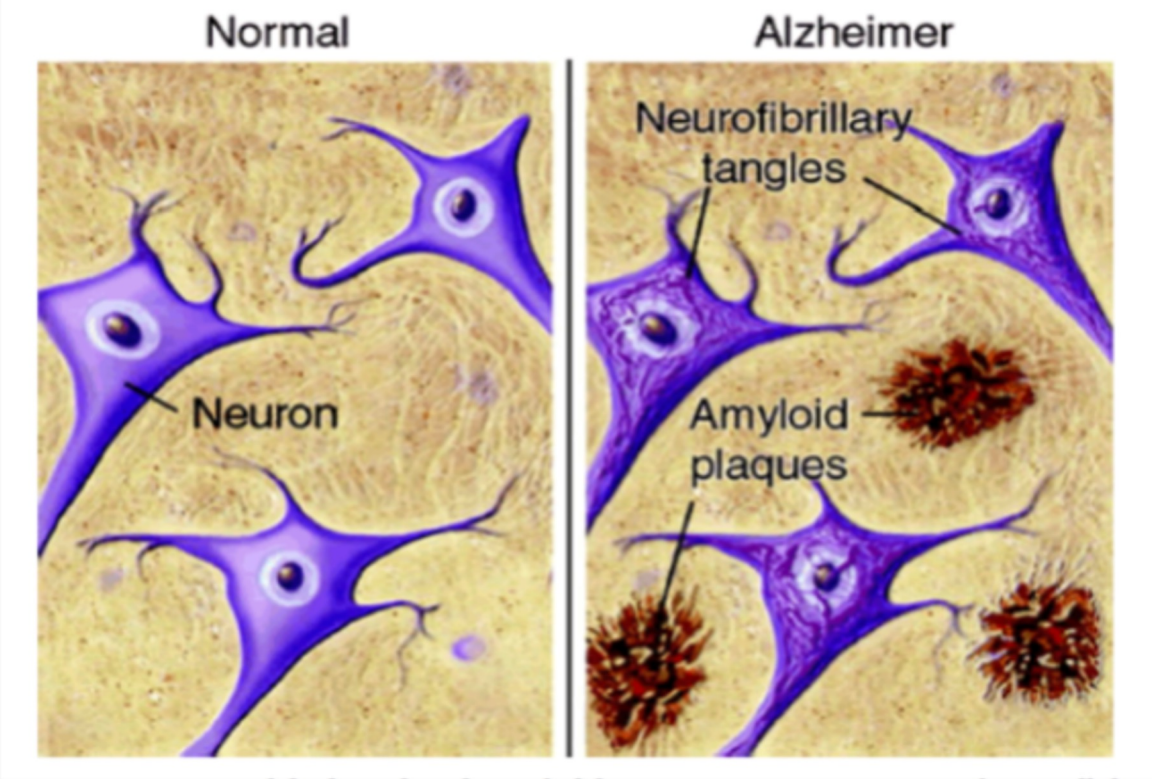
Investigating the Exocyst as an Insulin-Sensitive Regulator of Amyloid-beta Synthesis in Neurons

Amara Martin
Mentor: Ben Fogelgren, PhD
Coordinating Center: University of Hawai‘i at Mānoa

Aparna, M., et al., International Journal of Artificial Intelligence, 2023

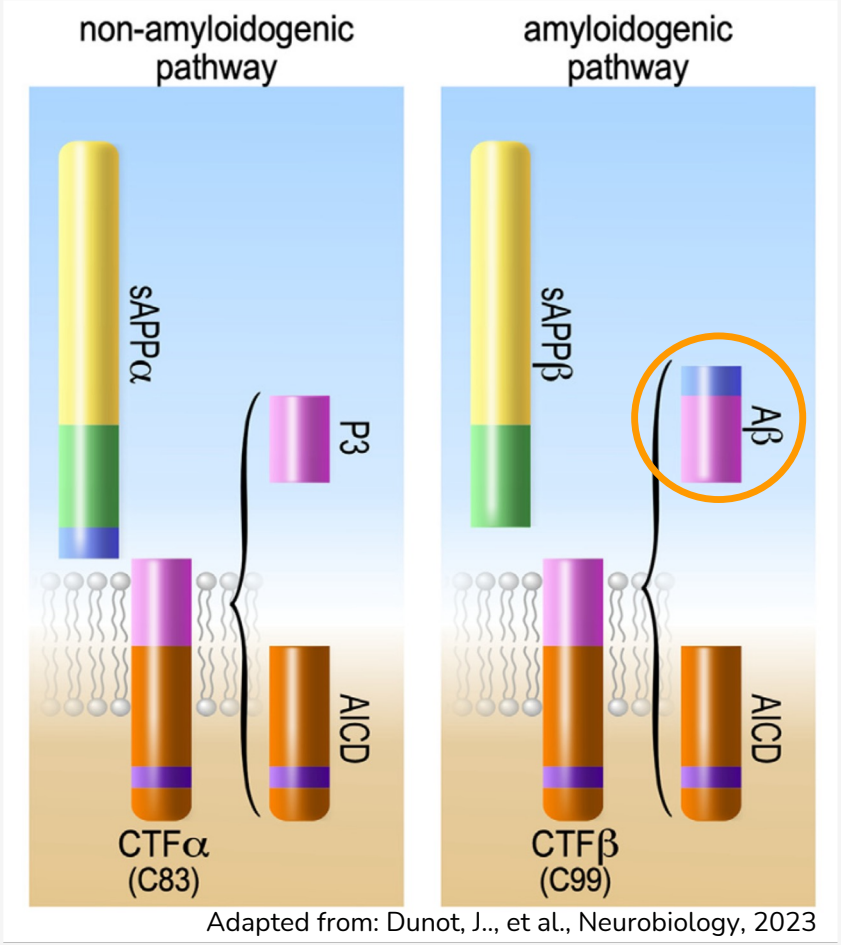
Pathological hallmarks of Alzheimer's disease



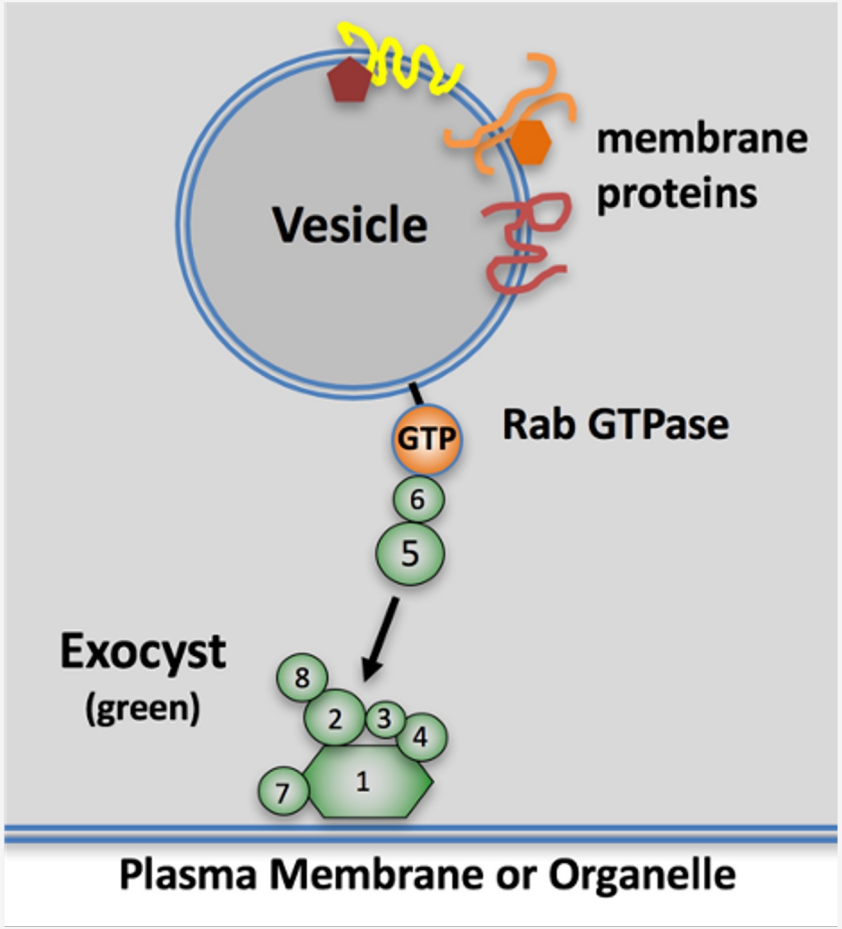
Silbert, LC, Neurology, 2007

Amyloid-beta is derived from proteolytic processing of amyloid precursor protein (APP) in neurons

The processing of APP in neurons is largely determined by regulating its intracellular trafficking



Adapted from: Dunot, J.., et al., Neurobiology, 2023



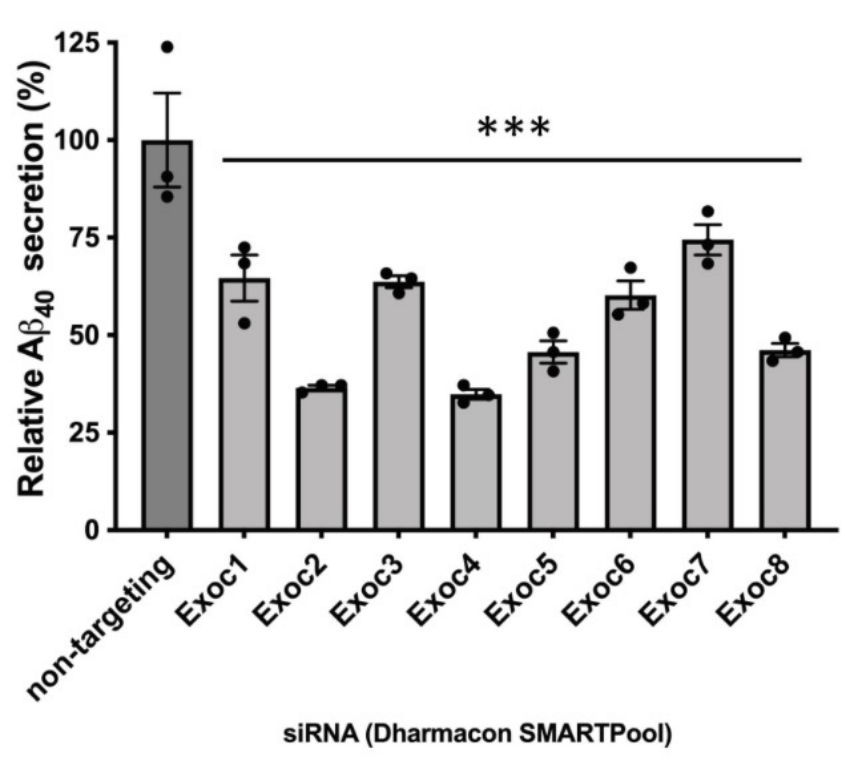
Exocyst: 8-protein complex facilitating targeted exocytosis and endocytosis of specialized cargo

- Highly conserved from yeast to humans, the exocyst binds to specific Rab GTPases on transport vesicles
- Highly expressed in neurons, although exocyst activities are still largely unknown.
- In adipocytes and muscle cells, insulin rapidly induces exocyst assembly to deliver more glucose transporters to the plasma membrane

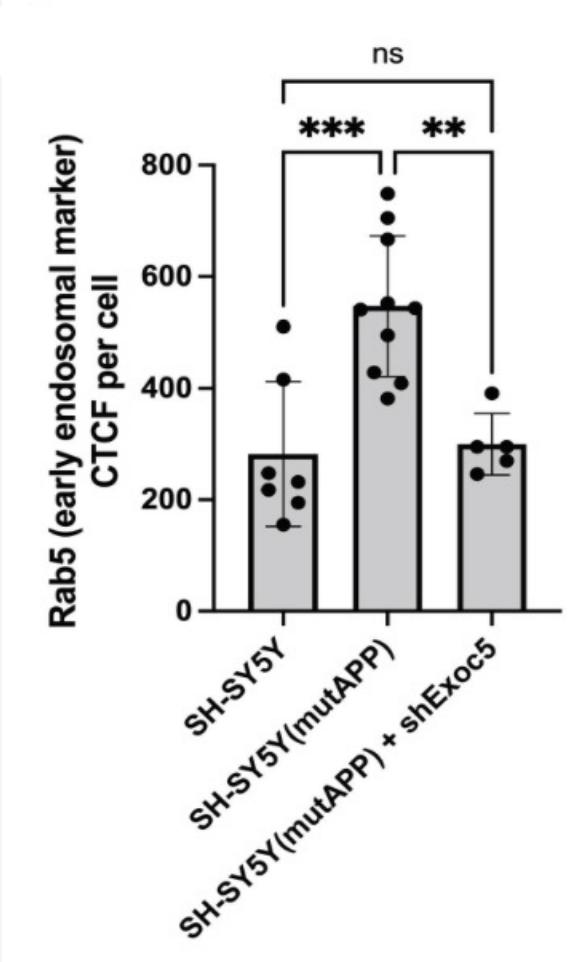
1st Question:

Does the exocyst have any role in regulating APP processing and Aβ production in neurons?

Knockdown of individual exocyst genes using siRNAs results in decreased Aβ secretion



Overexpression of mutant APP shows endosomal swelling, but knockdown of Exoc5 restores early endosome sizes to normal levels

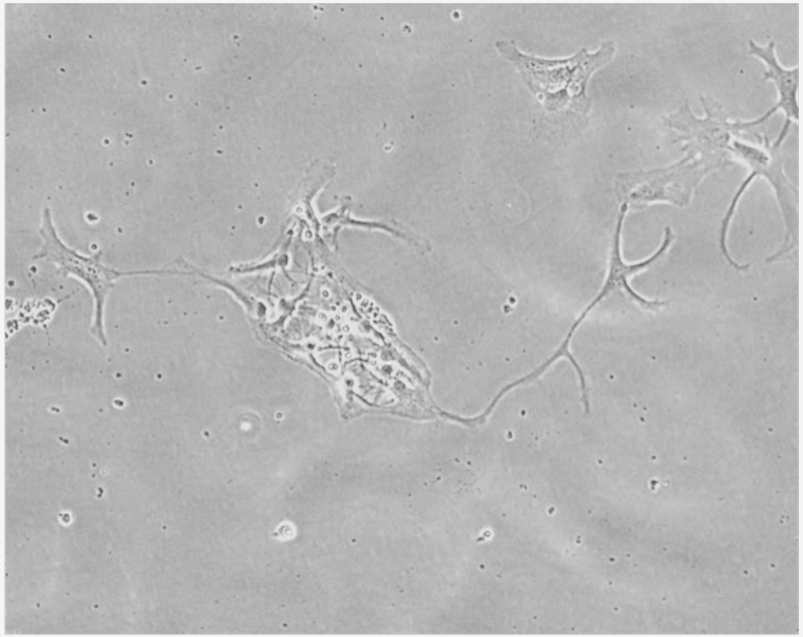


Current Questions:

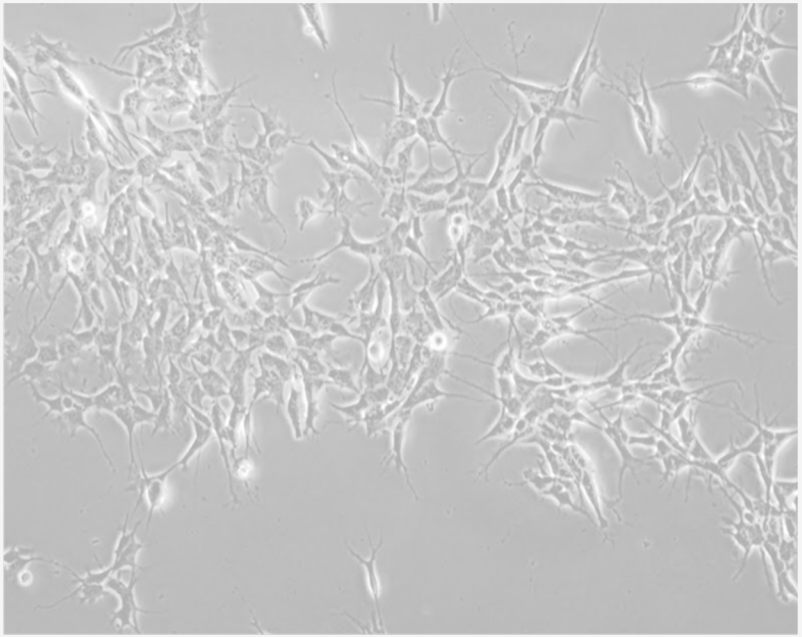
How does the exocyst regulate APP processing and Aβ production in neurons?

Is there a mechanistic connection between insulin signaling, the exocyst, and APP processing in neurons?

Two cell lines were used in all experiments



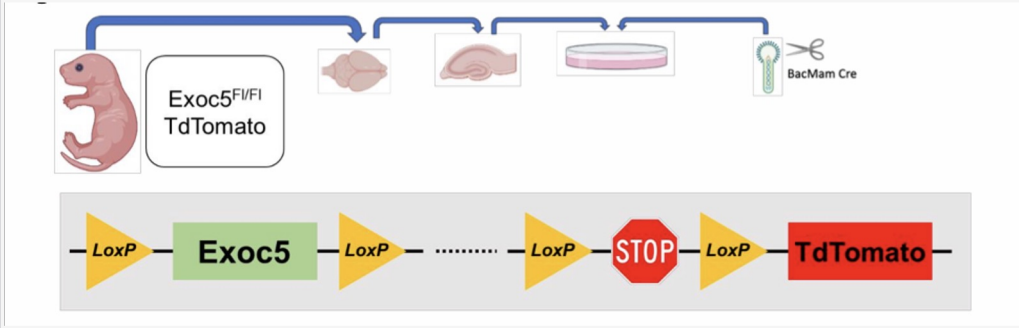
Mouse primary hippocampal neurons



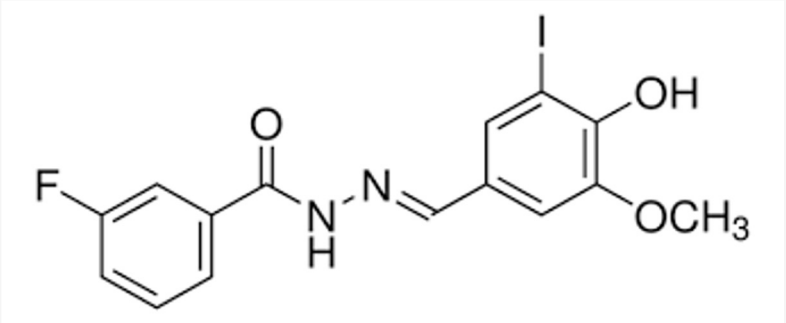
SH-SY5Y human neuroblastoma cell line
(differentiated into neurons with retinoic acid)

Two methods for loss-of-function of exocyst in these cell models

Genetic Knockout: floxed-Exoc5 mice + Cre

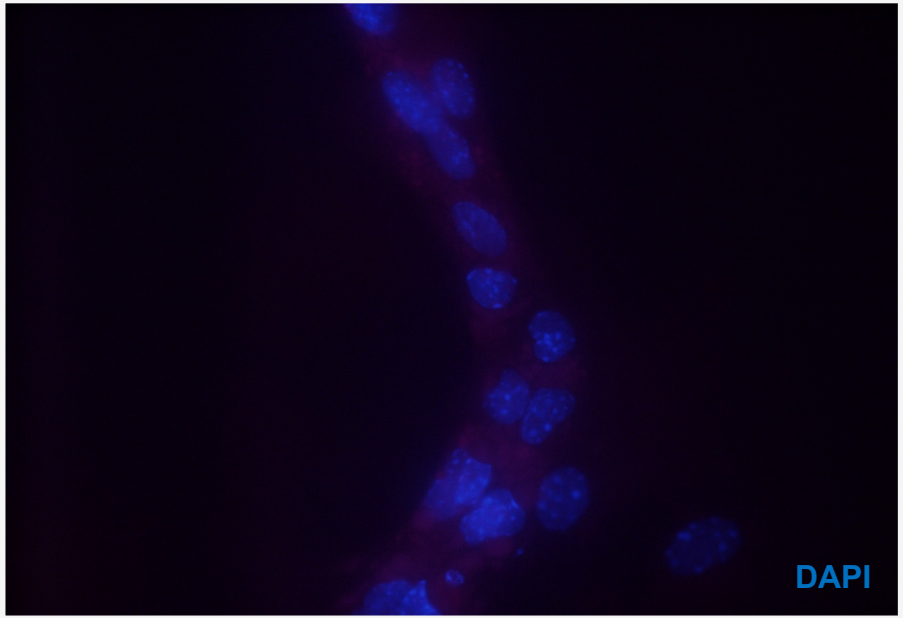


Chemical Inhibitor: Endosidin 2 (ES2)

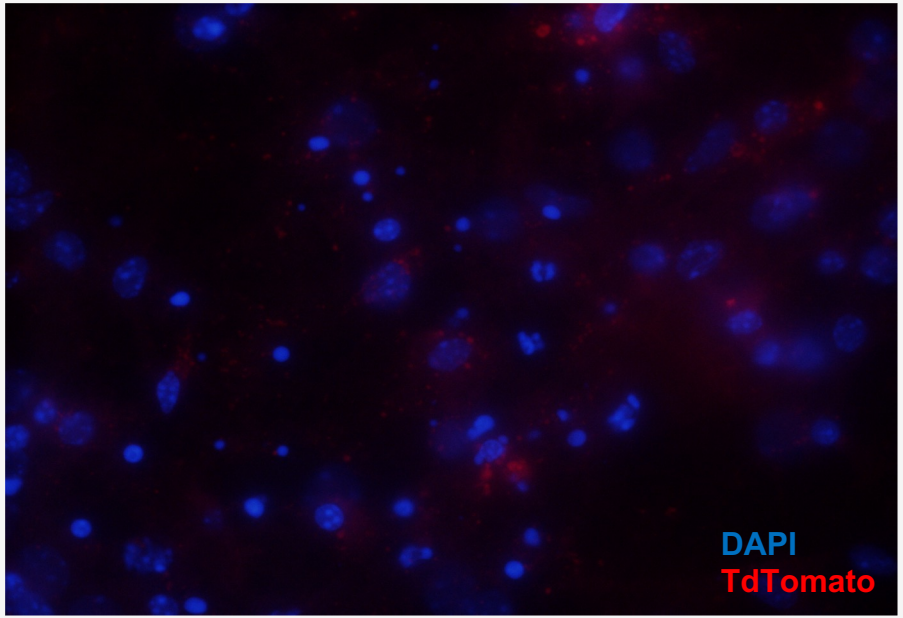


Knockdown of the exocyst via cre-recombinase

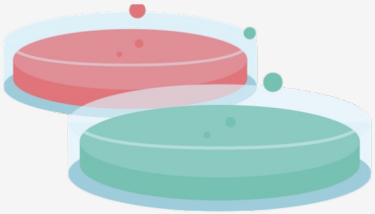
- Cre



+ Cre



Proximity Ligation Assay (PLA)



Seed Cells

Fix Cells

Block Cells

Treat with 1st Antibody

Treat with 2nd Antibody

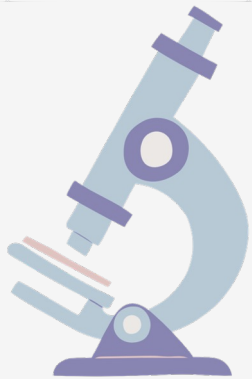
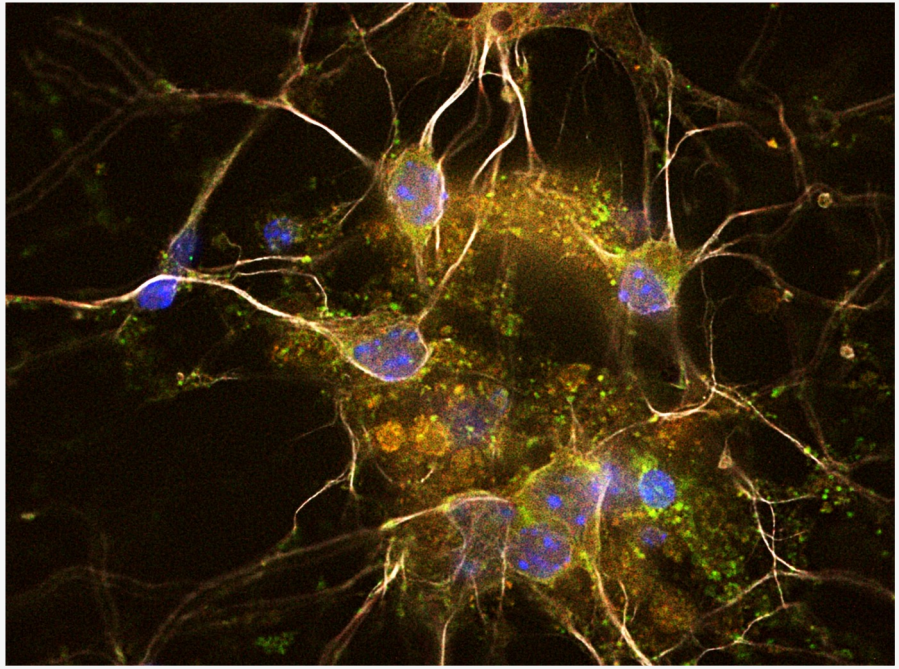
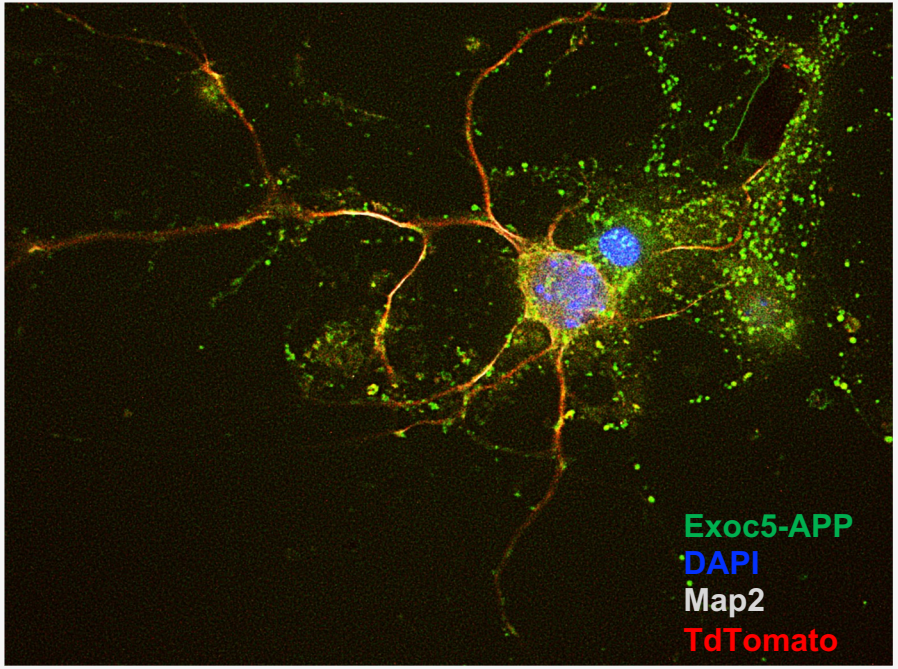


Image Cells

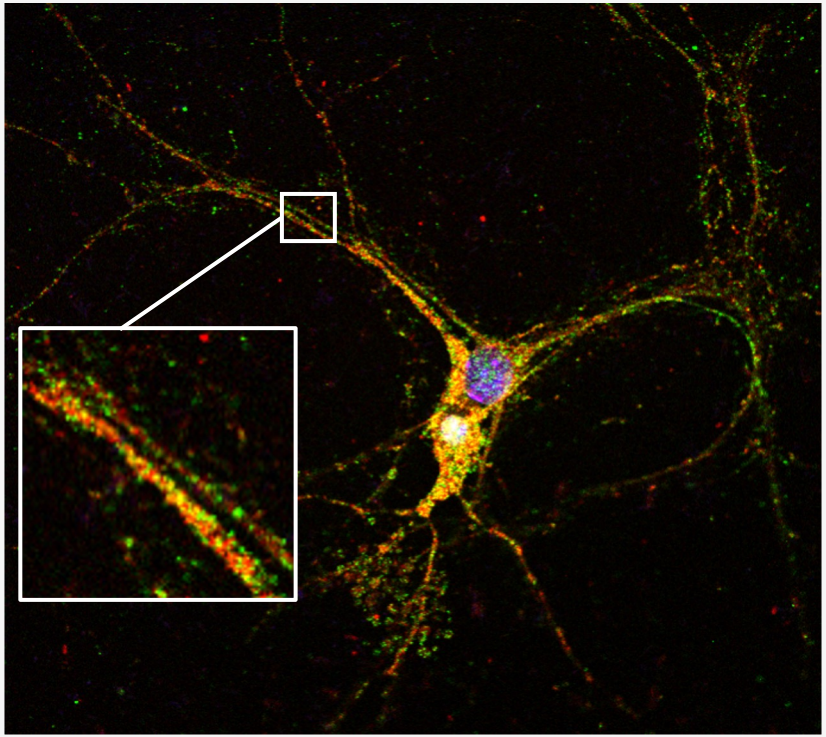
Colocalization of Exoc5 and APP in mouse primary hippocampal neurons



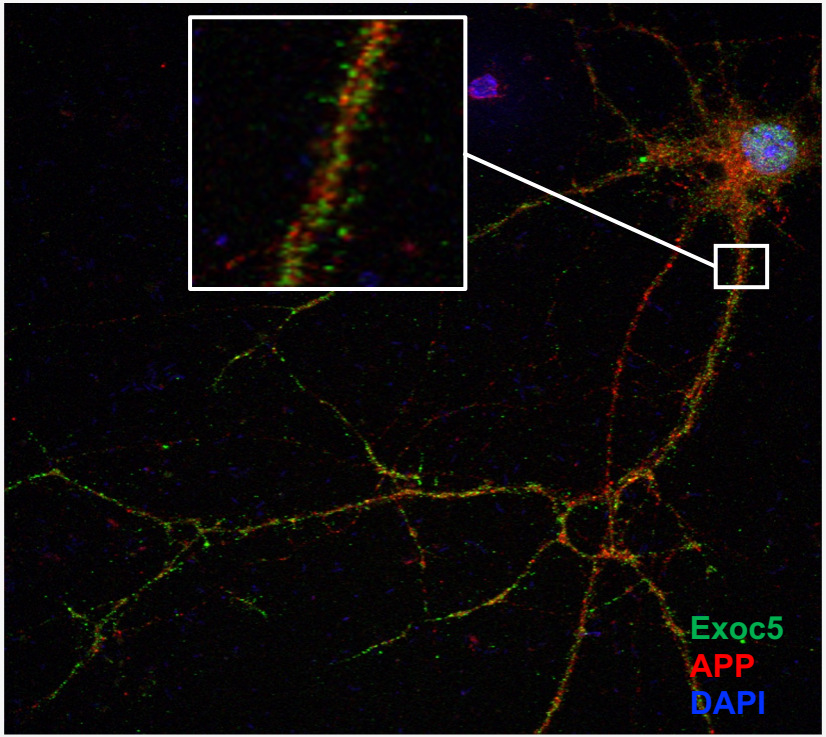


ICC shows colocalization of Exoc5 and APP appears decreased with insulin treatment, when compared to insulin starved cells

- Insulin

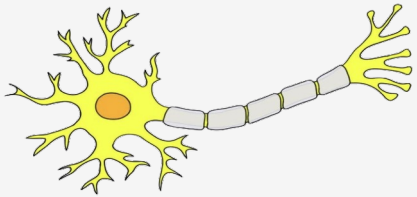


+ Insulin



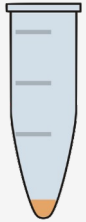
Proteomics

SH-SY5Y human
cell line

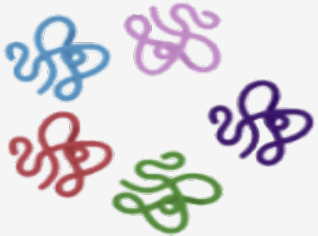


18-day neuron
differentiation protocol

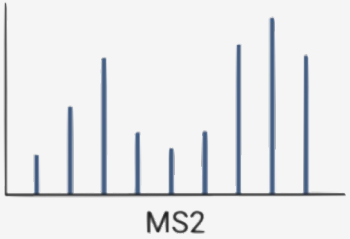
Biotinylation of cell
surface proteins



Capture with
streptavidin beads

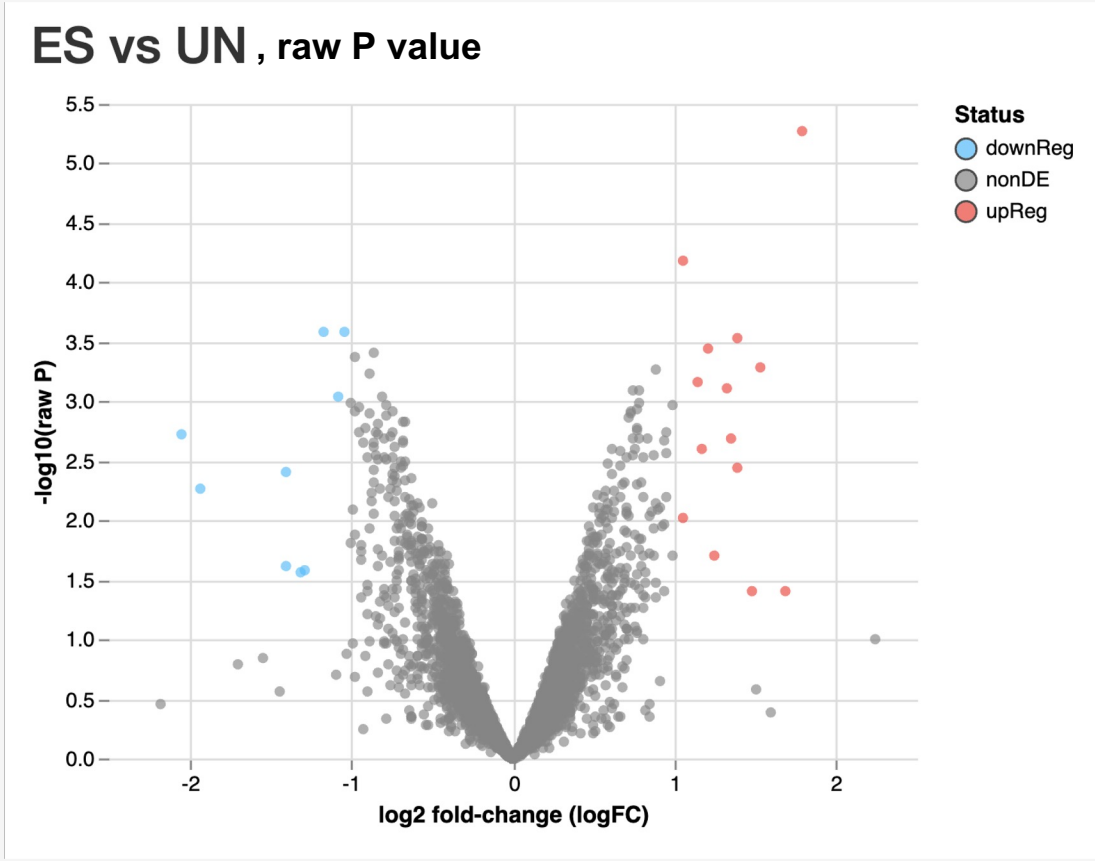


Intact Protein
Separation



Proteoform
Quantitative Analysis

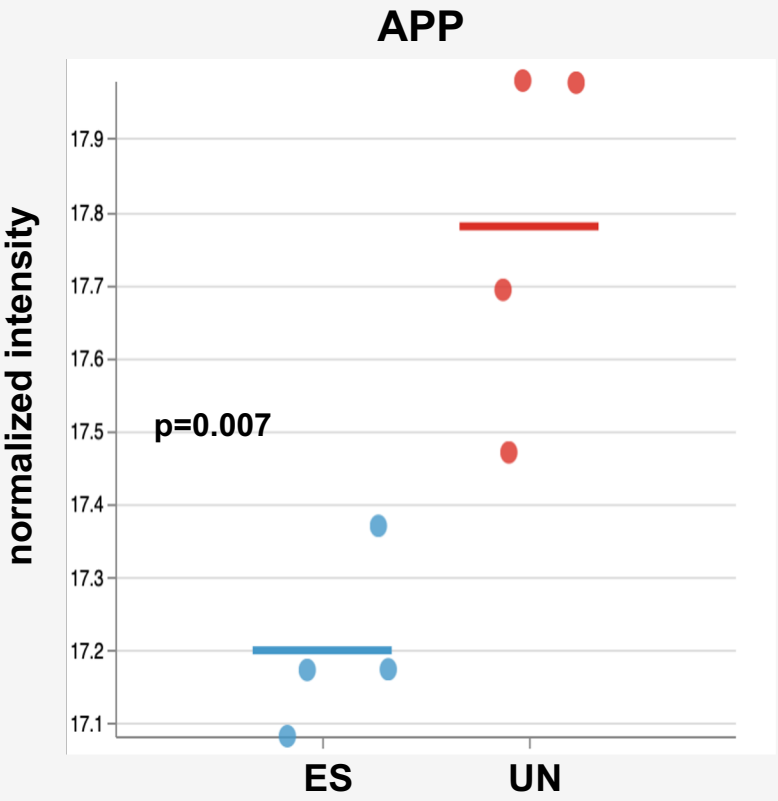
Increases, decreases, or no changes found in cell surface proteins

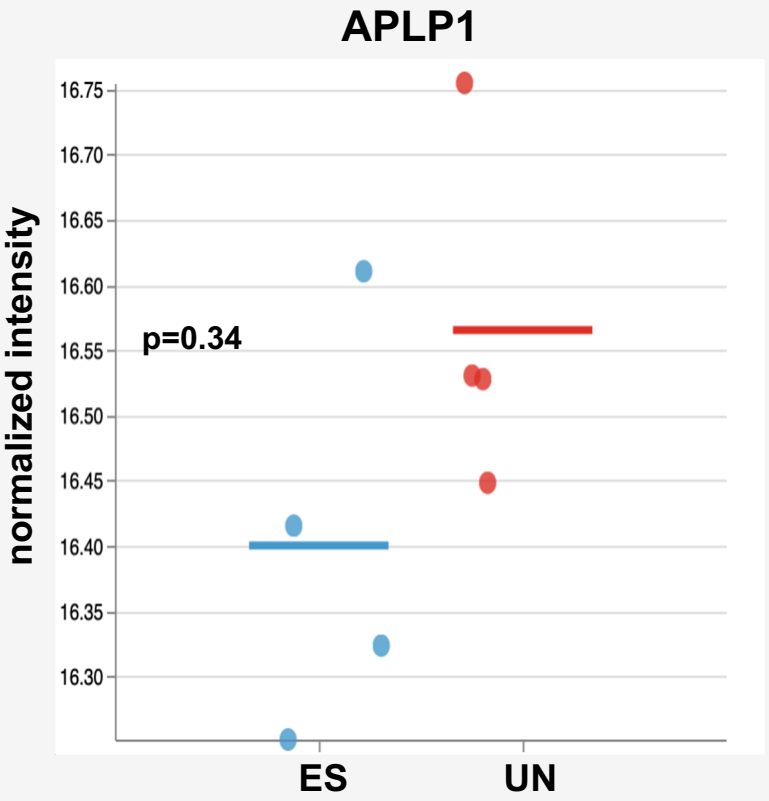


ES- endosidin treated

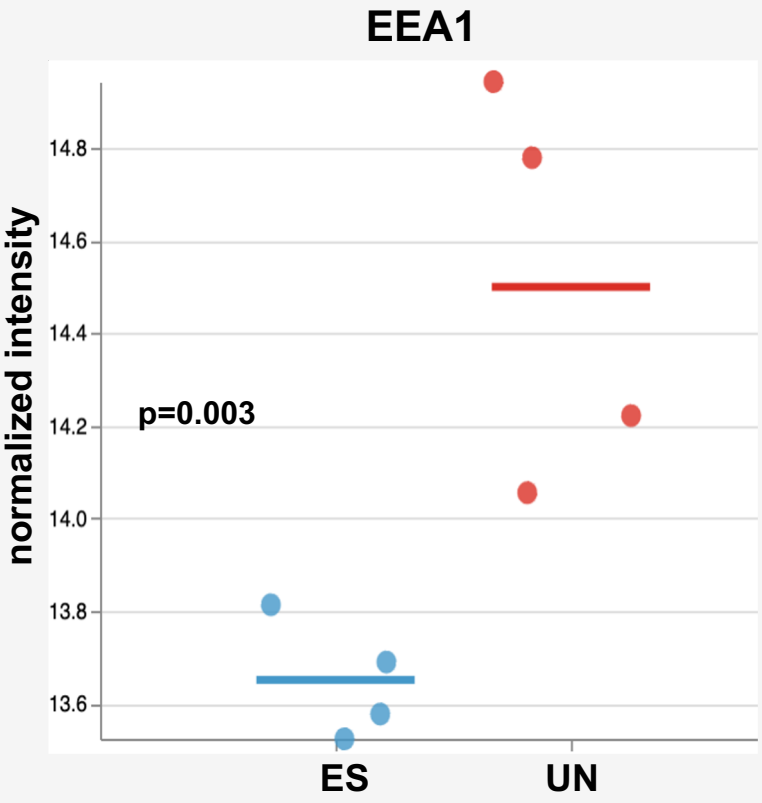
UN- untreated

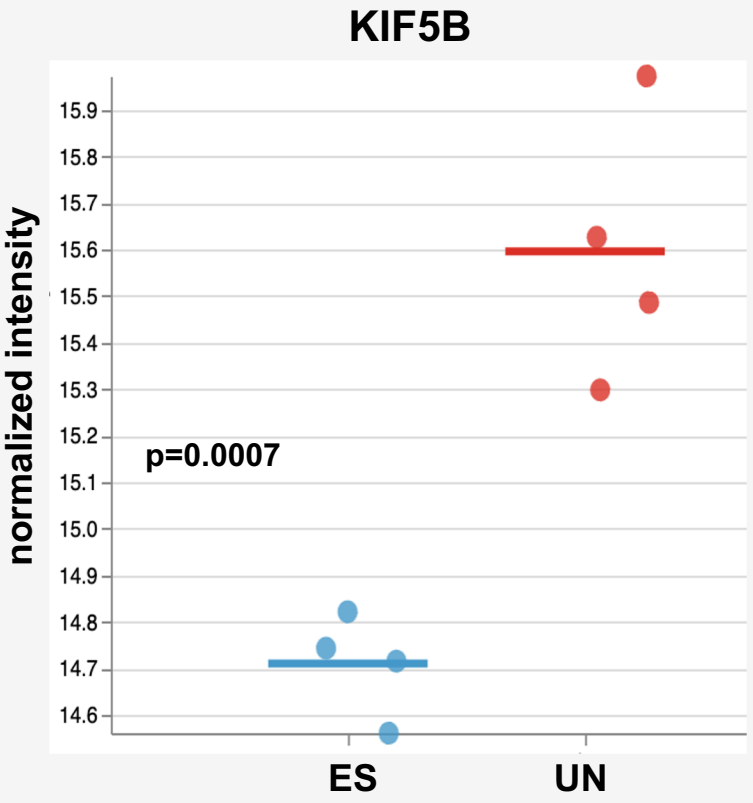
APP significantly decreased on the surface of neurons treated with ES2, while APLP1 is not.





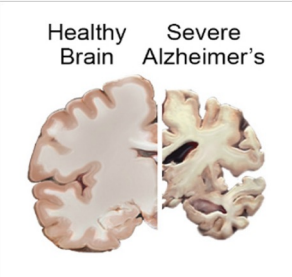
When treated with ES2, endosomal proteins EEA1 and KIF5B were significantly decreased





Conclusions

- Exocyst plays a role in Aβ production and is involved in intracellular trafficking of APP in neurons.
- Insulin may induce a re-targeting of the exocyst away from APP+ transport vesicles, reducing the production of Aβ
- Proteomics identified cell surface proteins (including APP) that are affected by exocyst inhibition, underscoring its role in trafficking neuronal plasma membrane proteins
- Greater understanding of exocyst function in the intracellular trafficking of APP and regulation of Aβ in neurons may reveal potential Alzheimer's therapeutics



National Institute of Aging

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Owens Lab