UC San Diego

SynCav1 Gene Therapy for CNS Disorders

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- Research Career Scientist VA Medical Center, San Diego

VA | U.S. Department of Veterans Affairs

Disclosure Slide

- Non-paid consultant with equity interest in Eikonoklastes Therapeutics (Cincinnati, Ohio)
- Eikonoklastes licensed the patented neuron-targeted caveolin gene technology from UCSD in December 2021
  - Received Orphan Drug Designation from FDA for ALS (2022)
  - **FDA approved the IND submission (October 2025)**
  - FIH Clinical Trial to commence in 2026 at Ohio State University

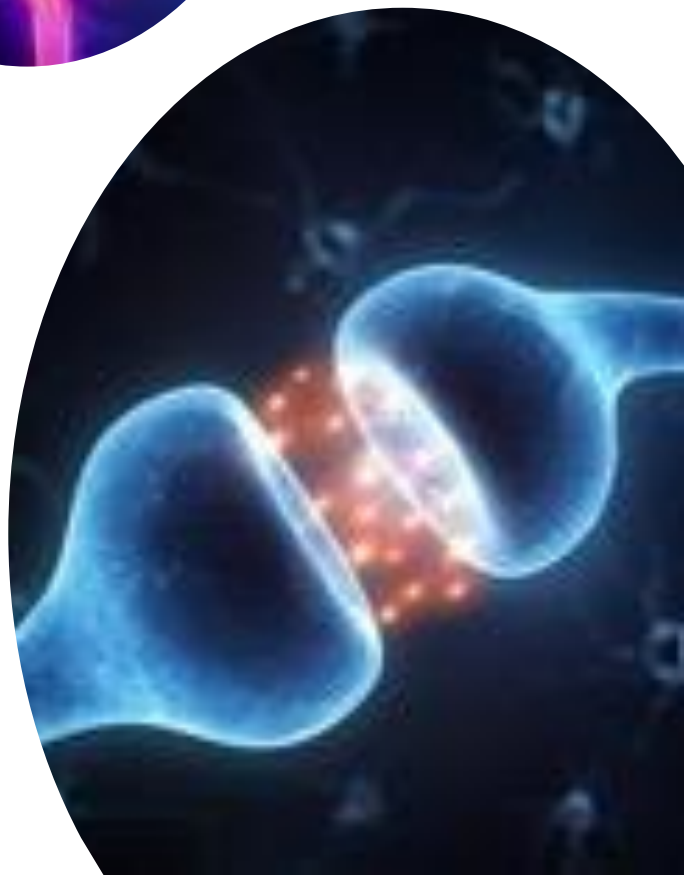
Aging, Nerve Injury and CNS Disorders

Aging & nerve injury increase risk of neurodegenerative diseases

- 87 mil people > 65 years by 2050; increased risk for Alzheimer's disease (AD) & Amyotrophic Lateral Sclerosis (ALS)

Associated cognitive and motor decline

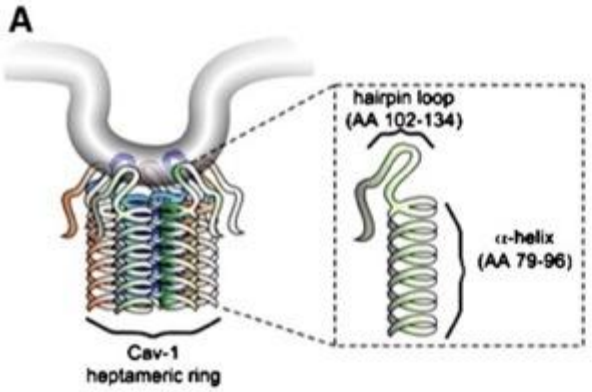
- Reduced synapses, deficits in cognition, sleep, executive function (AD)
- Loss of motor neurons in brain and spinal cord (ALS)

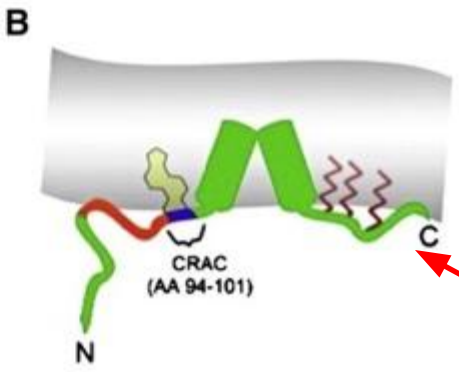


<u>Objective & Goals</u>

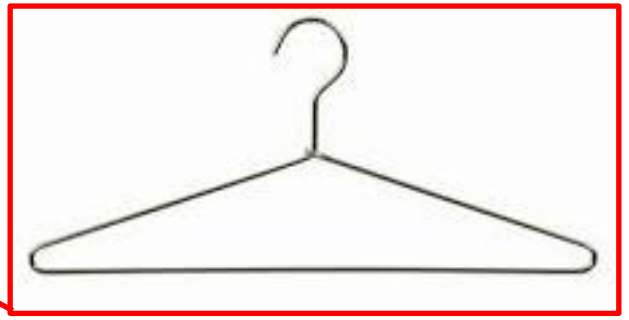
1. *Identify potential molecular targets.*
2. *Restore <u>functional</u> neuroplasticity in the degenerative CNS.*

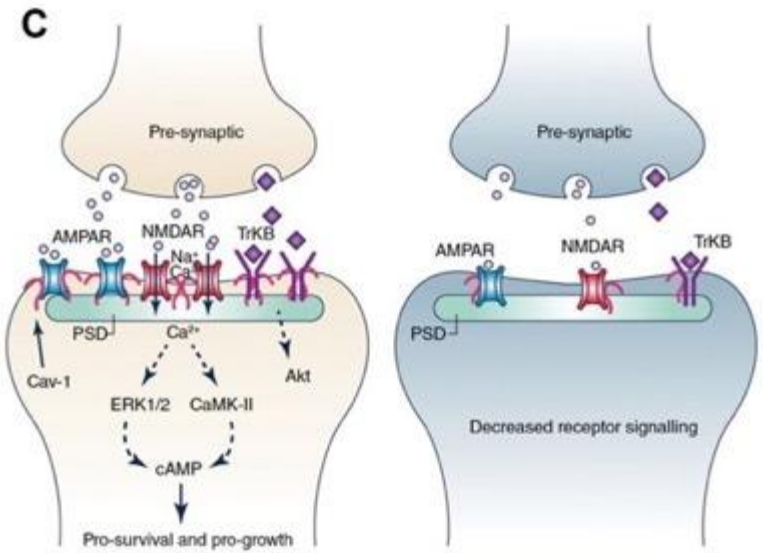
Caveolin: MLR Scaffolding Protein





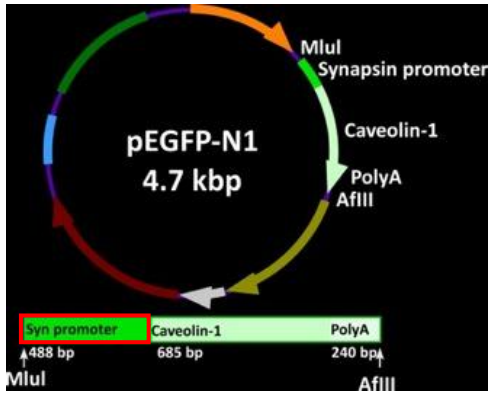
Cav-1 is akin to a "coat-hanger"



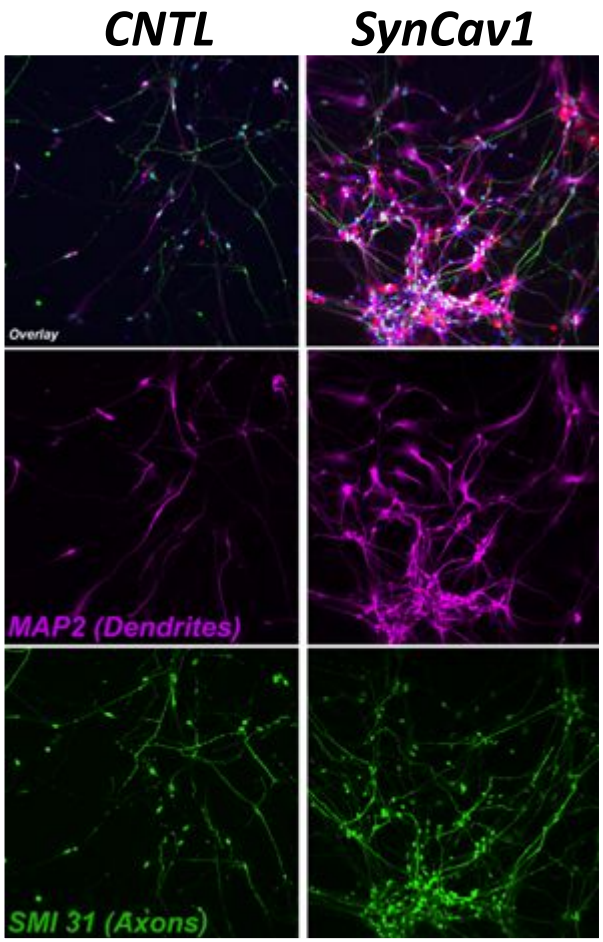


- Cav-1 is a membrane scaffolding (**A**) & cholesterol binding protein (**B**)
- Cav-1 organizes & regulates synaptic receptors & associated signaling components essential for neuronal signaling and neuroprotection
- Decreased Cav-1 causes ***early aging*** phenotype, loss of synapses & decreased synaptic plasticity (**C**)

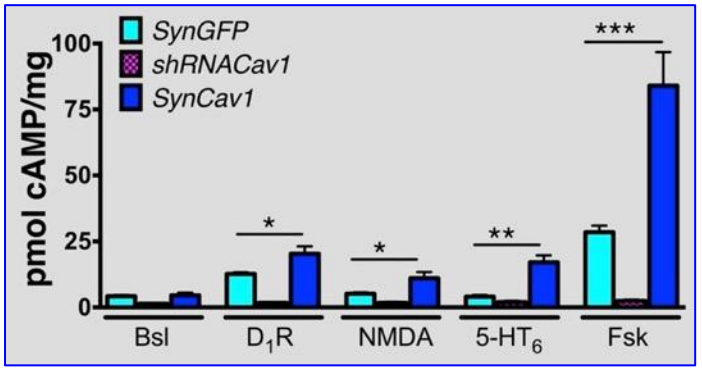
Synapsin-promoted Caveolin-1: ***SynCav1***



Human neurons derived from iPSCs

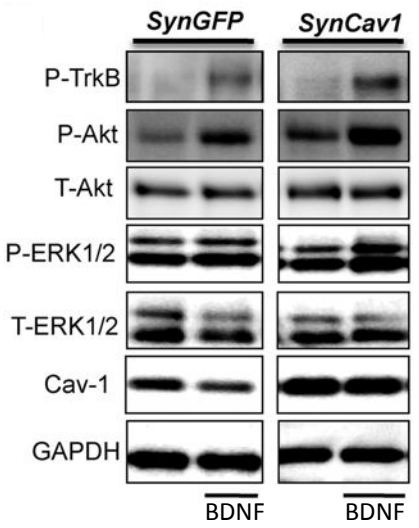


SynCav1 *increases dendritic and axonal growth*



SynCav1 augments receptor-mediated cAMP production

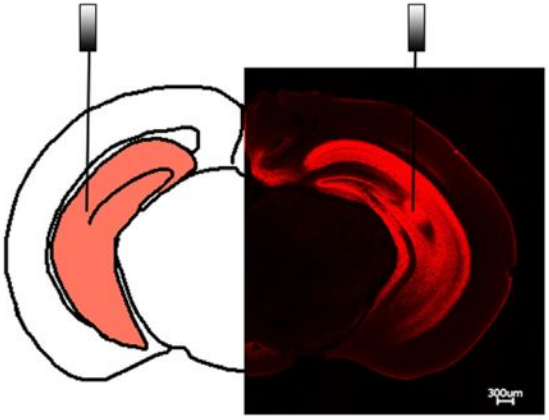
SynCav1 augments NMDA & BDNF-mediated signaling

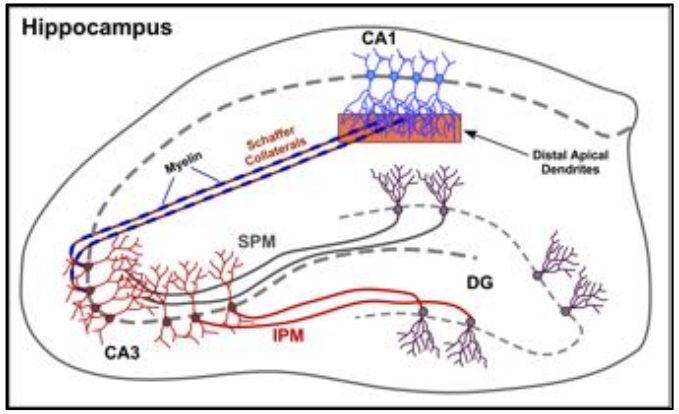


J Biol Chem 2011 & Frontiers Cell Neurosci 2019

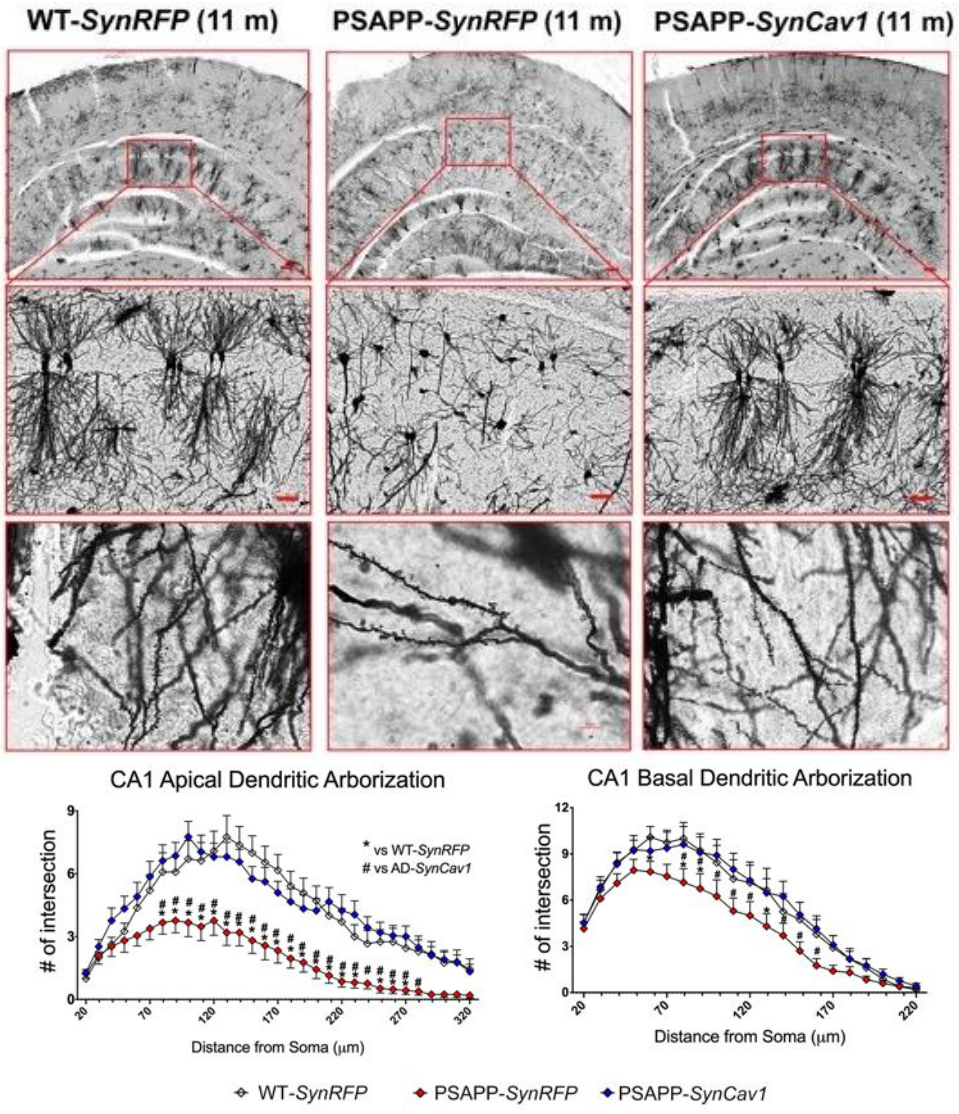
Can ***SynCav1*** evoke neuroprotection in models of neurodegeneration?

AAV9-SynCav1
PSAPP (AD) Mice



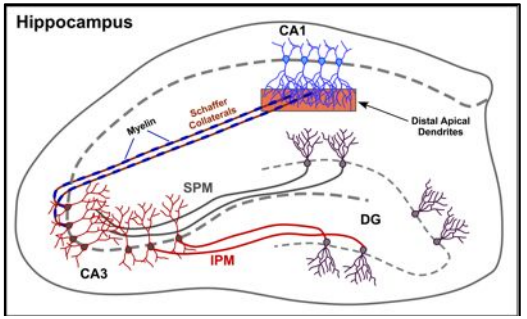


CA1 is central to learning & memory

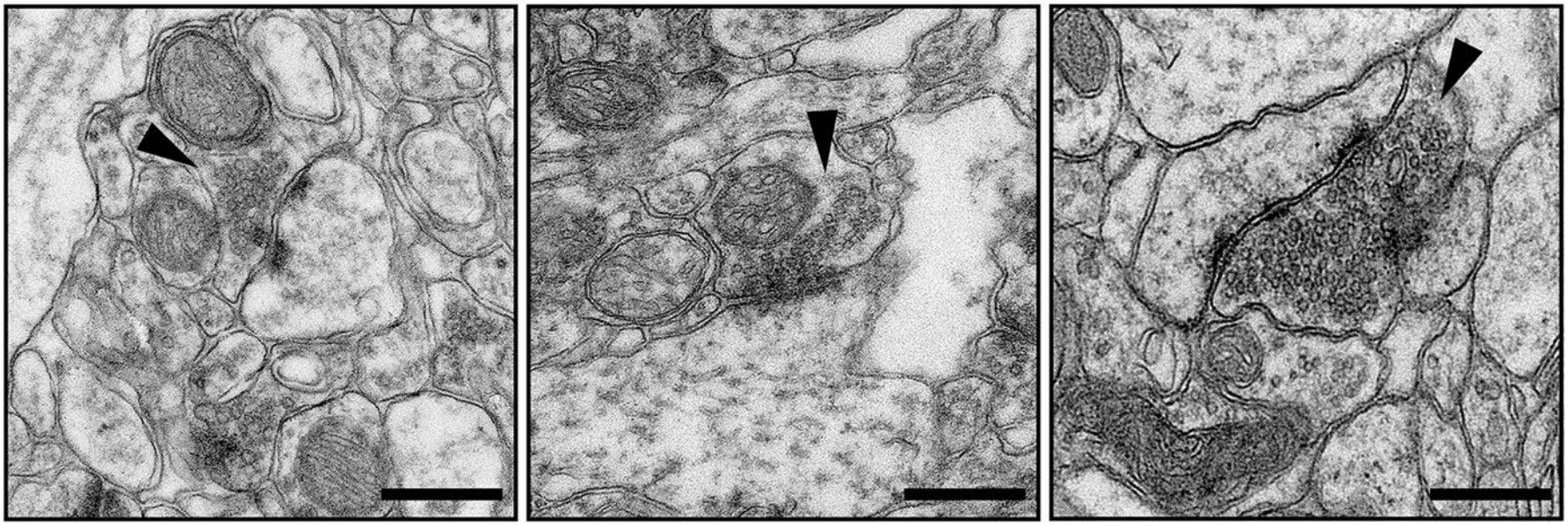


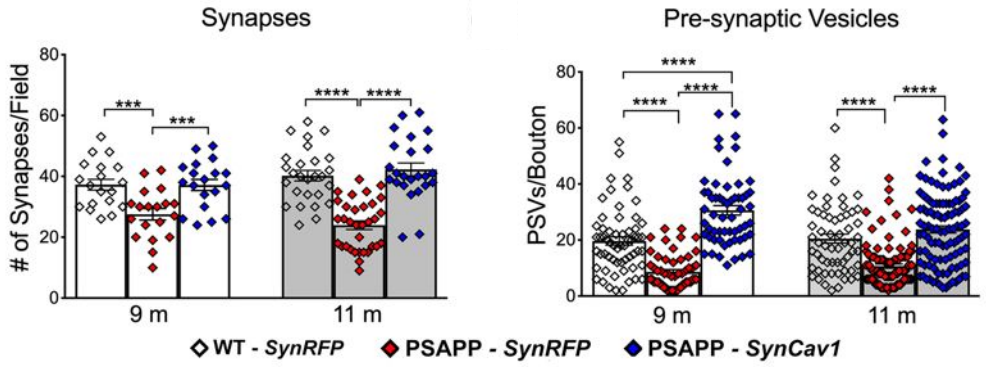
Mol Ther - Meth & Clin Dev 2021

SynCav1 preserves hippocampal synaptic ultrastructure in AD mice (PSAPP)



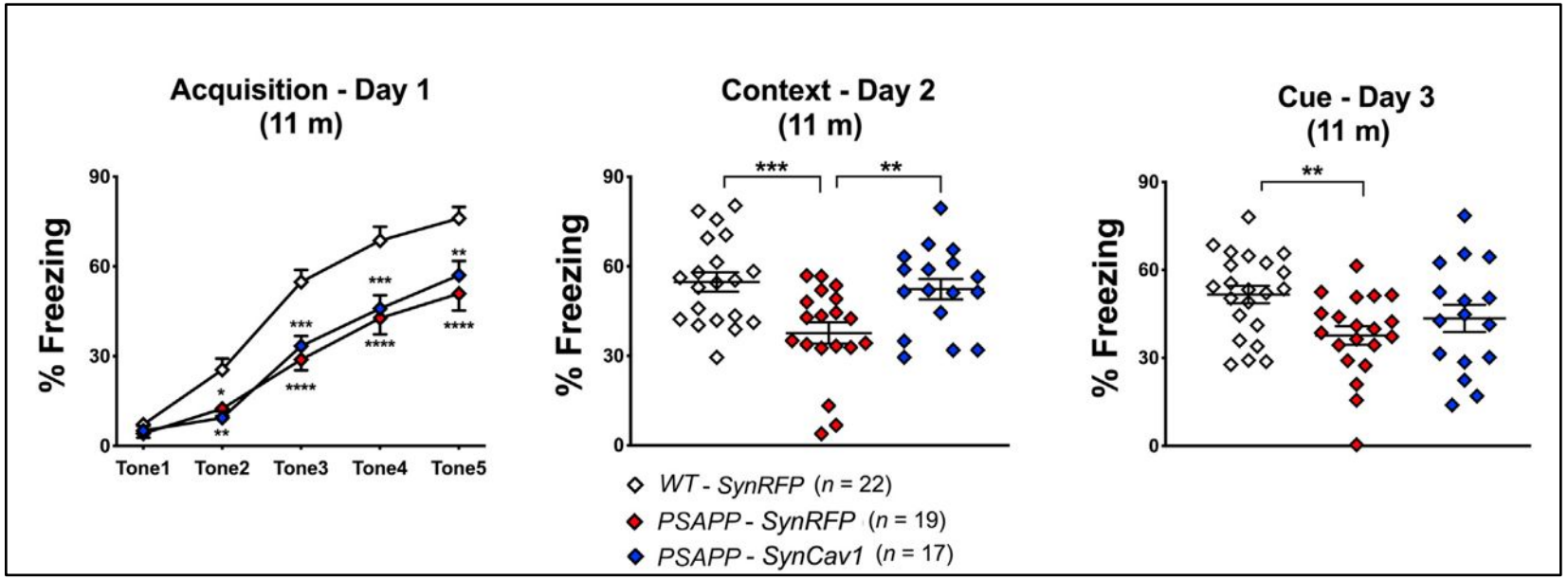
WT-*SynRFP* (11 m) | **PSAPP-*SynRFP* (11 m)** | **PSAPP-*SynCav1* (11 m)**





Mol Ther: Meths & Clin Dev 2021

AAV9-SynCav1 preserved hippocampal-dependent <u>memory</u> in 11 m old AD mice (PSAPP)



- ***SynCav1*** delivery to PSAPP mice *<u>preserved</u>* hippocampal
  - **dendritic arbor**
  - **synaptic ultrastructure (excitatory synapses, PSVs)**
  - **contextual memory recall**
  - **mitochondrial morphology and function**
    - ***Cells 2021 (data not presented)***

Mol Ther: Meths & Clin Dev 2021

Signal Transduction and Targeted Therapy www.nature.com/sigtrans

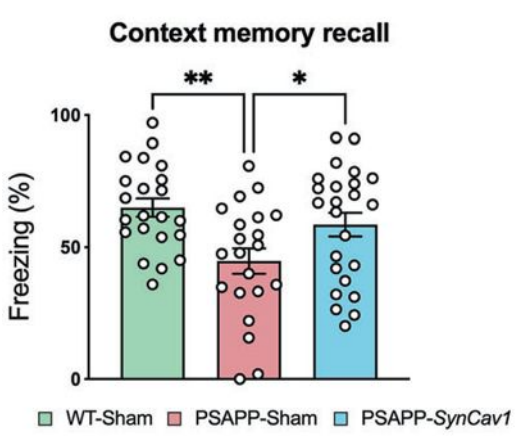
ARTICLE OPEN

Neuron-targeted caveolin-1 overexpression attenuates cognitive loss and pathological transcriptome changes in symptomatic Alzheimer's disease models

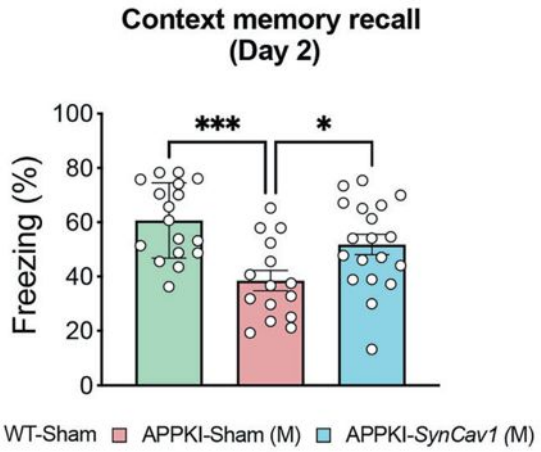
Dongsheng Wang[1,2], Andrei V. Chernov[1,2], Ryan Lam[1,2], Hongxia Wang[1,2], Wenxi Li[1,2], Xiaojing Li[1,2], Tiffany Duong[1,2], Shanshan Wang[1,2]✉ and Brian P. Head[1,2]✉

- ***Symptomatic*** PSAPP mice received *SynCav1* at **6 m**, behavior performed at 12 m
- ***Symptomatic*** APPKI mice received *SynCav1* at **9 m**, behavior performed at 12 m

PSAPP Mice



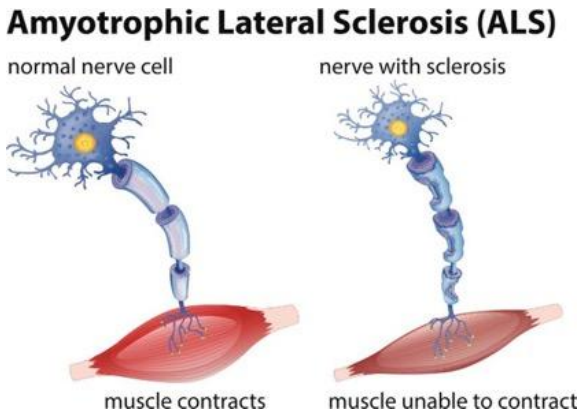
APPKI Mice



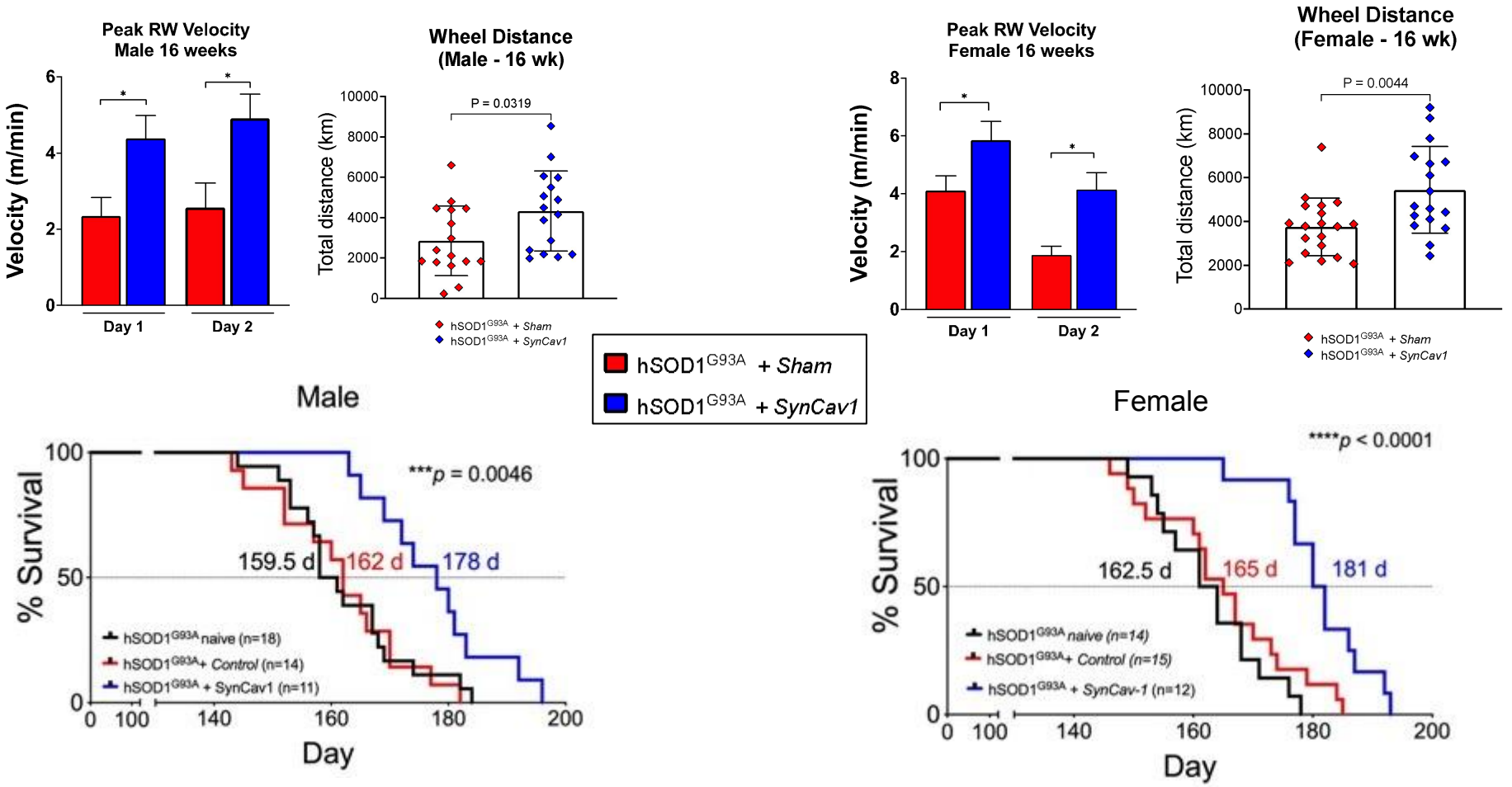
Signal Transduction & Targeted Therapy 2025

Can ***SynCav1*** protect against Lou Gehrig's disease (a.k.a. amyotrophic lateral sclerosis or ALS)?

- ***Amyotrophic*** – muscle atrophy in tongue, oropharynix, & limbs
- ***Lateral sclerosis*** – degenerating axons resulting in thinning & ***scarring*** of the ***lateral*** spinal cord



AAV9-SynCav1 delivery to the spinal cord improves motor function and extends survival and in male and female hSOD1^{G93A} mice



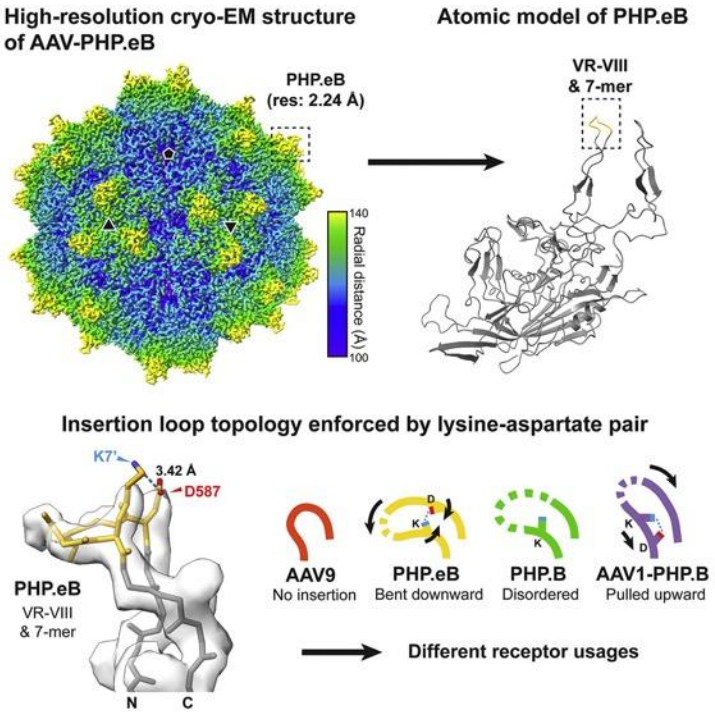
- ***SynCav1*** *delivery to male and female ALS mice*
  - *improved motor function (running wheel performance)*
  - *delayed disease onset and extended survival*

FDA recently approved the *SynCav1* IND submission for FIH Clinical Trial for ALS (October 2025)

Theranostics 2022

Study Limitations

- Direct brain & spinal cord administration requires surgical craniotomy and laminectomy respectively
- AAV9 requires direct CNS delivery (not systemic)
- AAV9 targets non-neuronal and neuronal cells, which limits neuron-specific CNS targeting



Gradinaru Lab, *Meth & Clin Dev: vol. 26, 2022*

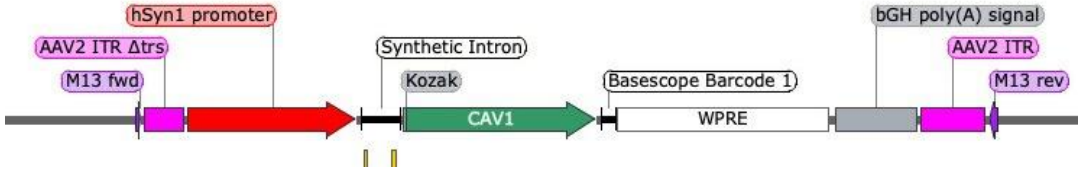
AAV-PhP.eB:

- *PhP.eB is derived from AAV9, modified viral protein 1 (VP1) in outermost capsid tip*
- *Interacts with LY6A on BBB to facilitate penetration & increase neurotopism*
- *Can be delivered systemically (intravenous)*
- *Has far greater neurotrophism and CNS biodistribution than AAV9*

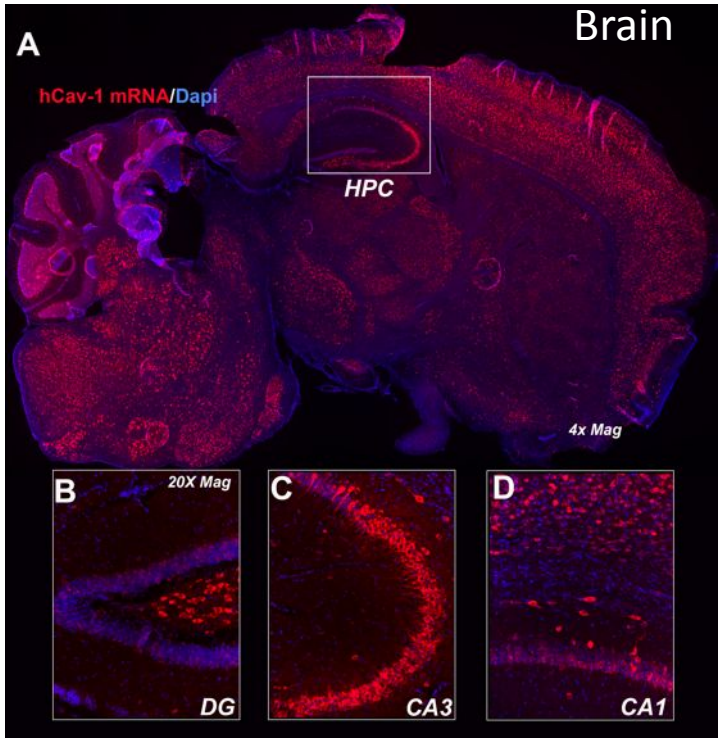
PhP.eB-SynCav1 achieves much broader neuronal tropism and CNS biodistribution

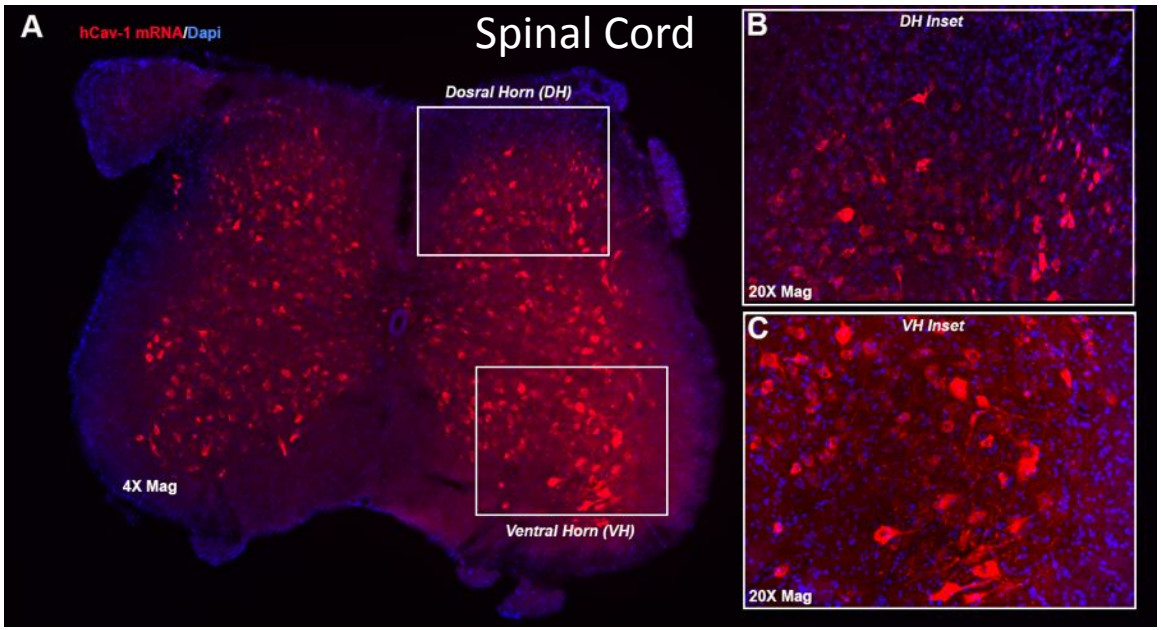
SynCav1 Modifications

- *Replaced AAV9 with PhP.eB*
- *WPRE to boost transgene transcript (mRNA)*
- *Replaced viral SV40 polyA with FDA required bovine growth hormone (bGH) polyA*



Human (h)Cav-1 mRNA detection using RNAscope



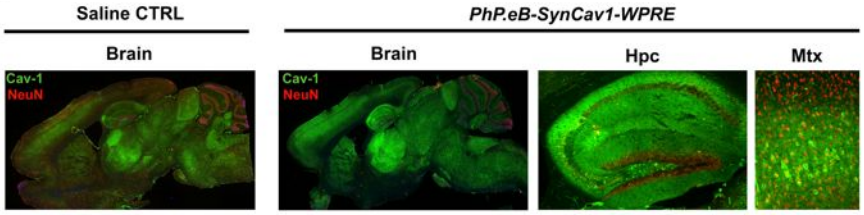


Unpublished

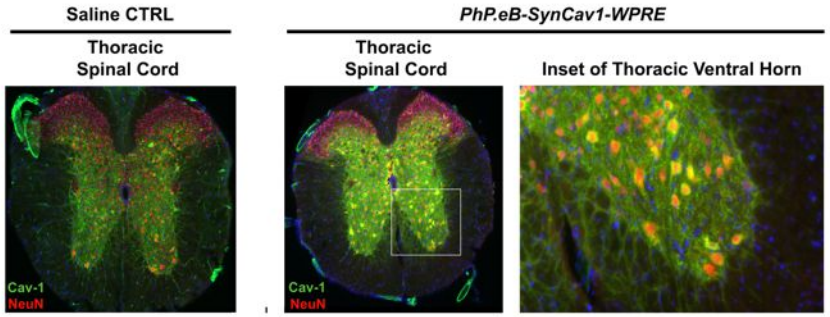
PhP.eB-SynCav1 improves RW performance and extends survival in hSOD1^{G93A} mice

hSOD1^{G93A} mice received *PhP.eB-SynCav1* at *post-symptomatic* age (13/14 wk)

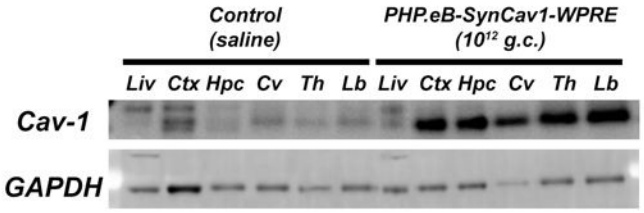
Brain



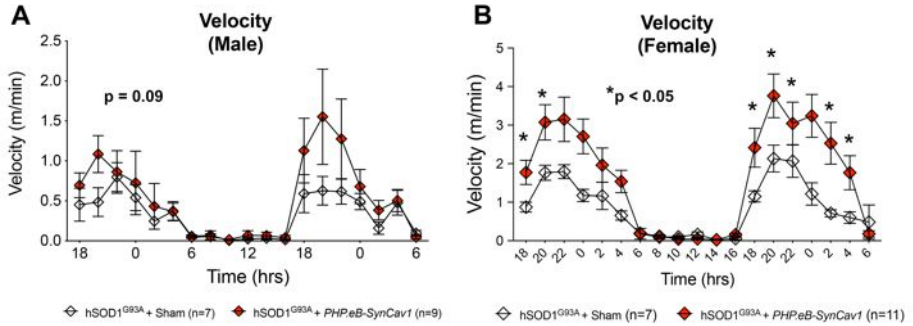
Spinal Cord

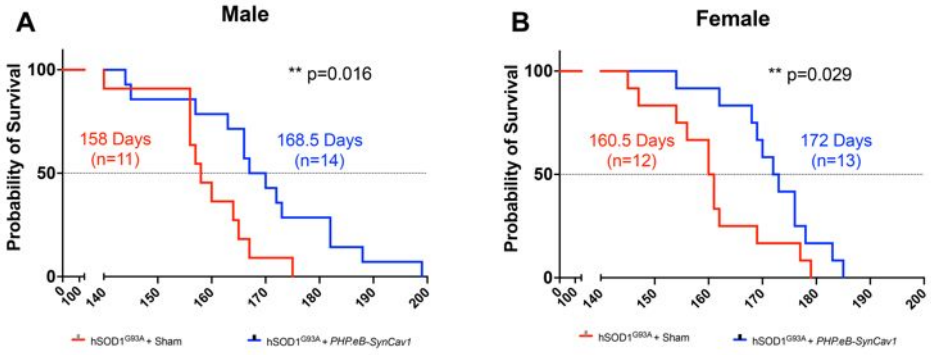


Cav-1 Immunoblot



Age 19 Weeks

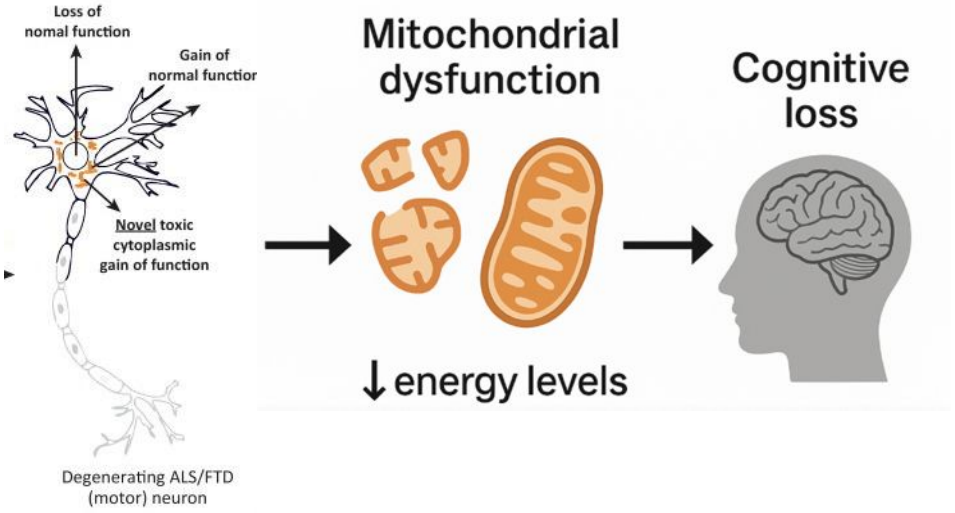




Unpublished

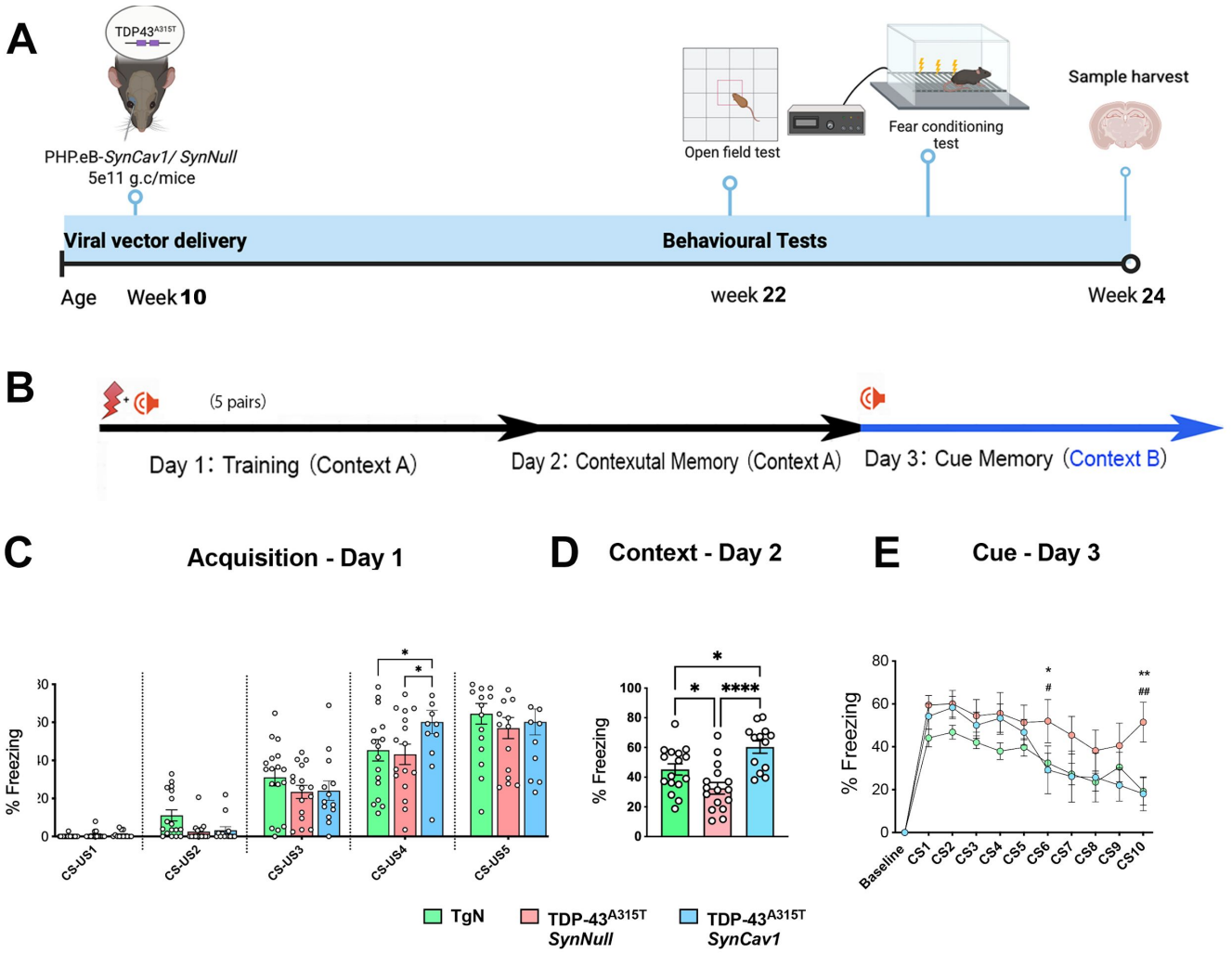
Mutant TDP-43 Causes Mito Dysfunction and Cognitive Loss Reflective of Frontotemporal Dementia (FTD)

- Impairs axonal mitochondrial transport and causes mitochondrial fragmentation
- Impairs cognitive function leading to dementia



Could *SynCav1* mitigate mitochondria damage and cognitive impairments in a mouse model of TDP-43/FTD pathology?

PhP.eB-SynCav1 preserves hippocampal memory in TDP-43^{A315T} mice (6 m)



- Hippocampal memory recall is preserved (Day 2 Context)
- Non-hippocampal memory recall is preserved with the ability to extinguish fear memory (Day 3 Cue)

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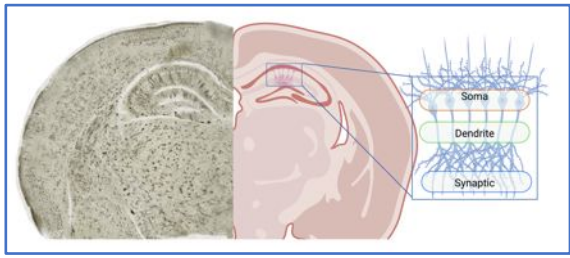
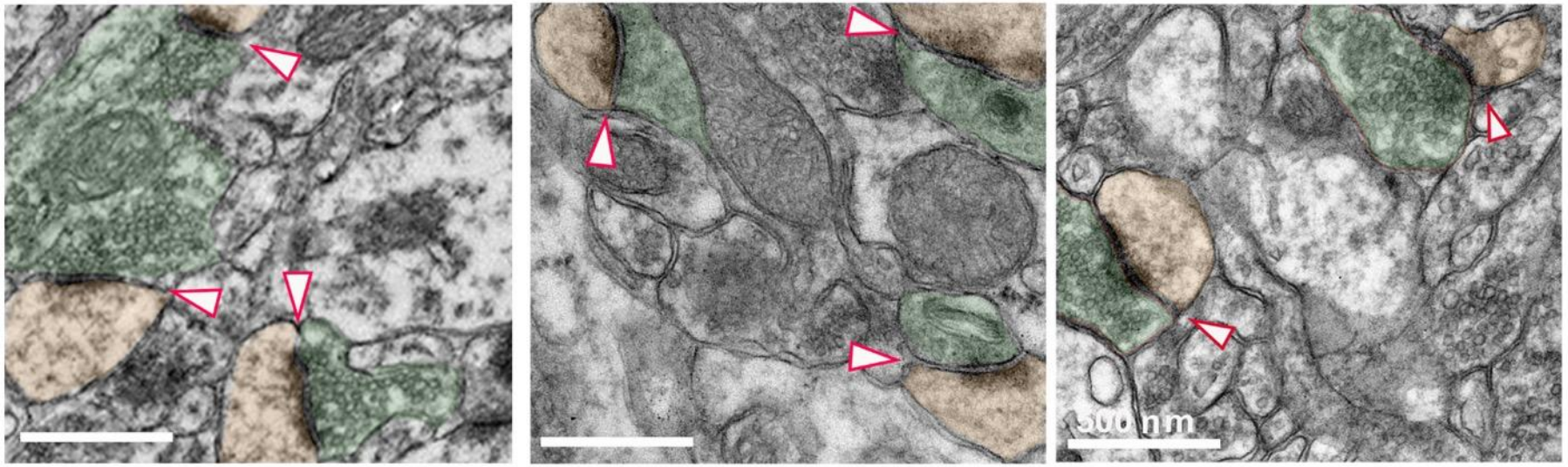
PhP.eB-SynCav1 preserves hippocampal synaptic ultrastructure in TDP-43^{A315T} mice (6 m)



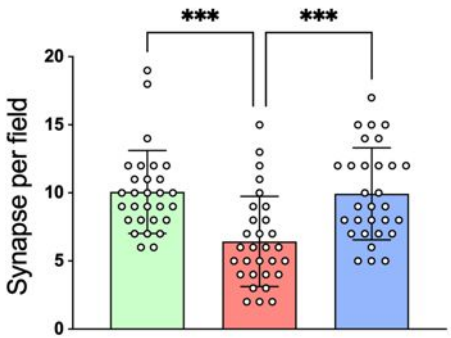
TgN

TDP-43^{A315T} *SynNull*

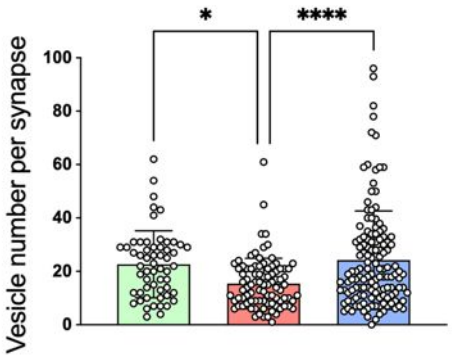
TDP-43^{A315T} *SynCav1*



Excitatory Synapses



Pre-Synaptic Vesicles



TgN

TDP-43^{A315T} *SynNull*

TDP-43^{A315T} *SynCav1*

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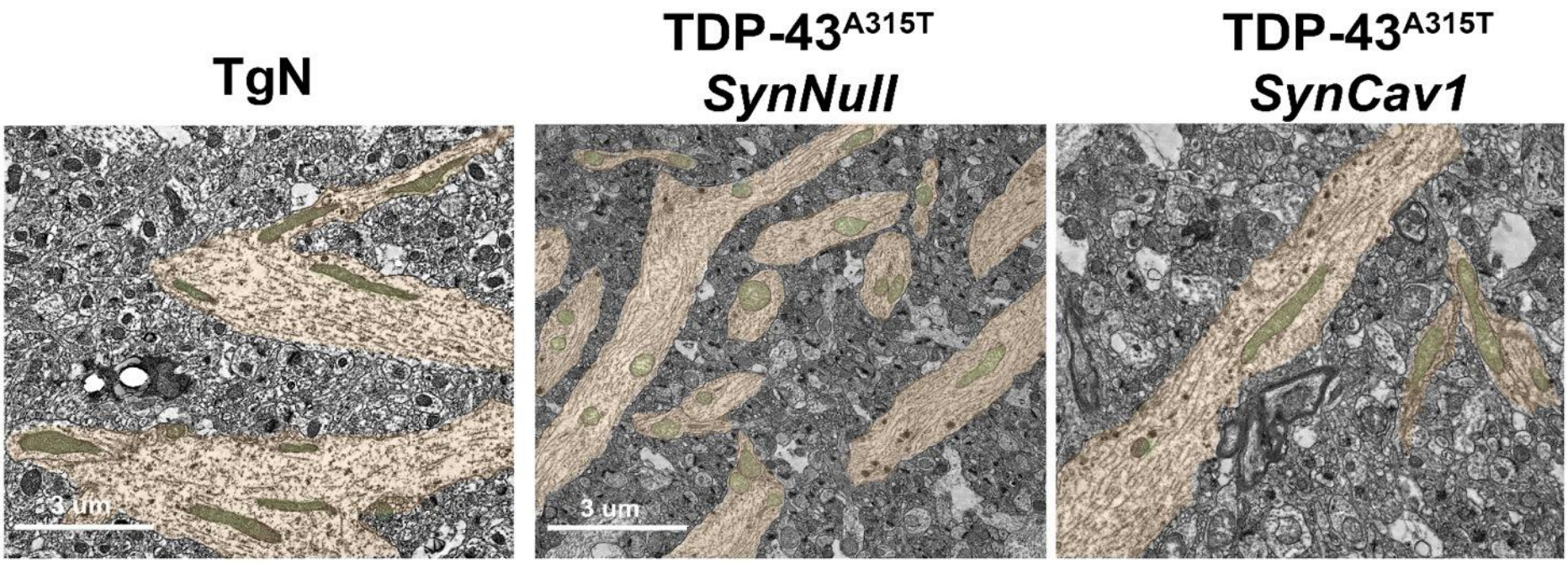
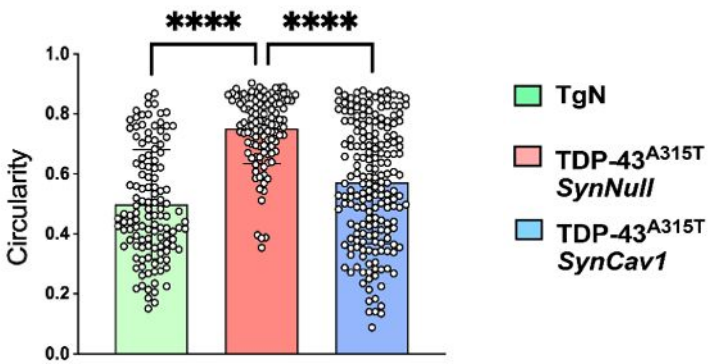
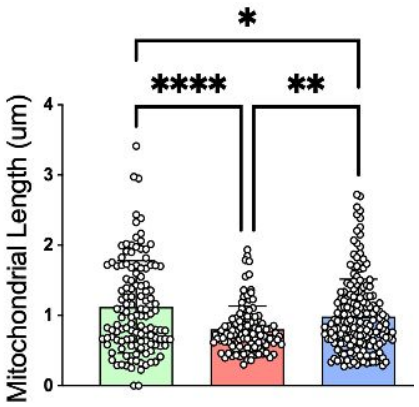
PhP.eB-SynCav1 preserves hippocampal mitochondrial morphology TDP-43^{A315T} mice (6 m)



Mito Circularity



Mito Length



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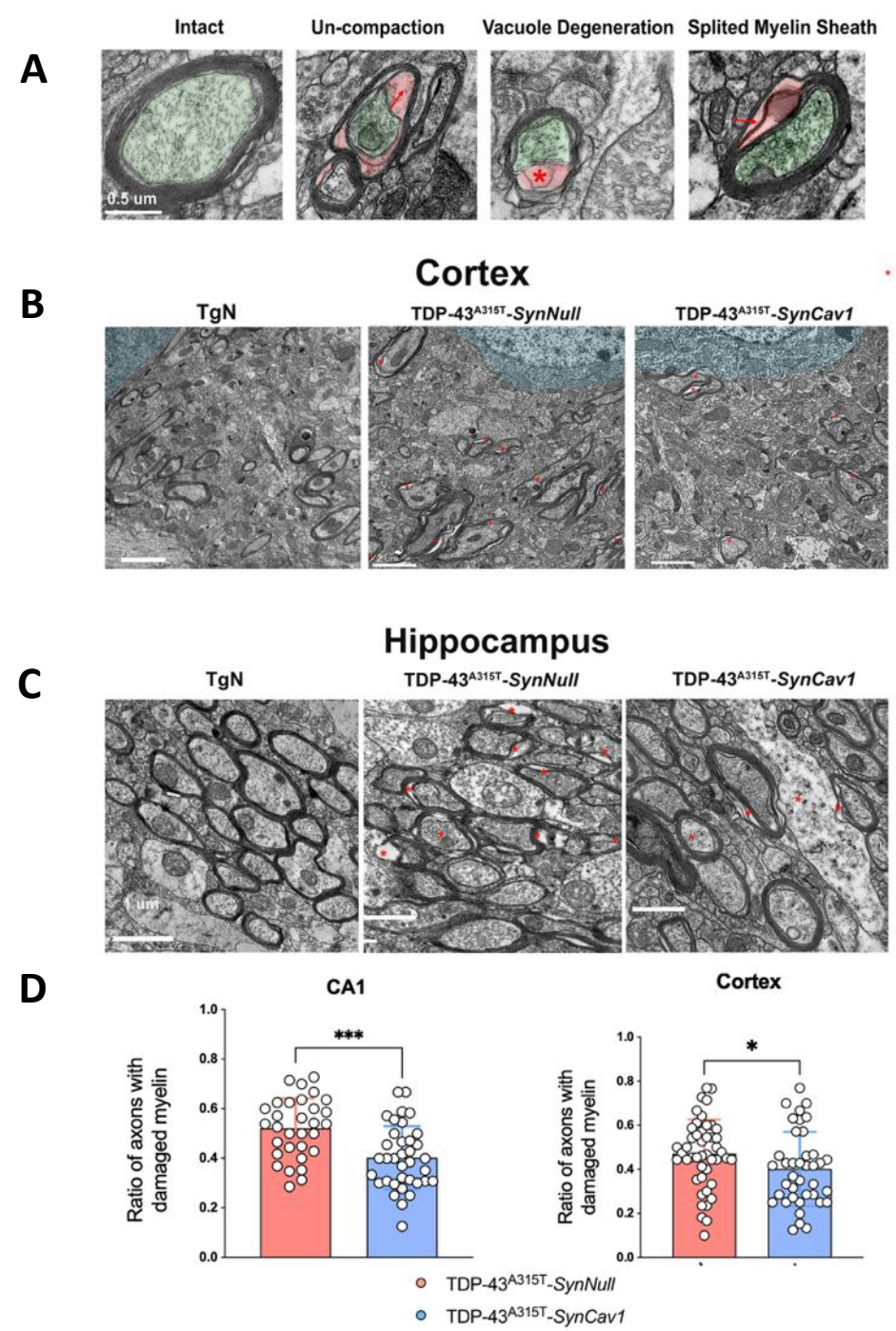

PhP.eB-SynCav1 mitigates axonal damage in the cortex and hippocampus of TDP-43^{A315T} mice (6 m)

(A) Representative image of various myelin sheath abnormalities.

(B) Representative EM images of **cortical layer V** showing myelin sheath damage in TDP-43 mutant mice.

(C) Representative images of axons in the **hippocampus** and quantification of damaged axons.

(D) Quantification of damaged axons shown in (A).

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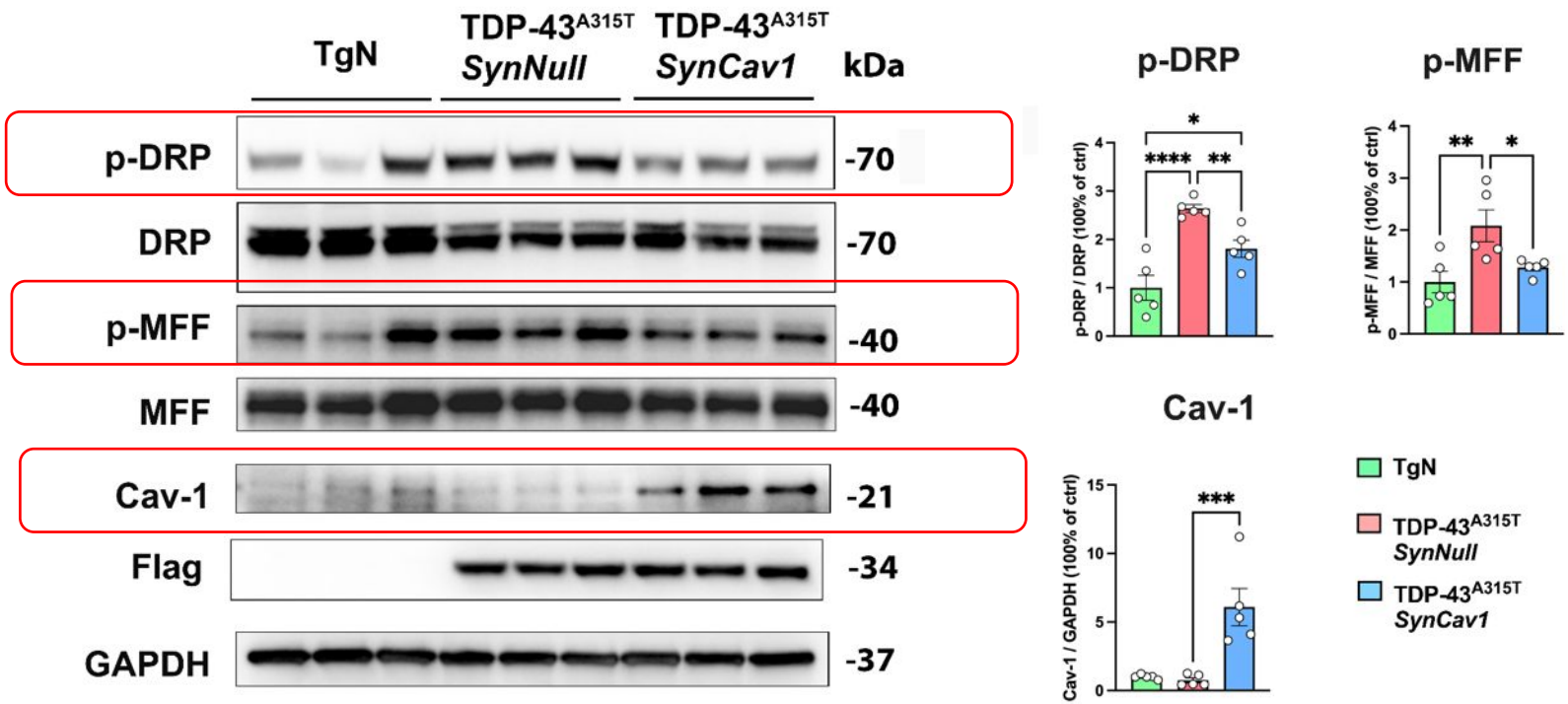
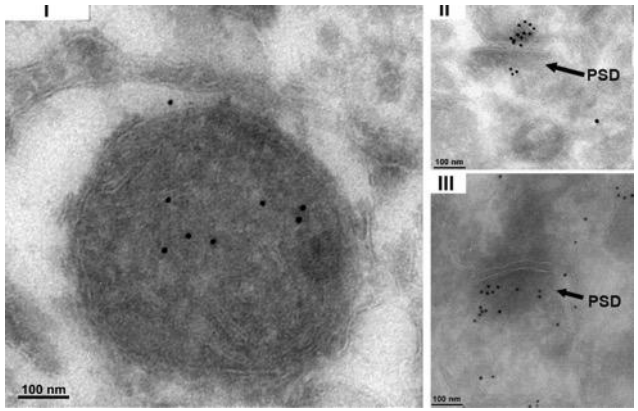
PhP.eB-SynCav1 inhibits mitochondrial hyper-fission signaling in TDP-43^{A315T} mice (6 m)



Cav-1 Immunogold



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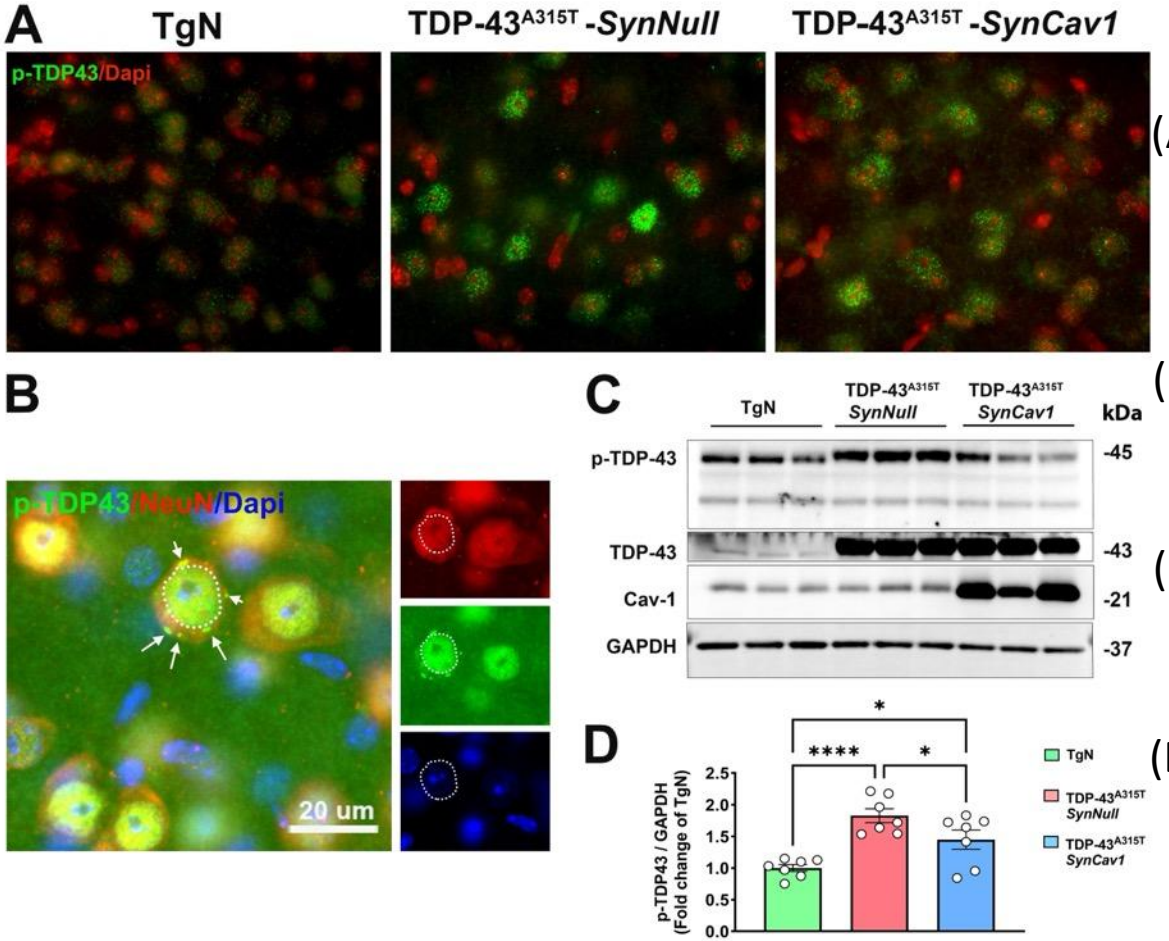
PhP.eB-SynCav1 reduces phosphorylated expression in TDP-43^{A315T} mice (6 m)



(A) Increased p-TDP-43 in both TDP-43-SynNull & TDP-43-SynCav1 mice relative to TgN

(B) Layer V neurons exhibit pathological cytoplasmic p-TDP-43 aggregates

(C) P-TDP-43 expression was significantly reduced in TDP43-SynCav1 mice

(D) Quantification of the IB shown in **C**.

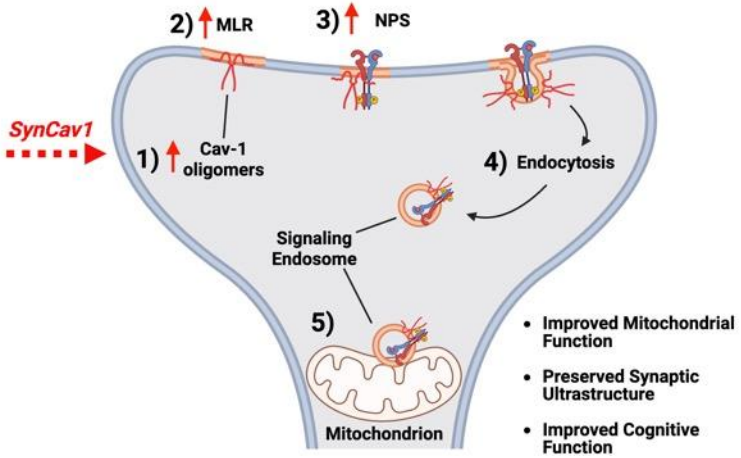
Under Revision
Alzheimer's & Dementia

Summary and Conclusion

- Hippocampal ***AAV9-SynCav1*** delivery *to* ***symptomatic*** *PSAPP & APPKI* mice preserved memory recall at 12 m

- Systemic ***PhP.eB-SynCav1-WPRE*** delivery to post-symptomatic male and female *hSOD1*^{G93A} mice
  - achieved much broader neuronal tropism and CNS distribution
  - improved motor function & extended survival

- Systemic ***PhP.eB-SynCav1-WPRE*** delivery to *TDP-43*^{A315T} mice
  - Preserved cognitive function, synaptic & myelin ultrastructure
  - Preserved mitochondrial morphology and mitigated mitochondrial hyper-fission signaling



SynCav1 *may serve as a novel gene therapy for AD, ALS & FTD and other forms of neurodegeneration due to unknown etiology*

FDA recently approved the *SynCav1* IND submission for FIH Clinical Trial for ALS

Translational Relevance of Systemic Gene Delivery for CNS Disorders

GENE THERAPY

An AAV capsid reprogrammed to bind human transferrin receptor mediates brain-wide gene delivery

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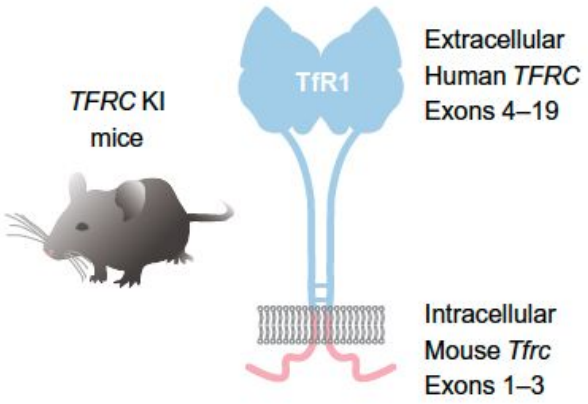

Deverman Lab (*Science* 384, 2024)

- AAV9-derived B1-hTFR1 binds human transferrin receptor (TfR1) on BBB
- 40-50x greater transgene expression than AAV9
- Future Direction:
  - Cross mice expressing TfR1 (TFRC KI mice) with with AD and/or ALS mouse models
  - Test *B1-hTFR1-SynCav1* biodistribution, expression, mitochondrial function, motor and cognitive function in these models
  - CMC manufacturing for GMP-like material for IND-enabling GLP dose escalation & toxicology studies

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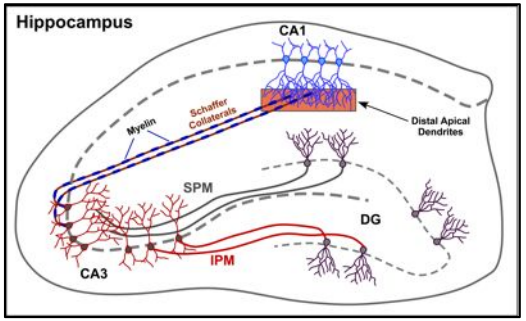
Funding since 2009:

1) VA CDA-2 (CNS Aging)
2) VA Merit Award (CNS Aging & cholesterol)
3) NINDS (TBI)
4) DoD (TBI & Tauopathy)
5) NIGM (Anesthetic neurotoxicity)
6) UC TRDRP (TBI & Nicotine)
7) VA SPiRE (ALS & Gene Therapy)
8) VA Merit Award (AD & Gene Therapy)

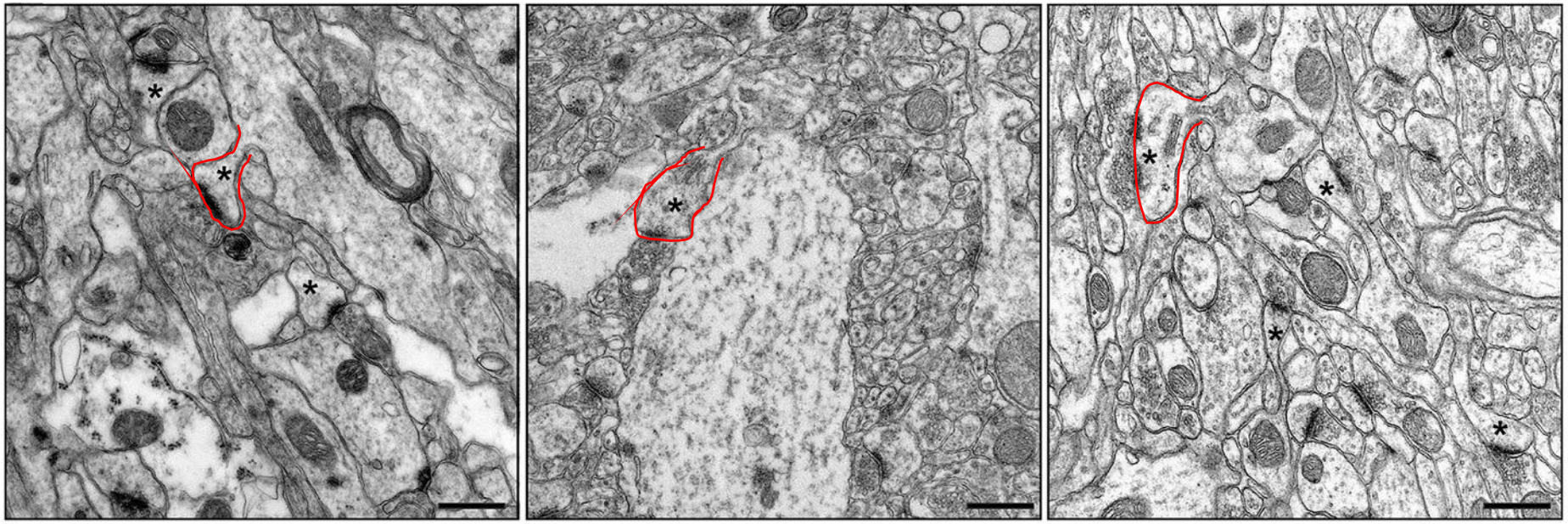
Current Funding:

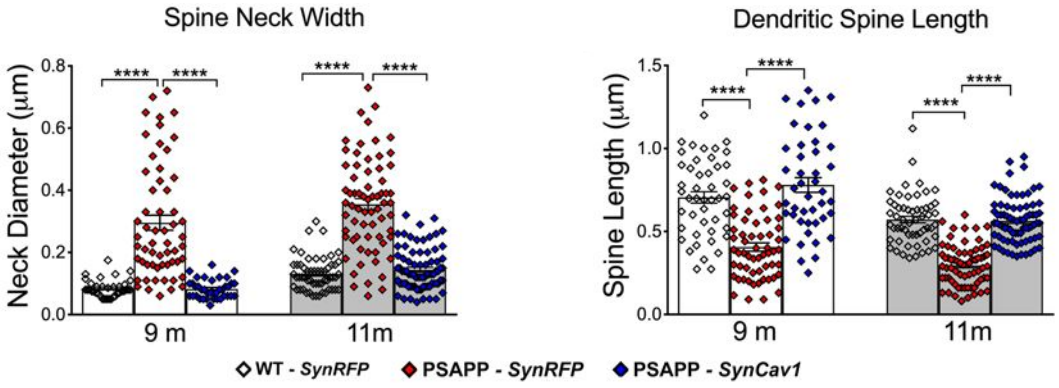
1) VA Merit Award (PhP in ALS)
2) VA Commercialization Supplement
3) CDMRP ALSTDA (DoD) (ALS & Gene Therapy)
4) KL2 (NIH) (Gene therapy for SCI)
5) UCSD GTI Seed Grant

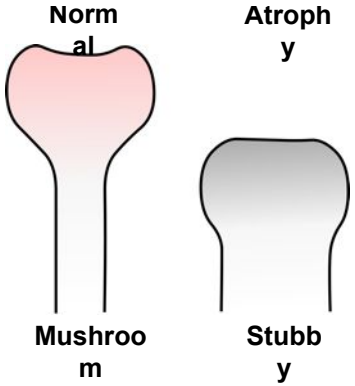
SynCav1 preserves hippocampal dendritic spine morphology in 11 m old PSAPP mice



WT-*SynRFP* (11 m) **PSAPP-*SynRFP* (11 m)** **PSAPP-*SynCav1* (11 m)**

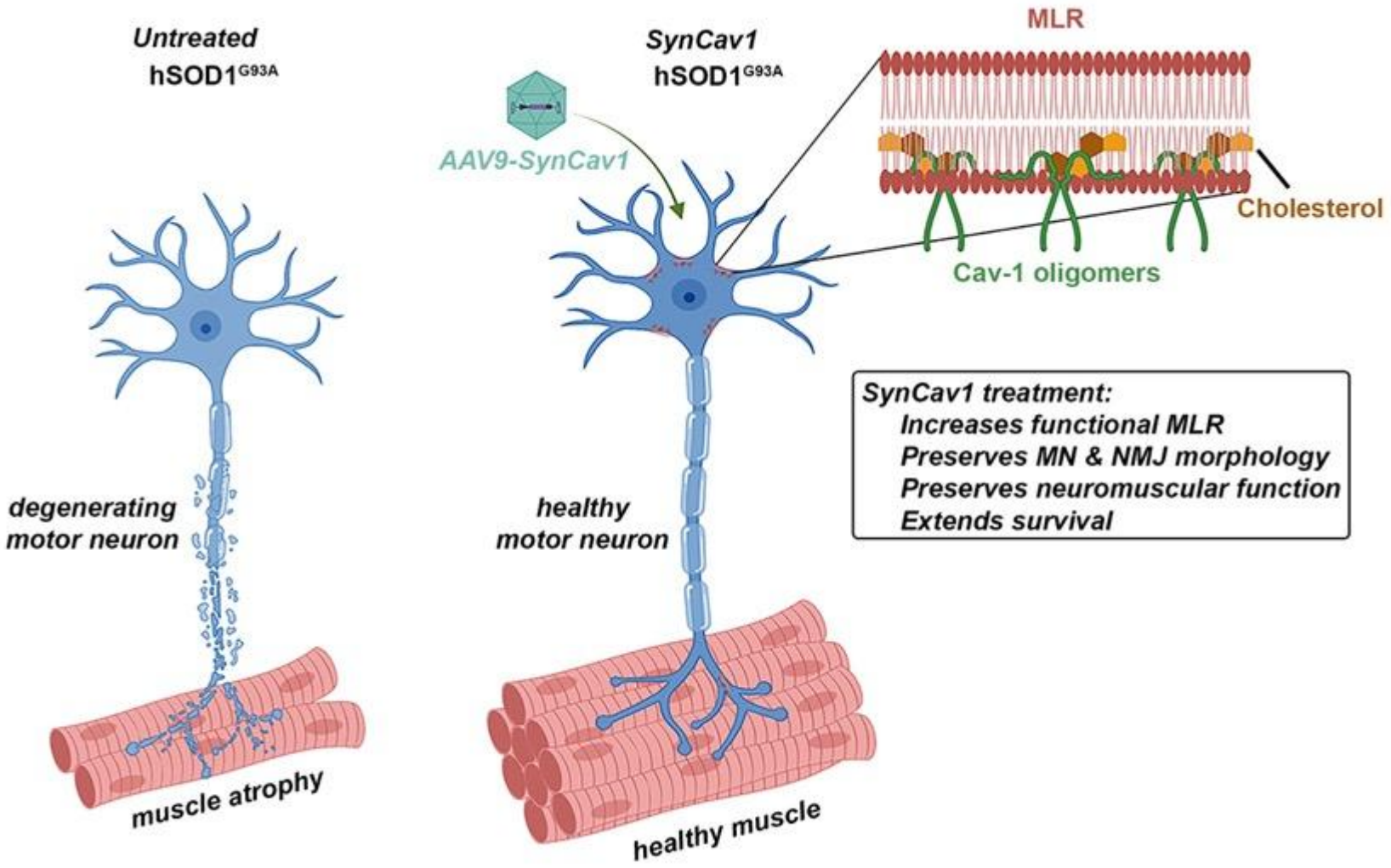






Mol Ther: Meths & Clin Dev 2021

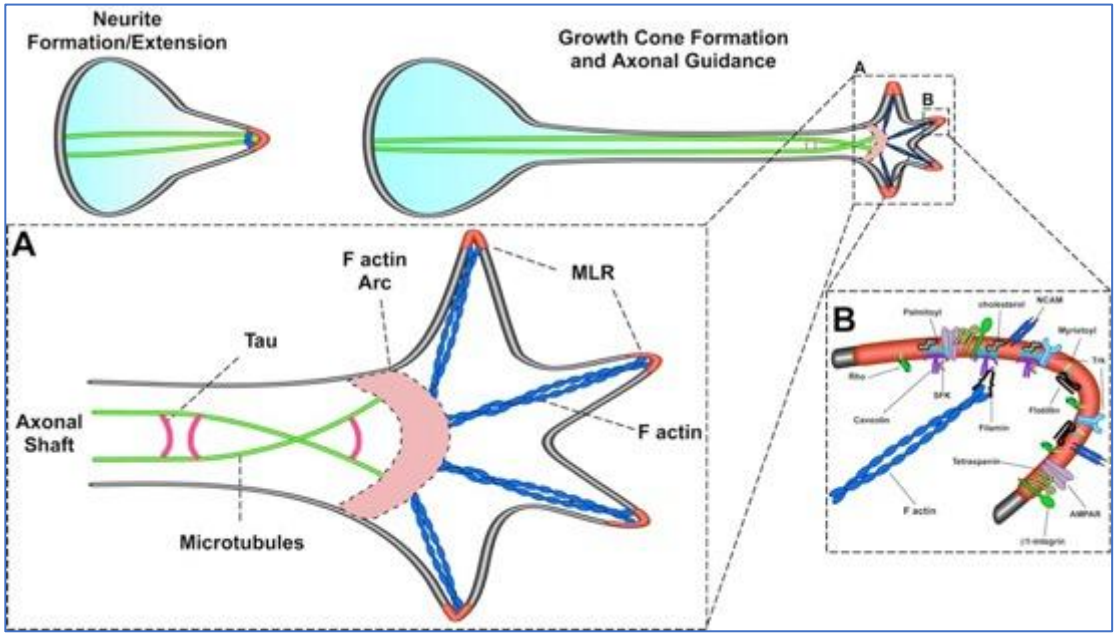
SynCav1 protects MNs in ALS



Theranostics 2022

Potential Target: Membrane/lipid rafts (MLRs)

- MLRs
  - ❑ Cholesterol-enriched signaling microdomains
  - ❑ Establish cell polarity & transduce extracellular cues to the cytoskeletal network
  - ❑ Localize Neurotransmitter & Neurotrophin receptors
  - ❑ Needed for synapse formation & maintenance



Head et al. 2013 *BBA*

MLR establish membrane polarity that translates extracellular cues to promote <u>functional</u> <u>neuroplasticity</u>