Using human induced pluripotent stem cells and CRISPR/Cas9 genome-editing to model autism spectrum disorders

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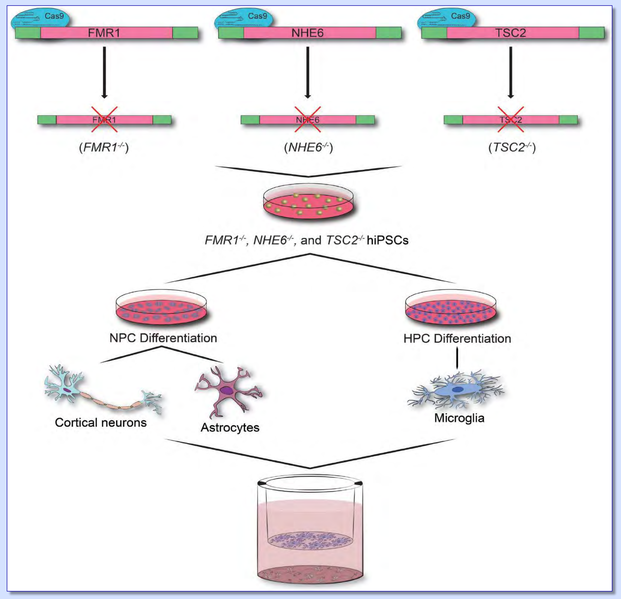
Introduction

Autism spectrum disorder (ASD) affects 1 in 68 children in the US and involves brain development with symptoms that are unable to be treated with current medicine. Although individual genomic variations strongly influence ASD, they are difficult to model *in vitro*, because patients show varying phenotypes. However, recent technological progress in genome editing offers an opportunity to investigate the genetic causes of ASD. The overall goal of this project aims to reveal upstream pathological mechanisms that cause synaptic and network dysregulation observed in ASD. In this study, we partnered a well characterized neurotypical human induced pluripotent stem cell (hiPSC) line with CRISPR-Cas9 genome editing to generate monogenic ASD hiPSC disease models.

Methods



Characterizing Control hiPSCs

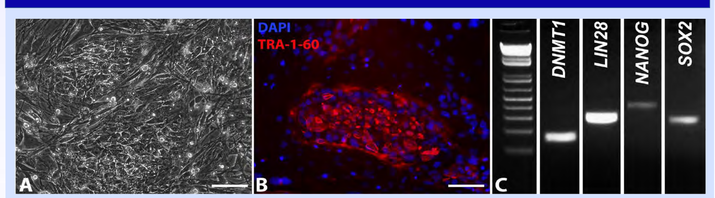


Figure 1: CVB wildtype hiPSC line. Human donor fibroblasts were reprogrammed using retroviruses containing the four Yamanaka Factors. The resulting hiPSCs were grown on MEFs **(A)** and expressed TRA-1-60 **(B)**, along with other pluripotency markers **(C)**.

Generating ASD hiPSC Lines

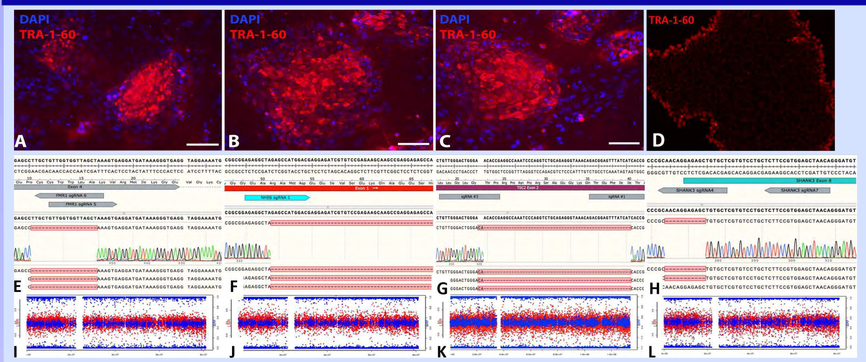


Figure 2: Generating FMR1$^{-/-}$, NHE6$^{-/-}$, TSC2$^{-/-}$, SHANK3$^{-/-}$, hiPSC lines. Control hiPSCs were nucleofected with sgRNA in a modified px458 vector targeting either FMR1, NHE6, or TSC2. The GFP^{+} hiPSCs containing the sgRNA were collected by cell sorting and expanded on MEFs. The CRISPR-edited hiPSC lines expressed TRA-1-60 **(A-D)**. The CRISPR lines were later genotyped and karyotyped to reveal a homozygous knockout of either FMR1 **(E, I)**, NHE6 **(F, J)**, TSC2 **(G, K)** or SHANK3 **(H, L)**.

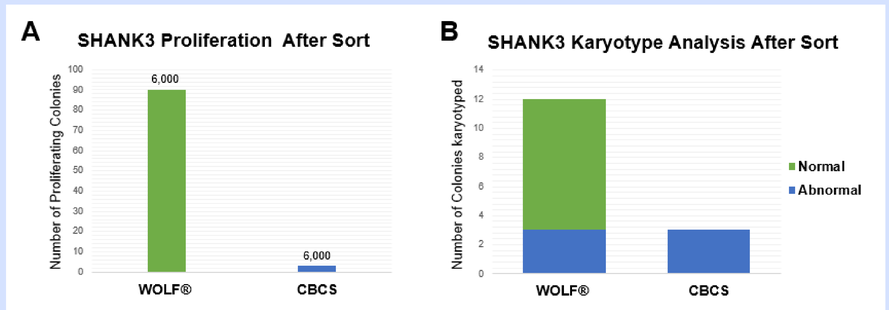
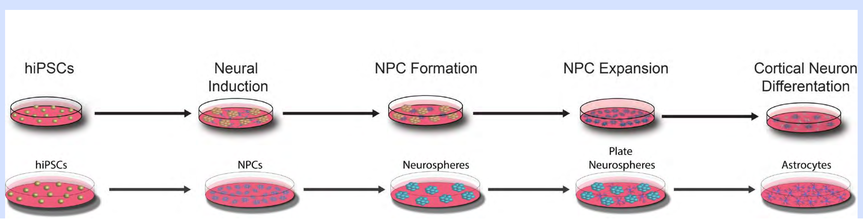


Figure 3: Gentle microfluidic Cell Sorting improves the yield and quality of the SHANK3, CRISPR-modified hiPS colonies. Same number of nucleofected hiPS cells were sorted in the NanoCellect's WOLF® Cell Sorter versus a Cuvette-Based Cell Sorter (CBCS). **A)** A higher number of colonies proliferated when the cells were sorted using NanoCellect's WOLF® Cell Sorter compared to a Cuvette-Based Cell Sorter (CBCS). **B)** A higher number of colonies maintained normal karyotypes when the cells were sorted with the WOLF® compared to the CBCS.

NPCs, Neurons, and Astrocytes



ASD NPCs, Neurons, and Astrocytes

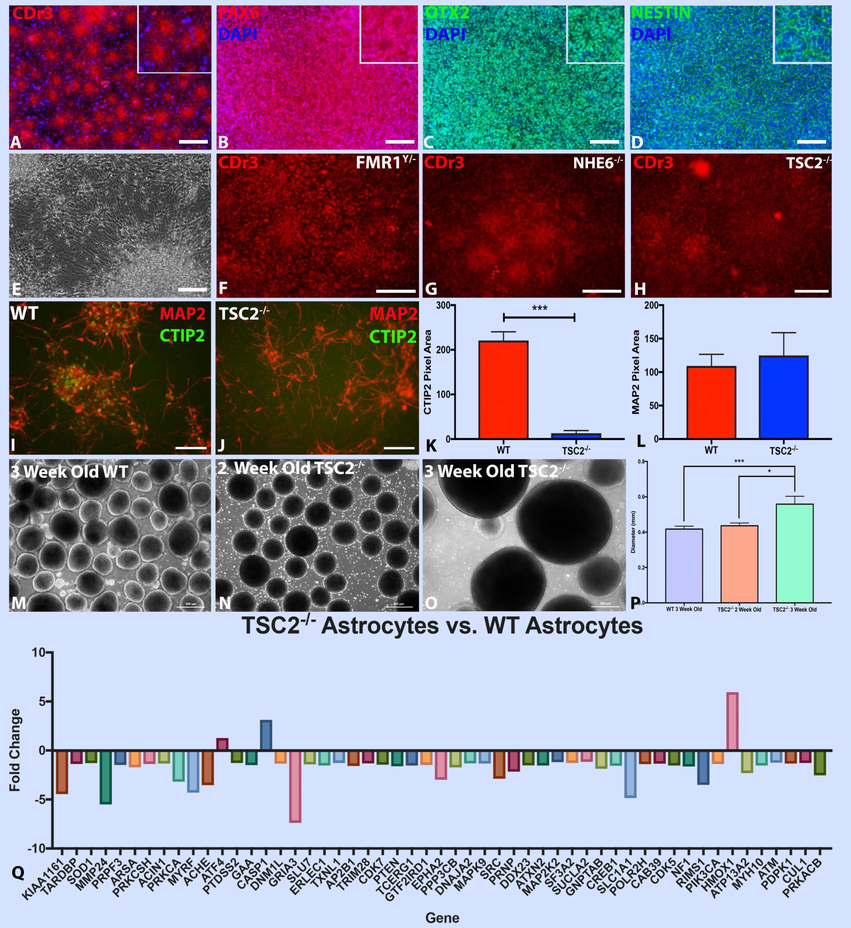


Figure 4: WT and ASD hiPSC-derived neural progenitor cells (NPCs), neurons, and astrocytes. WT **(A-D)** and ASD **(F-H)** hiPSC lines were differentiated into NPCs. WT **(E, I)** and TSC2$^{-/-}$ **(J)** NPCs were then differentiated into cortical neurons and CTIP2 and MAP2 pixel area was measured **(K, L)**. WT **(M)** and TSC2$^{-/-}$ **(N, O)** NPCs formed neurospheres and sphere diameter was measured **(P).** The neurospheres were then further differentiated into astrocytes and analyzed using the NanoString Neuropathology Gene Expression Panel **(Q)**.

Future Directions

1) Differentiate WT and ASD hiPSCs into microglia
2) Perform co-culture studies and investigate immune dysfunction and abnormal synaptogenesis

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