Single-Nuclei RNA Sequencing Reveals Inhibitory Network Dysregulation in Focal Cortical Dysplasia

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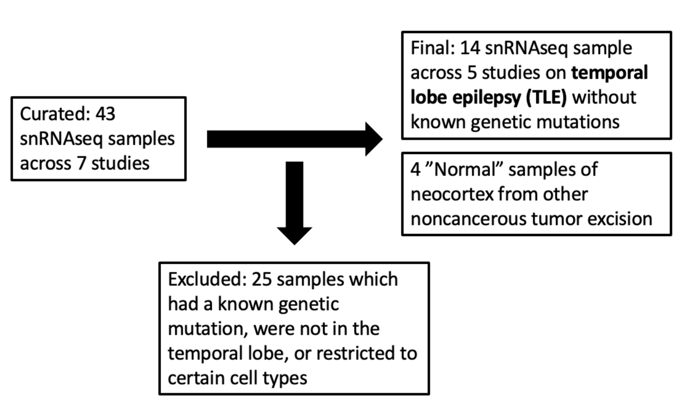
Background

- Focal Cortical Dysplasia (FCD) is a neurodevelopmental disorder resulting in a lesion that commonly causes intractable epileptic seizures. It is the most common reason for epilepsy surgery.
- FCD is caused by somatic mutations and aberrant upregulation of the mTOR pathway, causing focal disorganization of neural networks.
- Single-nuclei RNA sequencing of human epilepsy tissue has thus far been limited in scope with low sample sizes, largely due to high experiment costs and sample fragility.
- We aimed to compile data from recently published studies to investigate changes in rare cell populations of FCD patients with statistical rigor.

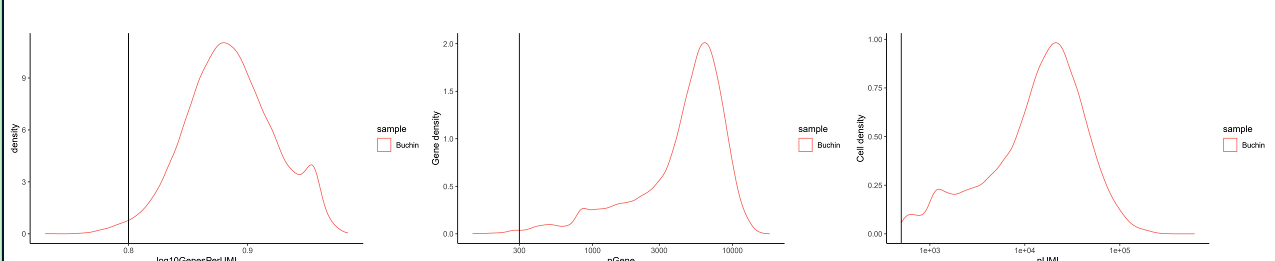
Methods

Sample selection: Single-nuclei RNA sequencing data (snRNAseq) was compiled from 5 studies. All samples were collected from the temporal lobe.

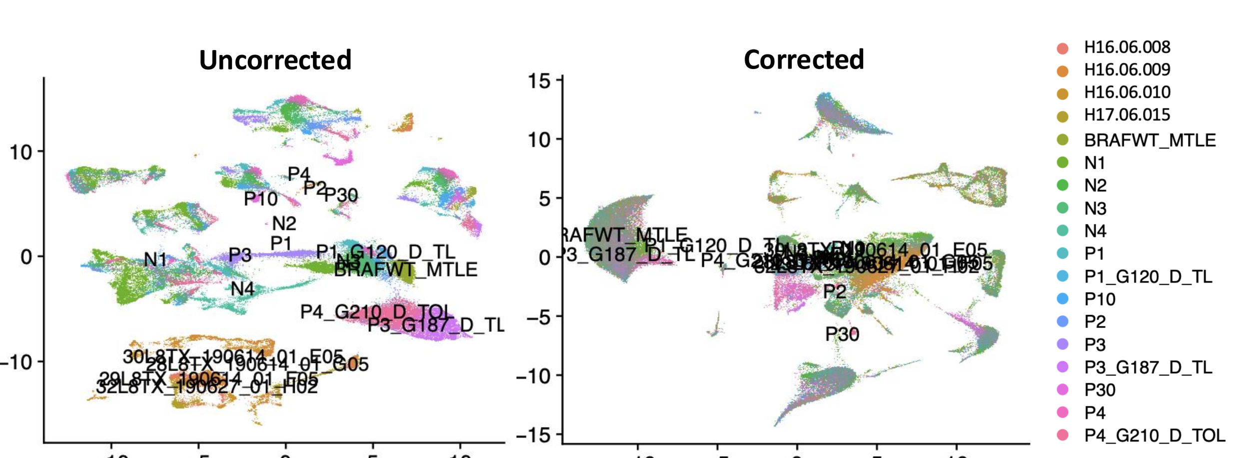


Sample	Diagnosis	Study
H16.06.008	TLE	Buchin 2022
H16.06.009	TLE	
H16.06.010	TLE	
H17.06.015	TLE	
P1	TLE, Hippocampal Sclerosis	Chen 2023
P2	TLE, Hippocampal Sclerosis	
P3	TLE, Hippocampal Sclerosis	
P4	TLE, Hippocampal Sclerosis	
N1	Intraventricular meningioma	
N2	Frontal meningioma	
N3	Right basal ganglia hemorrhage	
N4	Intracranial hematoma	
BRAFWT_MTLE	TLE	Lerond 2023
P10	TLE, FCD Type Ic	Miller 2023
P30	TLE, FCD Type Ic	
P1_G120_D_TL	TLE, FCD Type IIb	Galvao 2024
P3_G187_D_TL	TLE, FCD Type IIa	
P4_G210_D_TOL	TLE, FCD Type IIb	

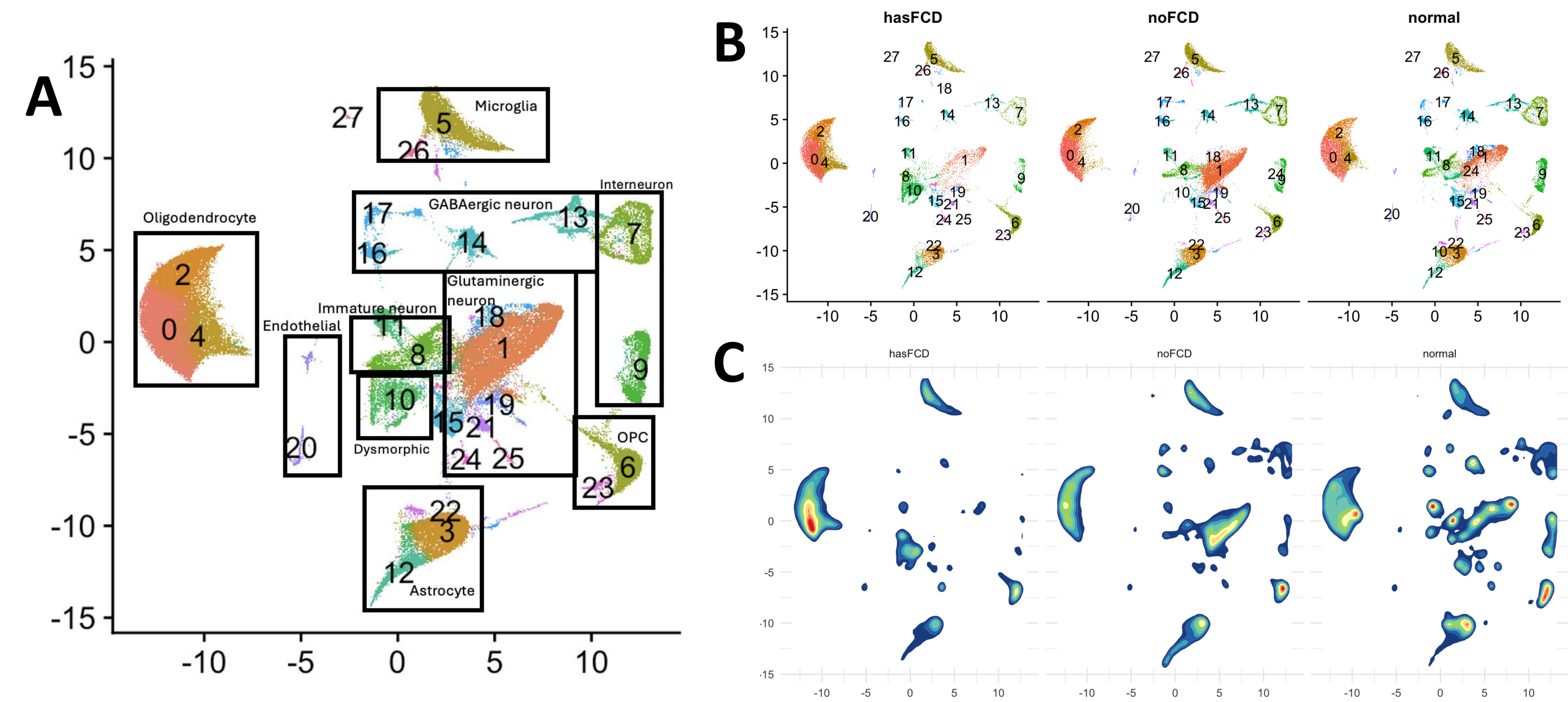
Data preprocessing: Quality control for low-quality cells



Data preprocessing: "Batch correction" for between-sample and between-study variance, allowing for legitimate cell-to-cell comparisons.



Cluster identification revealed a significant decrease of GABAergic neurons and inhibitory interneurons, and the rise of unique dysmorphic cell population.



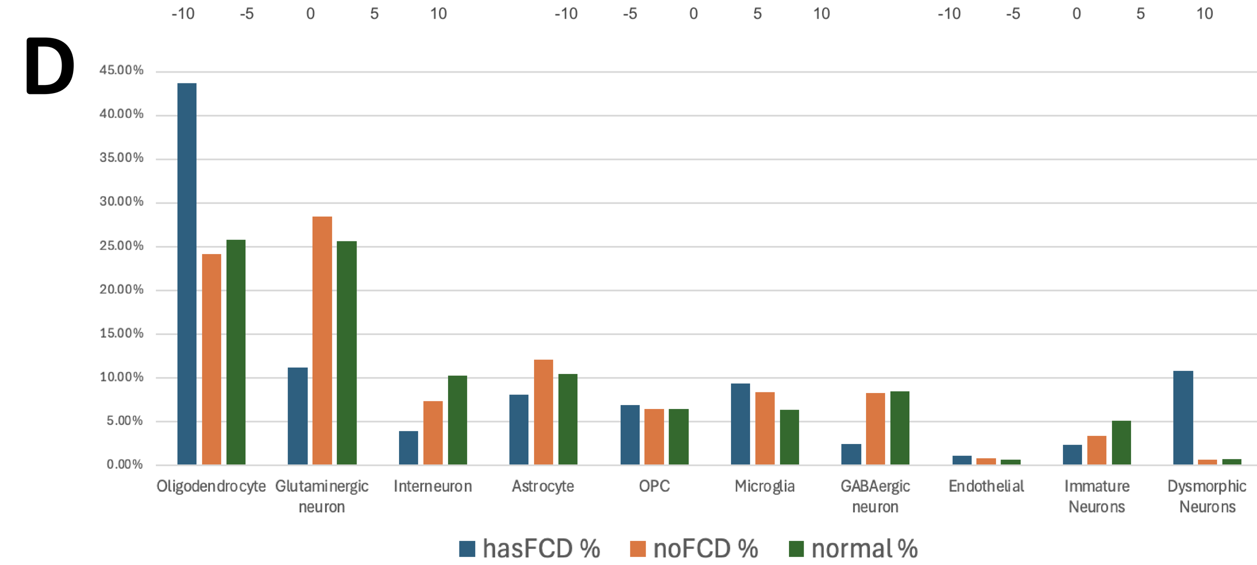
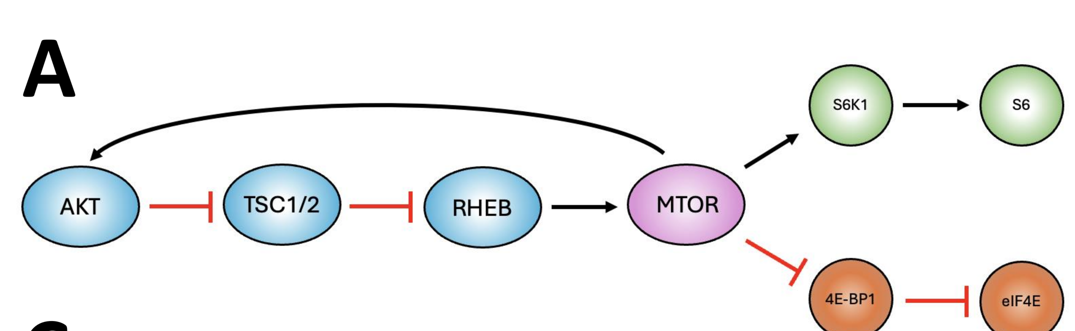
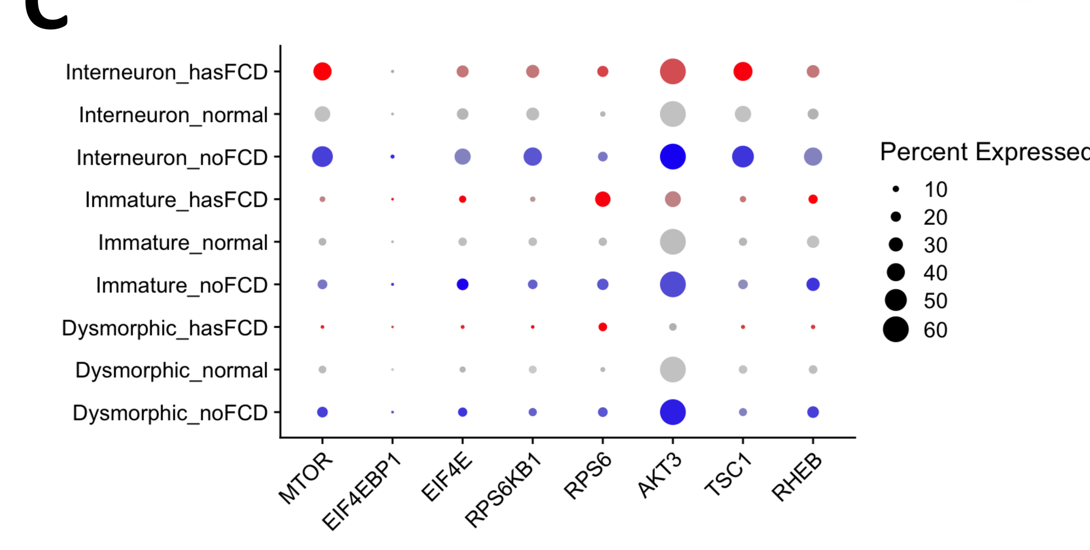


Fig 1. Uniform Manifold Approximation and Projection (UMAP) of all samples. (A) 18 pooled snRNAseq samples with labeled cluster assignments. (B) Samples split by clinical condition: FCD TLE, non-FCD TLE, and non-epileptic temporal lobe. (C) Density plots demonstrating cell population changes after normalization. (D) Bar graph showing significant statistical differences using a generalized linear mixed model.

mTOR pathway signaling is altered in FCD, resulting in broad dysregulation of downstream effectors.





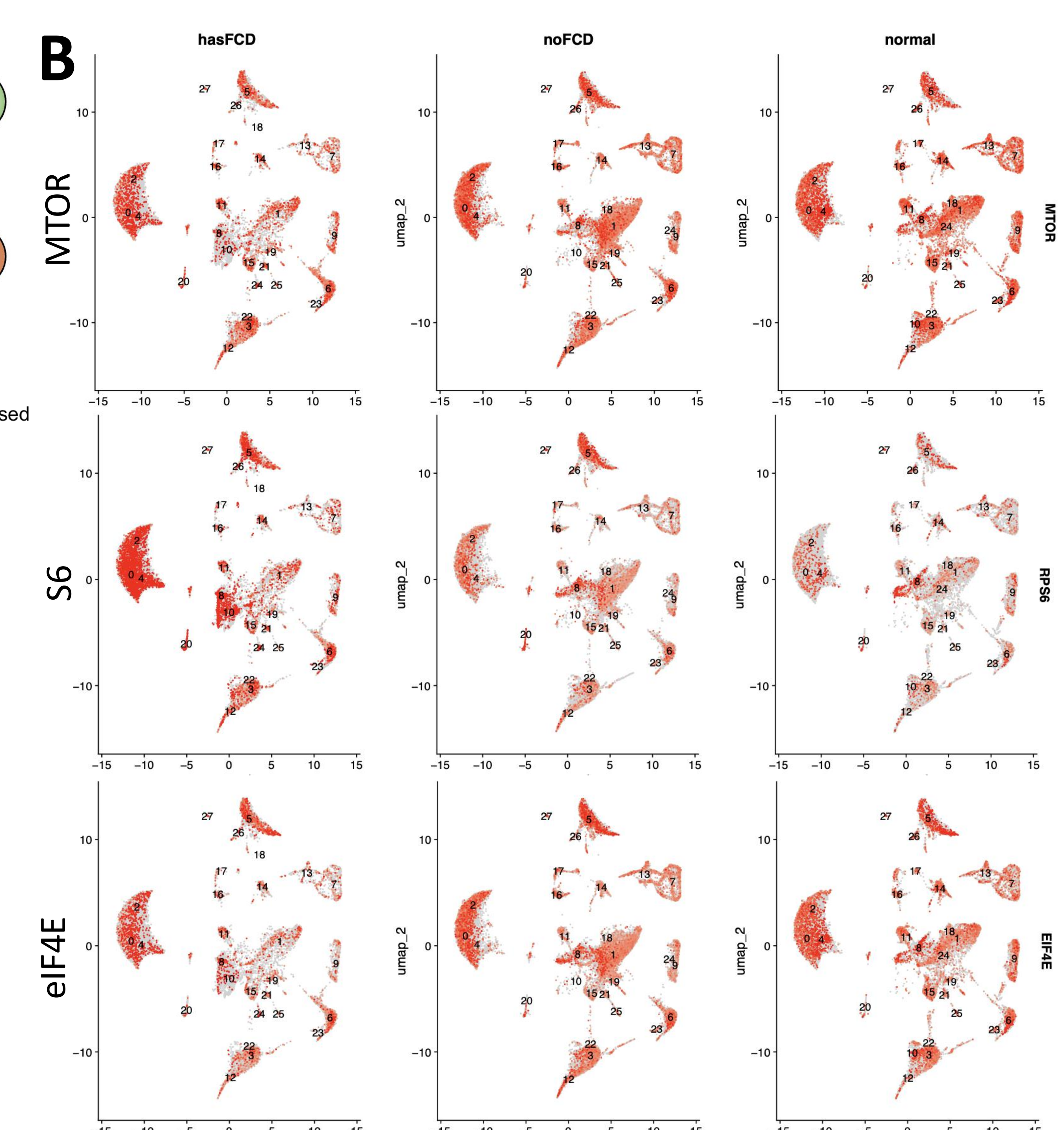


Fig 2. Nuclear RNA expression changes of mTOR signaling cascade elements. (A) Simplified schematic of the mTOR pathway containing primary proteins of interest. (B) Expression levels of wild-type mTOR, downstream effector S6 (a 40S ribosomal component), and downstream effector eIF4E (translation initiation factor) across UMAP cell populations. (C) Expression levels of mTOR genes in FCDII dysmorphic cells, immature neurons, and interneurons, by condition.

Conclusions

- GABAergic neurons and interneurons were significantly decreased in temporal lobe lesions of FCD epilepsy patients compared to non-FCD epilepsy and non-epileptic patients.
- Dysmorphic cells were identified as a distinct population only found in FCD type II. It expresses many early stem/progenitor genes.
- Wild-type mTOR and its upstream activators were suppressed, consistent with the mTOR-activating mutations reported in literature.
- The S6 effector protein, was very high in the dysmorphic neuron population.

Next Steps

- We will analyze the dysmorphic neuron population (cluster 10) for useful markers
- Immunohistochemistry with human FCD samples collected at Dartmouth-Hitchcock will additionally confirm the pathway changes we have observed.
- Investigation of markers of neuroinflammatory and extra-cellular matrix formation may reveal how mTOR dysregulation leads to seizure pathology.

References

1. Lim, J. et al. (Nat Med 2015). Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy.
2. François, L. et al. (Nat Commun 2024). Identification of gene regulatory networks affected across drug-resistant epilepsies.

Acknowledgements

This work was funded by the 2025 Dow Research Scholarship.