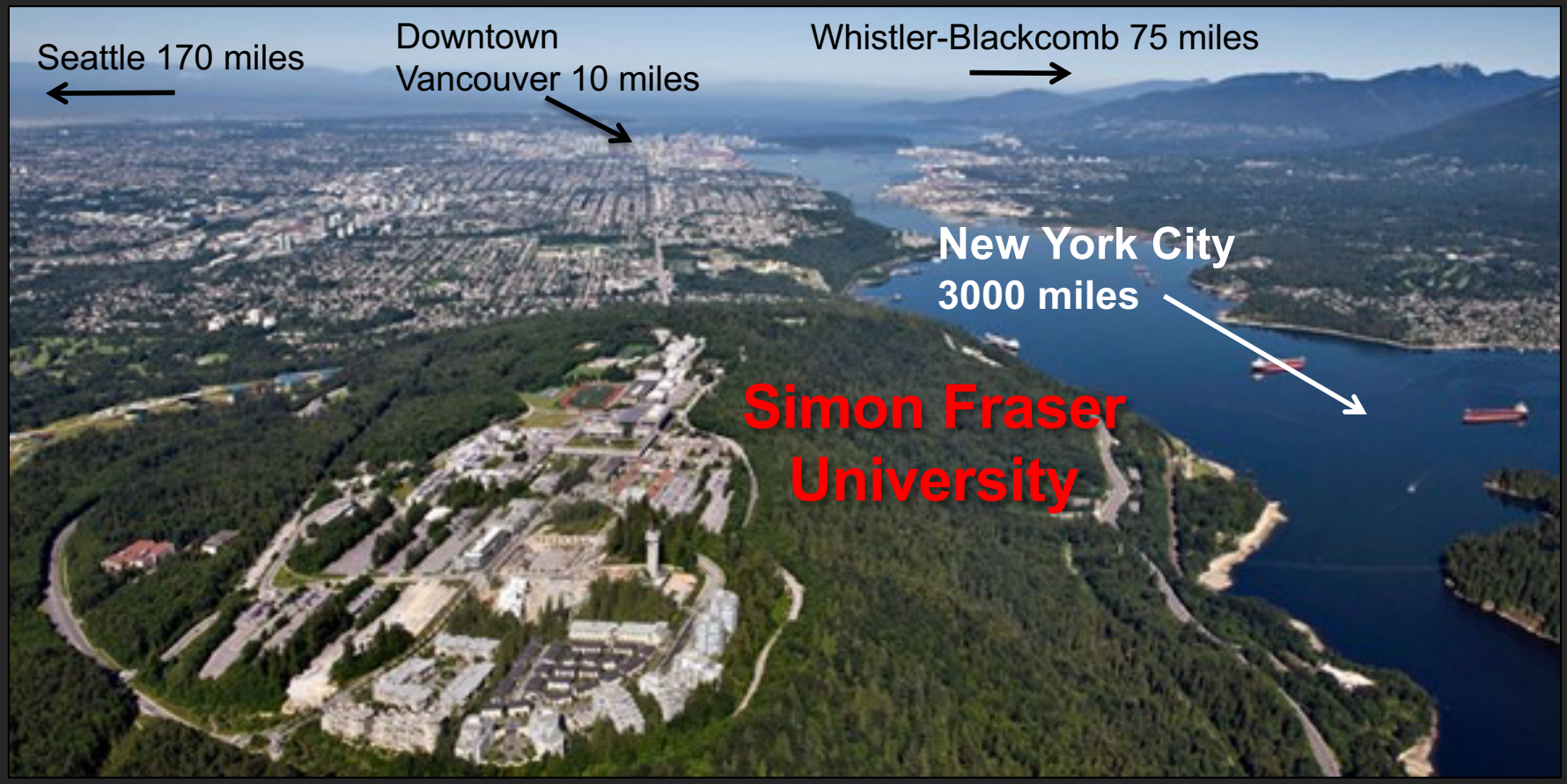
# A very brief introduction to the Silverman Lab




Michael Silverman and Troy Collins
Department of Biological Sciences
Department of Molecular Biology and Biochemistry

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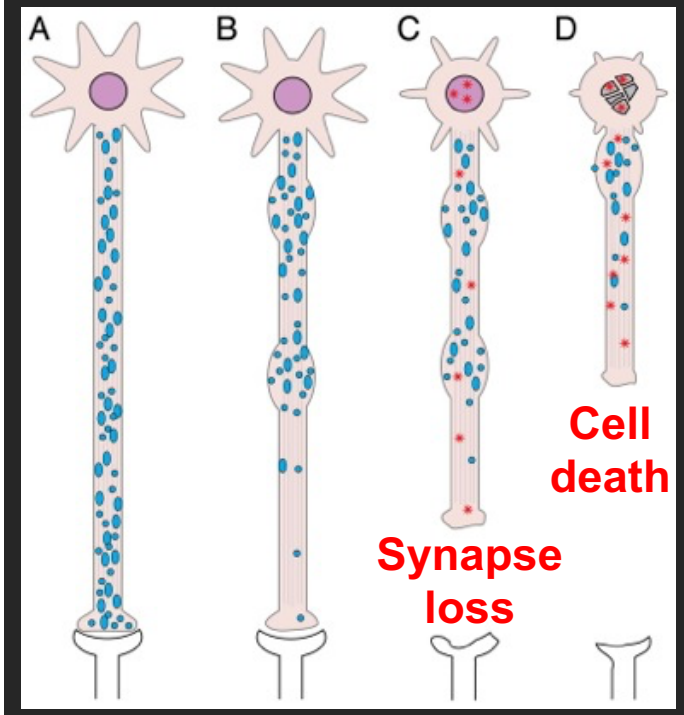
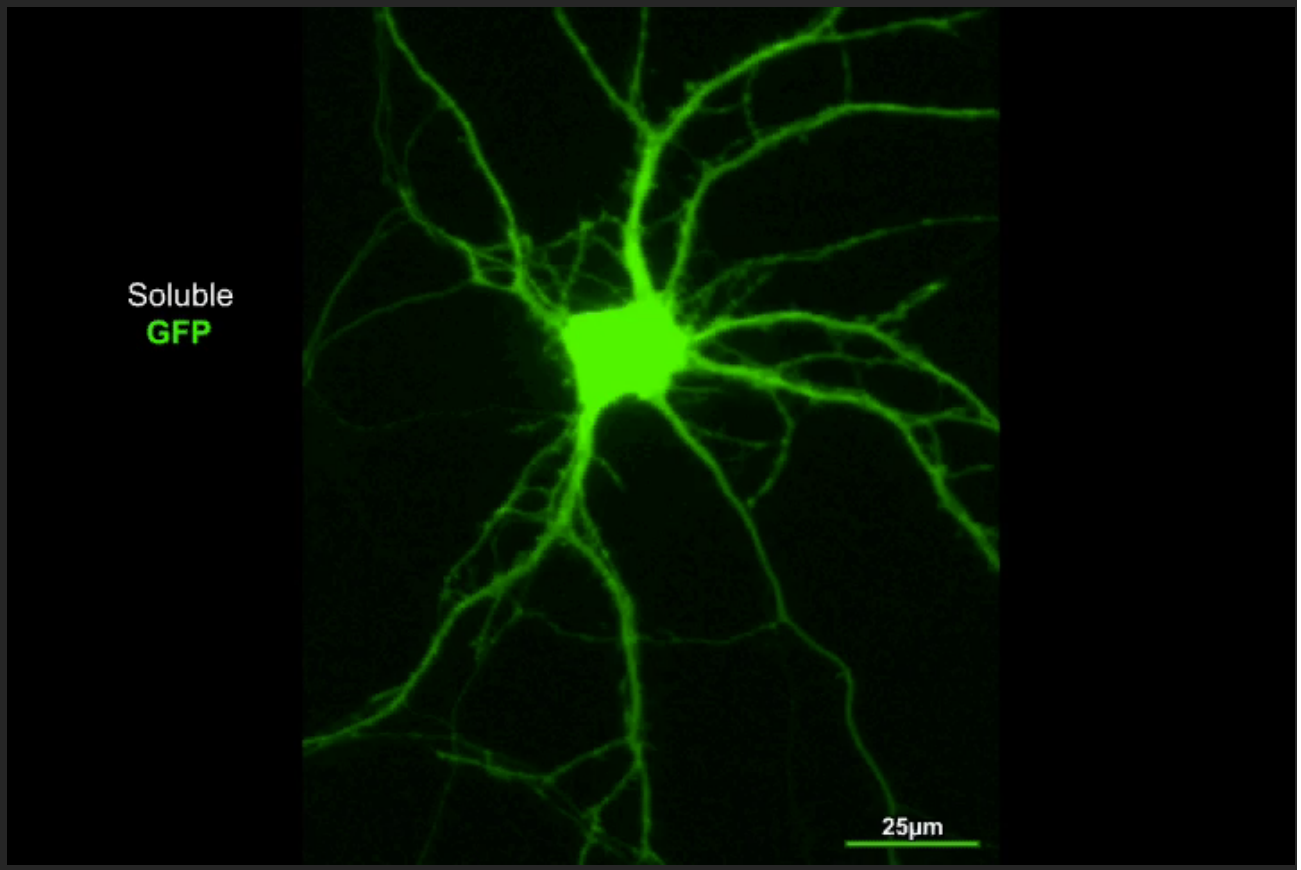
# We specialize in neuronal cell culture and imaging of KIF1A and its cargos

How does transport support neuron function and survival?




Han et al., 2010

Neuropeptide transport via KIF1A

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# Cargo Transport in R203S-KAND patient-derived induced neurons

## First Steps on the Transport Path

Troy Collins, MSc.
Silverman Lab, SFU

# Outline

- 1) Cargo: An important part of KAND?
- 2) The R203S KAND variant
- 3) Project Overview
  - Culturing iPSCs to Neurons
  - Cargo Localization to KIF1A
  - Live Cell Imaging and Cargo Transport Analysis

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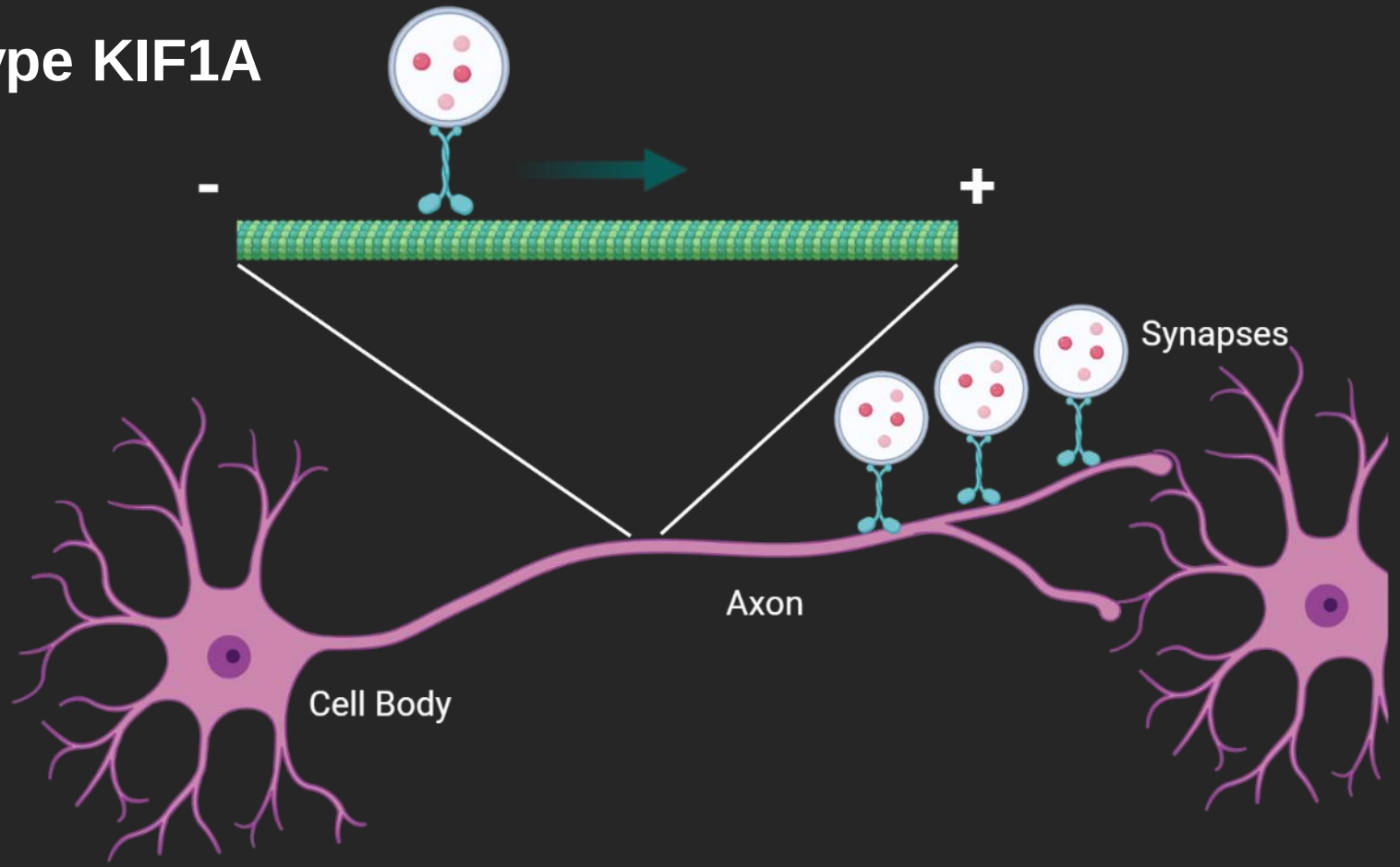
# Cargo: An important part of KAND?

**Wild Type KIF1A**

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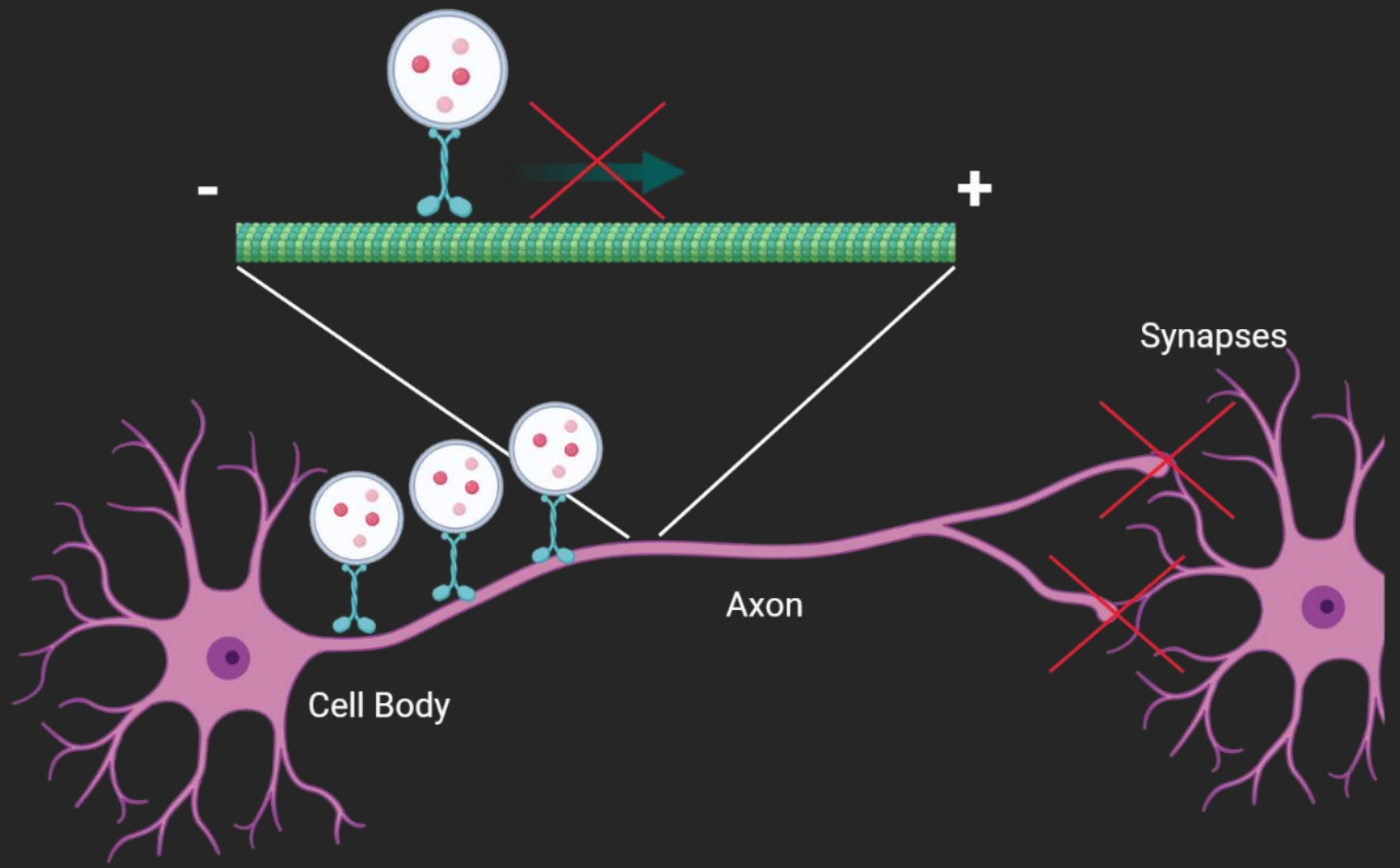
# Cargo: An important part of KAND?

**KAND-type KIF1A**




**Functional KIF1A requires:**

- **Cargo binding**
- **Microtubule binding**
- **ATP hydrolysis**
- **Motility**

**Dysfunction may lead to cargo mislocalization**

Lee et al., 2003
Hamdan et al., 2011
Ohba et al., 2015
Cheon et al., 2017

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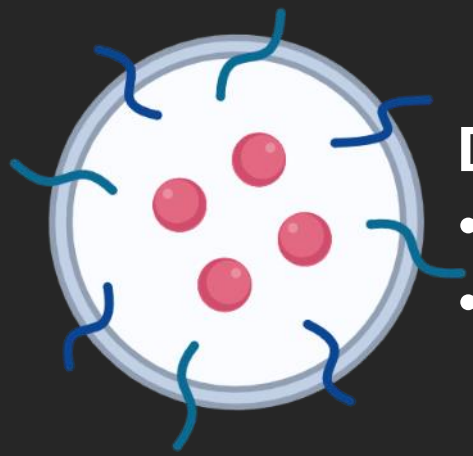
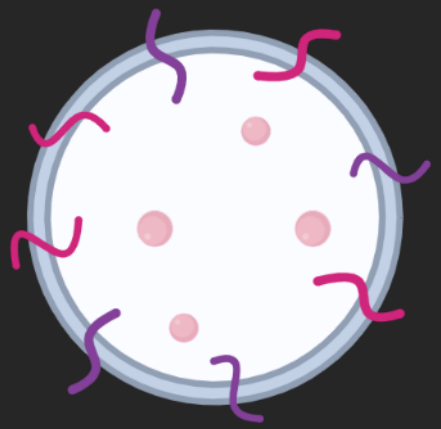
# Cargo: An important part of KAND?



**Dense Core Vesicles**

- Neuropeptides, e.g., BDNF
- VMAT2



**Synaptic Vesicle Precursors**

- Synaptophysin
- Synaptotagmin

**Cargo Mislocalization**

↓

**Cellular Dysfunction**

- Synapse formation and function
- Neuron survival

↓

**Neurodegeneration**

- Alzheimer's disease
- Huntington's
- Parkinson's

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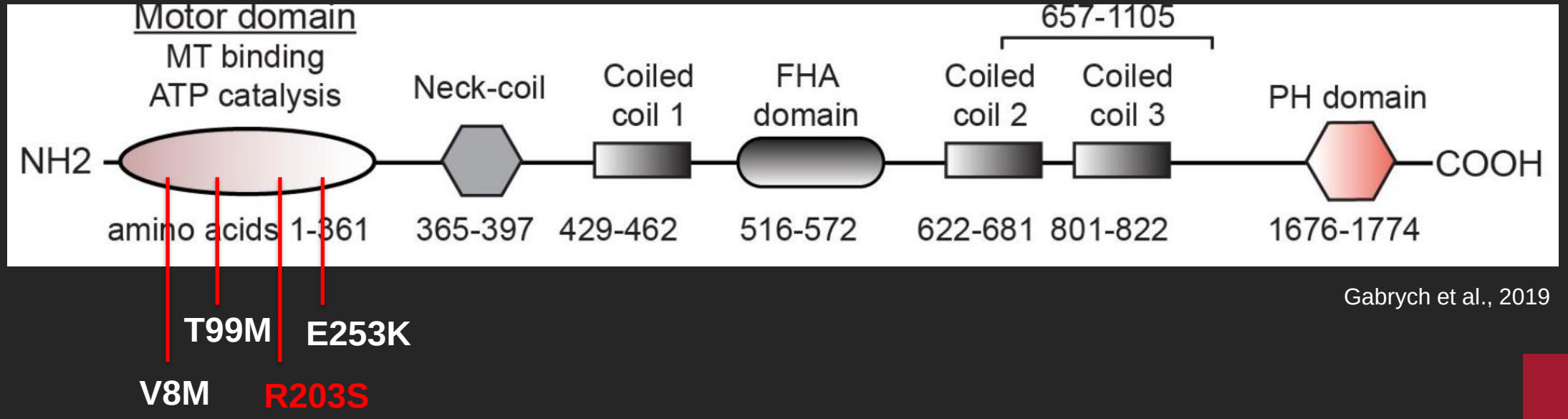
# R203S Variant

- One of many motor domain variants
- Human derived induced pluripotent stem cells (Chung Lab; Coriell)
- Patient is heterozygous and mosaic for R203S




Gabrych et al., 2019

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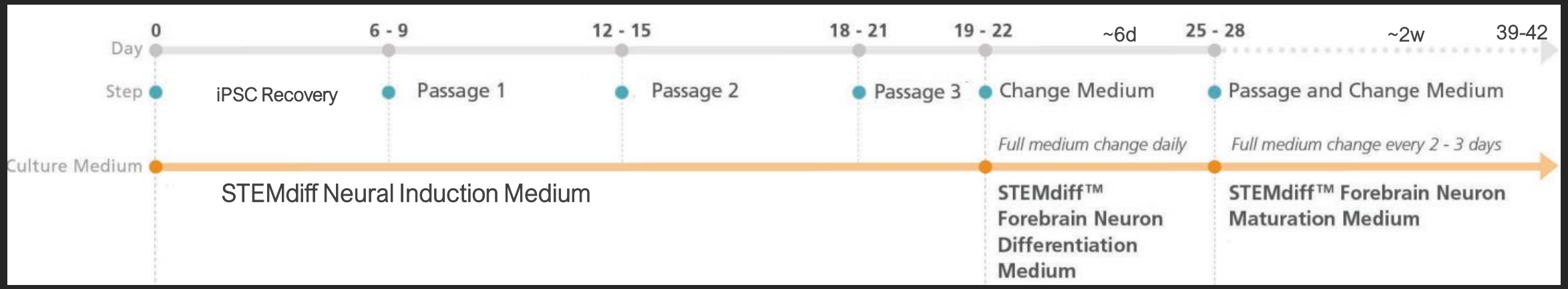
# Project Overview

1) Culture iPSCs to neurons
2) Characterize neurons via immunocytochemistry
3) Compare KIF1A and cargo localization via ICC
4) Live cell imaging and transport analysis of BDNF




STEMCELL Technologies

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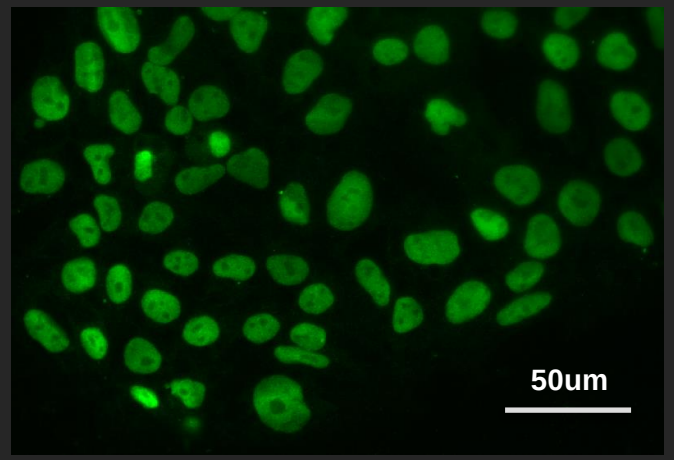
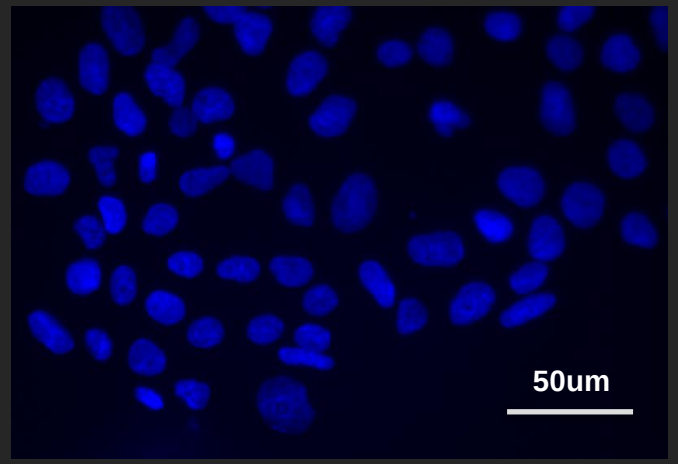
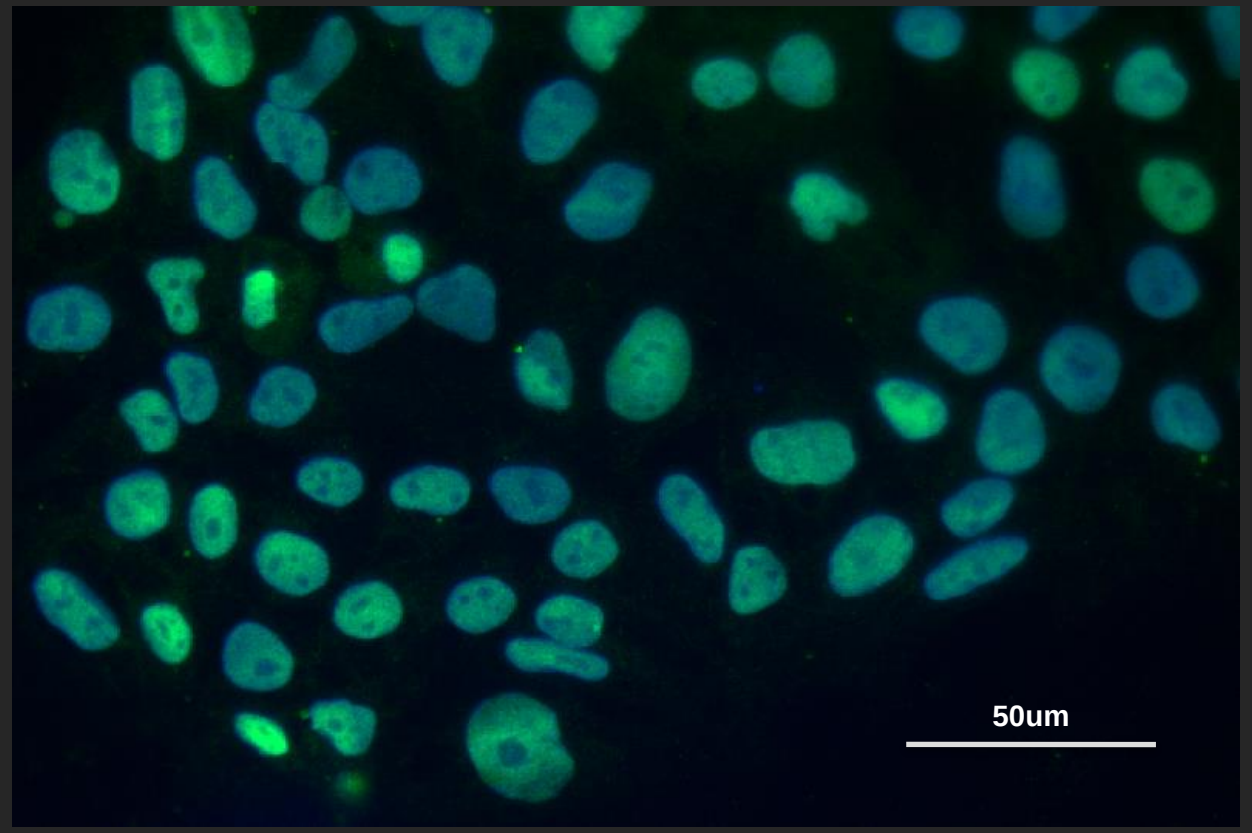
# 1) Culture iPSCs to Neurons
# Generation of iPSCs




Sox2




DAPI

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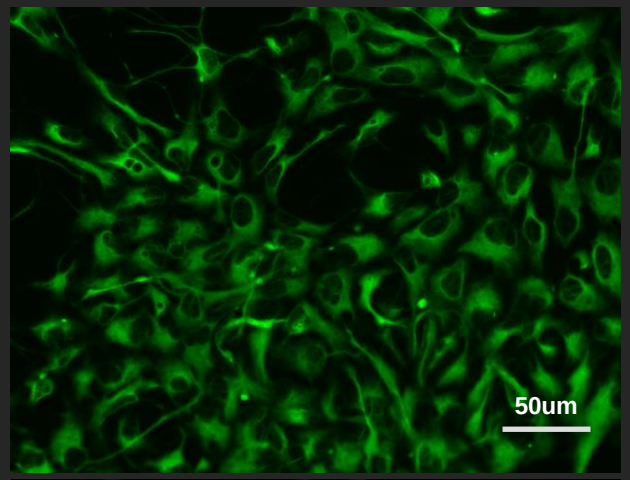
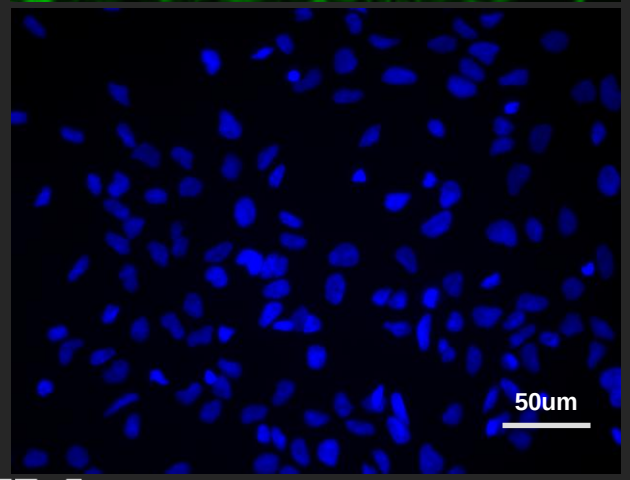
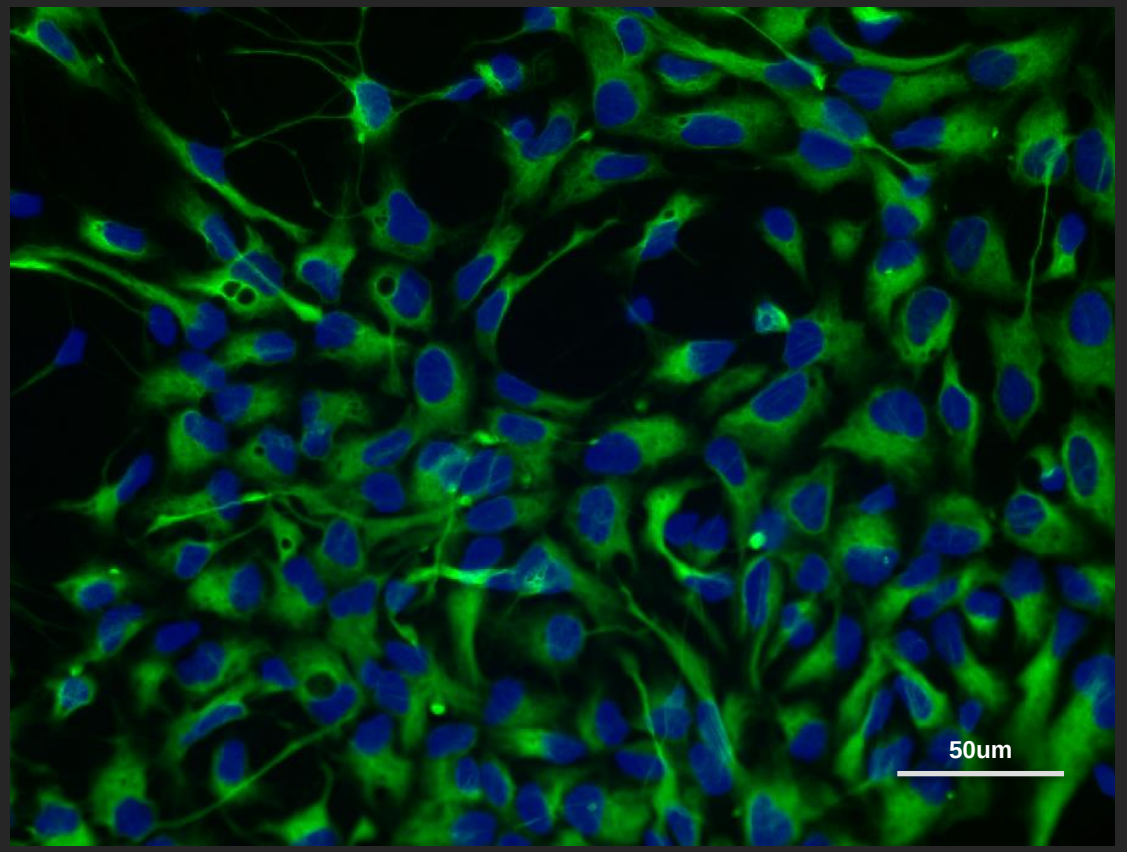
# 1) Culture iPSCs to Neurons
# Generation of Neural Precursors (NPCs)




Nestin




DAPI

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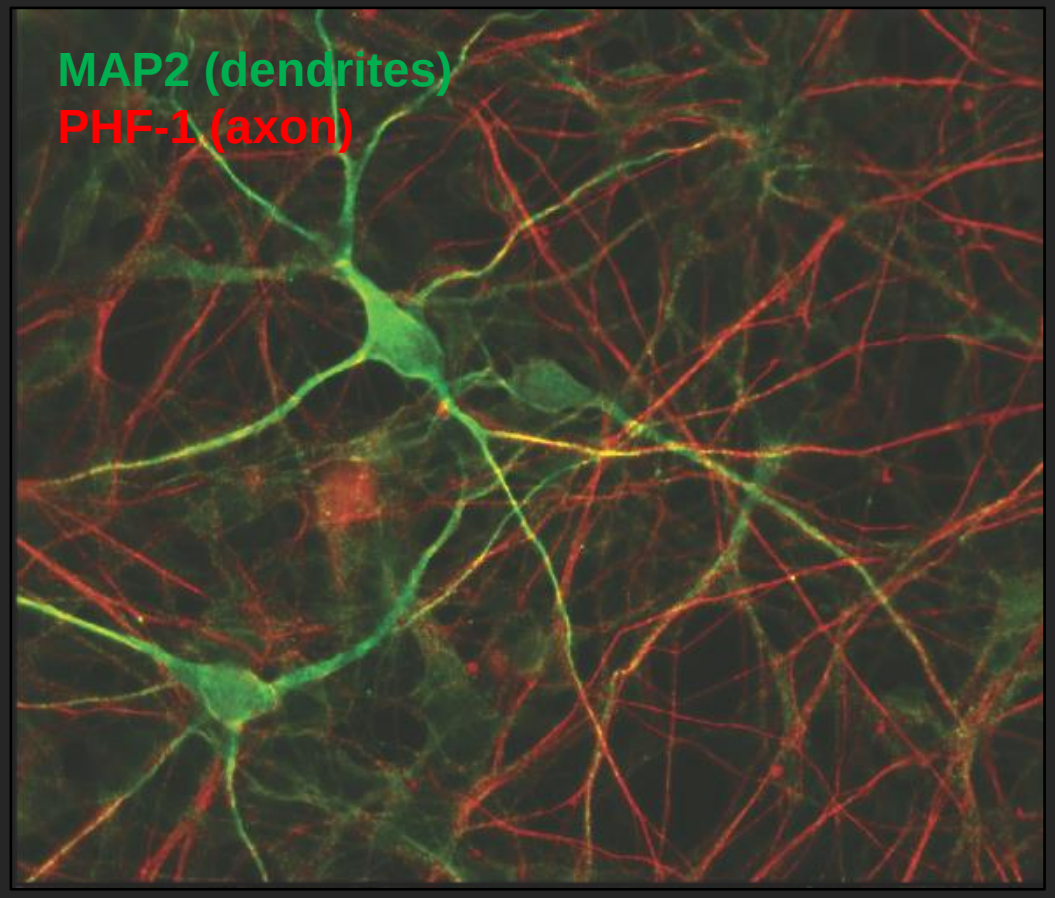
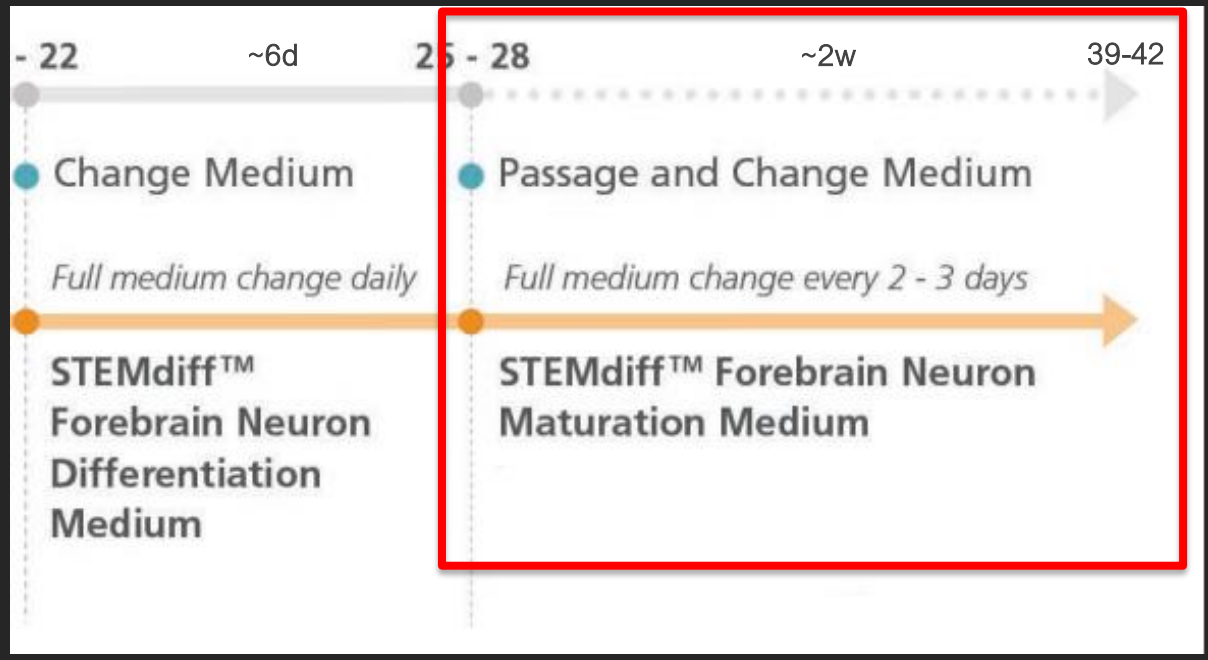
# 1) Culture iPSCs to Neurons
# NPCs to Neurons – Next Step




Human neuron Silverman lab

**Mature neurons to ~30d**

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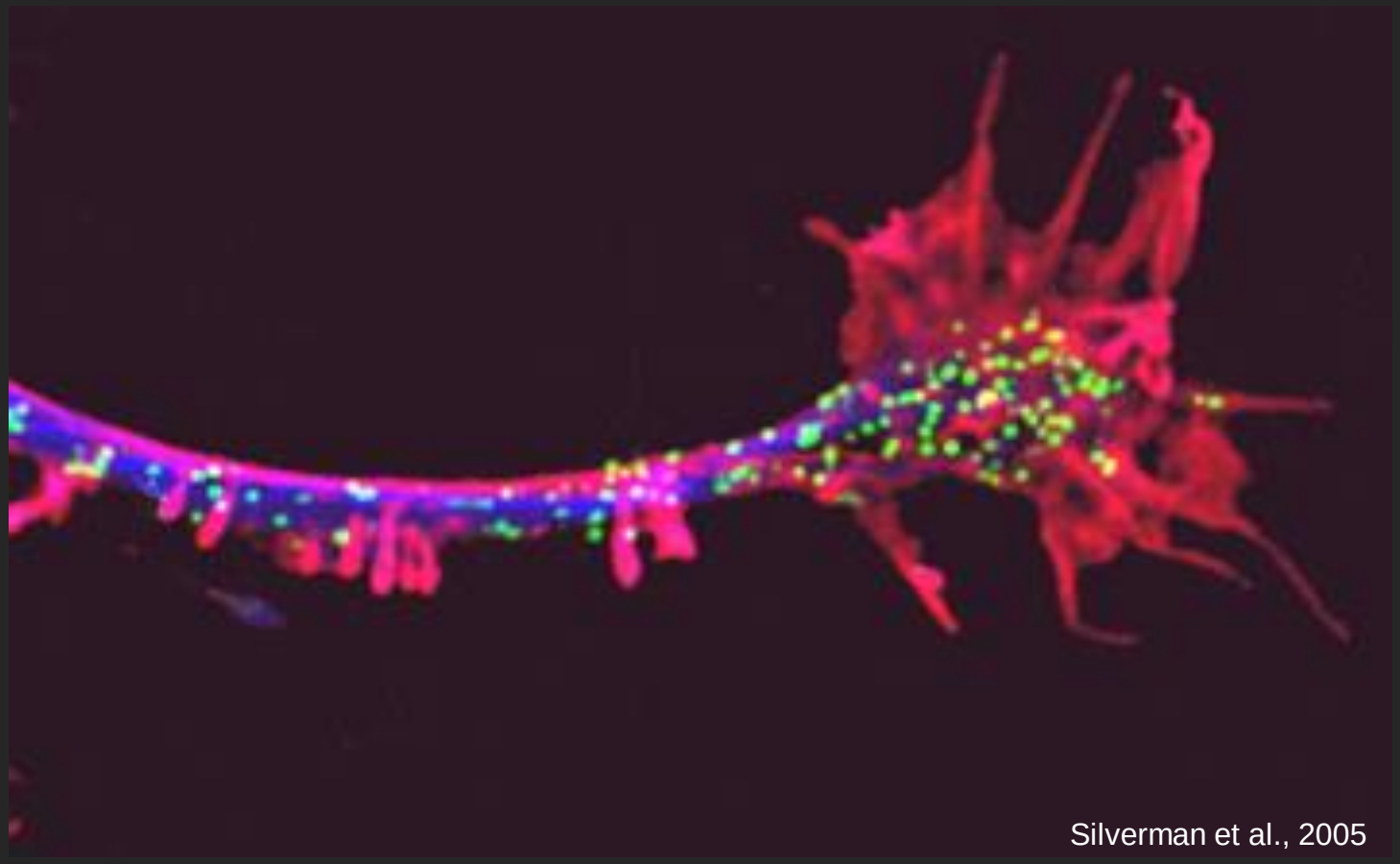
# Aim 2: Cargo Localization to KIF1A

- **Our lab has extensive background localizing cargo with ICC.**
- **R203S cargo will be proximal to cell body.**



Silverman et al., 2005

Hippocampal neuron growth cone. Actin, microtubules, Dense core vesicles

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# Aim 3: Live Cell Imaging and Transport Analysis

- **Transport is a dynamic process more fully captured by video**
  - **Directionality**
  - **Velocity**
  - **Run Length**

**Cell Body**


**Synapse**

Silverman M.

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# Future Directions

**Stay tuned for next year! Culturing neurons takes time, experiments will begin in ~1 month after differentiation is complete.**

**A comprehensive examination of cargo transport will elucidate the direct effects of KIF1A dysfunction on neuron health.**

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# Acknowledgements


**Thank you to**
**KAND patients and their families**

**KIF1A.org**

**The KIF1A Research Community and members of the Silverman lab**


**Team Nerve-ana 2021**