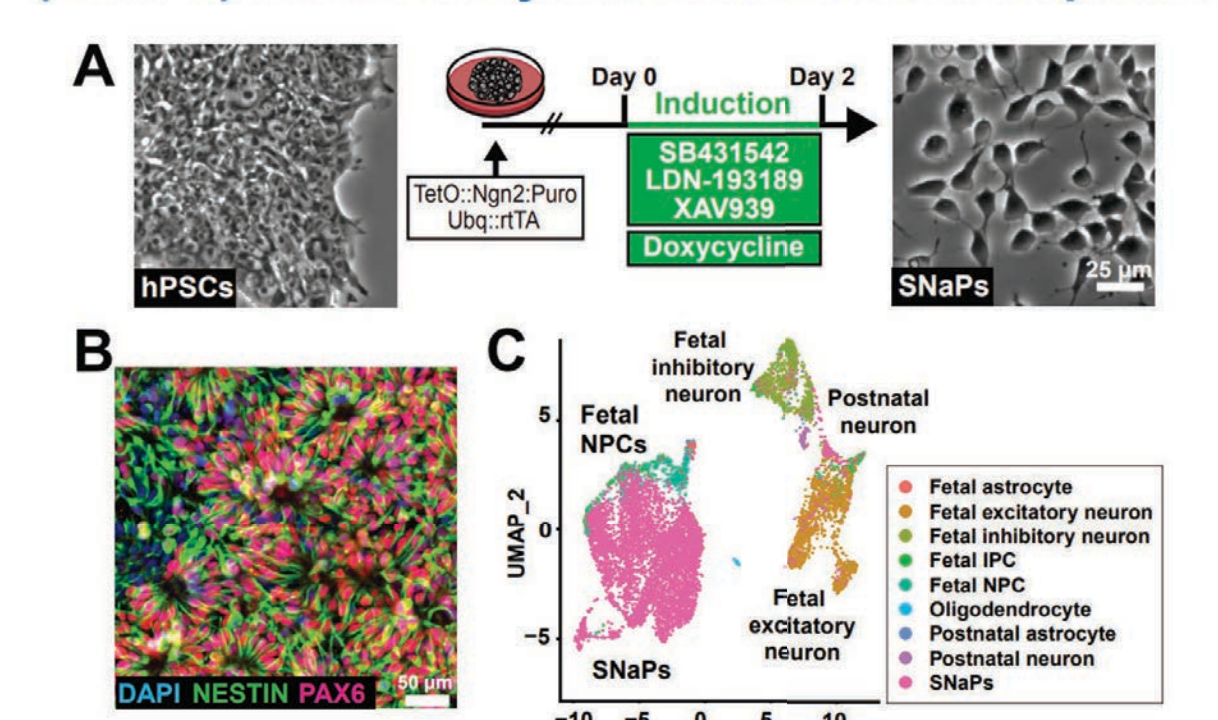
A. Stem cell-derived NGN2-driven neural induction with lentiviral TetO ::NGN2:T2A:PURO and Ubq::rtTA constructs and differentiation to NPCs (aka SNaPs). *B.* NESTIN+/PAX6+ NPCs can be induced from stem cells in two days. *C.* scRNA-seq based comparison of SNaPs to in vivo human brain tissue shows high similarity to fetal NPCs (integration with Darmanis et al 2015 and Nowakowski et al 2017 datasets).
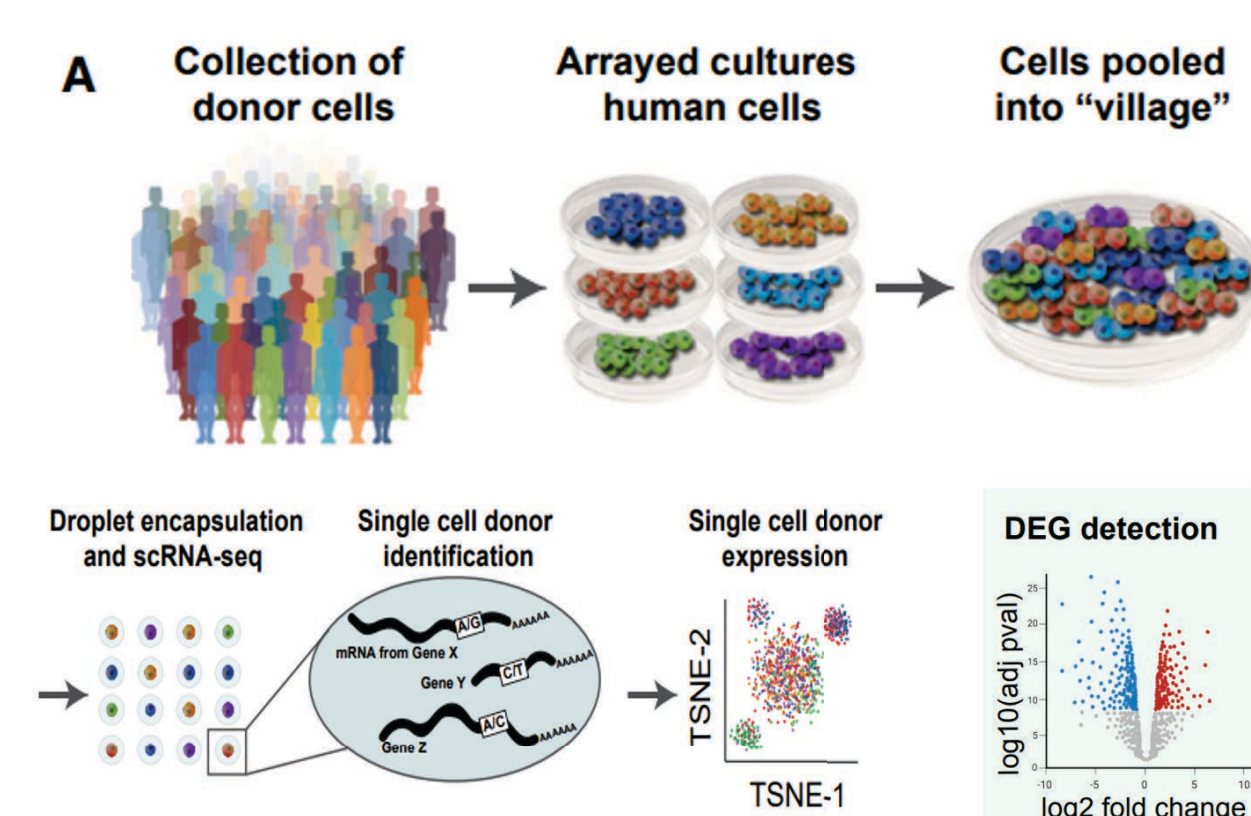Adapted from Wells et al 2023. *Cell Stem Cell*

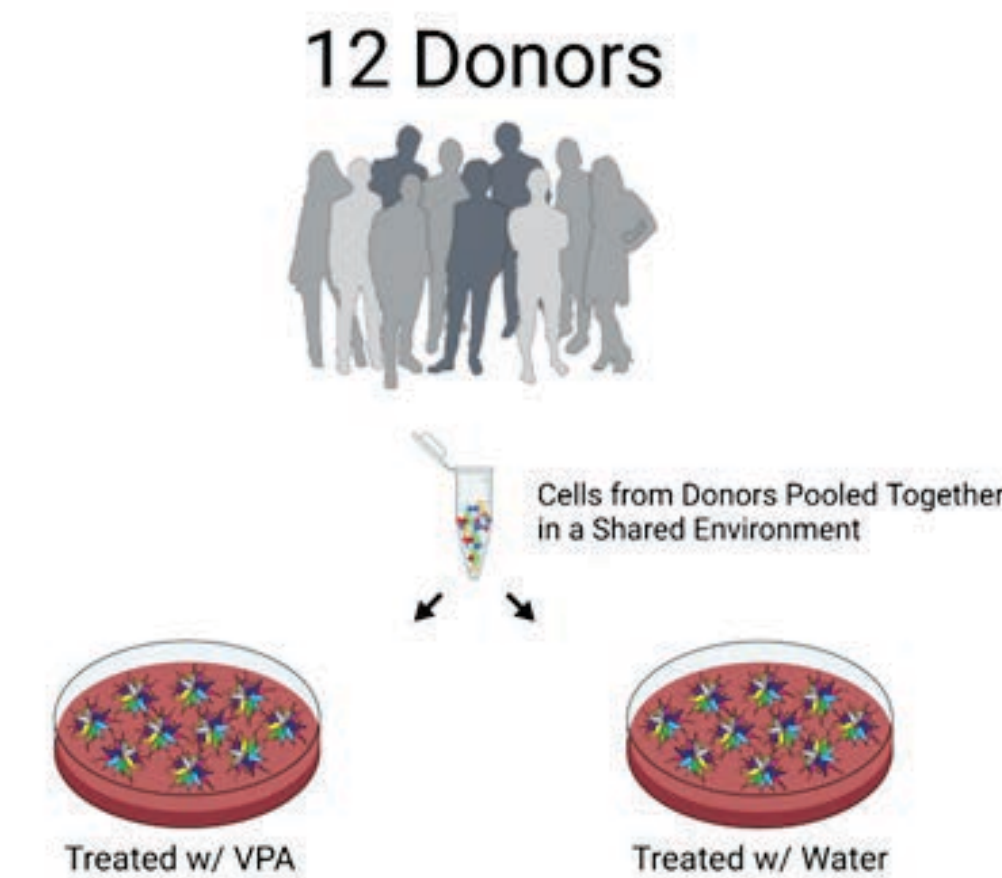**Figure 1.** Schematic of Cell Village workflow

Experimental Methods

- Village consisted of 12 donors
- One was treated with VPA and one with water
- Samples underwent single-cell RNA sequencing and Dropulation analysis

Figure 2. Experimental overview of the Cell Village used to treat samples with VPA and water

Analysis Methods

Gene Expression Analysis

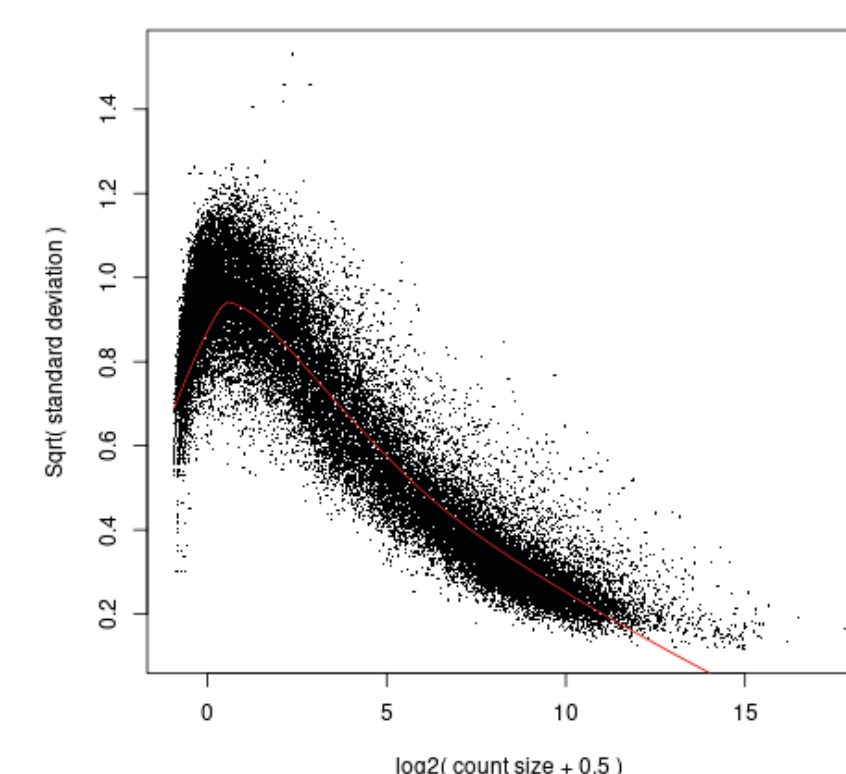1. **Preprocessing**
   - Filtering the Genes

Figure 3. Voom mean-variance trend plot of differentially expressed genes as a result of VPA *before* filtering
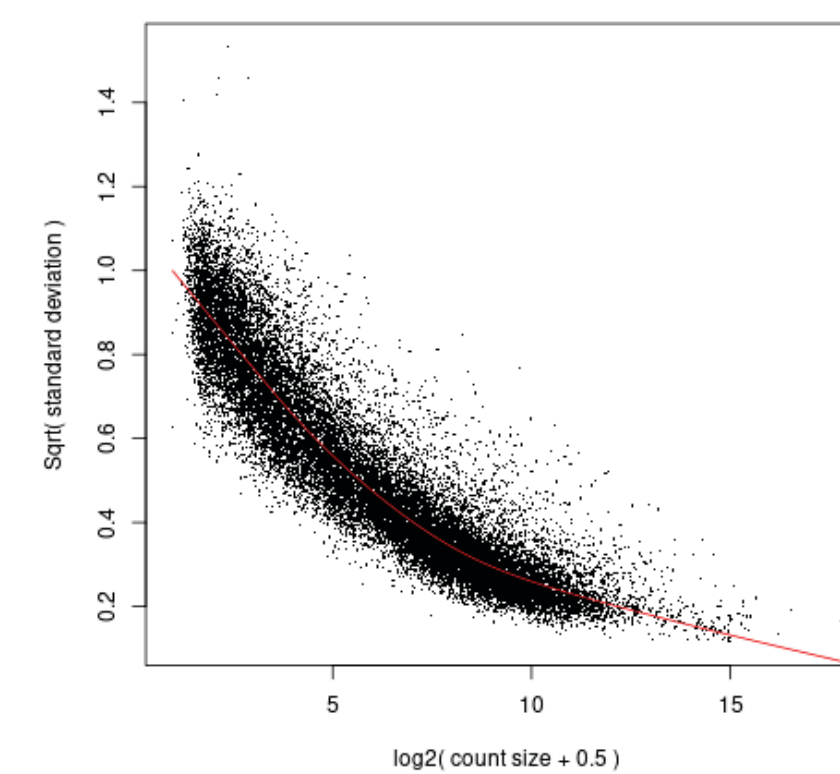
Figure 4. Voom mean-variance trend plot of differentially expressed genes as a result of VPA *after* filtering

2. **Modeling Variables**
   - Test - Treatment
   - Biological Variable - Sex
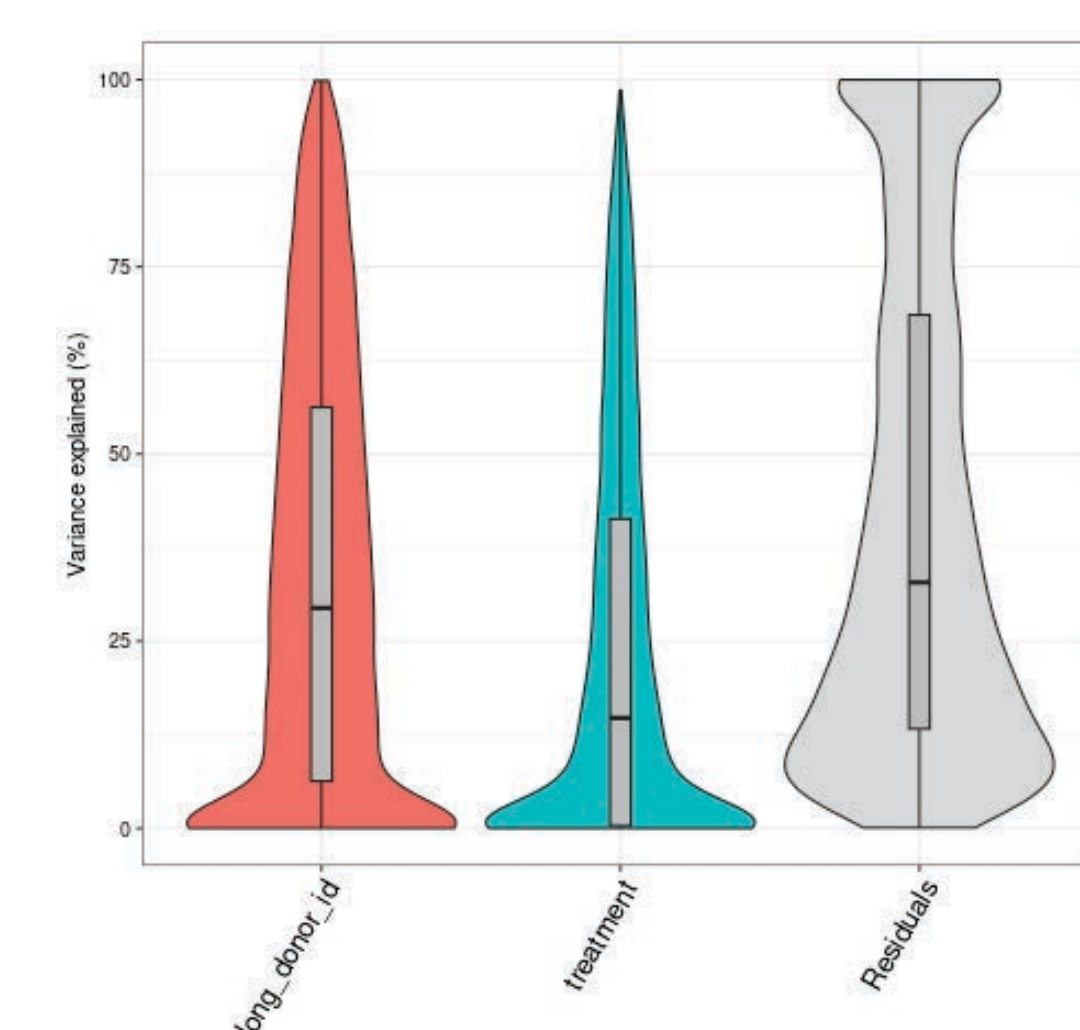   - "Random Effect" - Donor

Figure 5. Violin plot showing the density and distribution of genes
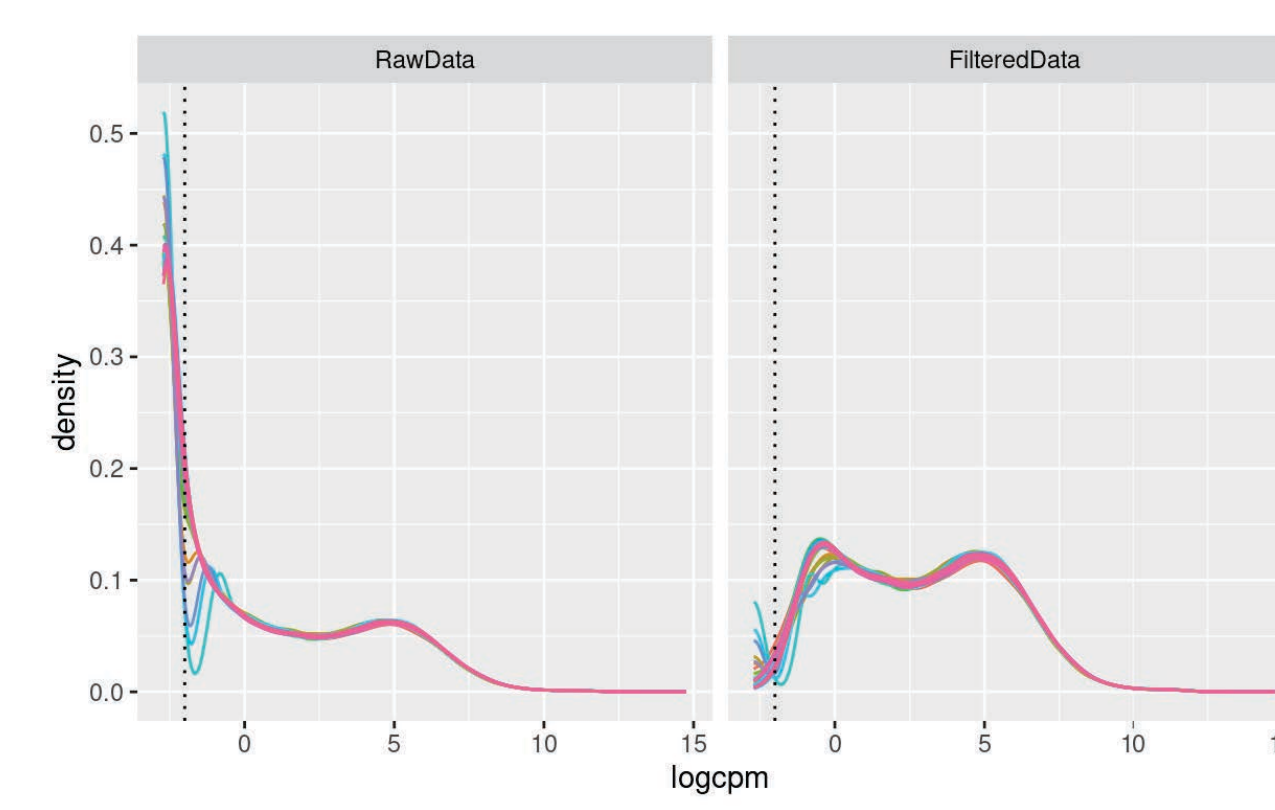
Figure 6. Digital Gene Expression (DGE) graph representing frequency of genes before and after filtering
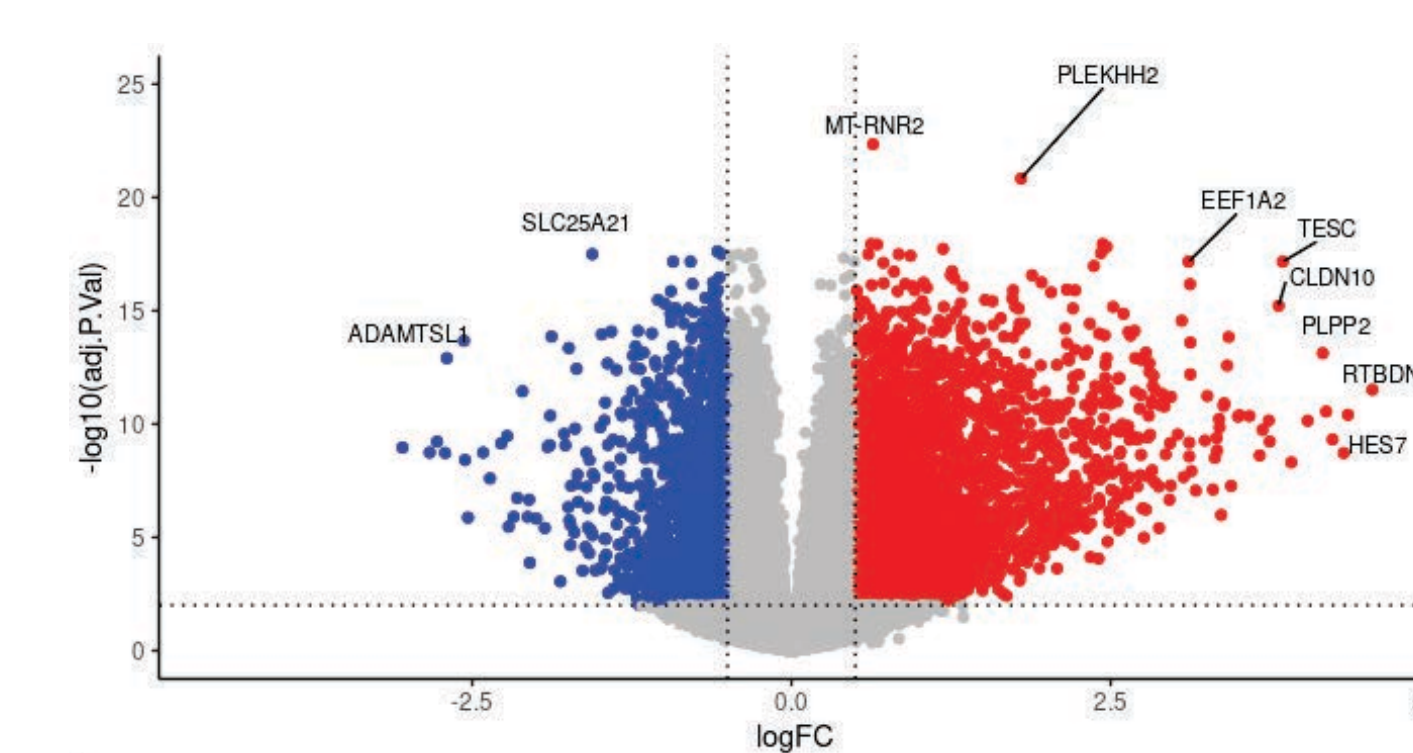
Results

Figure 7. Volcano plot with Benjamini-Hochberg adjusted p-values of less than 0.01 and logFC threshold, represented by colors blue (downregulated and significant) and red (upregulated and significant)
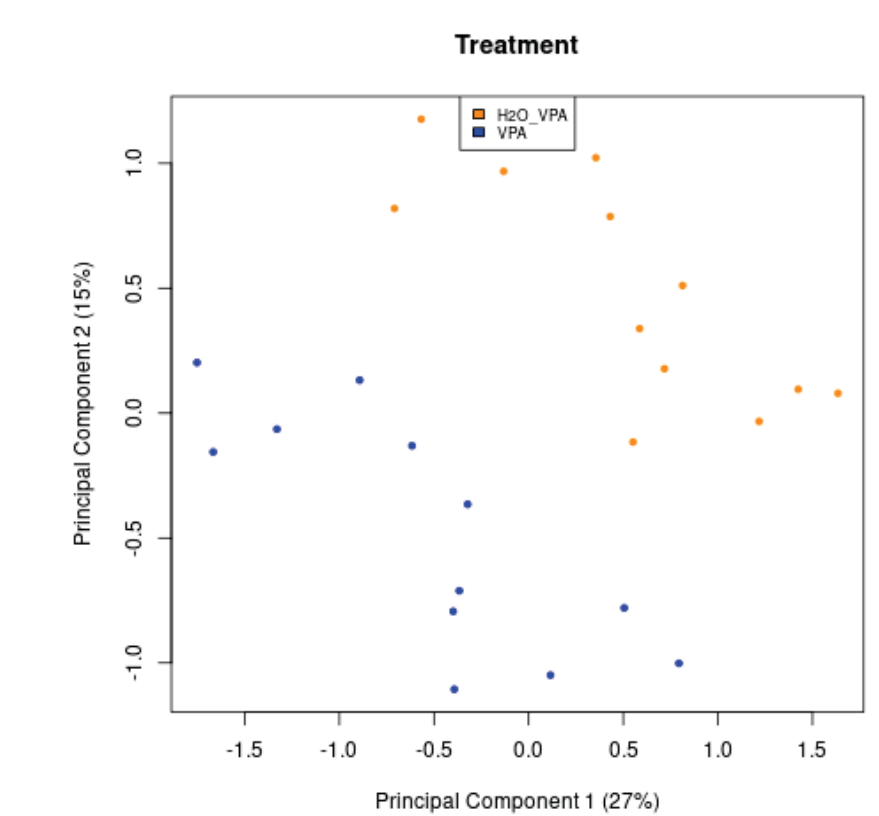
Figure 8. MDS plot of the effects of VPA on NPCs
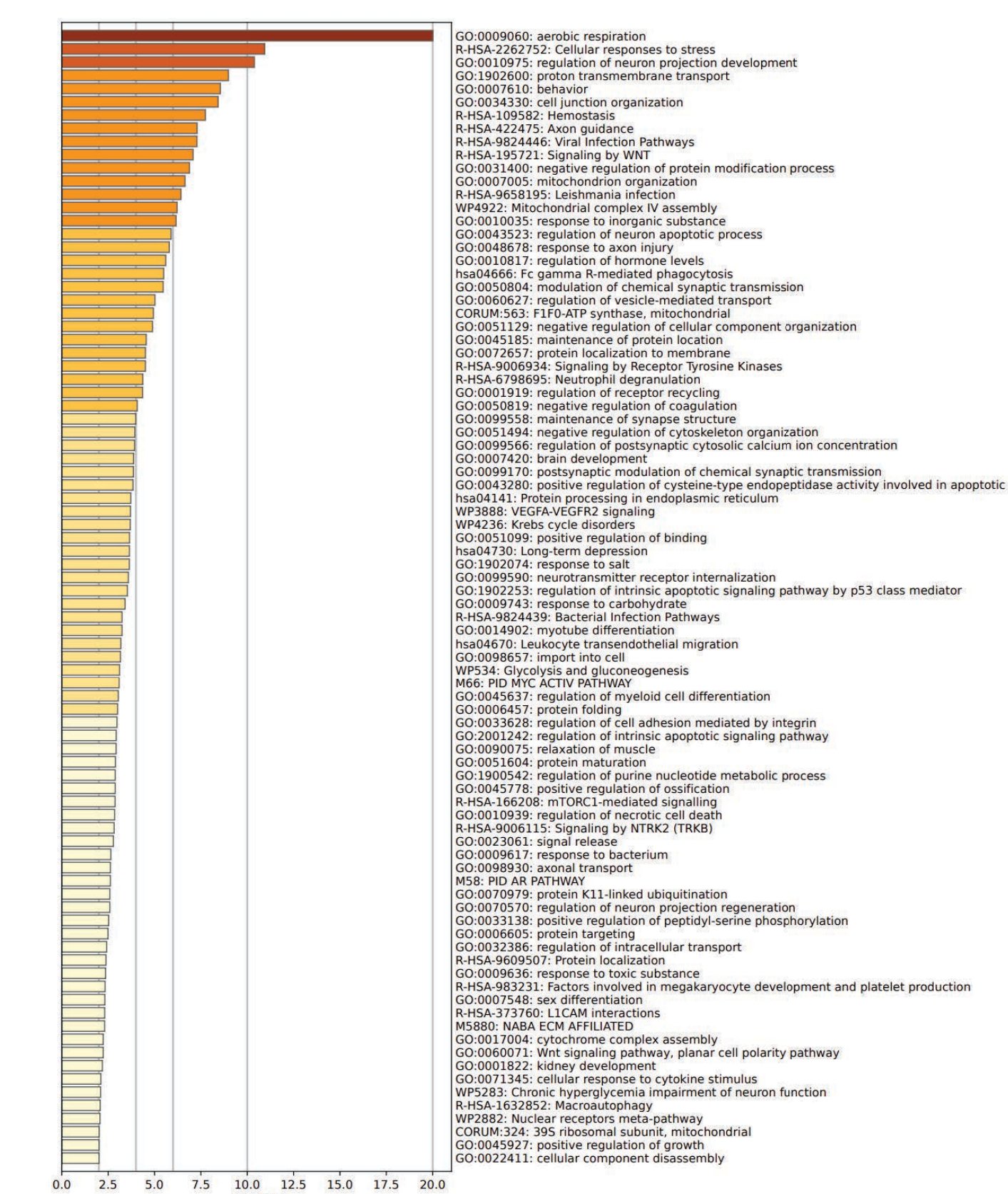
Upregulated Genes

Figure 9. Metascape bar graph created using the top 500 differentially expressed upregulated genes below adj. p-value of <0.01
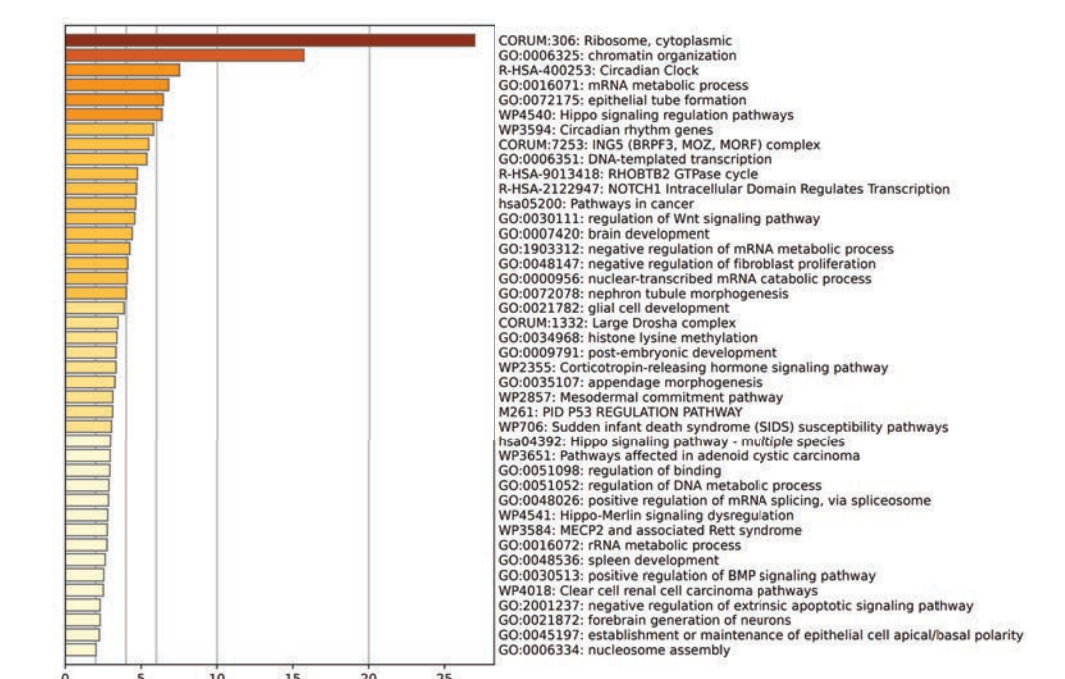
Downregulated Genes

Figure 10. Metascape bar graph created using the top 500 differentially expressed downregulated genes below adj. p-value of <0.01

Genes of Interest
Upregulated:
GO:0007610: behavior
R-HSA-195721: Signaling by WNT
GO:0007420: brain development
GO:0060071: Wnt signaling pathway, planar cell polarity pathway
GO:0099170: postsynaptic modulation of chemical synaptic transmission

Downregulated:
R-HSA-2122947: NOTCH1 Intracellular Domain Regulates Transcription;
GO:0021782: glial cell development;
WP3584: MECP2 and associated Rett syndrome;
GO:0021872: forebrain generation of neurons

Future Directions

- Test NOTCH1 and other GO terms experimentally
- Compare to other ASD genes that were shown to be affected
- Aim to reproduce results from this experiment

ACKNOWLEDGEMENTS

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