ADAM10 activity at the Huntington's Disease presynapse

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Background

ADAM10 is a major protease of the human brain and regulates the strength and activity of the glutamatergic synapse by cleaving a large repertoire of synaptic substrates. In Huntington's Disease (HD), active ADAM10 accumulates at the postsynaptic density and causes proteolysis of the cell adhesion protein Ncadherin, loss of excitatory synaptic contacts and cognitive decline.

Aims and Methods

To identify the molecular mechanisms through which ADAM10 is associated with synaptic dysfunction in HD, we performed a system-level study of ADAM10 interactors in the brain of HD mice. We then integrated our ADAM10 dataset with the HTT interactome curated in HDinHD database (www.hdinhd.org), to look for common protein partners.

Results

We found that ADAM10 and HTT share presynaptic proteins as putative interactors, including Bassoon, Piccolo, ERC2 and liprin-α3. We therefore speculated that ADAM10 may be involved in the organization of synaptic vesicle (SV) pools, and that this function is affected in HD. Here we show that, in the cortex, active ADAM10 forms a complex with Piccolo, a key player in the recycling and maintenance of SVs. Though the level of active ADAM10 increases in the HD cortical presynaptic fraction, we observed reduced ADAM10/Piccolo immunoprecipitation. We also found that SVs density is reduced in HD cortical neurons and depleted SVs stores are restricted to presynaptic regions actively engaged in SVs release and recycling. Notably, restoring ADAM10 activity to control level in the brain of HD mice restored the ADAM10/Piccolo complex and replenished SVs in HD cortical neurons.

Conclusions

We conclude that increased level of active ADAM10 in HD affects the cortical presynaptic terminal through the reduction of SVs pools involved in recycling and release. The results indicate that presynaptic ADAM10 is a relevant component of HD synaptic dysfunction and reinforce the role of the cortex and the presynaptic compartment in HD.

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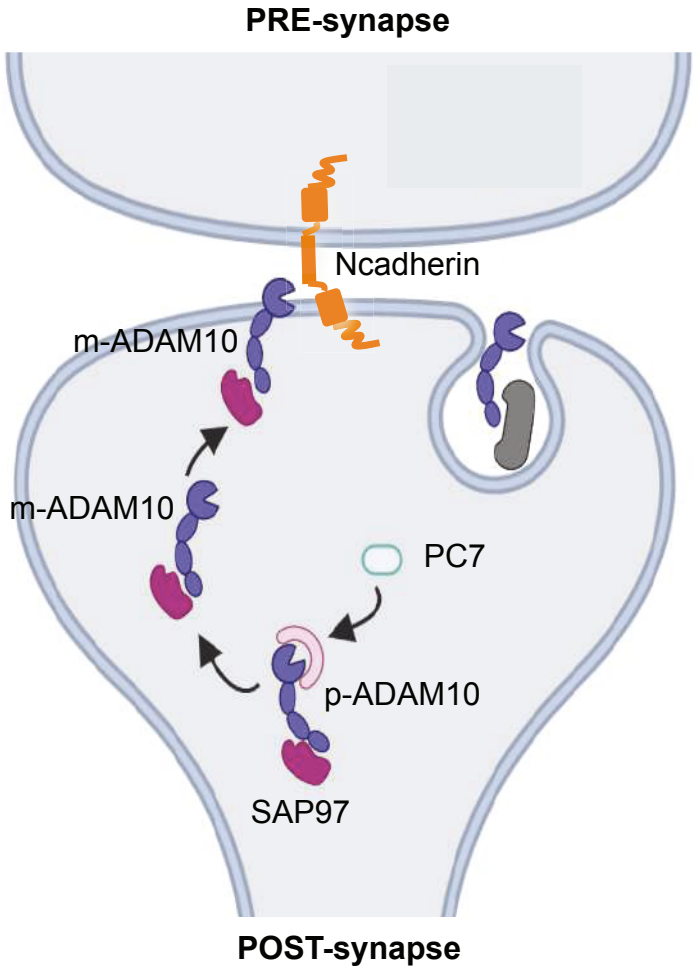
ADAM10 and synaptic dysfunction in HD



ADAM10 at the excitatory synapse

It regulates synaptic structure and function by cleaving cell adhesion proteins at the postsynaptic density (PSD) (*Saftig & Lichtenthaler Prog Neurobiol. 2015*).

ADAM10 in HD

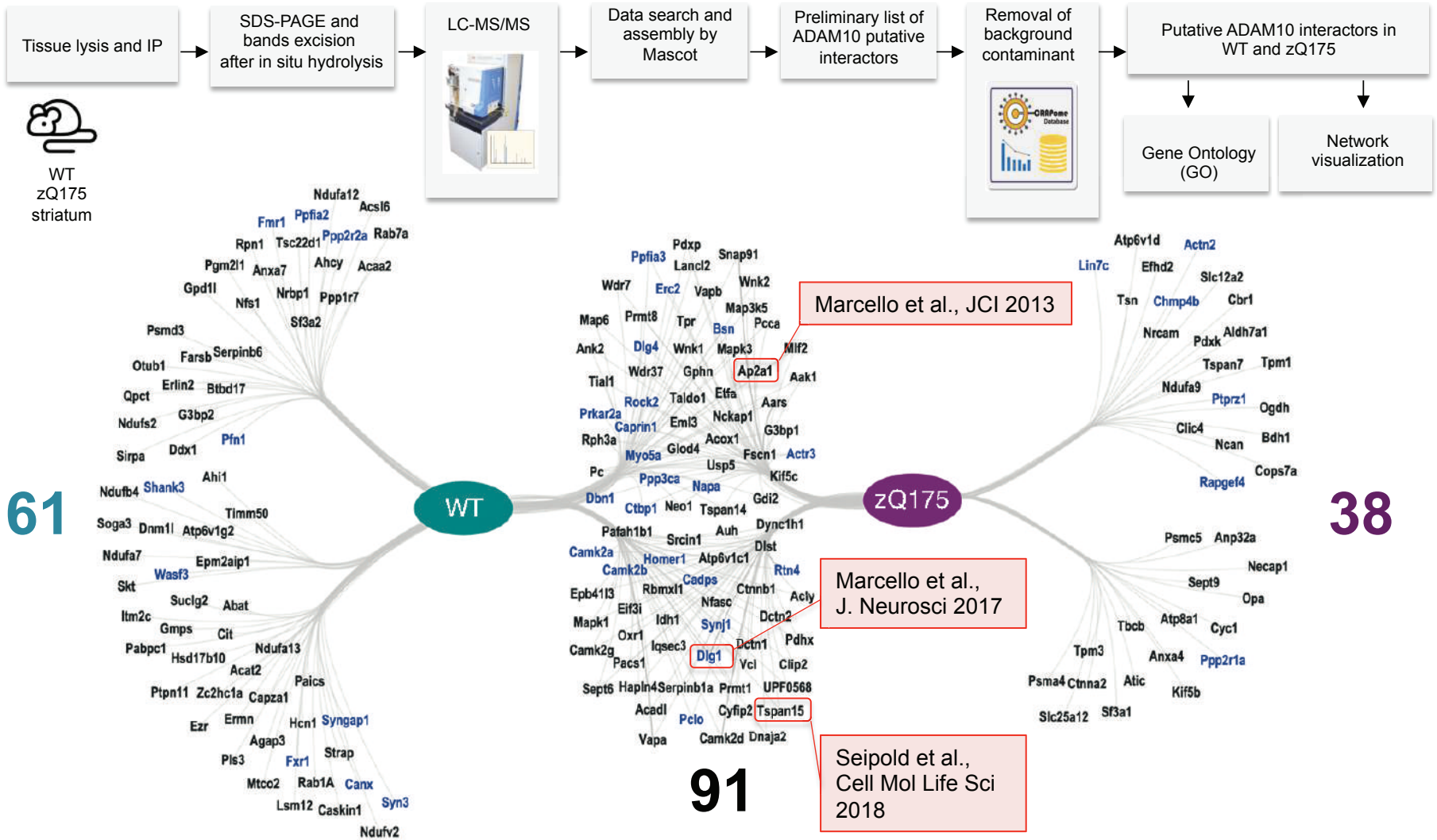
Normal HTT regulates ADAM10 activity and Ncadherin cleavage in the mouse brain (*Lo Sardo et al., Nat Neurosci, 2012*).

Active ADAM10 accumulates at the PSD and causes Ncadherin proteolysis, leading to loss of excitatory synaptic contacts, synapse deficiencies, and cognitive decline in HD mice (*Vezzoli et al., J Clin Invest 2019*).

What about the underlying molecular mechanisms?

To answer this question we adopted a **proteomic approach** to identify ADAM10 interactors in the WT and HD brain.

The ADAM10 interactome is enriched in synaptic proteins



Cytoscape network of ADAM10 interactors in WT and zQ175 striatum: proteins associated by GO with 'Glutamatergic synapse' are indicated in blue

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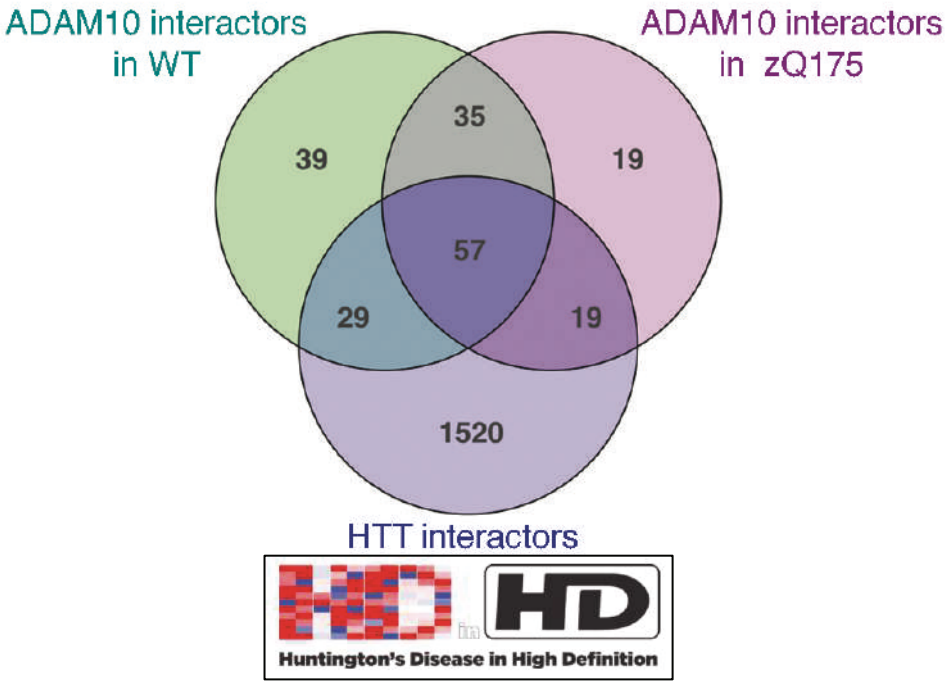
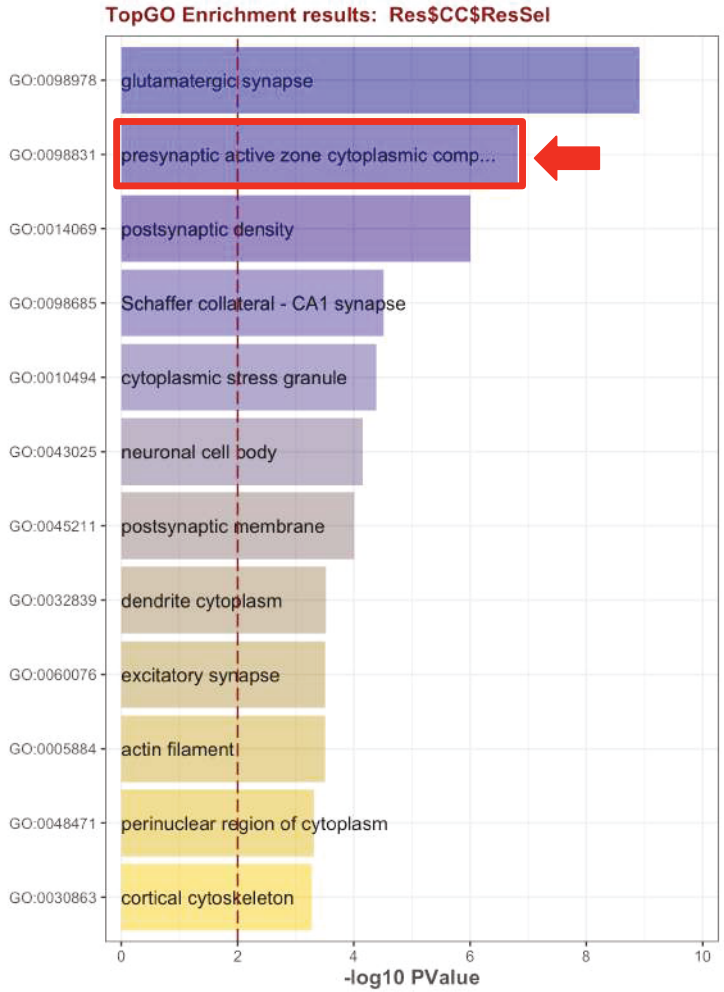
HTT and ADAM10 have common presynaptic partners



Merge between ADAM10 and HTT interactomes of WT and zQ175 mice:
105 common interactors



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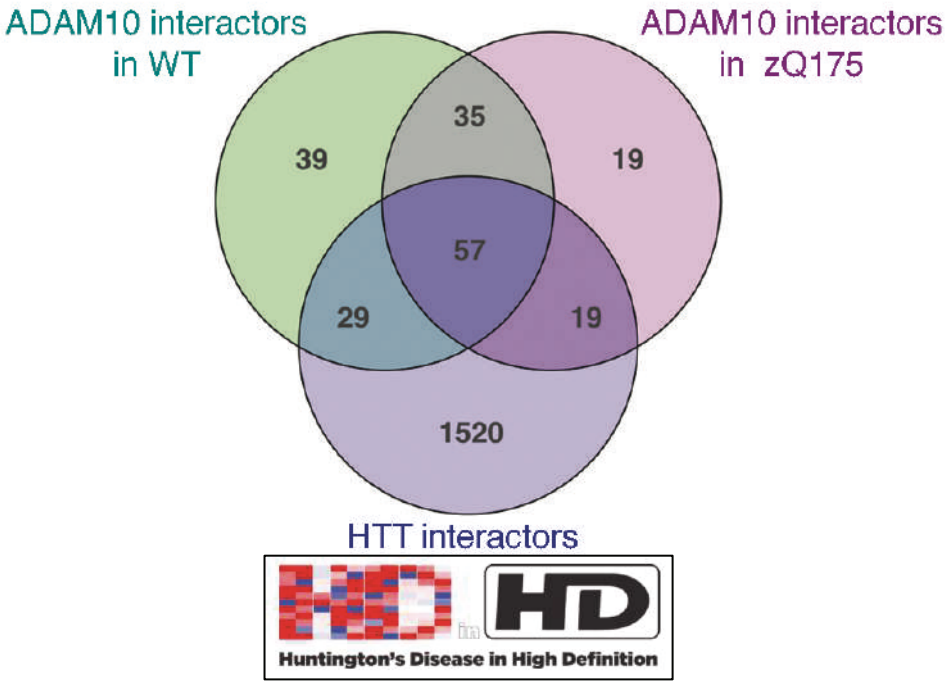

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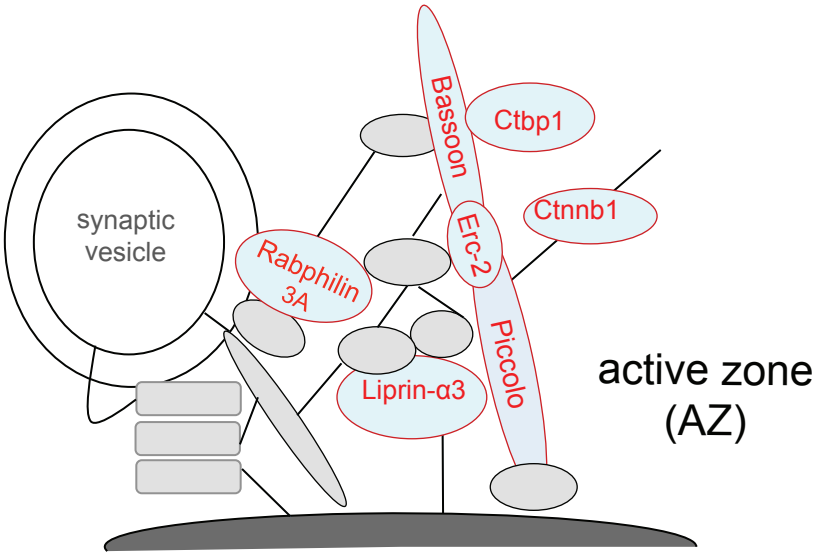

Red, presynaptic proteins shared by HTT and ADAM10 at the AZ

Has ADAM10 a role at the presynaptic terminal?
Is this affected in HD?

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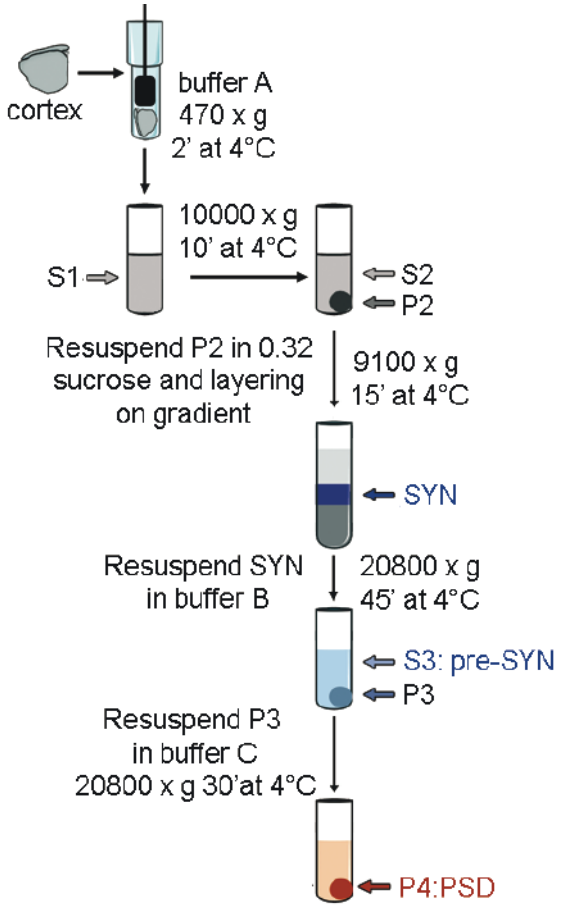
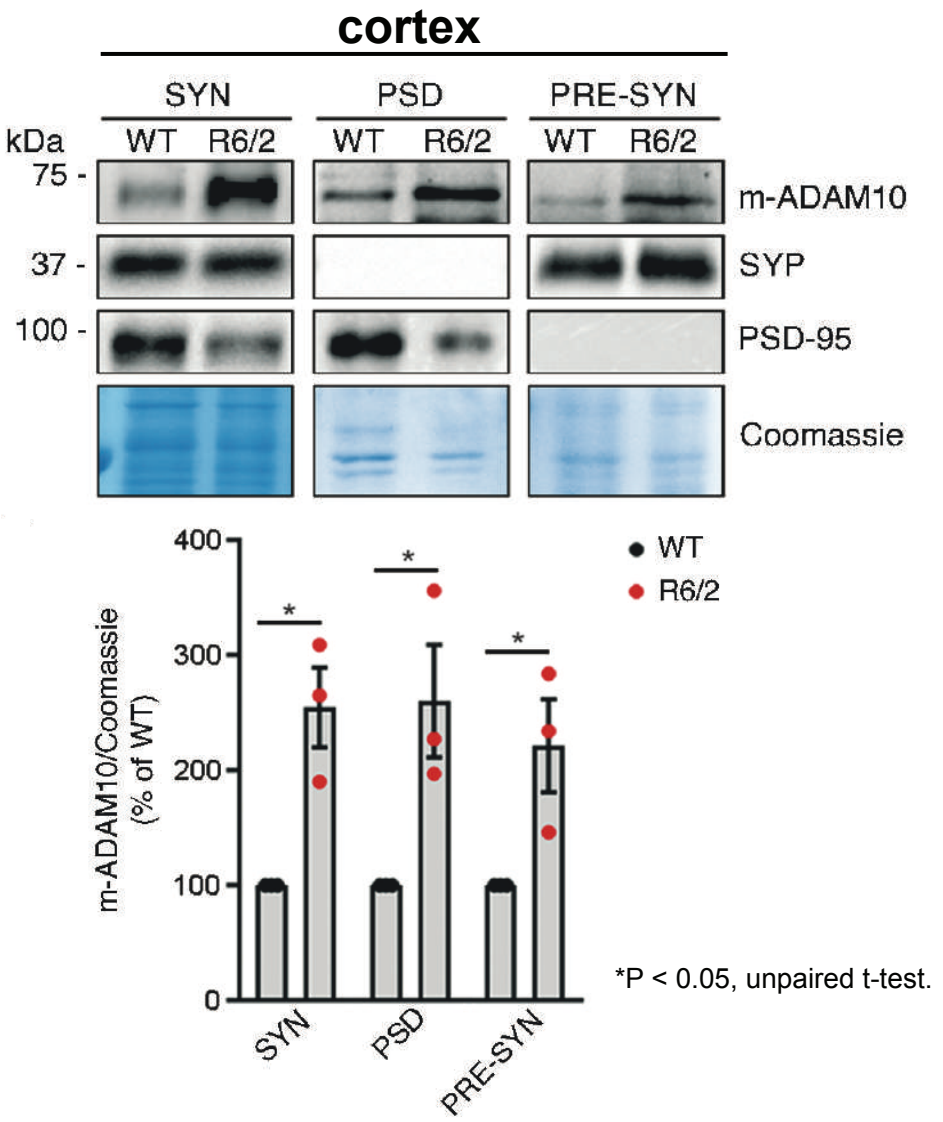
Level of m-ADAM10 increases in HD presynaptic fractions





*P < 0.05, unpaired t-test.

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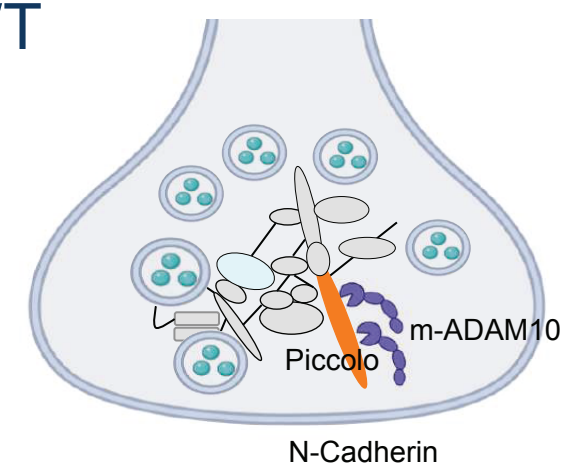
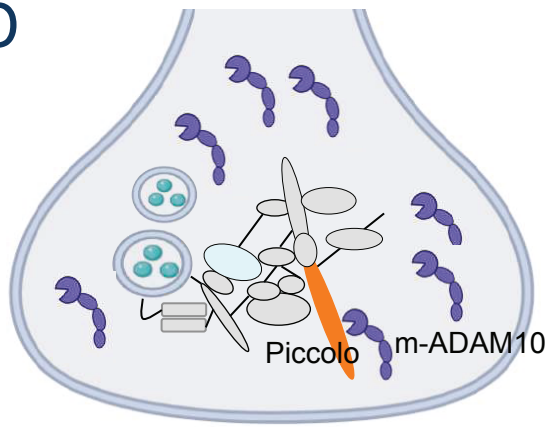
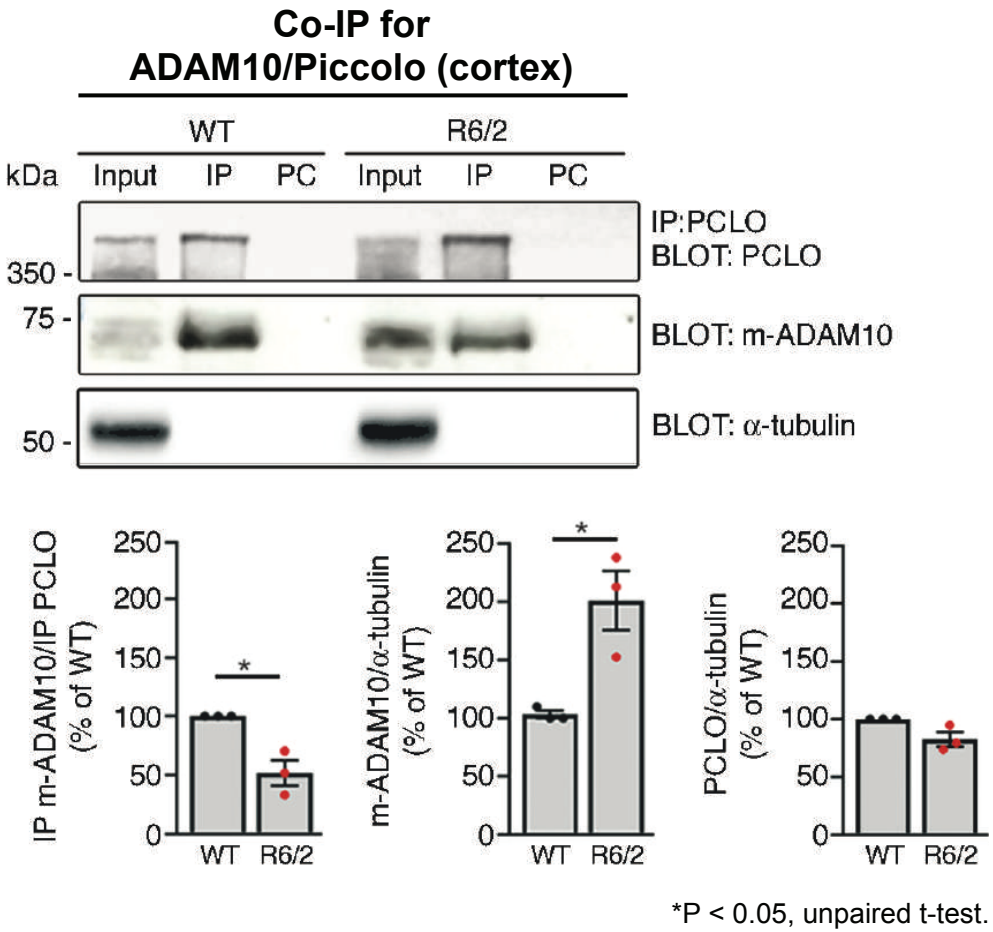
ADAM10 binds to presynaptic protein Piccolo

WT



HD





*P < 0.05, unpaired t-test.

Active ADAM10 binds to presynaptic **protein Piccolo (PCLO)** in the mouse cortex. Although m-ADAM10 increases in HD cortex, its binding to Piccolo is reduced.

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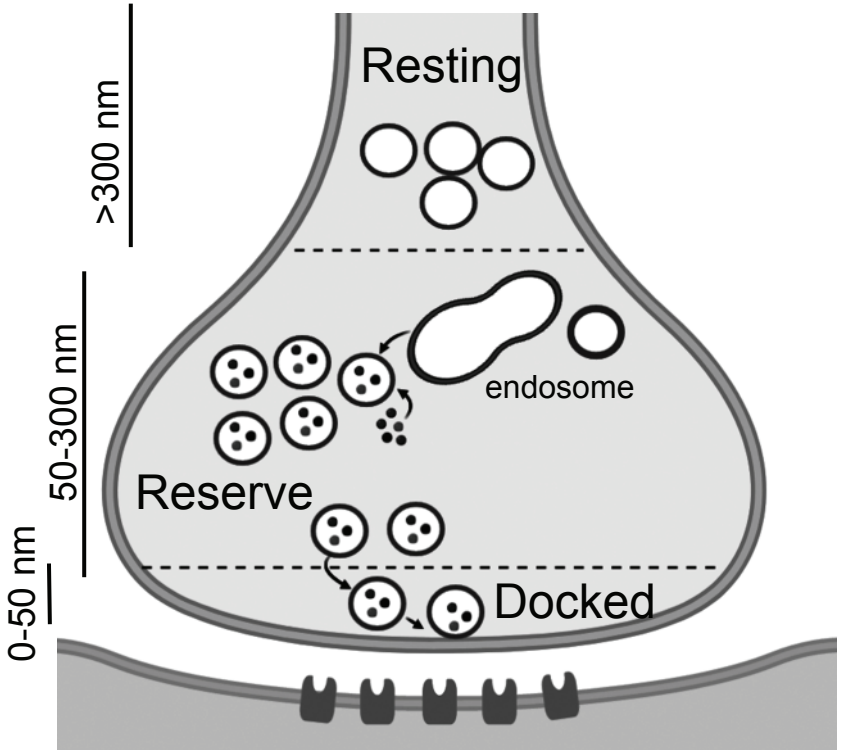
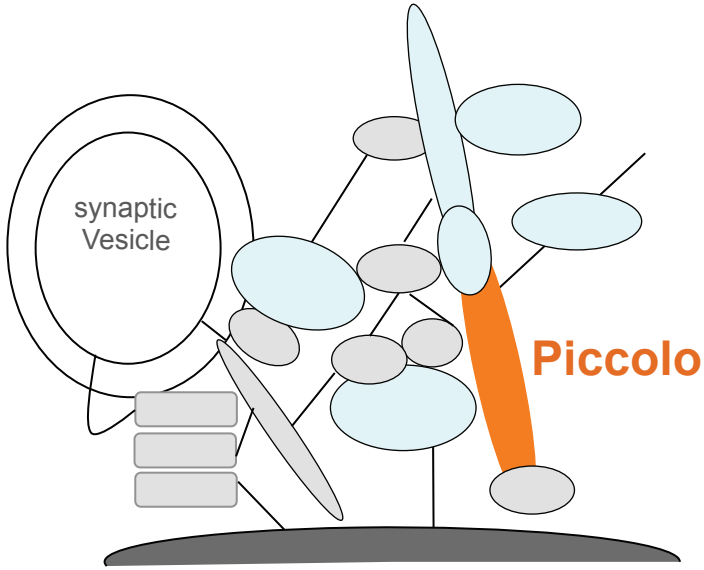
Piccolo's role at the presynaptic terminal

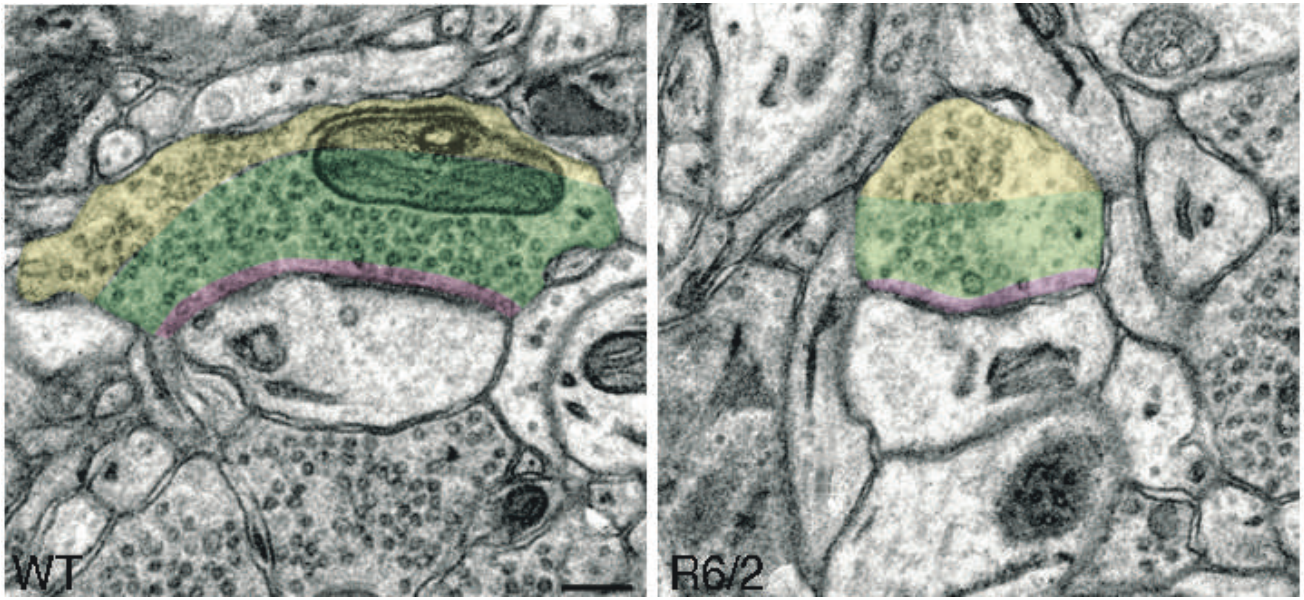


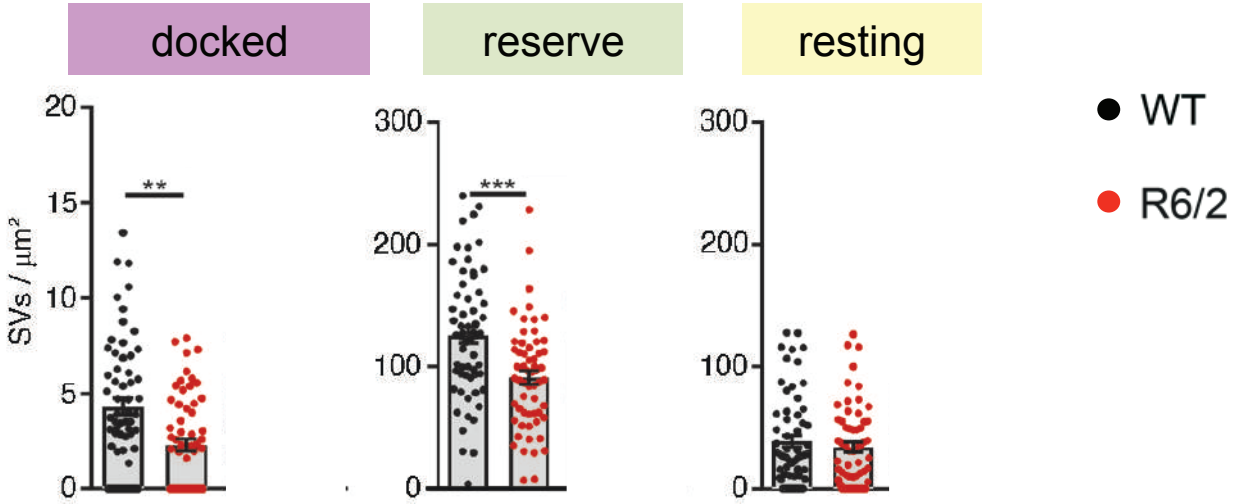


Piccolo loss of function causes decrease in SVs density (*Ackermann et al., Elife 2019*).

Piccolo is implicated in the maintenance of the SVs reserve pool (*Ackermann et al., Elife 2019*), and in the translocation of SVs from the reserve to the docked pool (*Leal-Ortiz et al., J Cell Biol. 2008; Parthier et al., J Physiol. 2018*).

Reduced SVs density in R6/2 cortical neurons





**P<0.01, ***P<0.001, ANOVA and Tukey's multiple comparison test.

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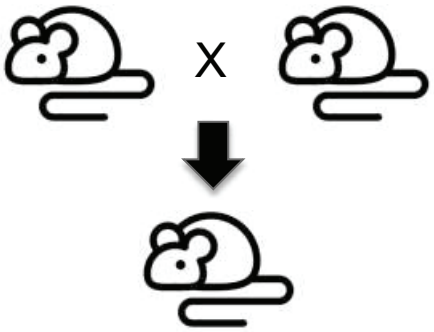
Genetic normalization of ADAM10 prevents reduction of ADAM10/Piccolo complex in the R6/2 cortex

CamKIIαCre;ADAM10$^{Flox/+}$

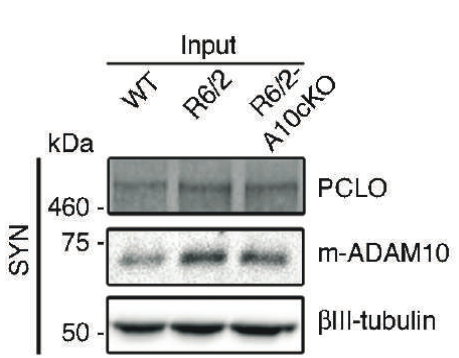
A10cKO **R6/2**

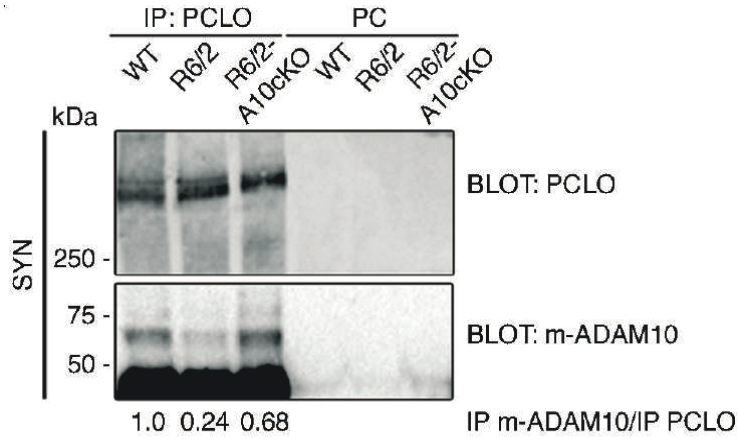


CamKIIαCre;ADAM$^{Flox/+}$;R6/2

R6/2-A10cKO

Co-IP for ADAM10/Piccolo complex in cortical synaptosomes





ADAM10/PCLO complex in **R6/2-A10cKO** mice is restored to the level of **WT** mice

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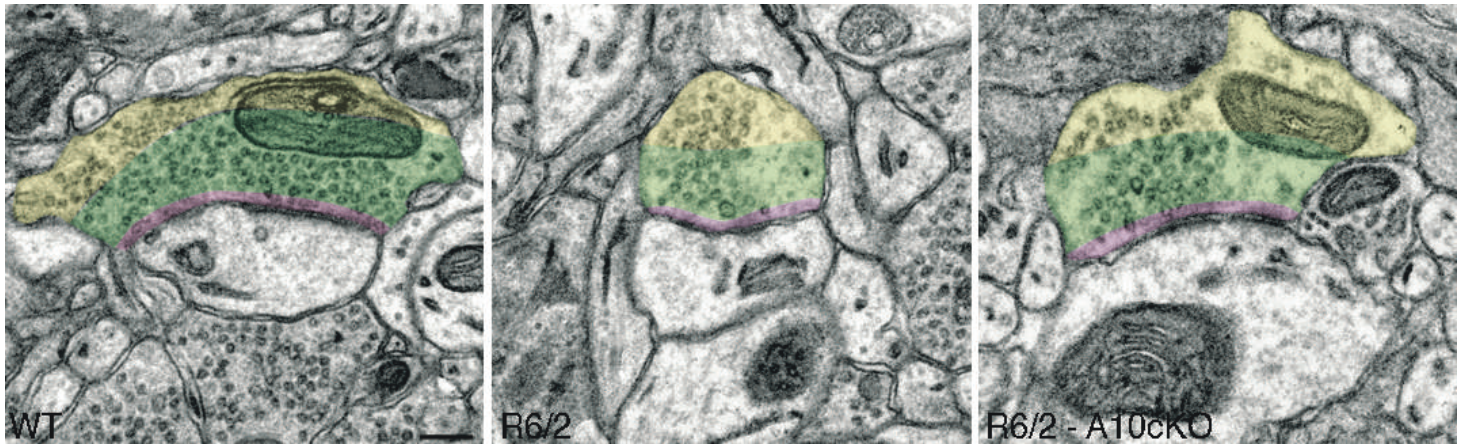
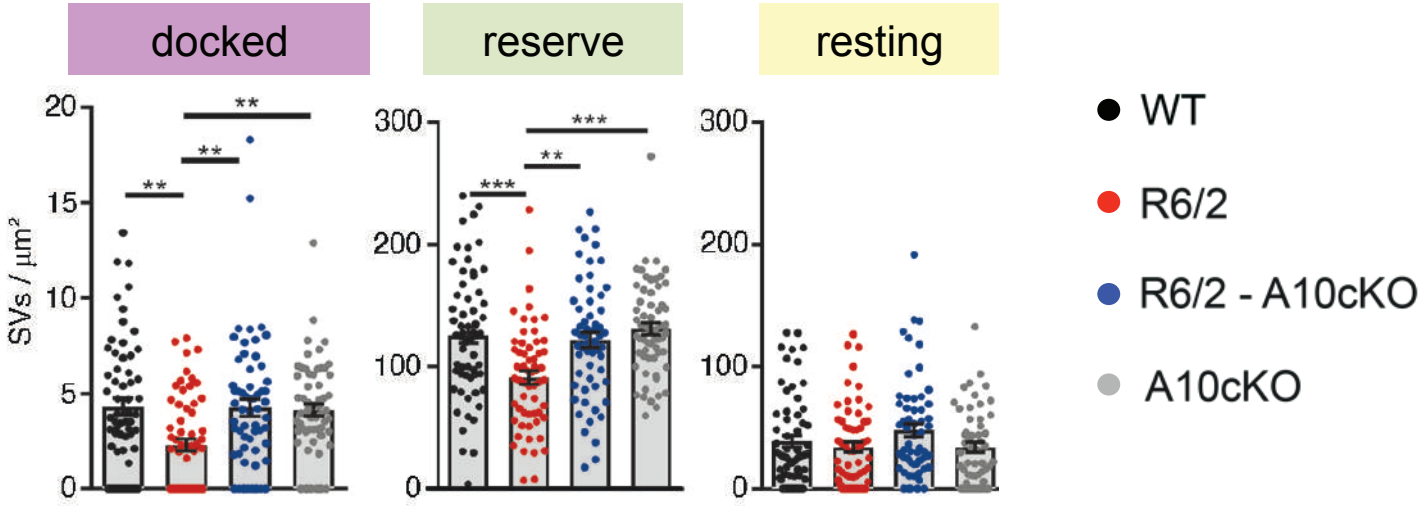
Genetic normalization of ADAM10 prevents morphological presynaptic defects in the R6/2 cortex





The density of SVs in the **docked** and **reserve** pools is restored to WT level in **R6/2-A10cKO**

**P<0.01, ***P<0.001, ANOVA and Tukey's multiple comparison test.

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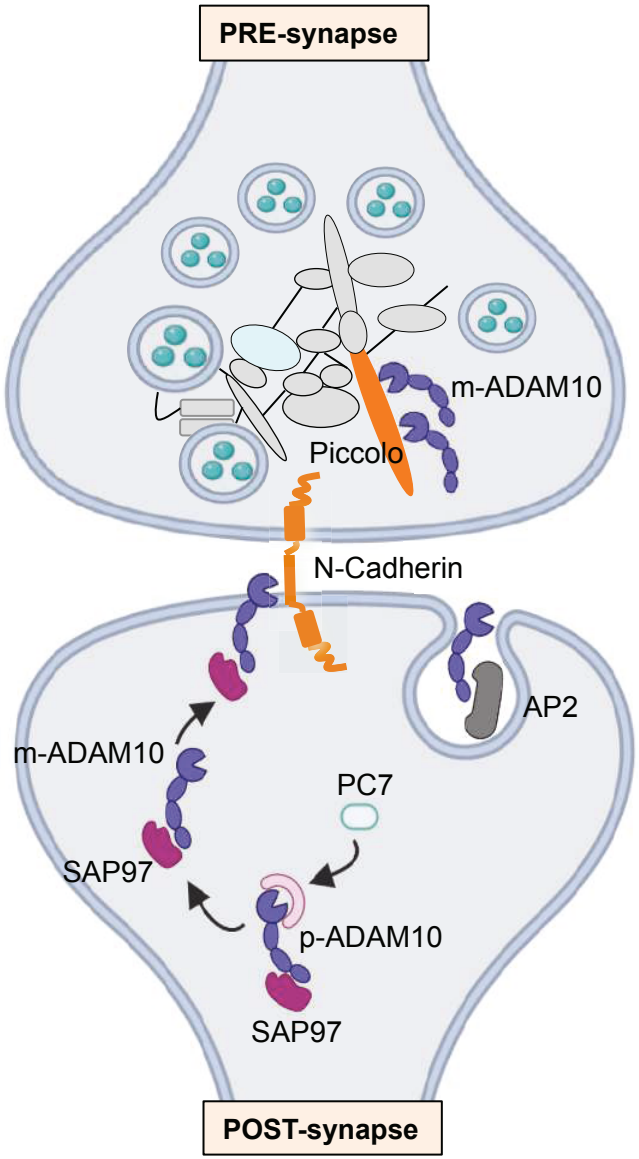
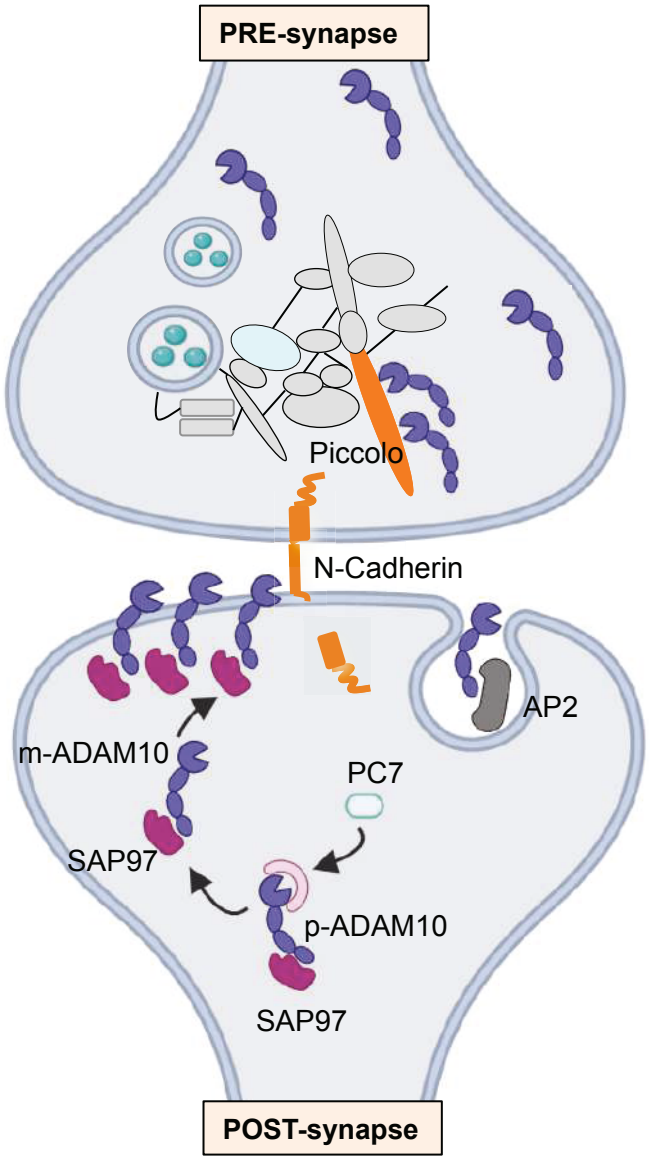
Conclusions – ADAM10 at the WT synapse



In the **presynaptic terminal** m-ADAM10 forms a complex with Piccolo and regulates the formation of SVs pools involved in recycling and release.

In the **postsynaptic terminal** m-ADAM10 regulates synaptic structure and function by cleaving cell adhesion proteins at the postsynaptic density (PSD).

Conclusions – ADAM10 at the HD synapse



Level of m-ADAM10 is increased in HD.

In the **presynaptic terminal** a decrease in the formation of the m-ADAM10/Piccolo complex is found in HD, reducing SVs pools involved in recycling and release.

Increased in **postsynaptic** m-ADAM10 causes synaptic cell adhesion defects in HD through excessive Ncadherin proteolysis.

Our results indicate that presynaptic ADAM10 is a relevant component of HD synaptic dysfunction and reinforce the role of the cortex and the presynaptic compartment in HD.