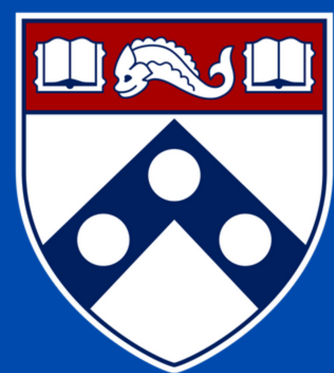

VALIDATING SYSTEMS OF INDUCIBLE GENE PERTURBATIONS IN STEM CELLS AND NEURONS

Matthew Liu, COL '23

Faculty Mentor: Ophir Shalem, Penn Medicine Department of Genetics

Funded by the College Alumni Society

Abstract

Due to the burdens of genetically engineering stem cells, inefficient and incomplete CRISPR gene perturbations in neurons have plagued the reliability of genetic screens in complex cell types such as neurons. The objective of this project was to establish a platform to generate iPSC cell lines with stable expression of constitutive and inducible CRISPR machinery for genetic screens with minimal genetic engineering. CRISPR integrated KOLF2.1J cells show stable dCas9 expression in long term culture and post freeze/ thaw following PiggyBac transfection. Immunofluorescence and western blot data show that dCas9 is expressed in the nucleus in iPSCs and expression is excluded from nuclei in differentiated neurons, and that cleavage of the dCas9 protein is occurring in neurons. This suggests that inability to localize at the nucleus and cleavage of the dCas9 protein are two potential explanations for loss of dCas9 expression and reduced gene silencing in neurons.

Introduction

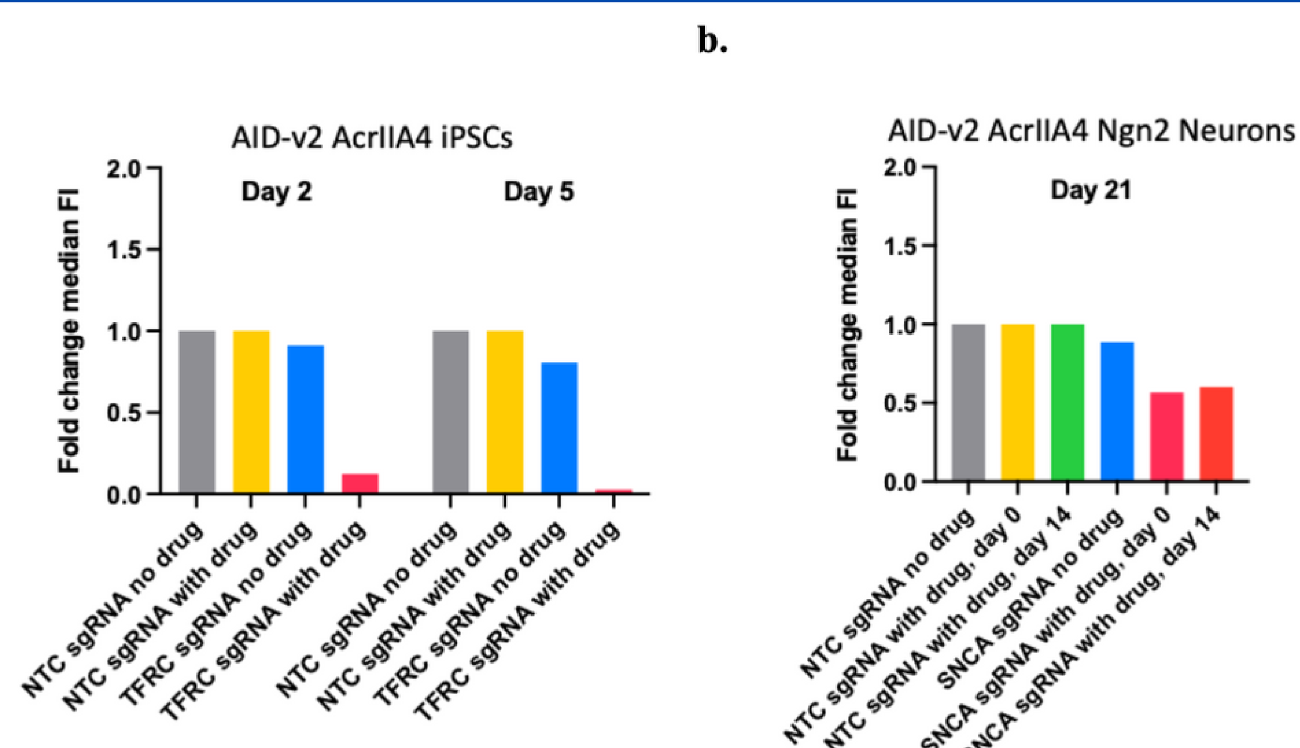

Figure 1: Existing inducible dCas9 systems in neurons show lackluster knockdown efficacy.

In order to devise a system of CRISPRi that is transferable between cell lines, we must first establish these CRISPRi cell lines in iPSCs, which have the potential to be differentiated into any somatic cell. Furthermore, dCas9 knockdown in WTC11 cells, the cell line that is currently used widely across the field, have shown to have gradual loss of knockdown efficacy as these cells are differentiated into neurons (Figure 1). Inducible knockdowns were performed on the Transferrin Receptor (TFRC) gene in iPSCs and on Alpha-Synuclein (SNCA) in neurons.

Results

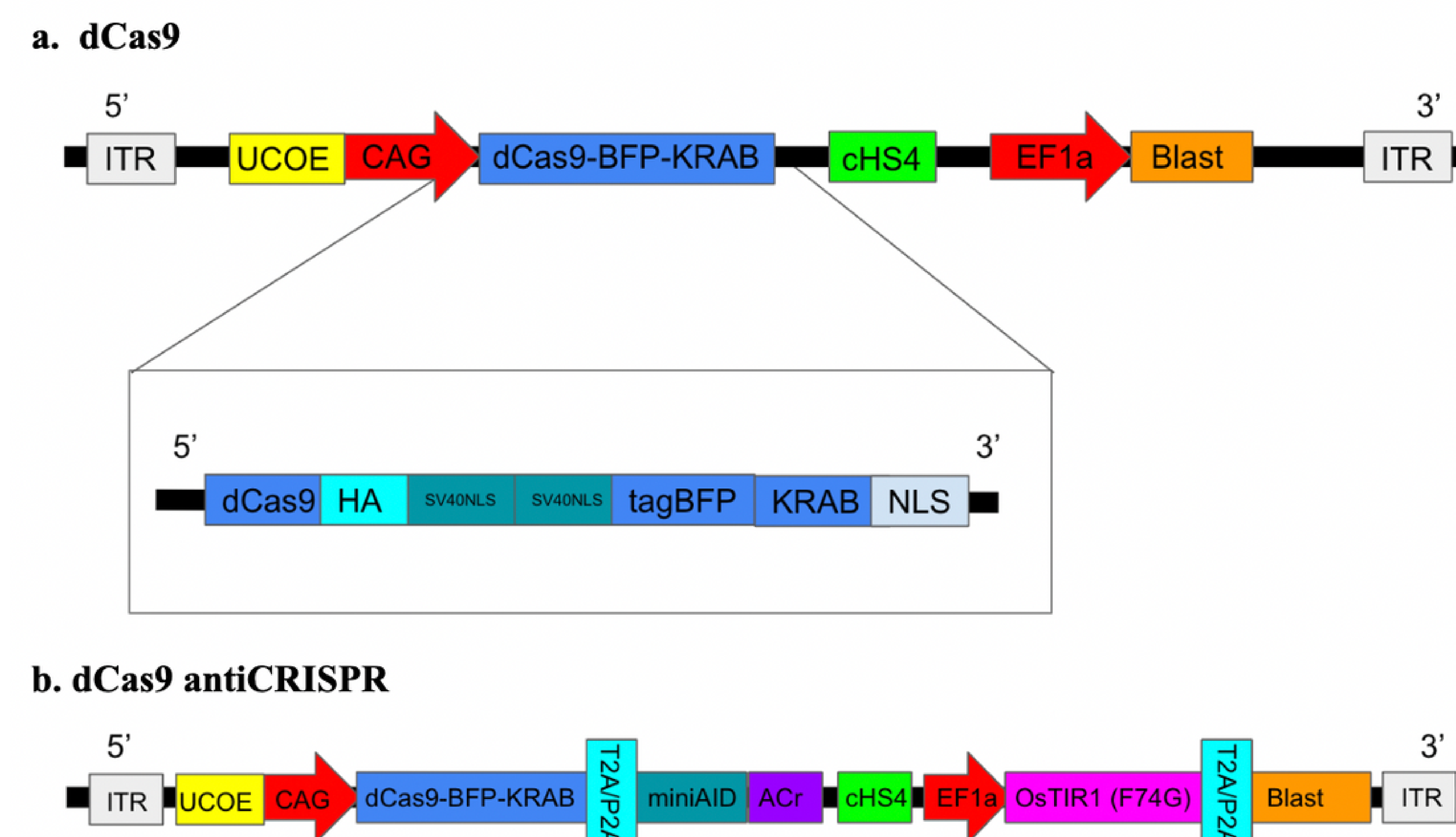

Figure 2: dCas9 constructs for new cell integration.

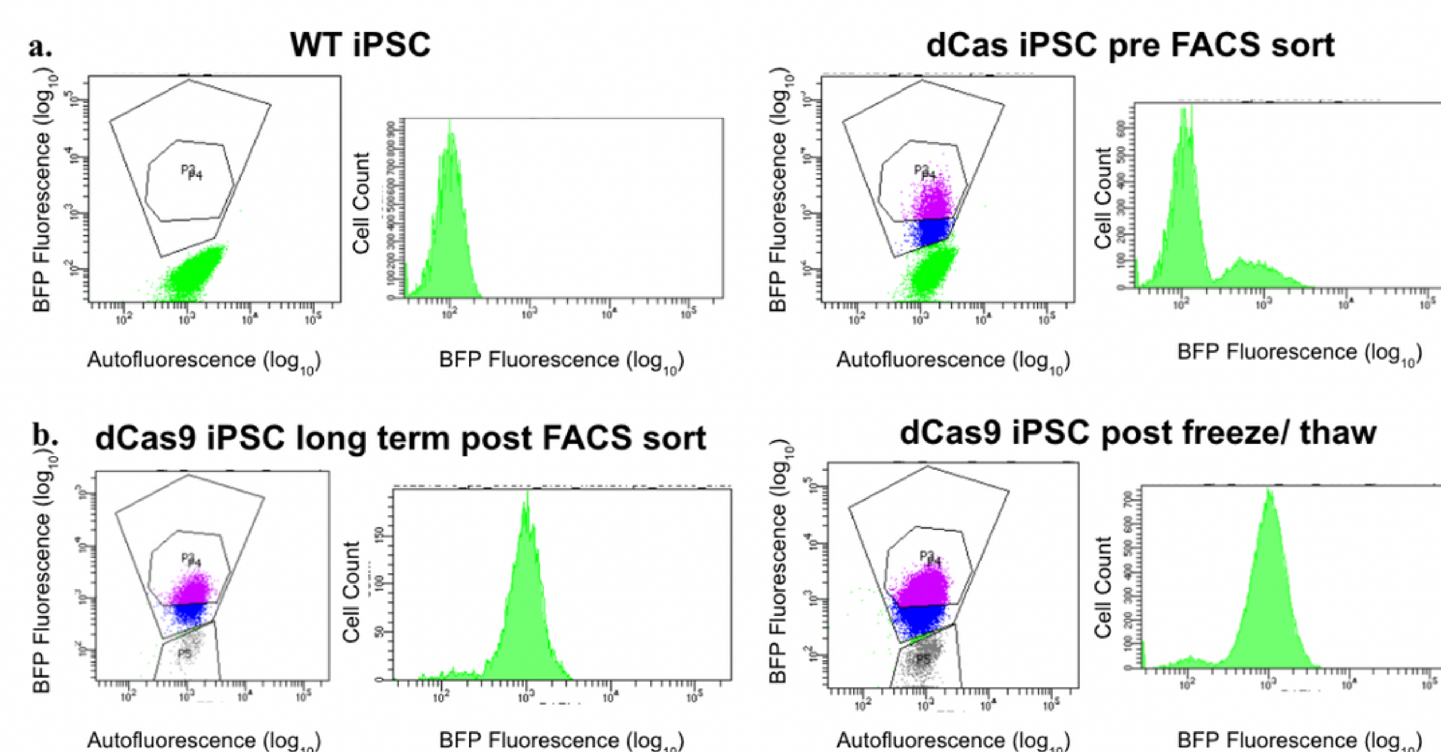

Figure 3: CRISPR integrated KOLF2.1J cells show stable dCas9 expression in long term culture and post freeze/ thaw.

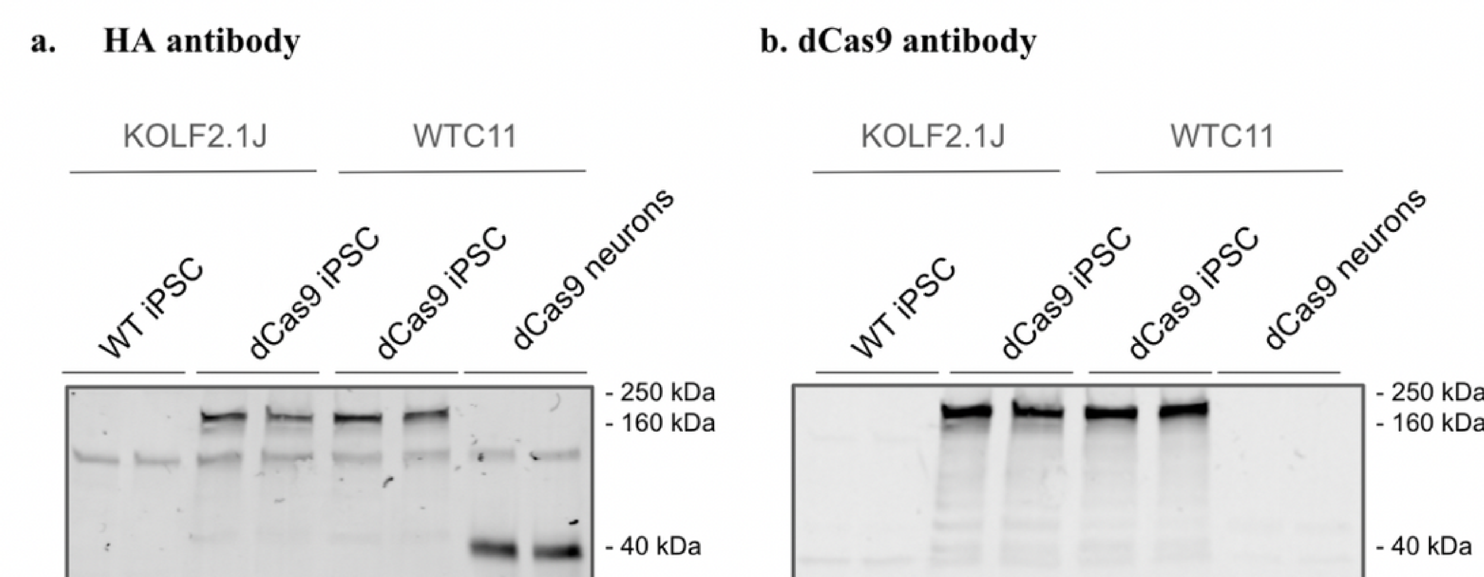

Figure 4: Western blots show clear cleavage of dCas9 only in differentiated neurons.

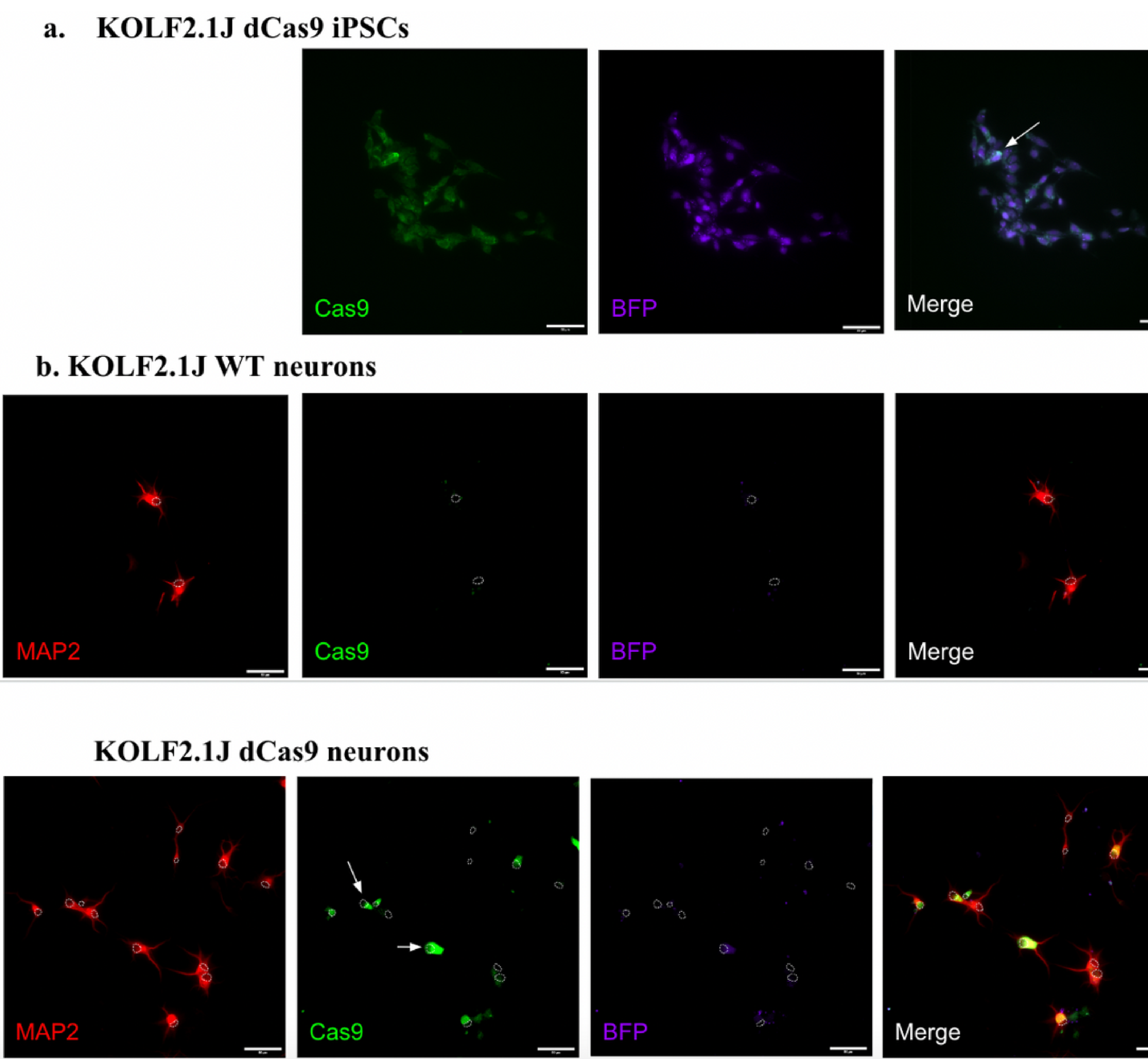

Figure 5: dCas9 is expressed in the nucleus in iPSCs and is excluded from the nucleus in differentiated neurons.

Future Directions

- Determine what is causing cleavage of the dCas9 protein in the neurons.
  - HA tag or Cas9
- Develop new cell lines will have to be cloned to be able to prevent this cleavage and retain Cas9 activity in neurons
- Testing constitutive and inducible knockdowns using dCas9 in neurons for large-scale genetic screens.

References

1. Wang, C. et al. Scalable Production of iPSC-Derived Human Neurons to Identify Tau-Lowering Compounds by High-Content Screening. *Stem Cell Reports*. **2017**.
2. Tian, R. et al. CRISPR Interference-Based Platform for Multimodal Genetic Screens in Human iPSC-Derived Neurons. *Neuron*. **2019**.
3. Fernandopulle, M. S. et al. Transcription Factor-Mediated Differentiation of Human iPSCs into Neurons. *Curr Protoc Cell Biol*. **2018**.
4. Akter, M. et al. Generation of two induced pluripotent stem cell lines with heterozygous and homozygous amyotrophic lateral sclerosis-causing mutation R521G (c.1561C > G) in FUS gene. *Stem Cell Research*. **2023**.
5. Pantazis, C. B. et al. A reference human induced pluripotent stem cell line for large-scale collaborative studies. *Cell Stem Cell*. **2022**.
6. Tian, R. et al. Genome-wide CRISPRi/a screens in human neurons link lysosomal failure to ferroptosis. *Nat Neurosci*. **2021**.
7. Feldman, D. et al. Optical Pooled Screens in Human Cells. *Cell*. **2019**.
8. Feldman, D. et al. Pooled genetic perturbation screens with image-based phenotypes. *Nat Protoc*. **2022**.
9. Shalem, O. et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*. **2014**.