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α-Synuclein is a functional interactor of the Exocyst complex

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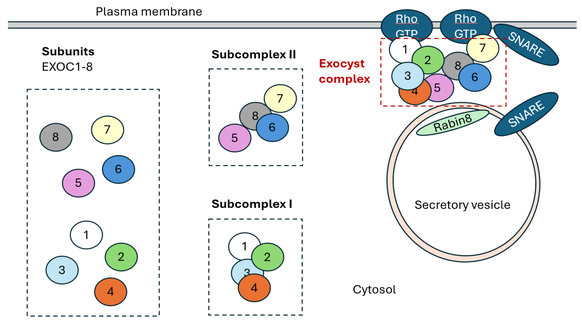
A-synuclein (α-Syn) function is associated with synaptic vesicle exocytosis where it promotes the assembly of the SNARE complex by directly interacting with VAMP2 and anchoring to the plasma membrane. However, little is known about the role of α-Syn in the exocytosis of the secretory vesicles (SVs) involved in the canonical secretory pathway. To address this gap in our knowledge, we have performed a series of RUSH, CRISPR-Cas9, immunofluorescence and biochemical experiments in α-Syn expressing SH-SY5Y cells and SNCA knock out mice.

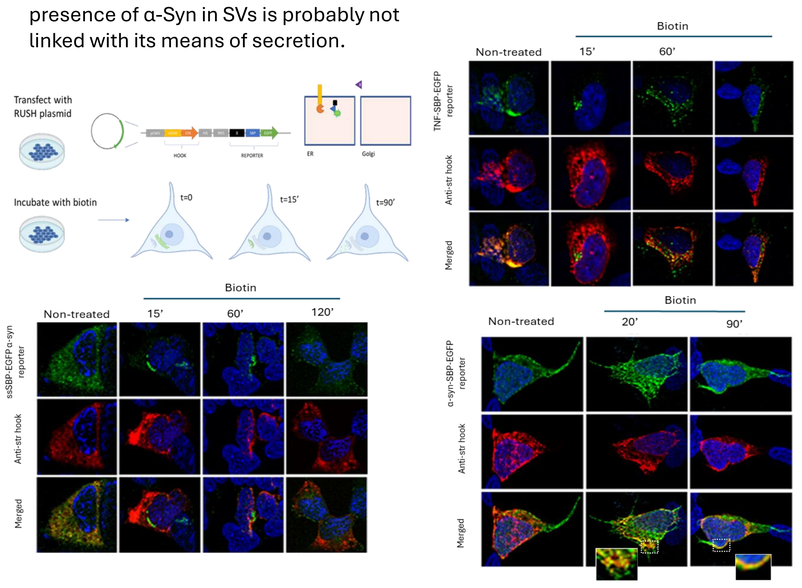
We found that α-Syn is a functional interactor of the Exocyst, a hetero-octameric complex, which is critical for the SV tethering to the plasma membrane. Our data provide strong evidence to support a yet unidentified physiological function of α-Syn in SV-mediated secretion and highlight the Exocyst as a novel regulator of intracellular α-Syn levels *in vitro* and *in vivo*.



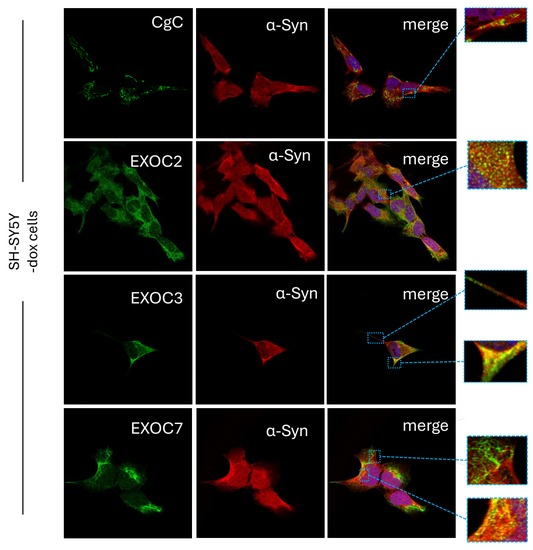
1. α-Syn is associated with Exocyst-positive SVs

A. α-Syn does not follow the classical secretory pathway

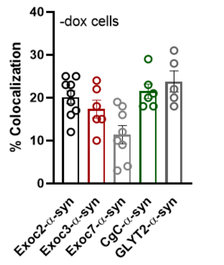
α-Syn does not traffic through the Golgi apparatus in SH-SY5Y cells, thus, the presence of α-Syn in SVs is probably not linked with its means of secretion.



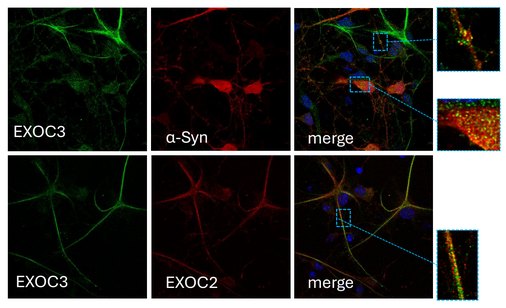
B. α-Syn colocalizes with SV markers and the Exocyst complex



In SH-SY5Y cells that overexpress α-Syn in the absence of doxycycline (-dox), α-Syn colocalizes SV marker, chromogranin C (CgC) and glycine transporter 2 (GLYT2) as well as with subunits of the Exocyst complex, EXOC2, EXOC3, EXOC7.

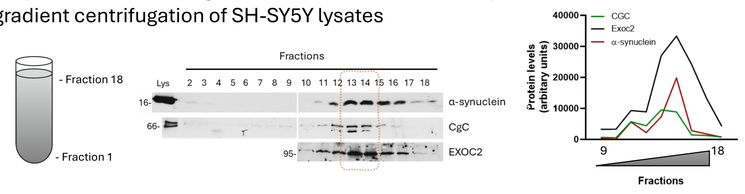


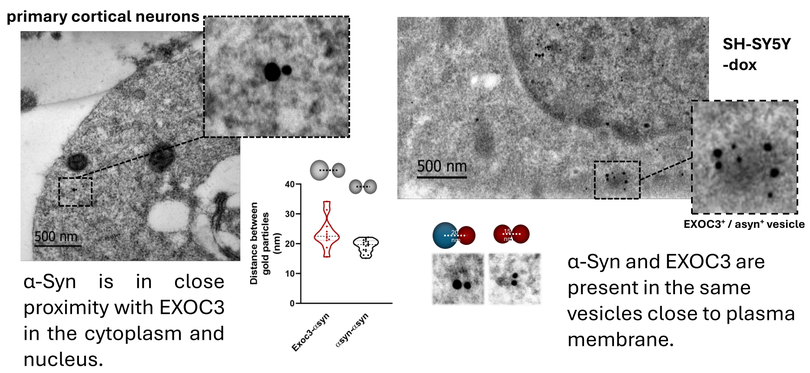
In mixed primary hippocampal neuron-glia culture, the Exocyst subunits EXOC2 and EXOC3 are expressed in the soma and neurites of neurons, where they colocalize with α-Syn. EXOC2 and EXOC3 are also abundantly expressed in astrocytes.



C. α-Syn colocalize with secretory vesicles that also contain the Exocyst complex

α-Syn co-elutes with CgC and EXOC2 after Percoll step gradient centrifugation of SH-SY5Y lysates





α-Syn is in close proximity with EXOC3 in the cytoplasm and nucleus.

α-Syn and EXOC3 are present in the same vesicles close to plasma membrane.

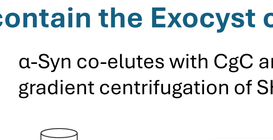
D. EXOC2, EXOC3, EXOC7 do not physically interact with α-Syn

Immunoprecipitation in crosslinked lysates of SH-SY5Y cells

WB: α-syn

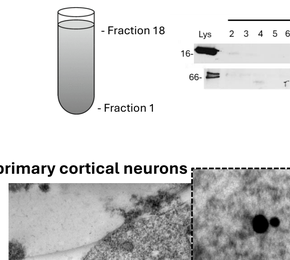
DBH was used as a control antibody

WB: EXOC3



WB: α-syn

Immunoprecipitation in pooled FPLC fractions of intermediate molecular weight proteins of SH-SY5Y cells containing Exocyst subcomplexes 1 and 2

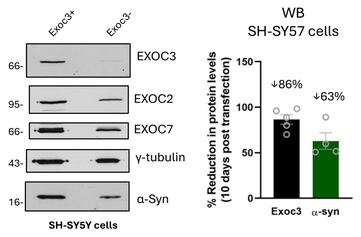


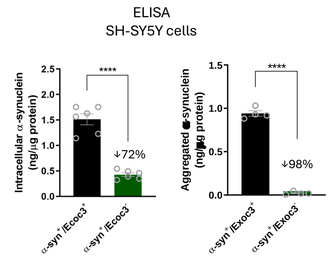
WB: EXOC3

WB: EXOC7

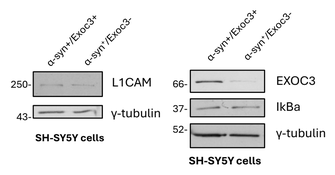
2. EXOC3 regulates intracellular α-Syn levels

A. CRISPR-Cas9-induced EXOC3 deletion results in a significant reduction of intracellular α-Syn

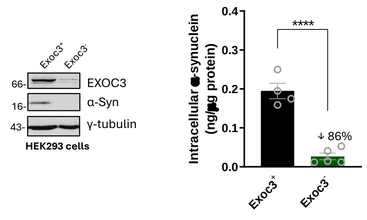


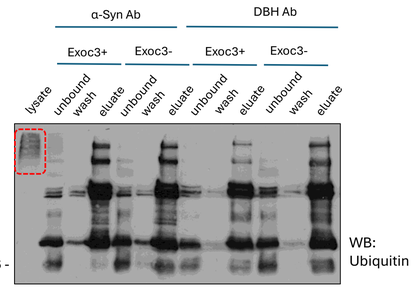


EXOC3 deletion does not affect other proteins such as L1CAM and IkBa

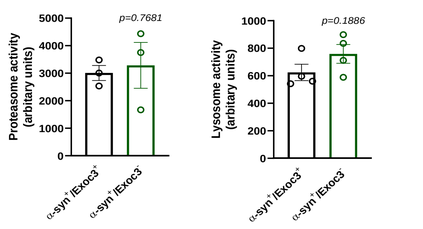


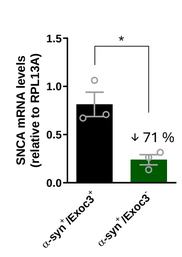
The reduction of α-Syn upon EXOC3 deletion is not cell type-dependent



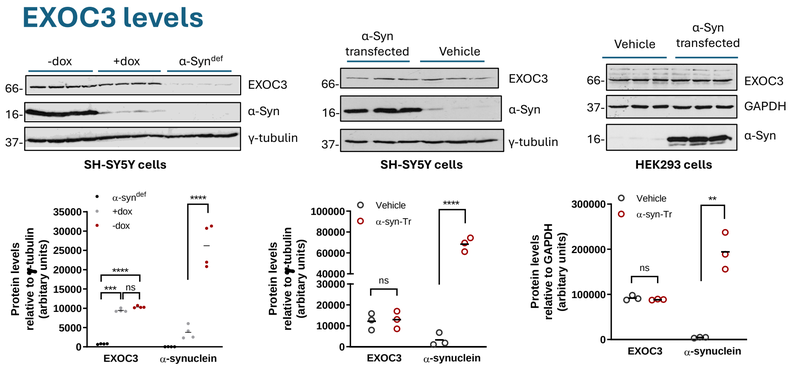


Reduction in EXOC3 expression causes a significant reduction in α-Syn mRNA levels, but does not affect proteasome or lysosome activities as shown by enzymatic measurements and assessment of the ubiquitinylated α-Syn.





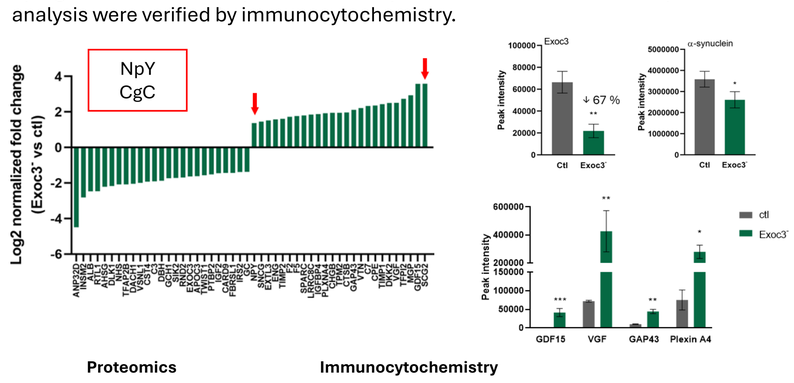
B. α-Syn is critical for the maintenance of EXOC3 levels even though over-expression has no effect in EXOC3 levels

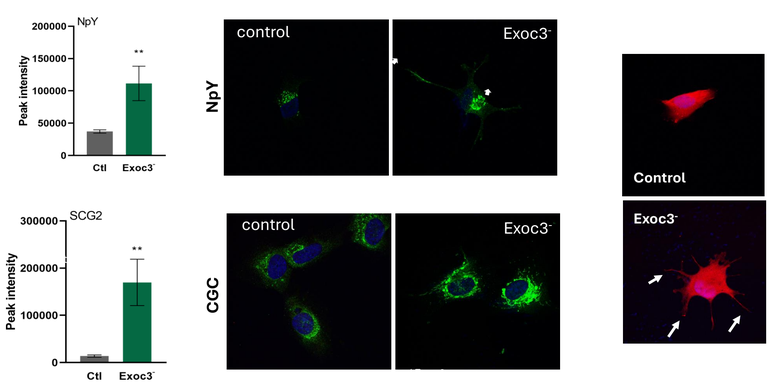


The levels of EXOC3 was compared in a clone of SH-SY5Y cells that does not express α-Syn (α-Syndef), as well as control SH-SY5Y cells and HEK293 cells transfected with α-Syn plasmid.

C. EXOC3 depletion promotes axonogenesis and impairs the secretion of NpY and CgC

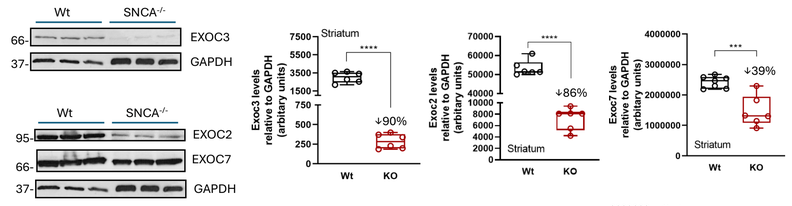
Proteomics in cells following CRISPR-Cas9-induced EXOC3 deletion showed an upregulation in secreted proteins and trophic factors. The results from proteomic analysis were verified by immunocytochemistry.



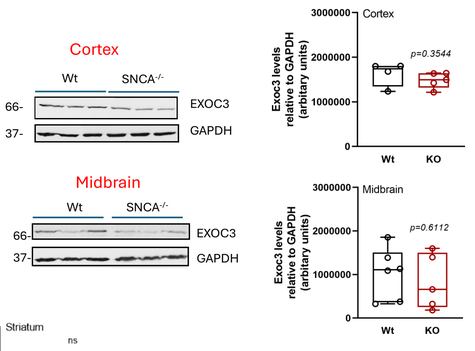


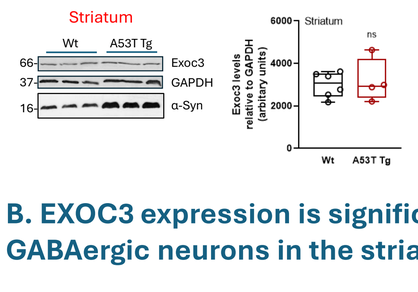
3. α-Syn depletion results in Exocyst complex downregulation in mice

A. α-Syn deletion results in a significant reduction of the Exocyst complex only in the striatum



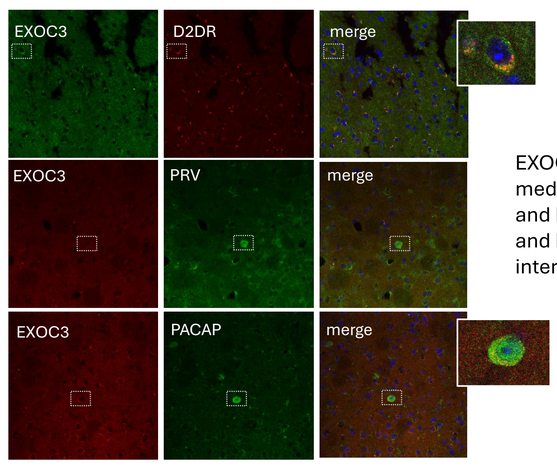
EXOC3 downregulation in SNCA -/- mice is restricted to the striatum, compared with cortex and midbrain tissue



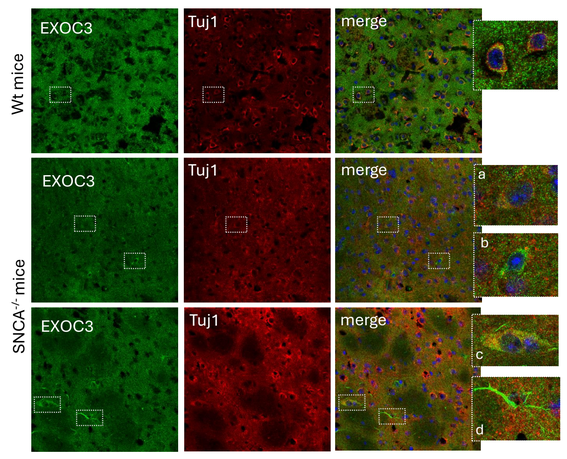


Increased expression of α-Syn does not affect EXOC3 levels in mice

B. EXOC3 expression is significantly reduced in GABAergic neurons in the striatum of SNCA-/- mice

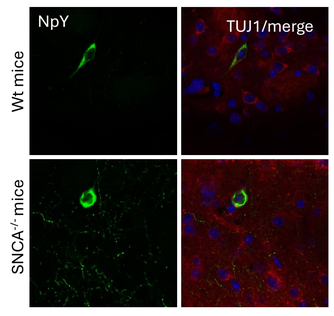


EXOC3 is expressed in medium spiny neurons and Parvalbumin (PRV) and PACAP positive interneurons in striatum



EXOC3 expression in striatum is maintained in cholinergic neurons and elevated in astrocytes.

C. Exocyst downregulation in the striatum of SNCA-/- mice reduces the secretion of the neuropeptide NpY from GABAergic interneurons



NpY-positive GABAergic interneurons in mouse striatum of SNCA-/- exhibit intracellular NpY accumulation possibly due to the Exocyst complex dysfunction.

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