

Identifying New Regulators of Neuronal Amyloid-Beta Production in Alzheimer's Disease

Amara Martin

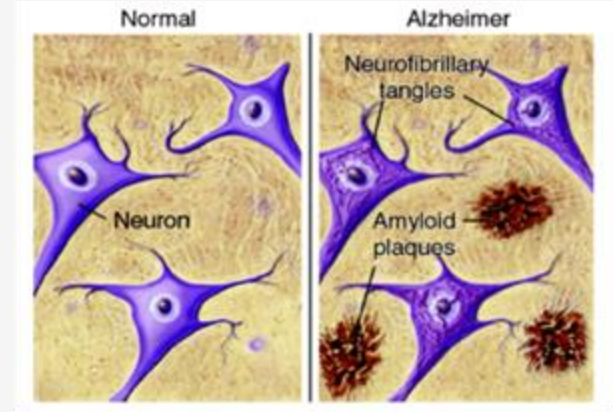
Kamehameha Schools Kapalama

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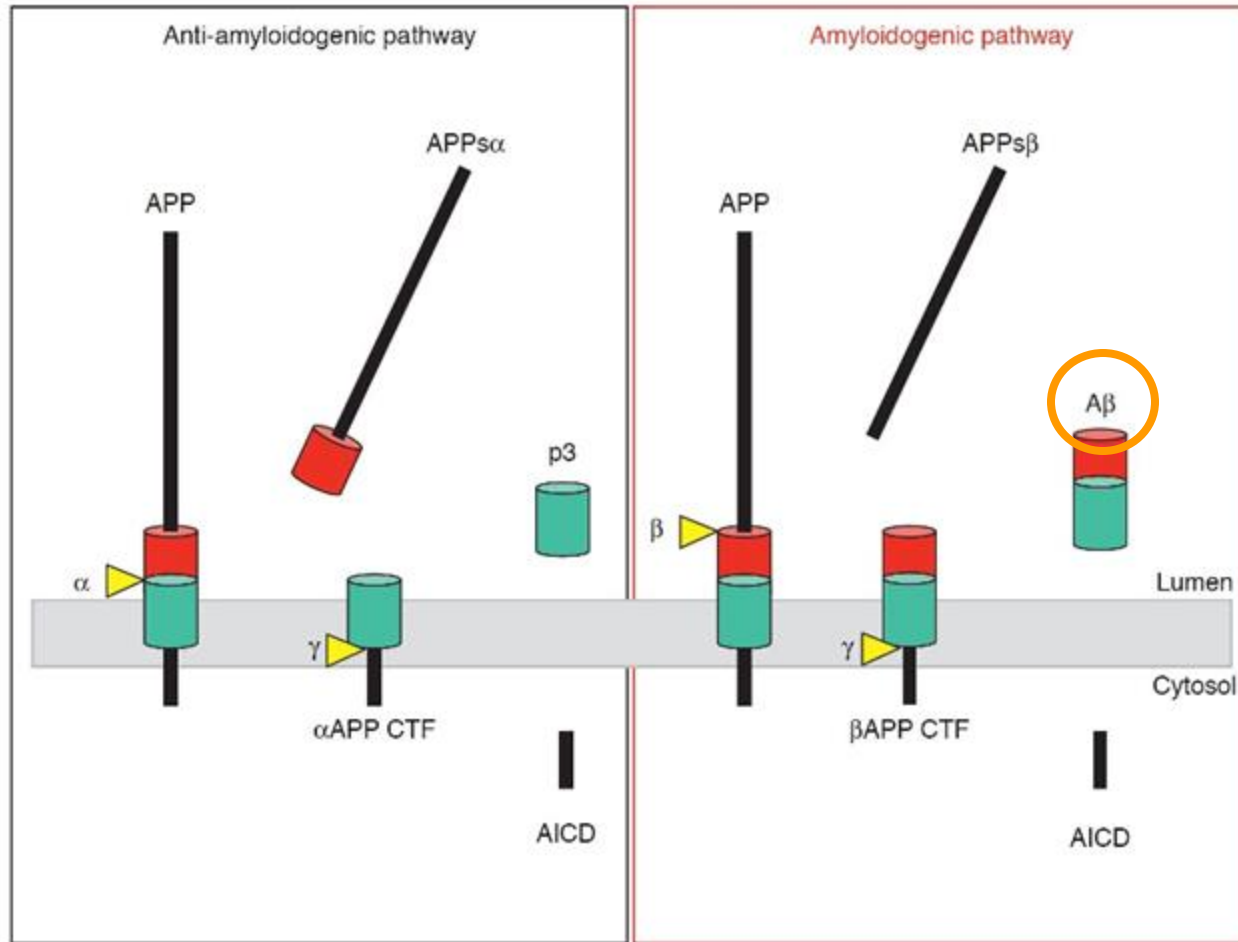
Coordinating Center: University
of Hawai'i at Mānoa School of
Medicine

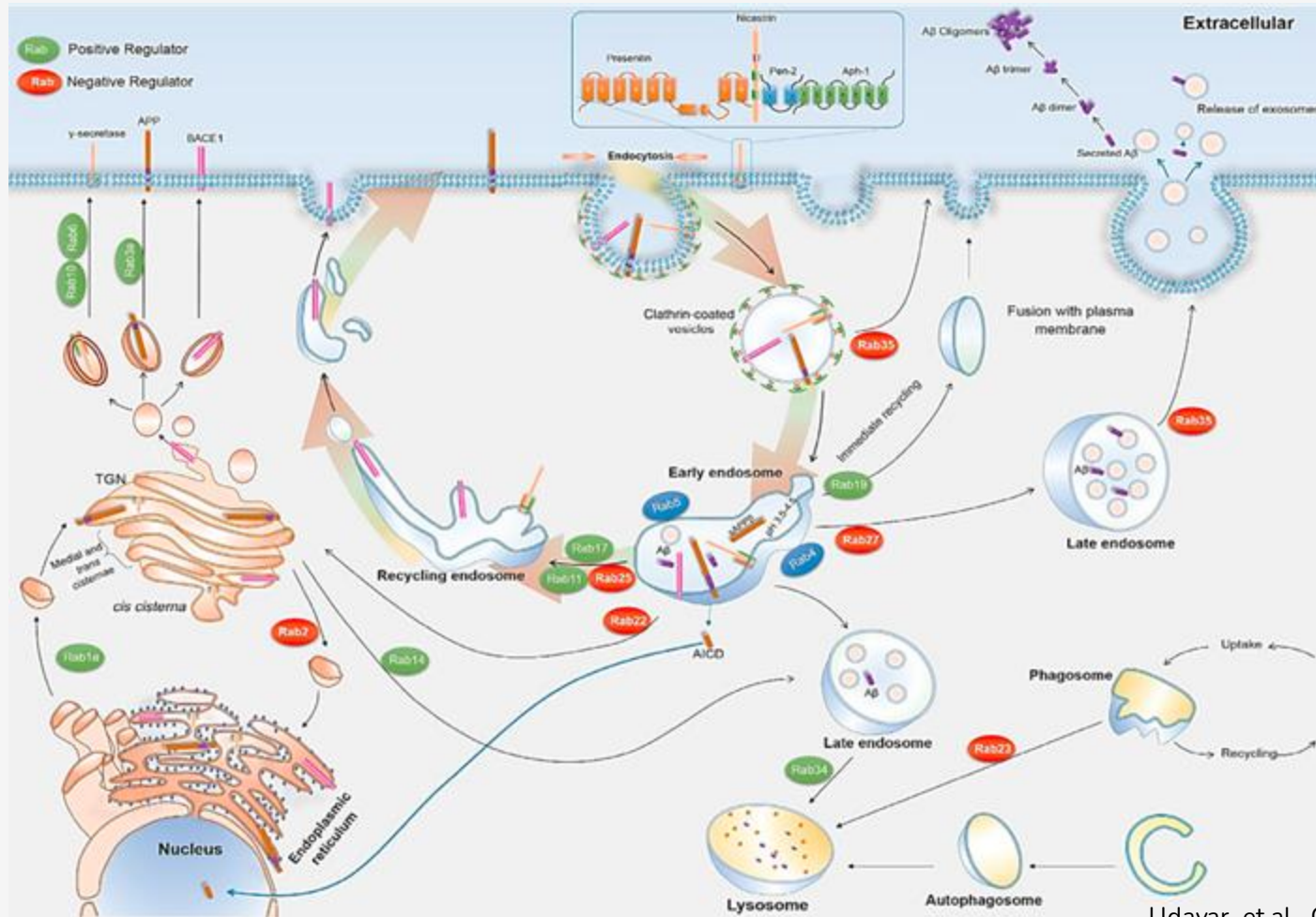


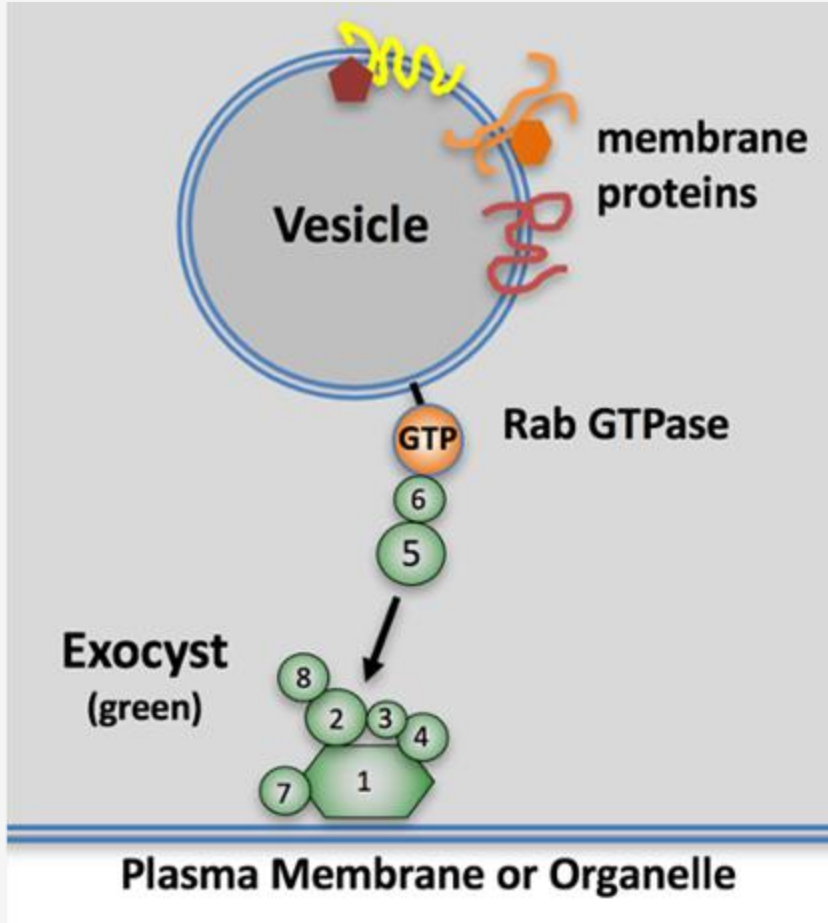
National Institute of Aging



Silbert, LC, Neurology, 2007.







Exocyst: 8-protein trafficking complex for polarized exocytosis of specialized cargo

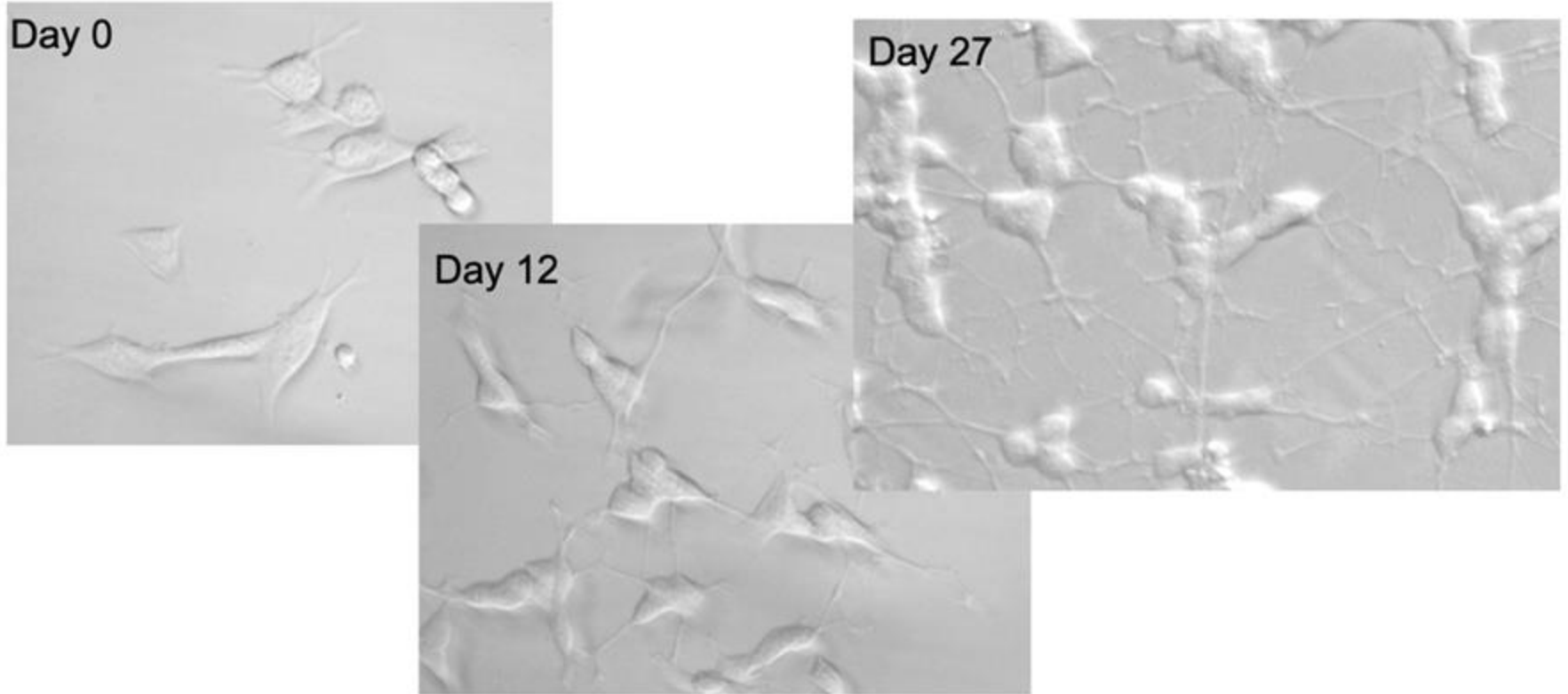
- Expressed in neurons, participating in vesicle delivery to the growth cone of developing neurons

Hypothesis/Question:

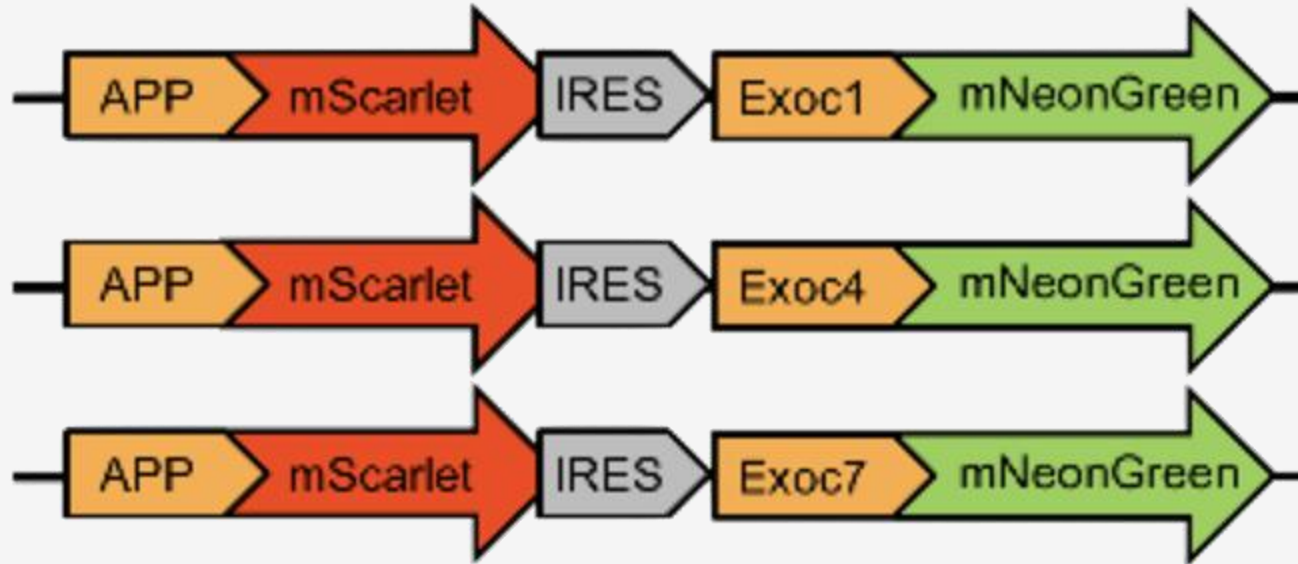
Does the exocyst have any role in A β production?

If so, HOW does the exocyst have a role in A β production?

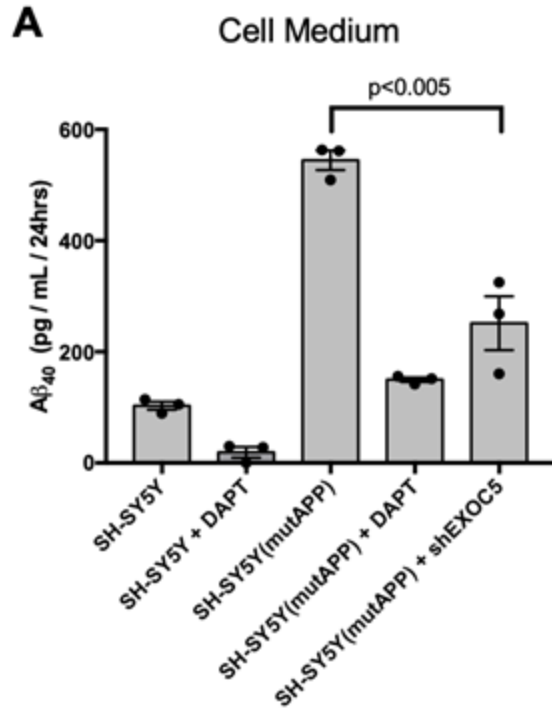
SH-SY5Y human neuroblastoma cells were differentiated into neurons with retinoic acid and other factors



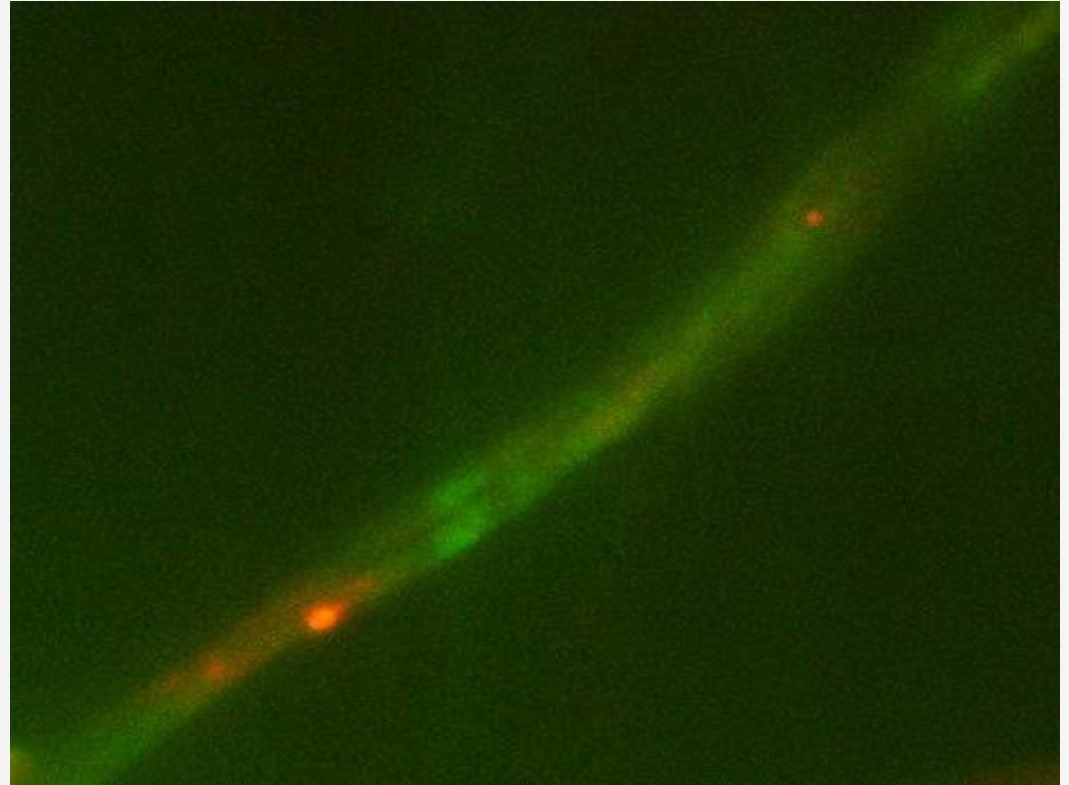
3 Cell Lines were made.



explain cell lines. how do i explain the exoc???????



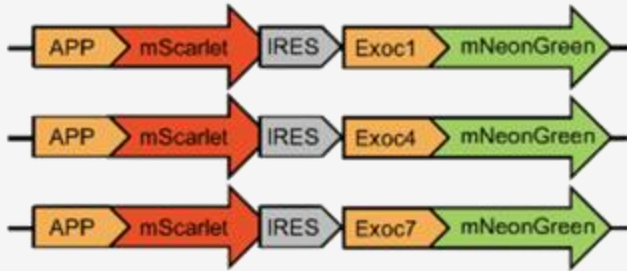
SH-SY5Y(mutAPP) cells secrete high levels of Aβ, which is decreased with shExoc5 knockdown



The **APP** and **Exocyst** are shown moving together in the SH-SY5Y cells

Plasmids were used to create 7 new cell lines

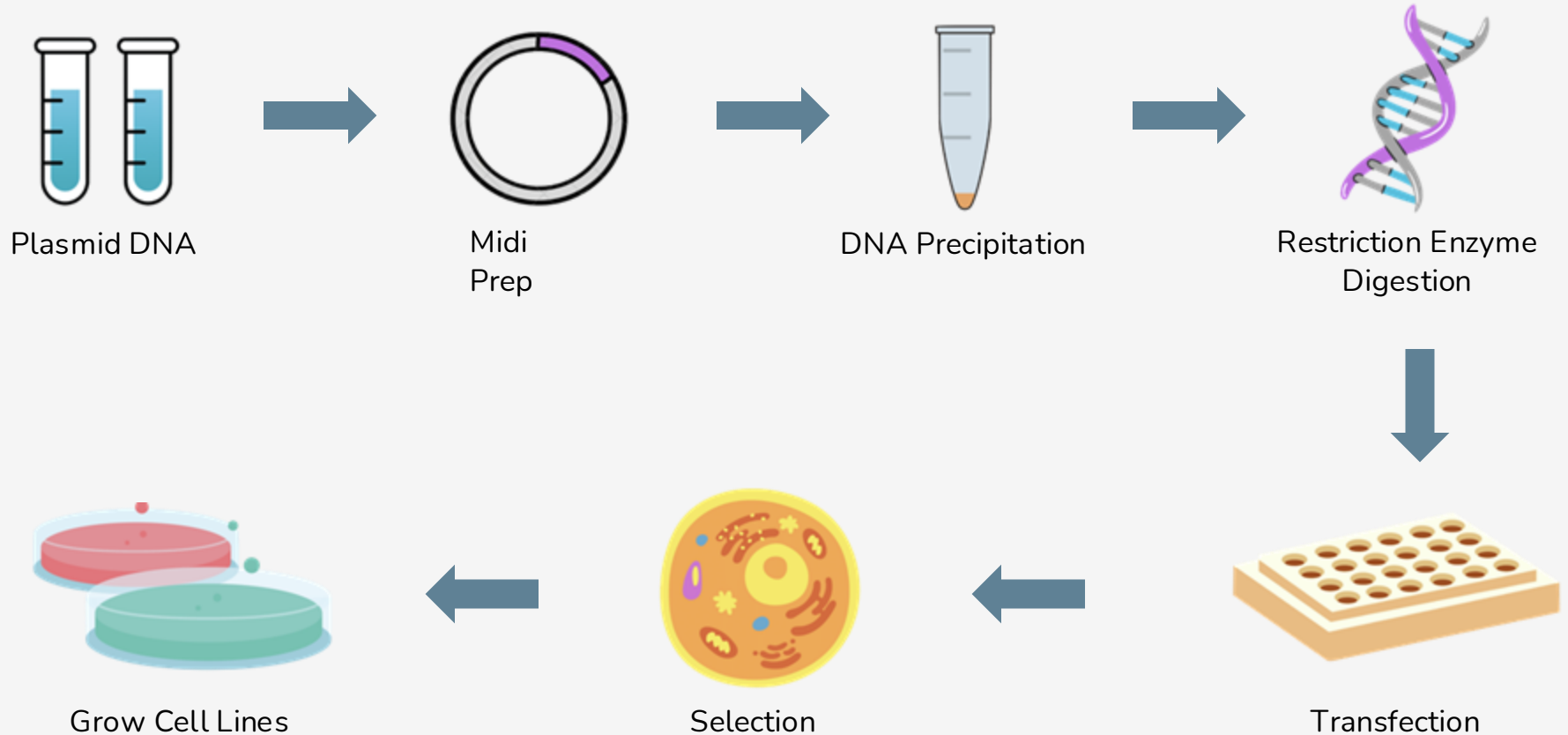
Original Three Cell Lines



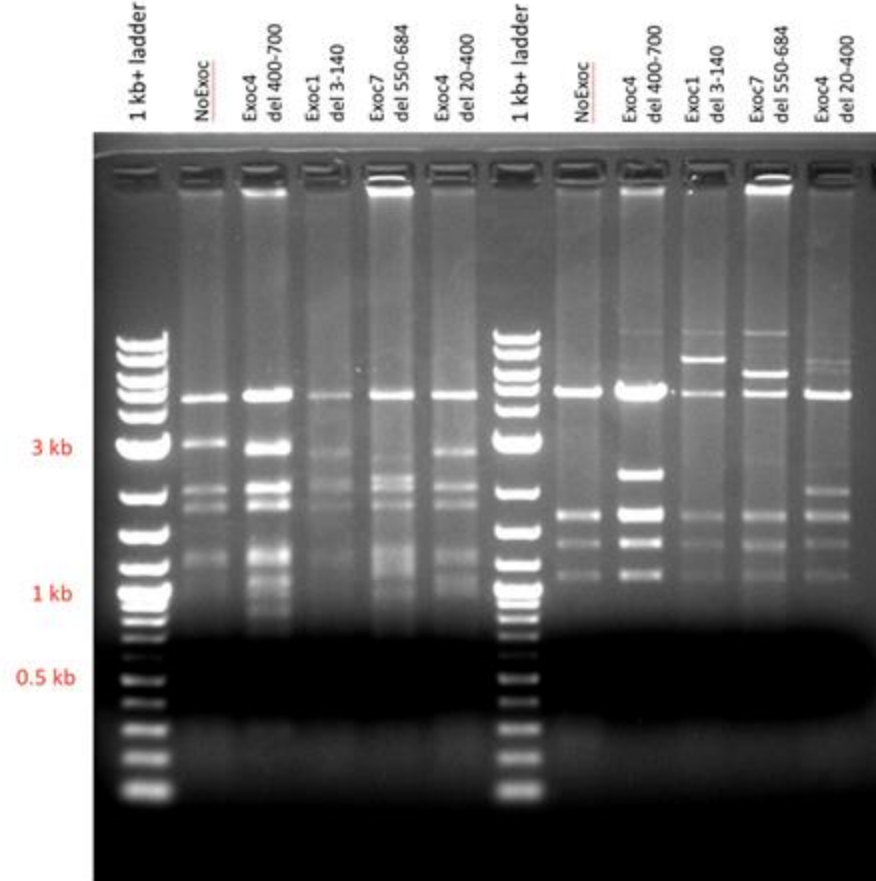
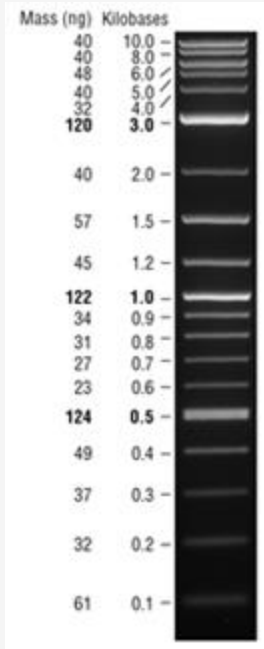
Plasmids Used to Create Several New Cell Lines

hypG3-CAG-APP-mScarlet-Exoc4 Δ 20-400-neonGreen
hypG3-CAG-APP-mScarlet-Exoc4 Δ 700-974-neonGreen
hypG3-CAG-APP-mScarlet-Exoc4 Δ 400-700-neonGreen
hypG3-CAG-APP-mScarlet-Exoc1 Δ 3-140-neonGreen
hypG3-CAG-APP-mScarlet-Exoc7 Δ 550-684-neonGreen
hypG3-CAG-APP-mScarlet-Exoc7 Δ 2-384-neonGreen
hypG3-CAG-APP-mScarlet-NoExoc-neonGreen

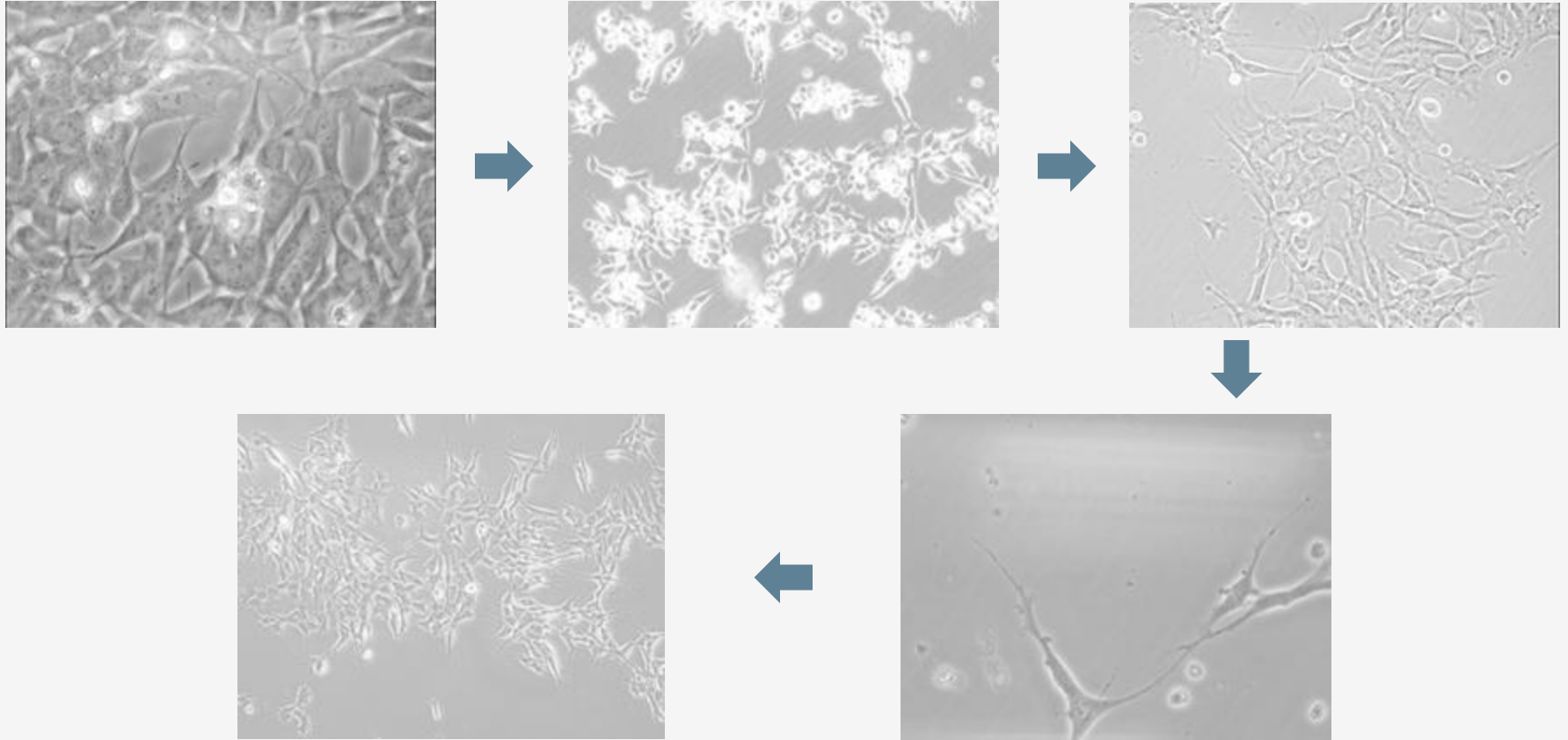
Creation of 7 New Cell Lines



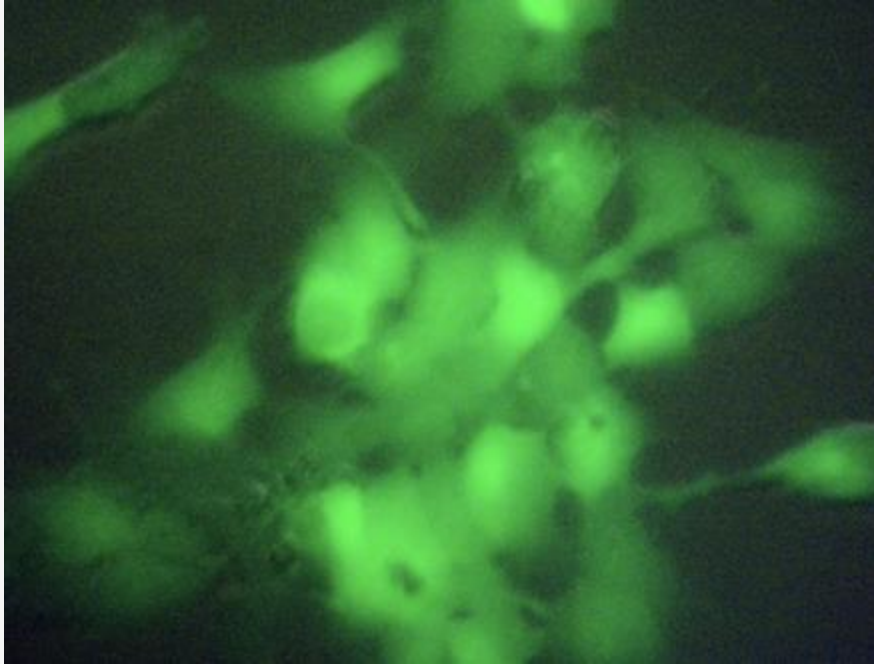
Restriction Enzyme Digest



Selection of SH-SY5Y cells transfected with the Exoc1 $\Delta 3-140$ plasmid

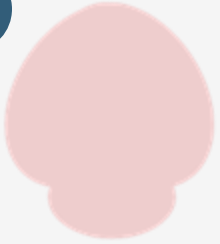


Confirmation of Transfection in SH-SY5Y No Exoc Cells



Primary Mouse Hippocampal Neuron Cell

1



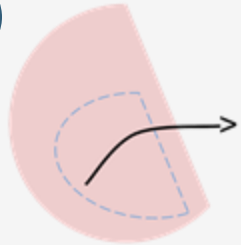
Day 0-3 old mouse brain

2



Remove cerebellum and split cerebrum

3

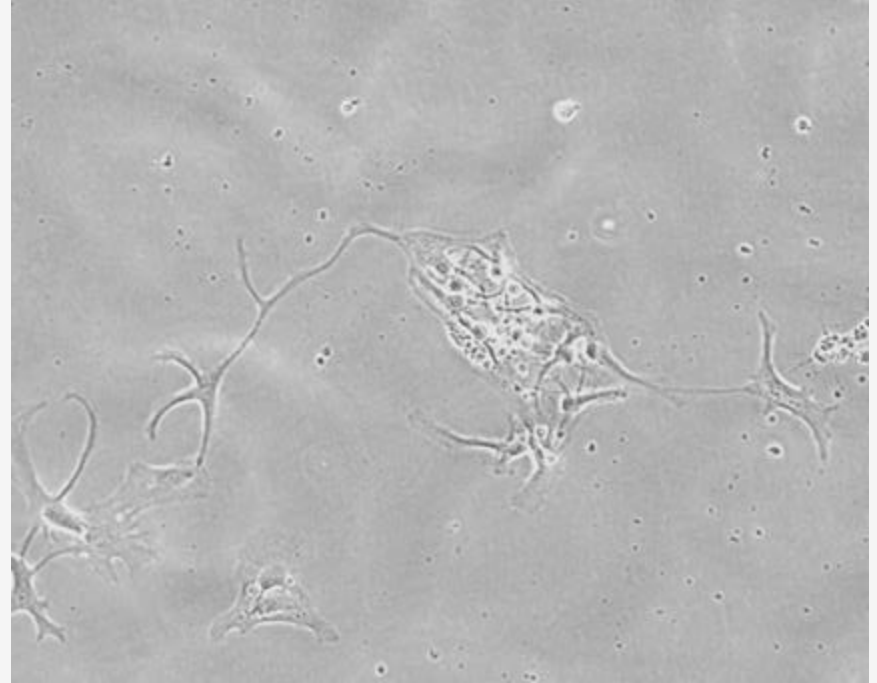


Flip the hippocampus over

4

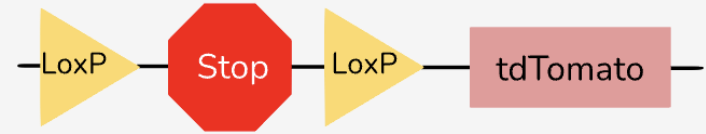


Cut out hippocampus to culture



Primary Hippocampal Neuron Cells Treated with Cre-Recombinase

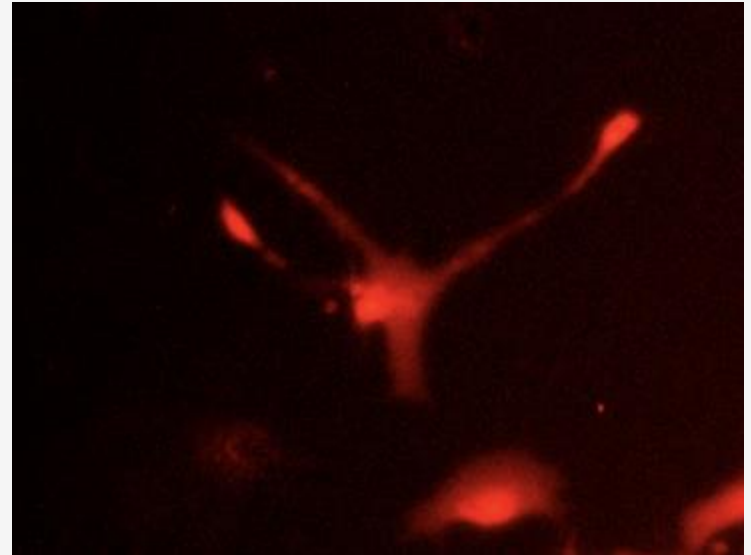
Transgenic Mice:



Brightfield



Red Fluorescence



Conclusions

- ❑ The exocyst does have a role in A β production, with strong evidence that the exocyst is involved in intracellular trafficking of APP in neurons
- ❑ We successfully created several new cell lines for particle tracking in neurons to enable more rigorous experiments and narrow down which protein domain is important for coordinated movement between APP and the exocyst.
- ❑ We successfully isolated primary hippocampal neuron cells and used Cre-Recombinase to downregulate the Exoc5
- ❑ A greater understanding of exocyst function in the intracellular trafficking of APP and regulation of A β in neurons may reveal potential Alzheimer's therapeutics



National Institute of Aging

Acknowledgements



Fogelgren Lab

Ben Fogelgren

Chantell Balaan

Hanna Kumasaka

Swasthita Sadagopan

Kelsey Zane

NIDDK/NIH

The STEP-UP High School program is supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the national Institutes of Health, Grant Number: R25DK78386-17



Owens Lab