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Cell type-specific effects of C9orf72 repeat expansion in the motor cortex of mouse models of fALS at single cell resolution.

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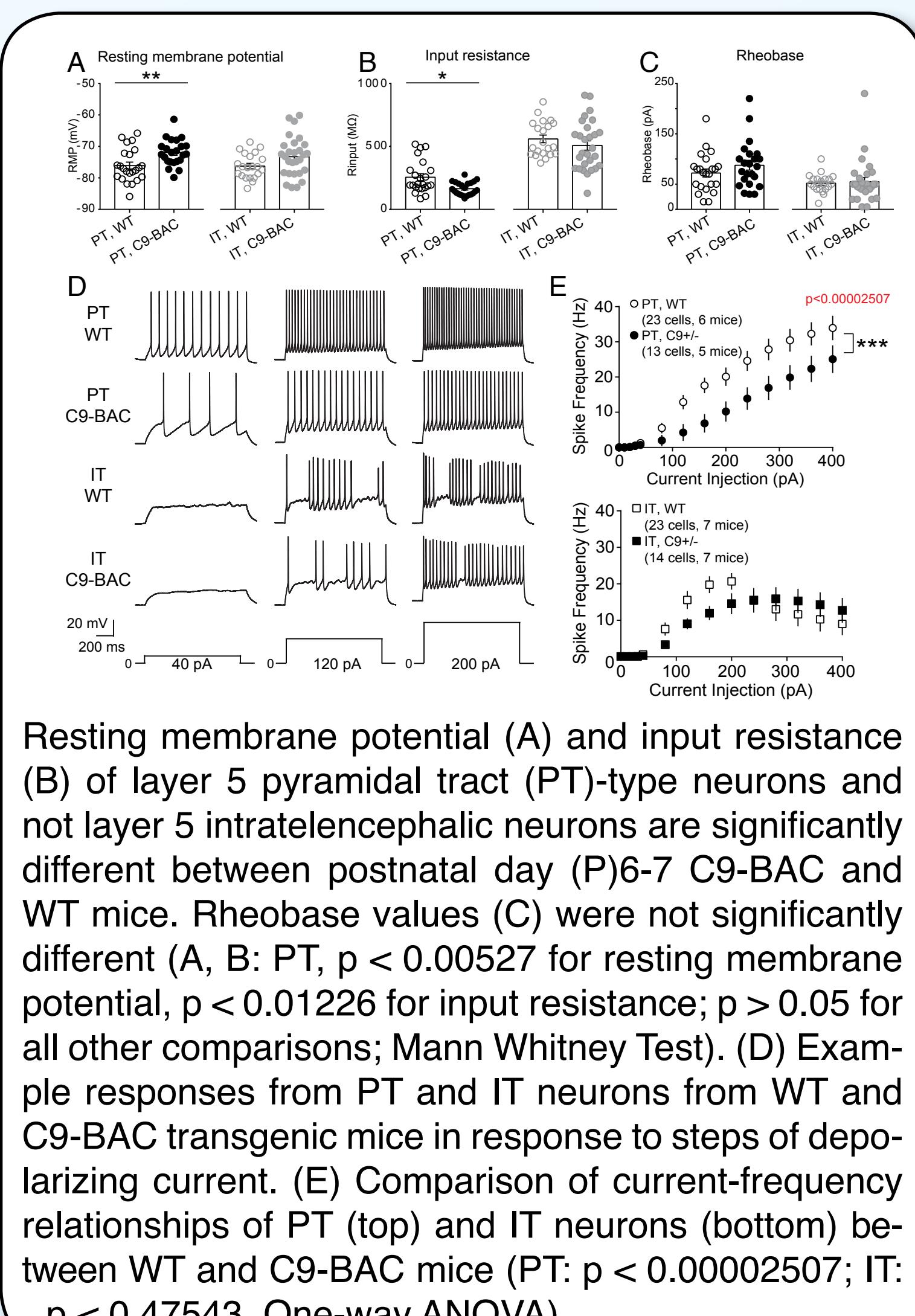
Abstract

Neurodegenerative disorders (ND) are characterized by neuronal loss often diverging based on the selective vulnerability of different neuron types. Amyotrophic Lateral Sclerosis (ALS) is a progressive and selective loss of motor neurons in the motor cortex or spinal cord. Several pathological features of ALS, including neuronal hyperexcitability, are observed across many different cortical cell types in mouse models. While bulk gene expression studies across models have revealed both distinct and common features among NDs, suggesting commonalities in the underlying pathological processes, these studies do not describe the distinct pathological responses of discrete cell types; a hallmark of selective vulnerability. Here we use single-cell RNA-Seq (scRNA-Seq) to examine the cell-type-specific transcriptional responses in the motor cortex of two different mouse models of C9orf72 repeat expansion, a causal variant for familial ALS. We identify a subset of differentially expressed genes capable of modulating the observed electrophysiological response of layer 5 pyramidal tract (PT) neurons to C9orf72 repeat expansion. We further identify distinct and shared molecular pathological features across discrete cell types in the motor cortex. These gene expression differences represent both common cellular responses to the pathogenic C9orf72 repeat expansion as well as responses that are unique to subpopulations of cells, and can be used to enhance understanding of the selective vulnerability to this pathogenic repeat. Future work will involve recursive analysis of major cell types, and exploration of the cell type specific genetic architecture of C9orf72 disease. This analysis provides a template for single cell-resolution analysis of complex NDs as part of a larger, integrated study across time points and select models of ALS.

Experimental Questions

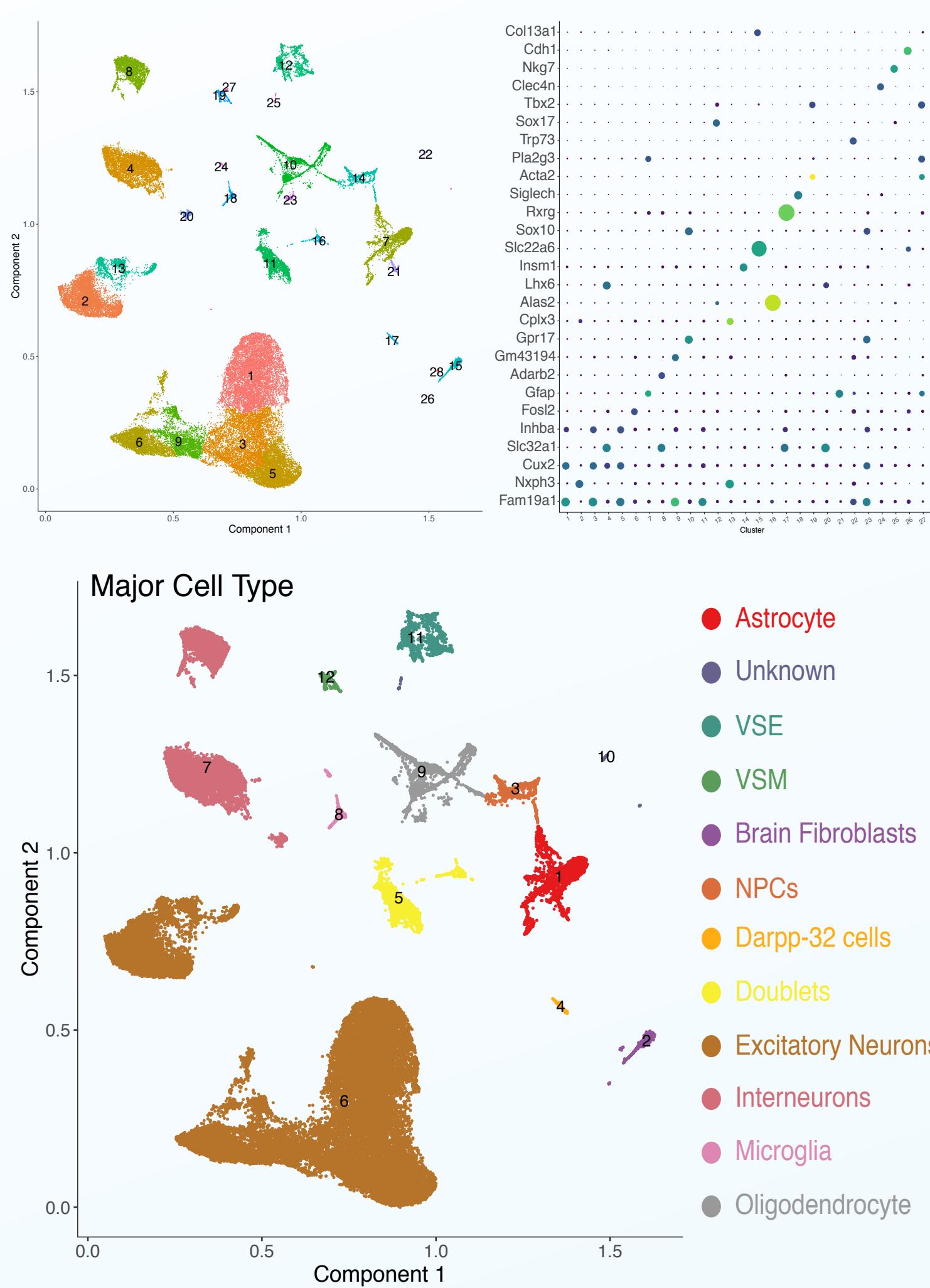
- Can we identify common and divergent effects of pathogenic C9orf72 expression in distinct mouse motor cortex cell types?
- Does C9orf72 repeat expansion affect M1 tissue composition in presymptomatic developmental stages?
- Is there a bias in differentially expressed gene burden across cell types?
- Can we identify DE genes that may contribute to PT hyperexcitability?
- What is the extent of nuclear pore gene expression heterogeneity in the motor cortex?

Cortical Hyperexcitability in a mouse model of C9orf72 disease



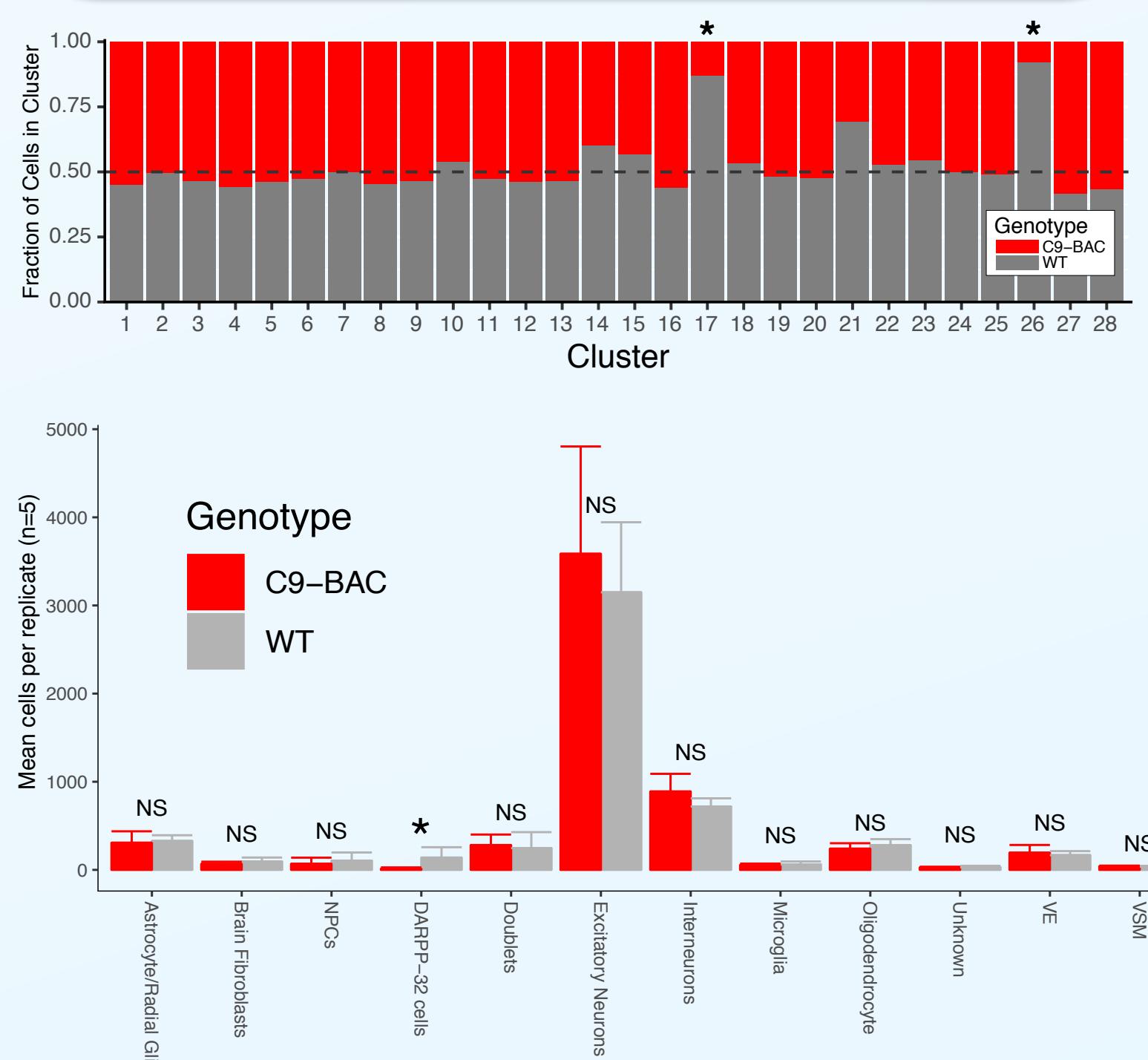
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Annotation of Major Cell Types

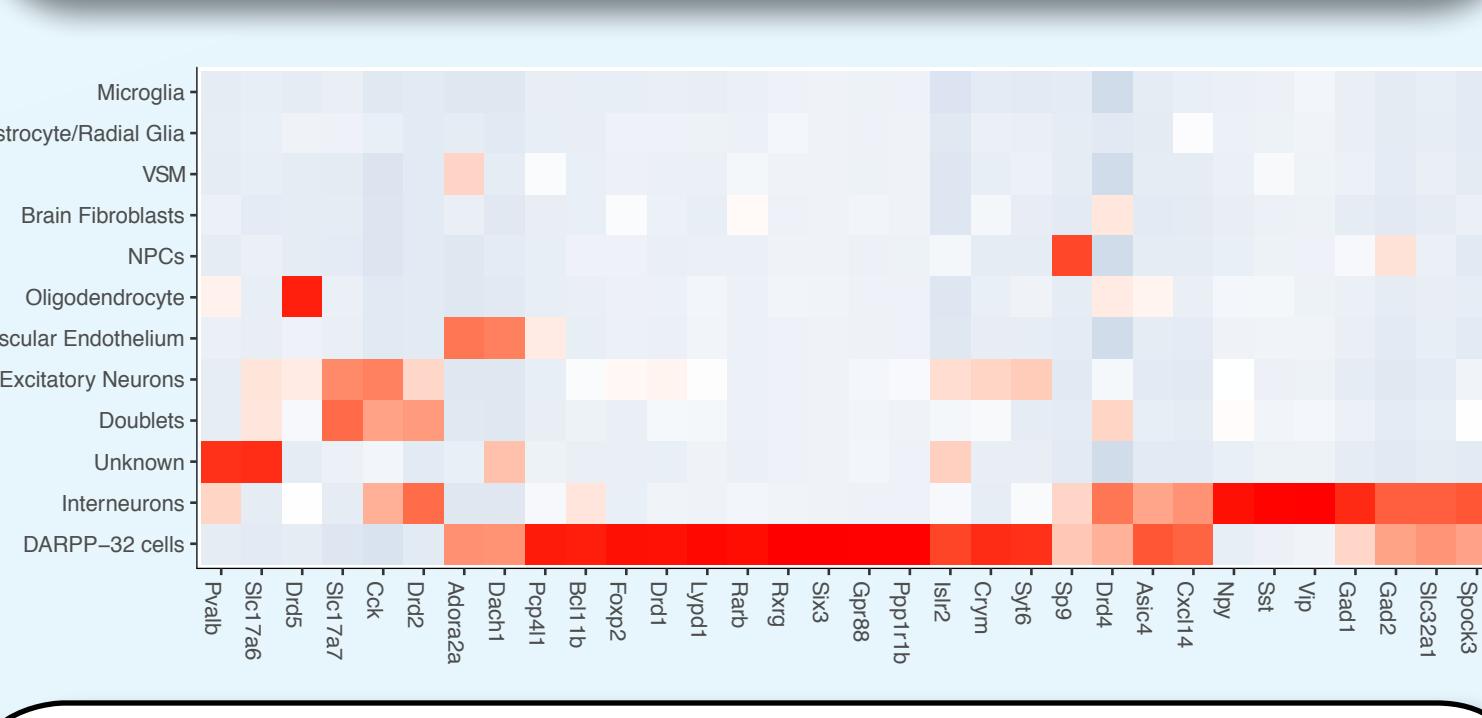


Dissected motor cortex from WT and C9-BAC repeat-expanded mice (Liu, et al 2016) were enzymatically dissociated ($n=5$) and processed using the 10x Genomics single cell RNA-Seq platform. Mice were balanced w.r.t. litter, sex, and genotype. 55,371 cells passed QC with a mean of 34,389 UMI per cell and a median of 2,341 genes detected per cell. A subset of high-variance genes (positive residuals to negative binomial fit of coefficient of variation \sim mean copies per cell) was selected, log-transformed, and used as input for PCA. The top 25 PCs were embedded in 2D via Uniform Manifold Approximation and Projection (UMAP). Louvain clustering of the 2D UMAP identified 28 distinct clusters. Clusters were associated with major cell types using a combination of manual annotation and identification of spatially autocorrelated genes via Moran's I test (Monocle3).

Differential Tissue Composition in C9-BAC mice

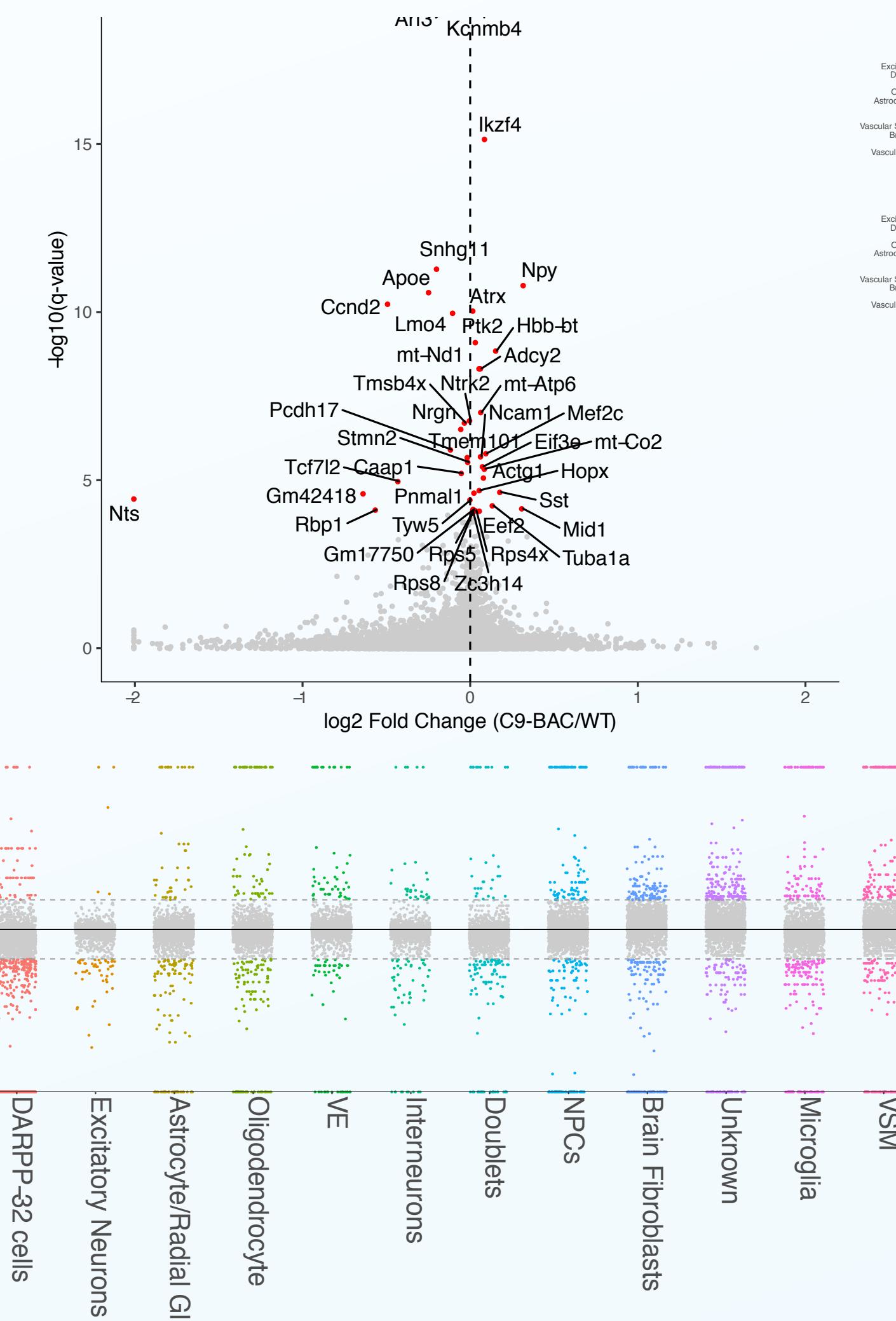


- All major cell types in M1 are represented
- Most with consistent proportions across genotype
- Two distinct clusters exhibit a significant reduction in the proportion of cells derived from p6-7 C9-BAC mice including the DARPP-32+ cellular cluster (Chi-Sq test for equal proportions; $p < 2.2e-16$)
- A significant reduction in the number of DARPP-32+ neurons in the C9-BAC mouse model at p6-7. (Student's T-test, $p < 0.0231$, $n=5$)

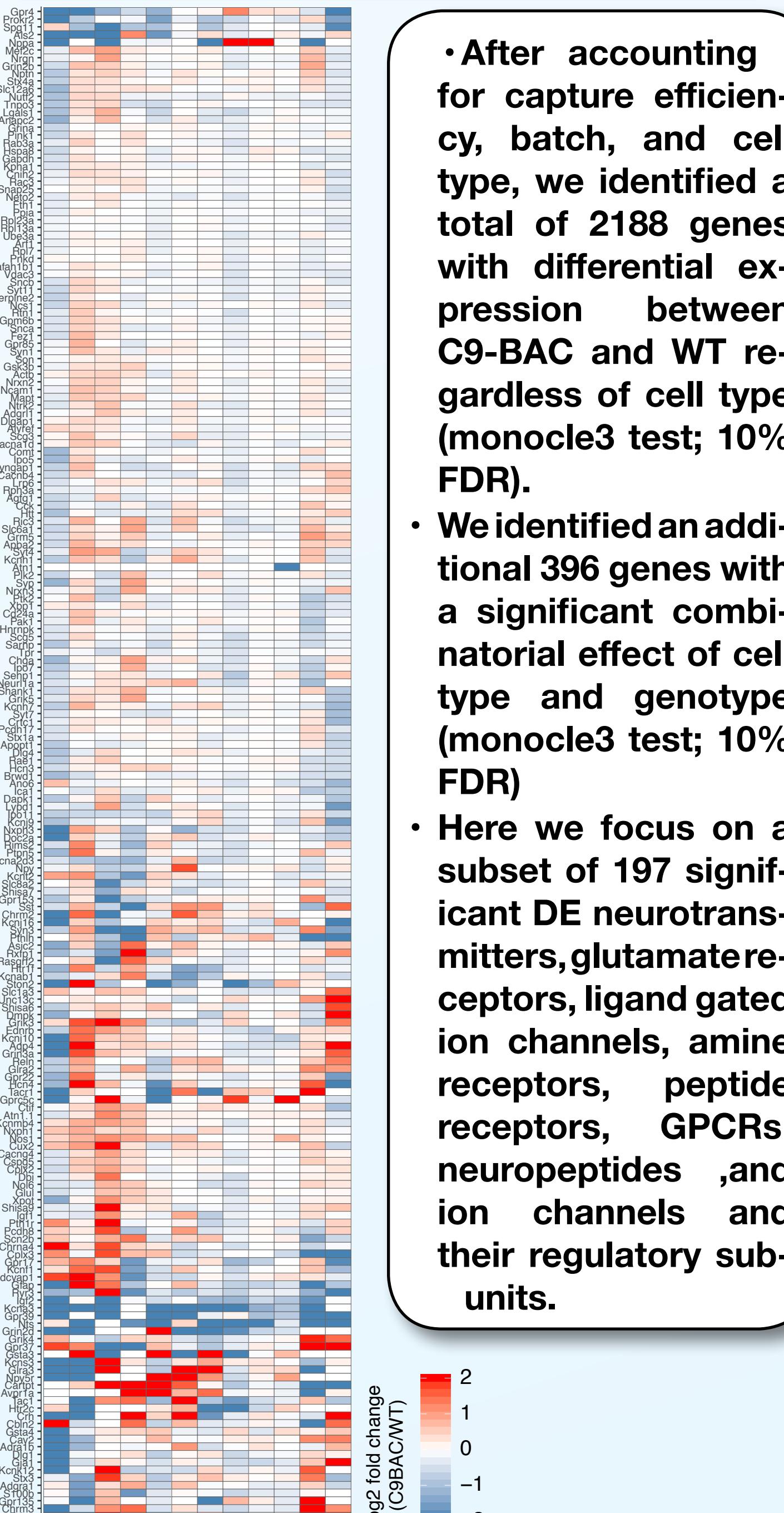


The significantly reduced DARPP-32+ cellular population consists of GABAergic interneurons expressing a unique combination of marker genes including the neuronal cytokine *Cxcl14*, RA receptors *Rxrg* and *Rarb*, DA receptors *Drd1* and *Drd4*, and the layer V subcerebral projection neuron markers *Bcl11b*, *Crym*, and *Foxp2*.

Cell Type Distribution of DE Genes

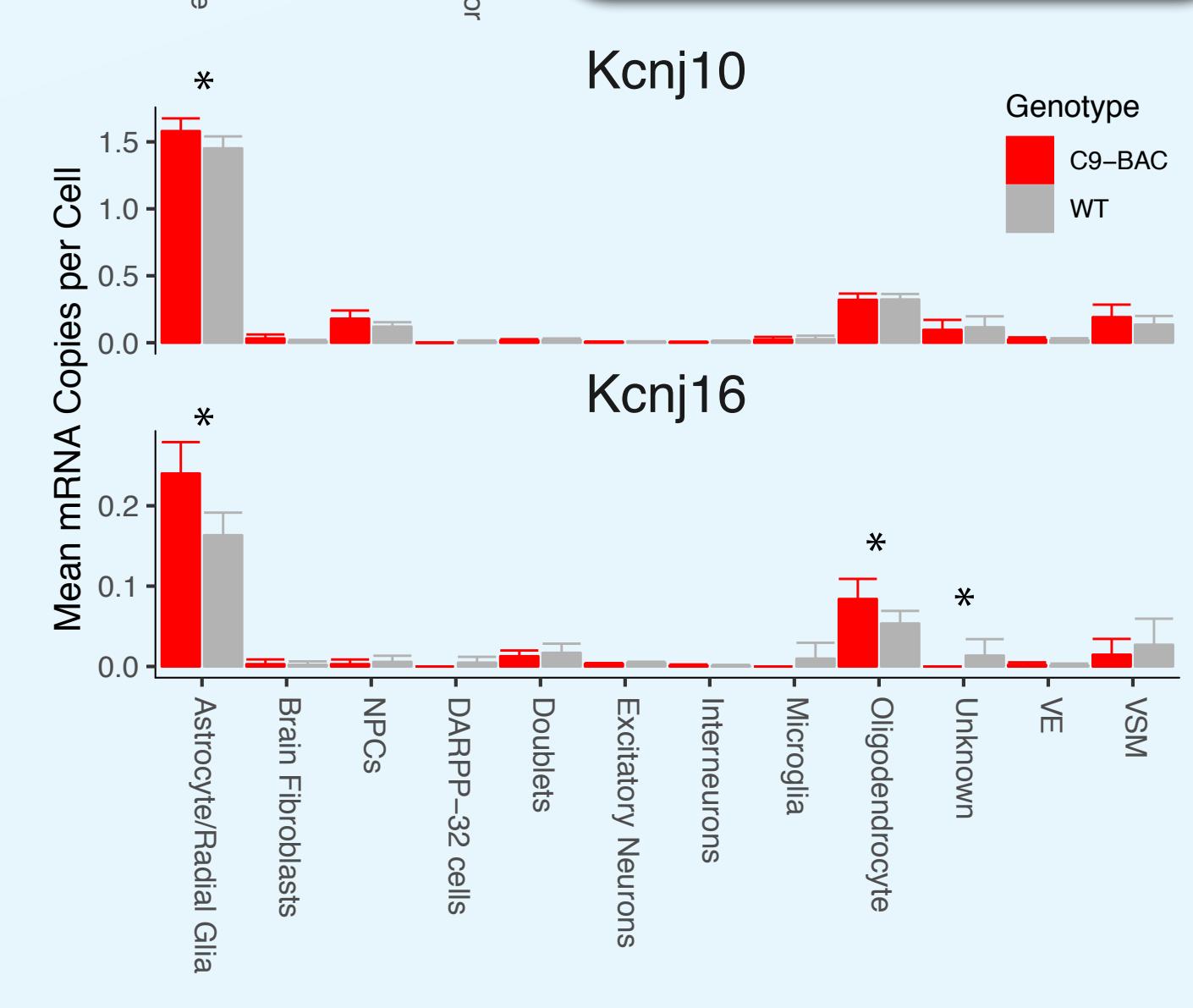


Cell Type Specific Differential Gene Analysis



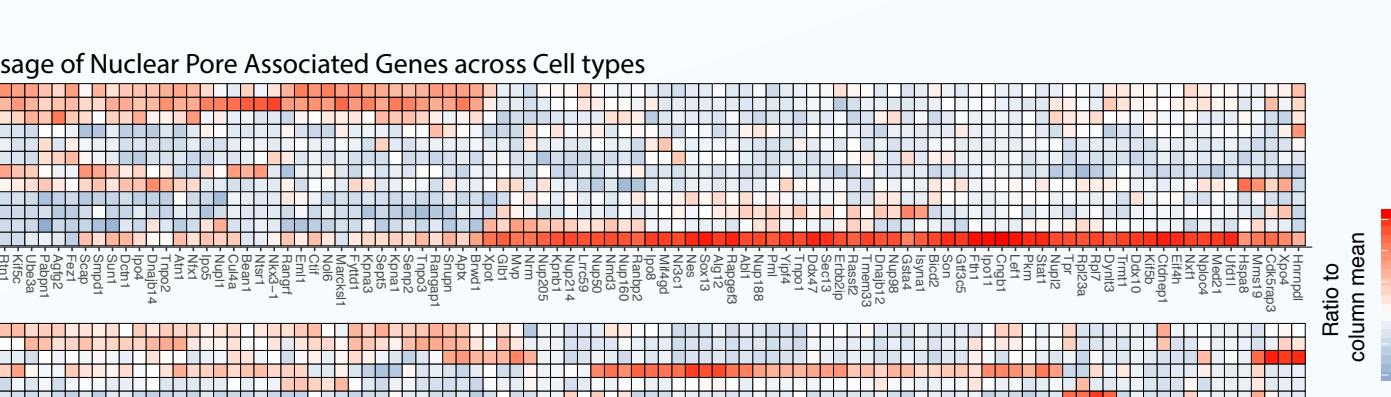
- After accounting for capture efficiency, batch, and cell type, we identified a total of 2188 genes with differential expression between C9-BAC and WT regardless of cell type (monocle3 test; 10% FDR).
- We identified an additional 396 genes with a significant combinatorial effect of cell type and genotype (monocle3 test; 10% FDR)
- Here we focus on a subset of 197 significant DE neurotransmitters, glutamate receptors, ligand gated ion channels, amine receptors, peptide receptors, GPCRs, neuropeptides, and ion channels and their regulatory subunits.

K_{ir} Channels



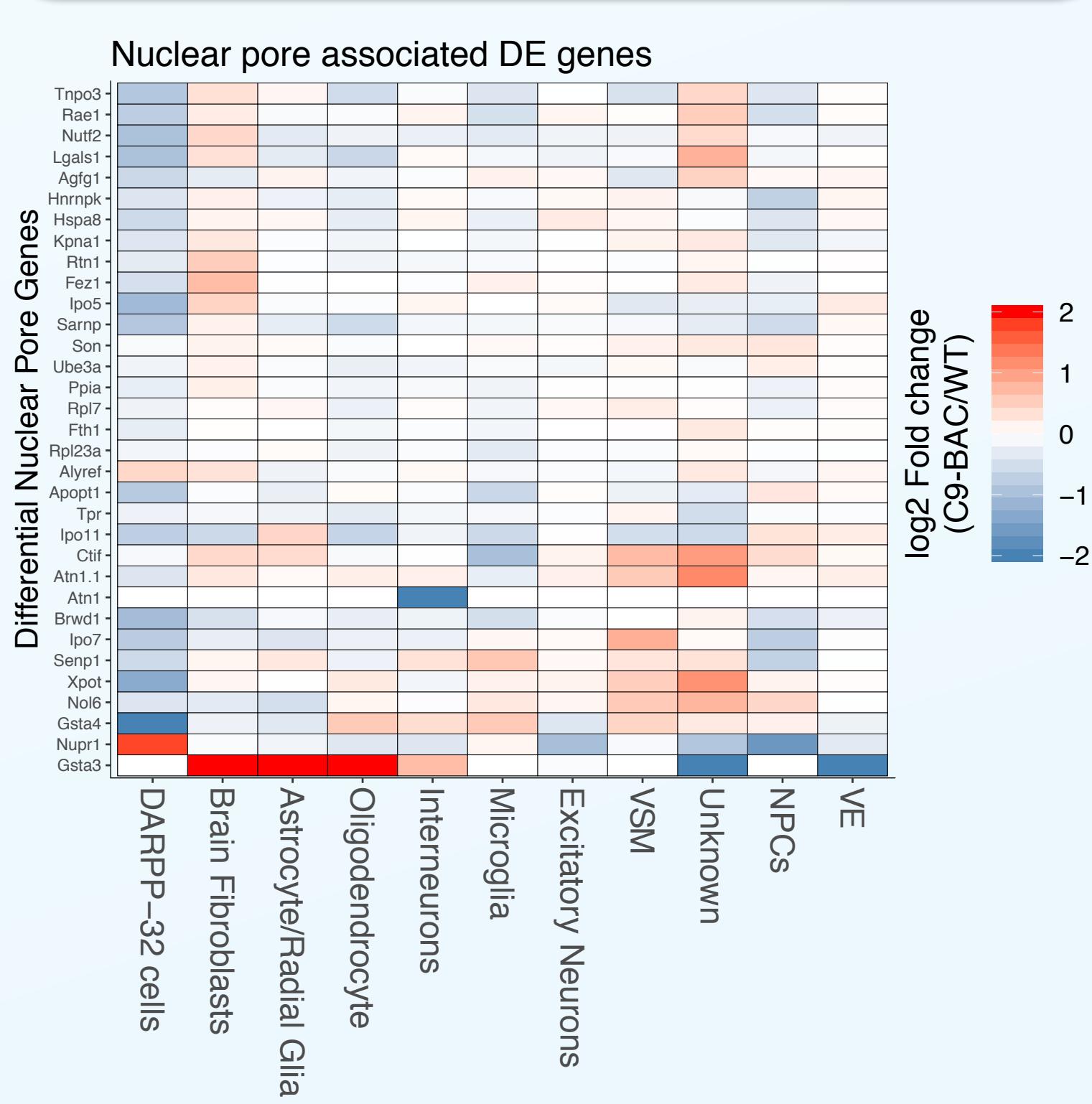
- The inwardly rectifying K⁺ channel genes *Kcnj10* and *Kcnj16* are significantly elevated in the glial cell population of P6-7 C9BAC mice
- Glial *Kcnj10* gene encodes Kir4.1 channels and modulates resting membrane potentials in excitable cells. (Larson, et al, eLife 2018).

Nuclear Pore Gene Expression



- Dysfunction in nucleocytoplasmic transport may be a fundamental pathway for C9orf72-ALS pathogenesis.
- Mutations in various nucleoporins are associated with tissue-specific diseases.
- Expression map of nuclear pore-associated genes shows significant heterogeneity across major cell types in M1 cortex.
- We are exploring the cell type-specific roles for nuclear pore-associated genes in C9orf72 repeat expansion disorder

Nuclear Pore Differential Expression



- In presymptomatic (P6-7) C9BAC mouse motor cortex, we identify 33 nuclear pore-associated genes with significant differential expression in at least one major cell type.
- No nucleoporin genes are differentially expressed across major cell types at this developmental time point.
- Future work will include recursive analysis of cell types and more advanced disease states.

Conclusions

- A putative dopamine-responsive GABAergic interneuron population is significantly reduced in P6-7 C9BAC M1 cortex.
- 2188 DE genes identified in presymptomatic P6-7 C9-BAC mouse model of fALS.
- Neuronal populations exhibit lowest number of significantly DE genes in C9orf72 P6-7 mouse motor cortex.
- Enhanced K⁺ clearance by glial K_{ir} channels may be responsible for higher resting membrane potential in C9-BAC.
- Significant heterogeneity in nuclear pore-associated gene expression and differential expression across major cell types in mouse motor cortex.

References

- Liu, Y., et al. C9orf72 BAC Mouse Model with Motor Deficits and Neurodegenerative Features of ALS/FTD. *Neuron* 90, 521–534 (2016).
- Larson, V.A., et al. Oligodendrocytes control potassium accumulation in white matter and seizure susceptibility. *eLife* 7, (2018) doi: 10.7554/eLife.34829.

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We are looking for motivated postdocs for a variety of funded single cell projects in neurodevelopment and disease!

