Characterization of a Prime-Edited *BIN1* Knockout Human Induced Pluripotent Stem Cell Line for Alzheimer's Disease Modeling

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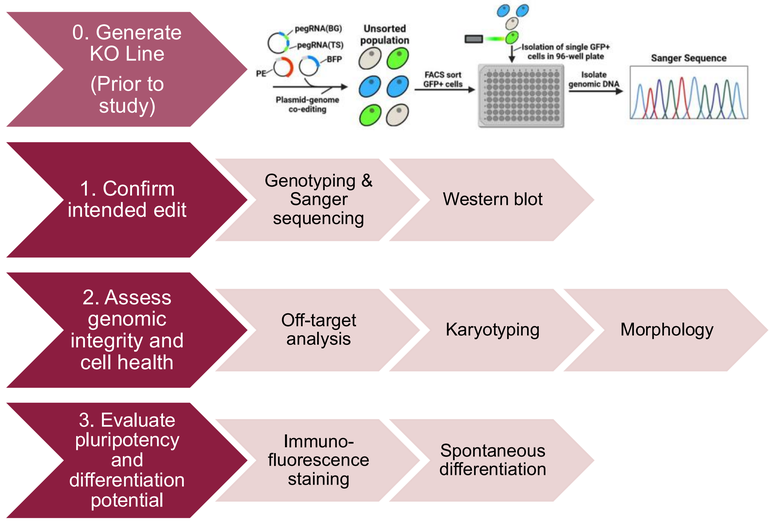
Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases, affecting over 7.2 million people in the United States and contributing to significant healthcare burden [1]. It is characterized by impairments in memory and cognition, as well as the accumulation of neurofibrillary tangles and amyloid plaques that drive neuronal dysfunction and cell death [2]. Sporadic Alzheimer's disease (SAD), which typically presents after age 65, is multifactorial, with genetic factors contributing an estimated 60–80% of disease risk [3]. The Bridging Integrator 1 (*BIN1*) gene is the second strongest genetic risk factor for SAD after APOE. BIN1 plays important roles in endocytosis, intracellular trafficking, and cytoskeletal organization [4], and has been implicated in AD-related pathways including tau and amyloid pathology, inflammation, and calcium homeostasis [3]. Despite these associations, the mechanistic role of *BIN1* in AD pathology remains unclear. Human induced pluripotent stem cells (hiPSCs), combined with CRISPR-based editing technology, provide a unique platform to study gene function in relevant cell types [5]. Using this technology, isogenic *BIN1* knockout hiPSC lines were generated to investigate its role in AD. The objective of this project is to validate and characterize this *BIN1* knockout iPSC lines by confirming successful gene disruption and ensuring the cells remain genetically stable, healthy, and pluripotent for downstream experiments.

Methods

BIN1 knockout hiPSC lines were generated using the PINE-TREE platform. Both homozygous (-/-) and heterozygous (+/-) clones were selected for validation to enable future dose-dependent studies of *BIN1* loss in Alzheimer's Disease.



Results

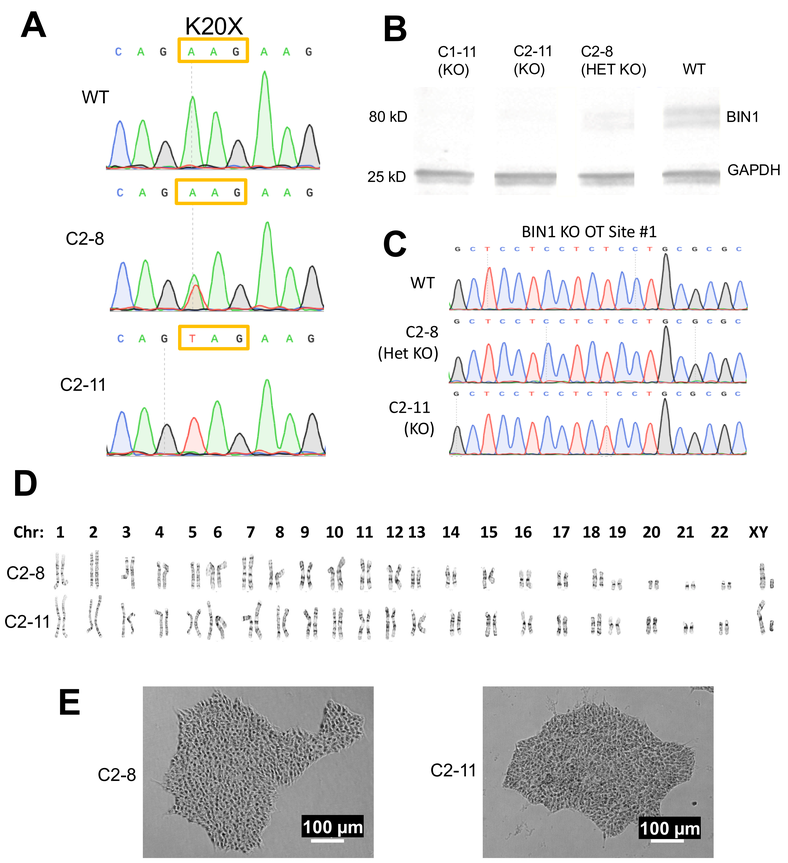


Figure 1. Verification of edit and cell health. (A) Edited cell lines were assessed via genotyping. (B) Western blot confirming loss of BIN1 protein, (C) off-target analysis of top predicted locus, (C) G-band karyotyping, (E) morphology/phase imaging. These analyses confirm the intended edits, absence of major off-target effects, normal karyotype, and typical cell morphology.

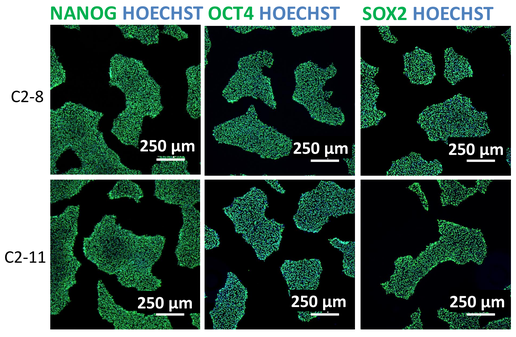


Figure 2. Pluripotency marker expression in edited cell lines. Immunofluorescence staining of homozygous and heterozygous cell lines showing Hoechst (blue) and pluripotency markers NANOG, OCT4, and SOX2 (green).

Results

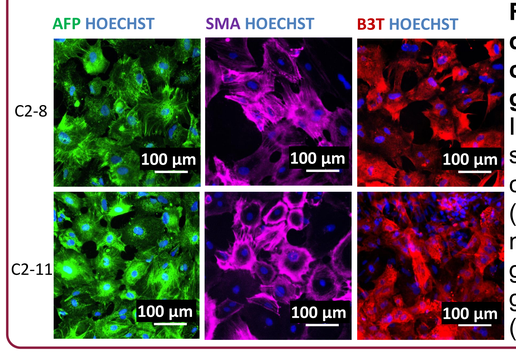


Figure 3. Spontaneous differentiation of edited cell lines into the three germ layers. Immunofluorescence staining of differentiated cells showing Hoechst (blue) and lineage-specific markers: AFP (endoderm, green), SMA (mesoderm, green), and β3-Tubulin (ectoderm, green).

Summary, Conclusions and Future Directions

- A prime-edited BIN1 knockout hiPSC line was successfully validated through genotypic and functional characterization
- Edited cell lines maintained genomic integrity, pluripotency, and differentiation potential
- Establishes a reliable human cell model for studying the role of BIN1 in Alzheimer's disease

Future Directions:

- Apply validated BIN1 knockout lines in neuronal, astrocytic, and co-culture systems to study BIN1-dependent trafficking
- Assess effects on APP processing (APP internalization, BACE1 localization) and tau pathology (phosphorylation, uptake)
- Enable comparisons across WT, KO, and variant lines to define BIN1-associated disease mechanisms

References

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Acknowledgements

I would like to express my sincere gratitude to my PI, Dr. David Brafman, for his mentorship and for providing the resources necessary to complete this project. I am deeply grateful to Salma Leyasi for her tireless patience and for teaching me the fundamental techniques required for this work. This project builds upon the initial prime-editing and cell line generation successfully completed by Salma, and I thank her for her guidance throughout the characterization process.

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