



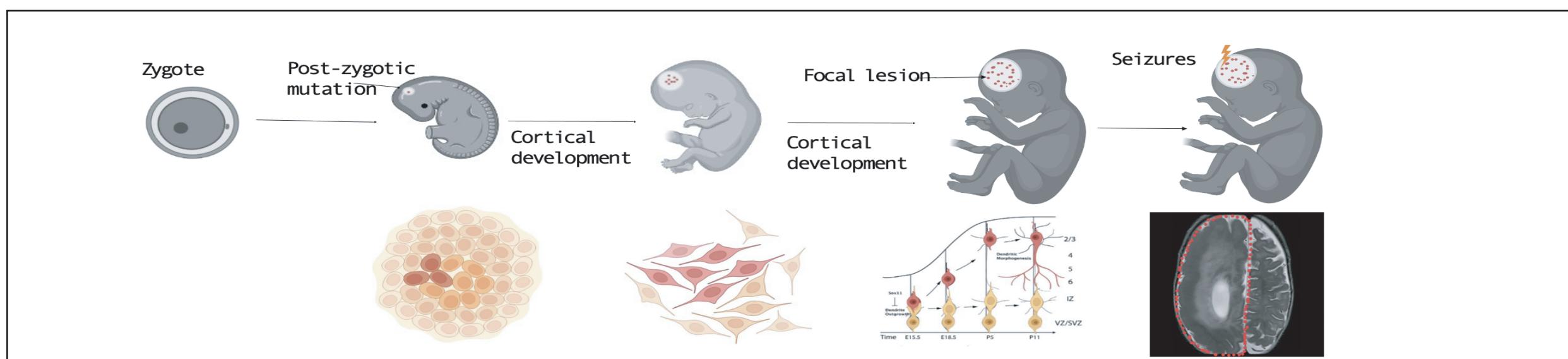
# Leveraging mosaic epileptogenic human brain tissue to study cell-type specific transcriptional changes associated with a pathogenic PIK3CA variant

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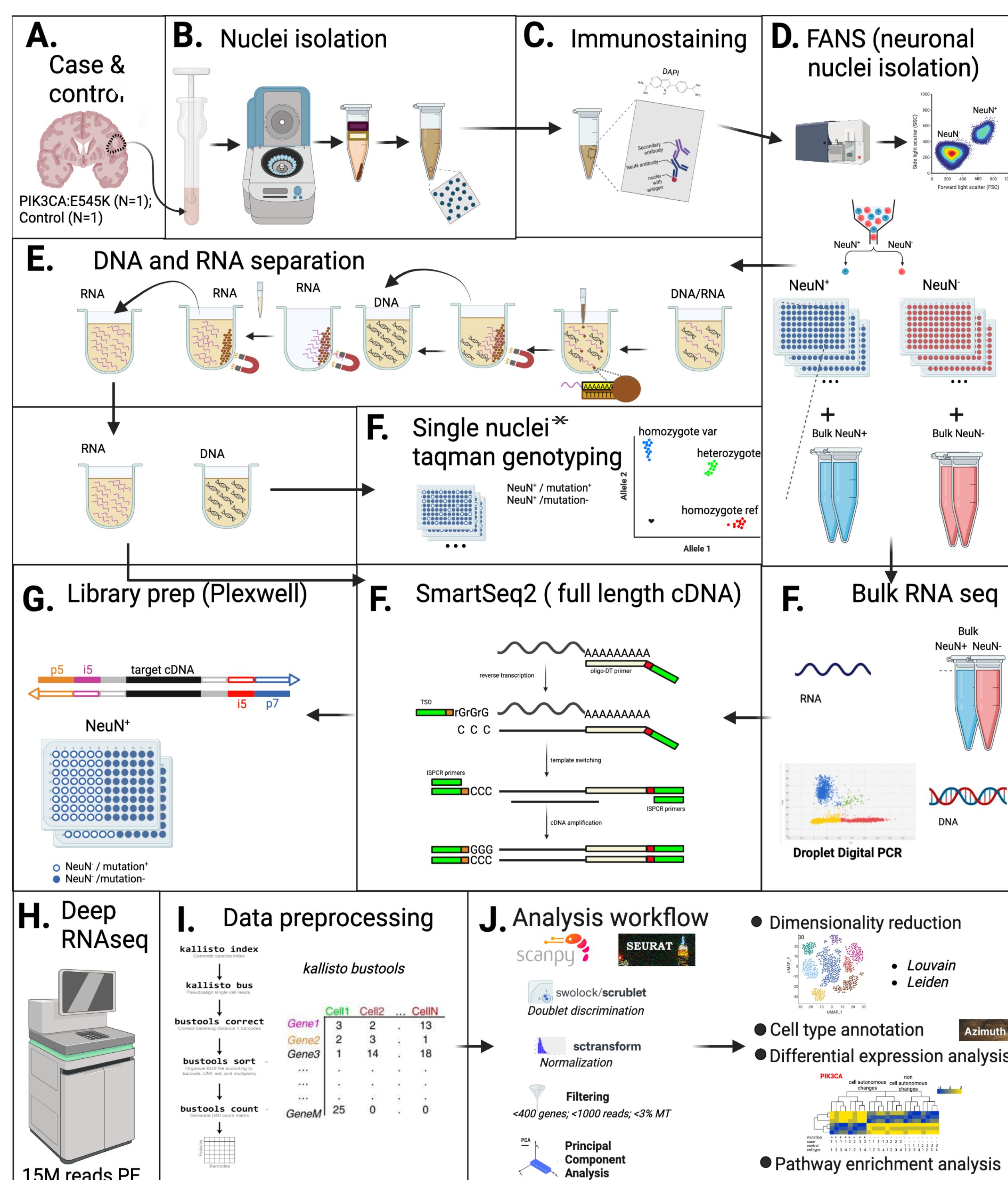
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# Background



Post-zygotically acquired mutations that arise during embryonic development can lead to abnormal neuron morphology and/or migration defects, both of which can result in seizures and developmental disabilities. Somatic mutations that result in abnormal activation of the PI3K-AKT-mTOR signaling pathway have been implicated in focal cortical dysplasia type 2 and hemimegalencephaly (HMEG). Surgically resected mosaic brain tissue from the affected individuals offers a unique opportunity to determine the transcriptional effects of the mutation across cell types.

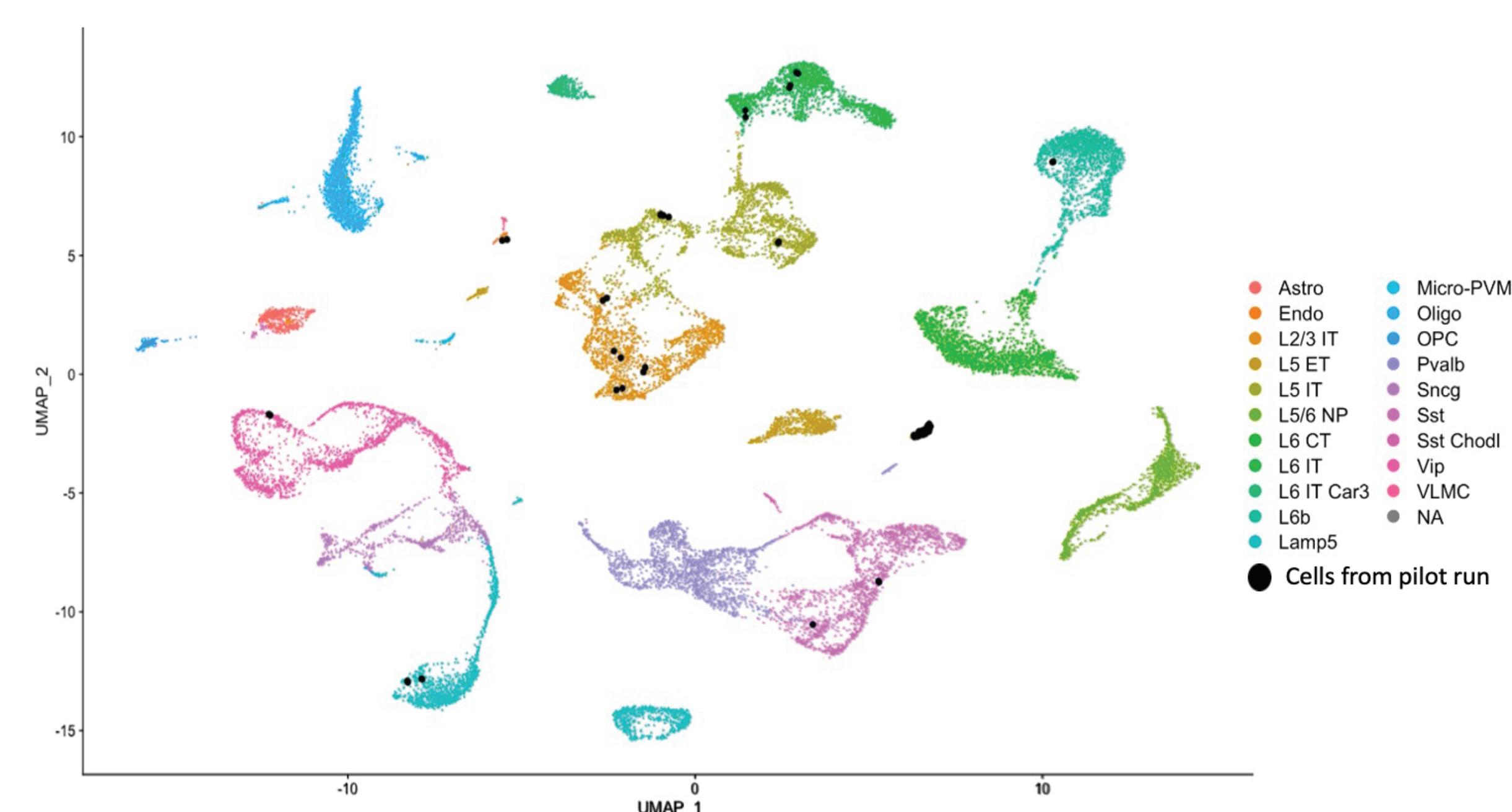
# Methods



**Figure 1. Simultaneous single-nuclei genotyping and transcriptomic profiling workflow performed on brain tissue resected from an individual with HMEG, harboring a pathogenic somatic mutation in PIK3CA (E545K) in 60-70% of cells and an age matched control.**

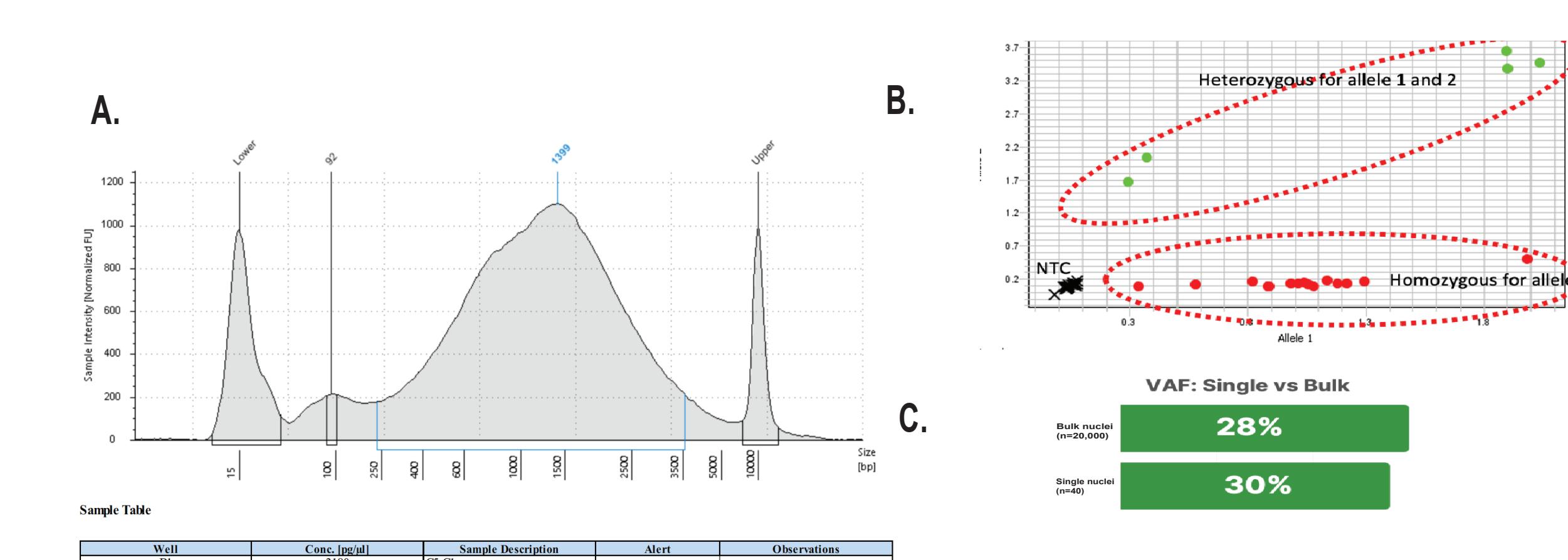
\*In this preliminary study, we attempted to genotype the missense mutation from nuclear cDNA transcripts but were unsuccessful due to low expression of the gene. Following this, a separate pilot was performed using the above method to demonstrate our ability to simultaneously genotype and perform full length RNA seq on single nuclei.

# Key Findings



**Figure 2. Cell type identification.** A UMAP showing nuclei from the pilot run comprising of 88 neuronal nuclei ( $n=44$  cases and  $n=44$  controls) projected over a publically available 10x genomics dataset of the motor cortex (Bakken et al, bioRxiv 2020). Most cells ( $n=70$ ) fall into the glutamatergic cluster, primarily ‘Layer 2/3 Intratelen-  
cerebral neurons’.

# Quality Control



**Figure 5. Evidence of successful cDNA synthesis and genotyping from single nuclei.** A) A typical cDNA synthesized from nuclear RNA using SMARTseq2 gives an average yield of 2ng/ $\mu$ L in 10 $\mu$ L. B) Genotyping results from genomic DNA isolated from single nuclei isolated from the mosaic brain sample. The variant positive nuclei (heterozygous for allele 1 and 2) and variant negative nuclei (homozygous for allele 1) segregate into two distinct axes. The variation in the y-axis translates to the varying amount of DNA present in individual nuclei. C) Comparable genotyping results from bulk ( $n=20,000$ ) and single nuclei ( $n=44$ ) with a VAF of 30% and 28% respectively.

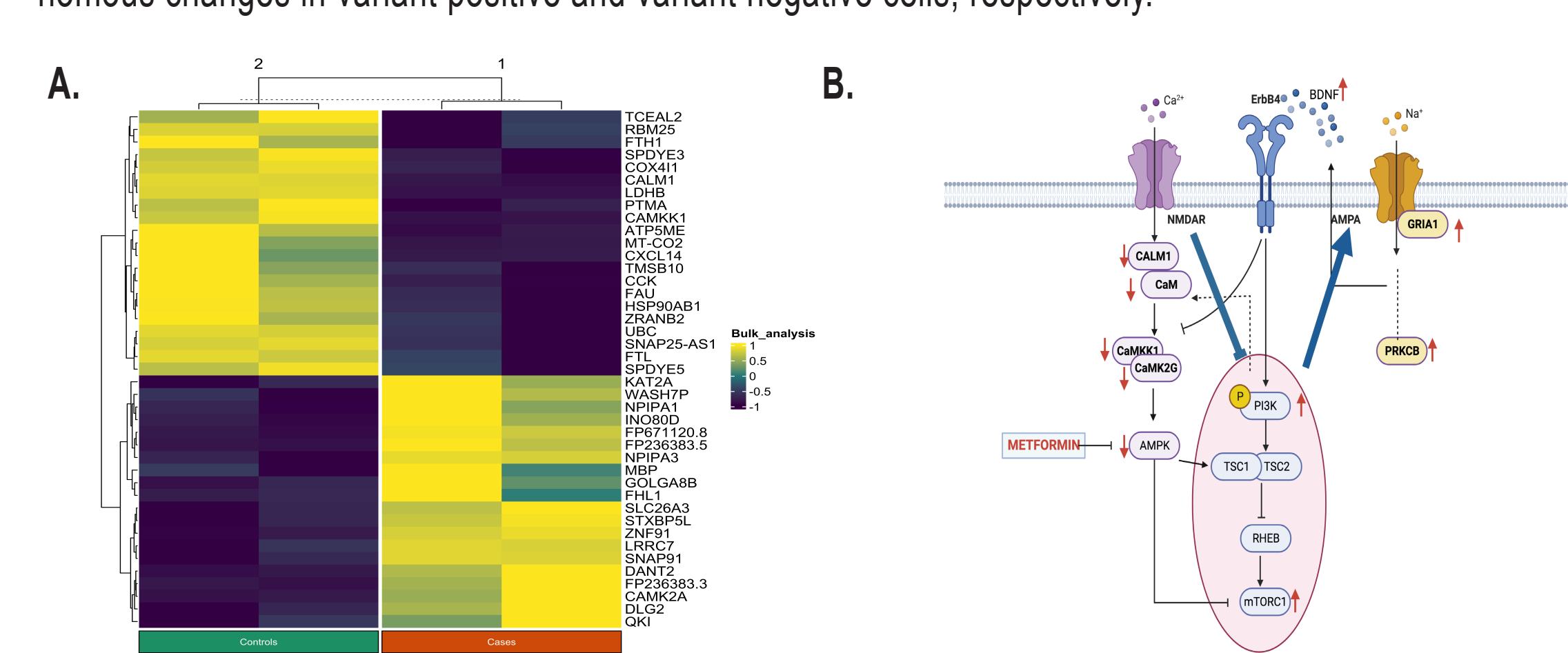
# Conclusions

This is the first study to show simultaneous single-cell genotyping and comprehensive cDNA sequencing from single nuclei in a HMEG case. While genotyping was not possible in the preliminary study, results reflect two clusters that likely represent mutation positive and mutation negative cells suggesting the autonomous and non-cell autonomous effects of the mutation. Because this was a small proof of concept study with an N=1 (number of cells=44), we were unable to assess cell-type specific gene expression differences. We are currently validating these results using a larger sample size with biological replicates.

# Acknowledgements

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# References



**Figure 4. Gene expression correlates well between bulk and single nuclei.** A) A heatmap showing the top differentially expressed genes in bulk nuclei ( $n=20,000$ ) isolated from case compared to control (log fold-change $>0.5$ ). B) Downregulated genes present in both bulk and single nuclei include many involved in long term potentiation and the calcium signaling pathway. Downregulation of AMPK through inhibition of calcium signaling and upregulation of AMPA are the dominant pathways altered in cases.

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