Genetic Approaches to Tackle Dominantly Inherited Ataxias

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Spinocerebellar Ataxia Type 1 (SCA1)

Dominantly inherited

Affects 1/100,000 people

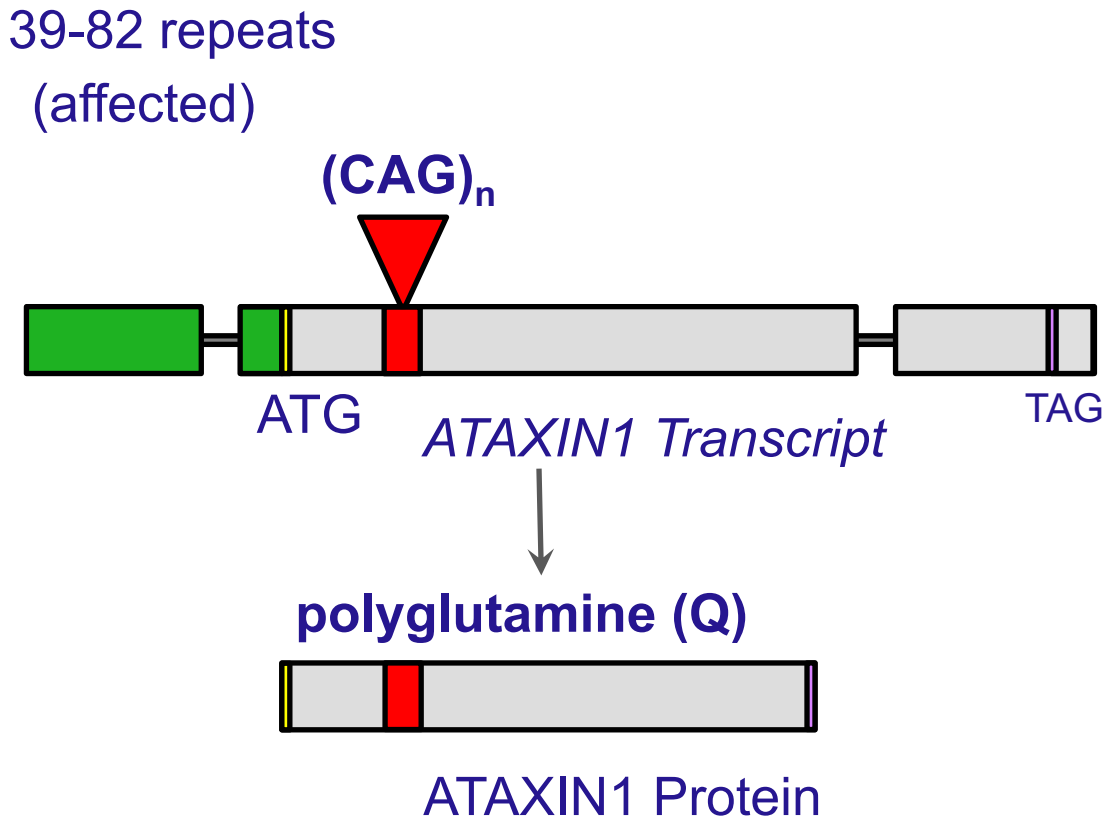
Loss of balance and coordination

Difficulty with swallowing & breathing

Loss of cerebellar Purkinje cell and brainstem neurons

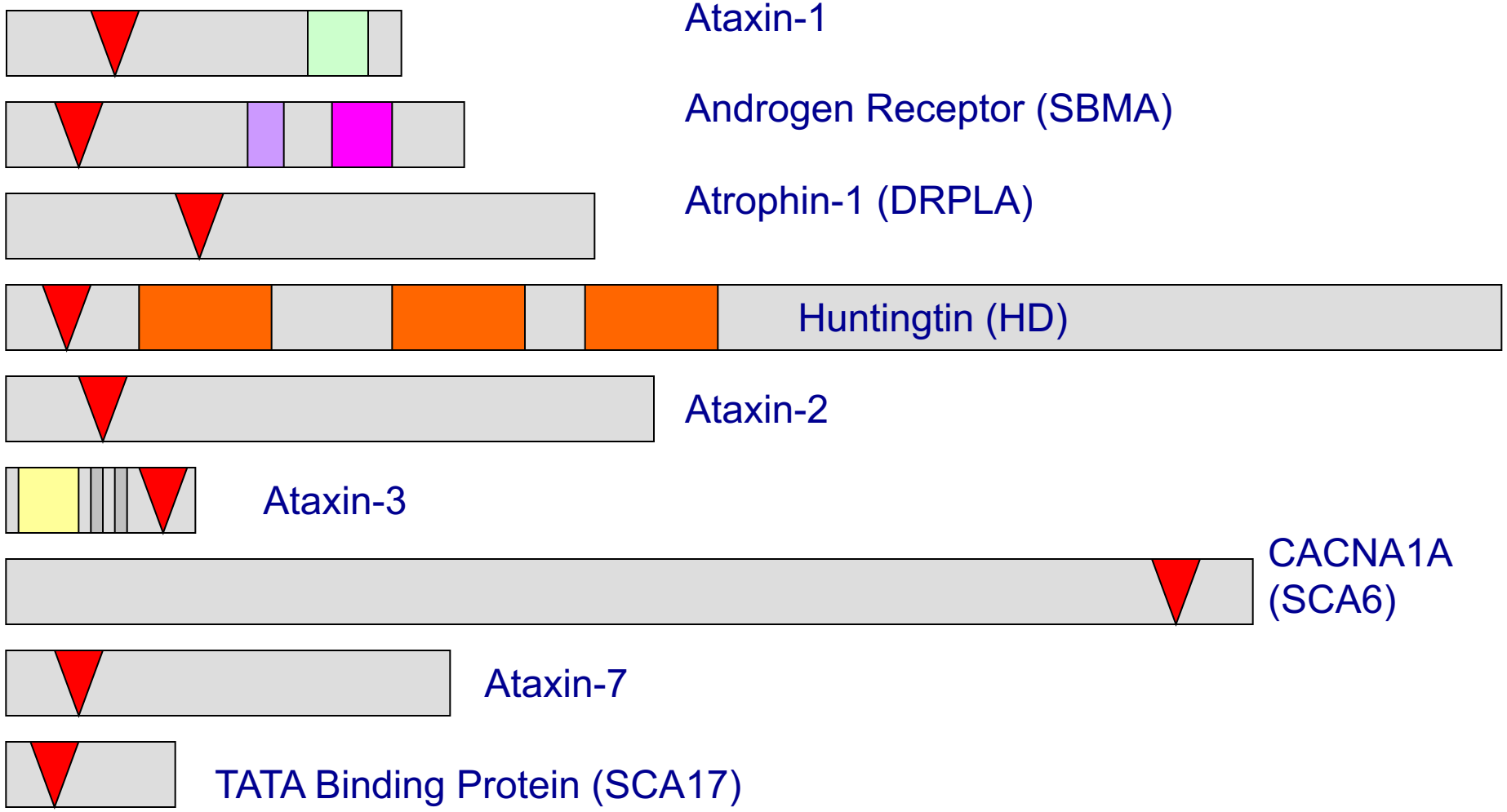
Premature death

SCA1 is caused by expansion of unstable CAG repeats



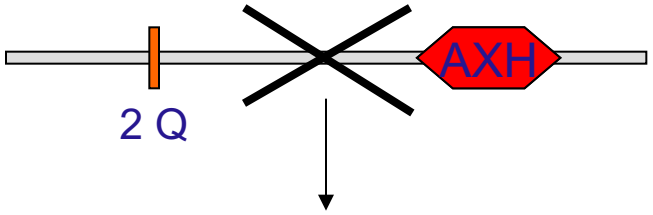
Orr *et al*, *Nat. Genet.*, 1993

SCA1 is one of nine polyglutamine diseases



SCA1 pathogenesis is caused mainly by a gain-of-function mechanism

Atxn1$^{-/-}$



No SCA1 phenotypes, but some neurological deficits

SCA1 pathogenesis is caused mainly by a gain-of-function mechanism

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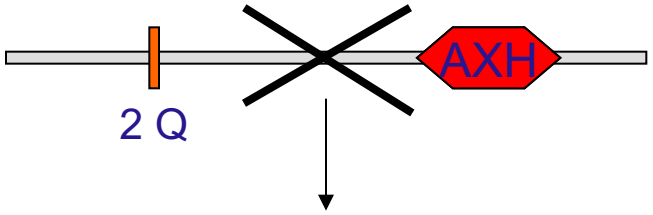

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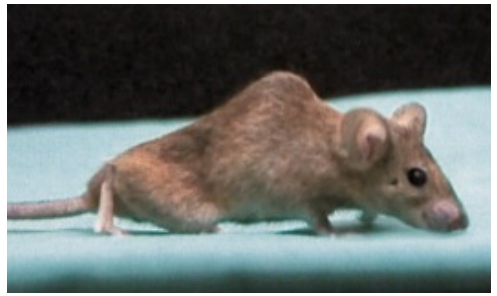
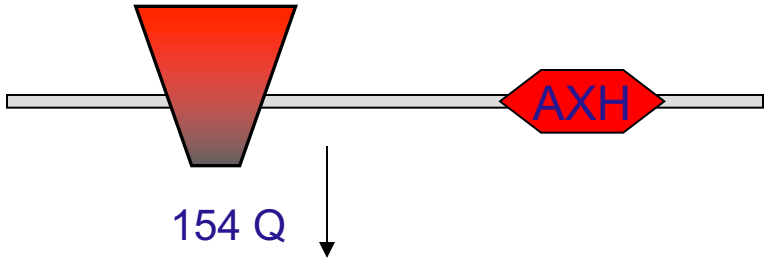


Atxn1$^{154Q/+}$



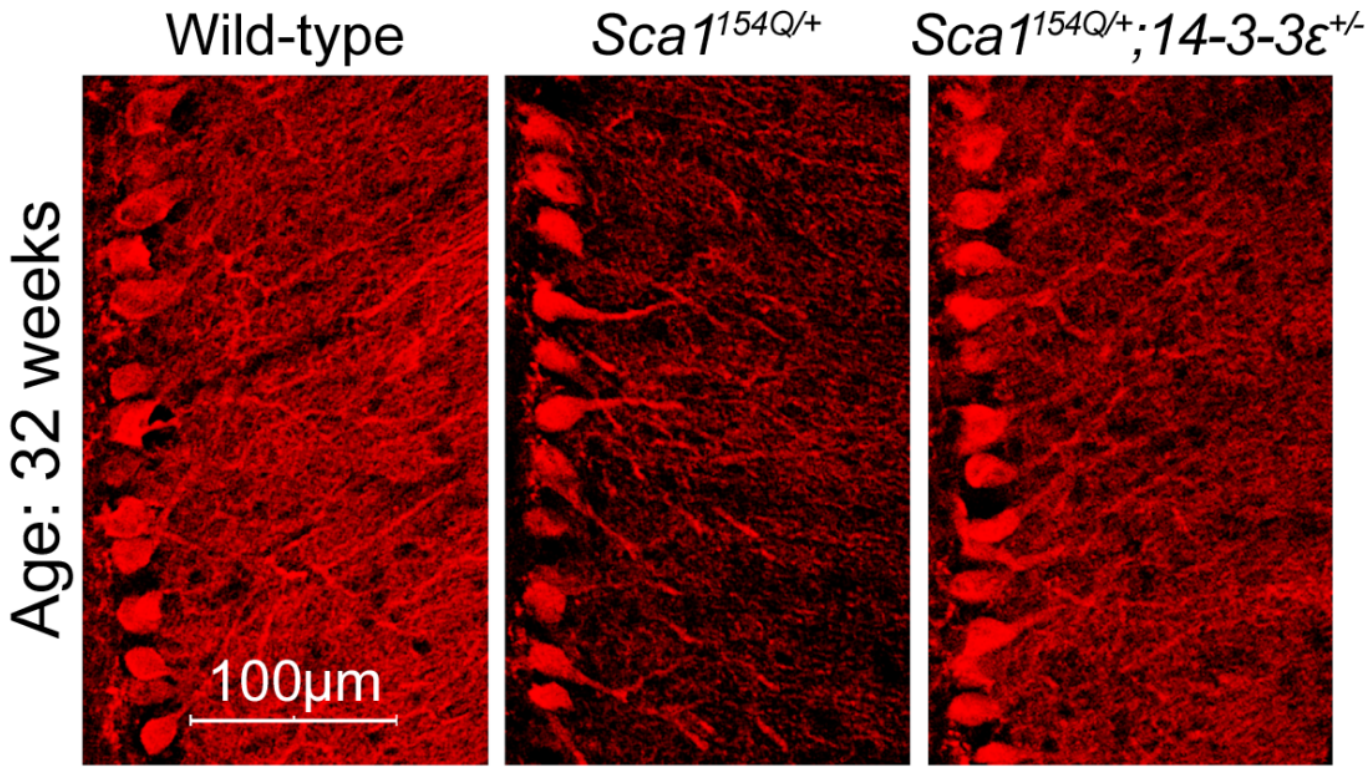
Recapitulate SCA1 phenotypes

- *Ataxia*
- *Brain stem dysfunction*
- *Purkinje cell loss*
- *Reduced lifespan*

Insights from genetic & biochemical studies

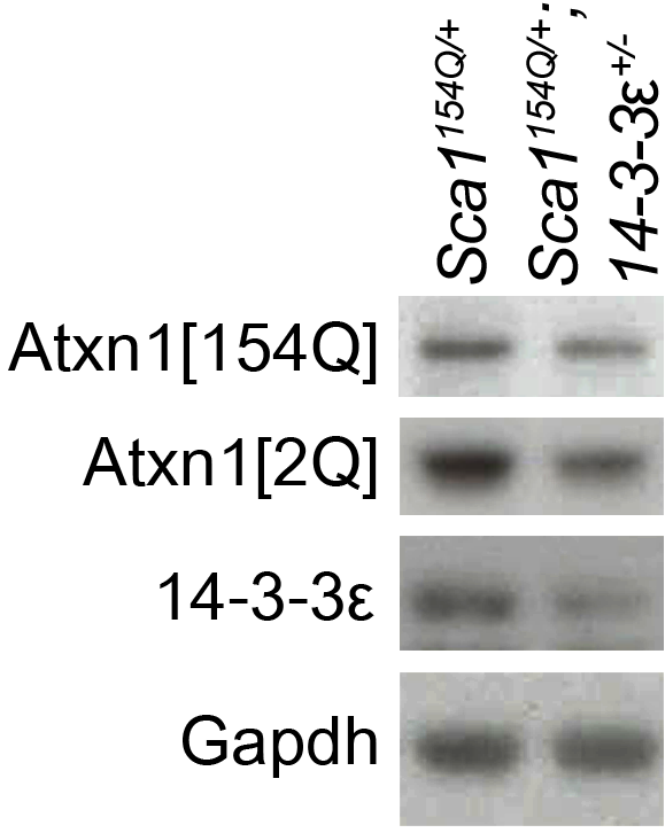
- The glutamine (Q) expansion in Ataxin-1 (ATXN1) makes ATXN1 more stable (resistant to degradation), so its interactions with its protein partners are prolonged and ATXN1 gradually accumulates in cells
- Increased levels of even wild-type ATXN1 cause neurodegeneration
- Phosphorylation at serine 776 in ATXN1 is necessary for SCA1 pathogenesis

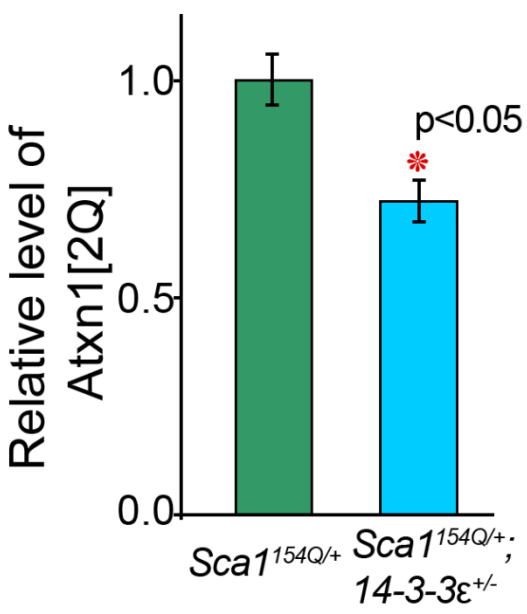
Reducing 14-3-3ε rescues Purkinje cell degeneration in SCA1 mice

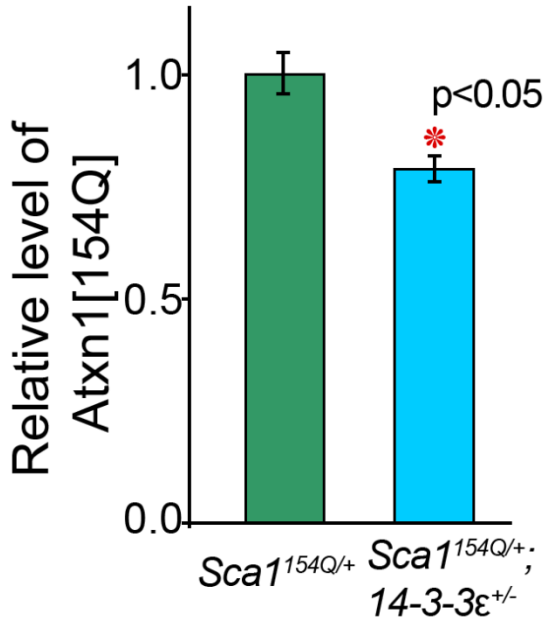


Calbindin immunofluorescence

Reducing 14-3-3ε decreases the levels of ATXN1 in SCA1 mice





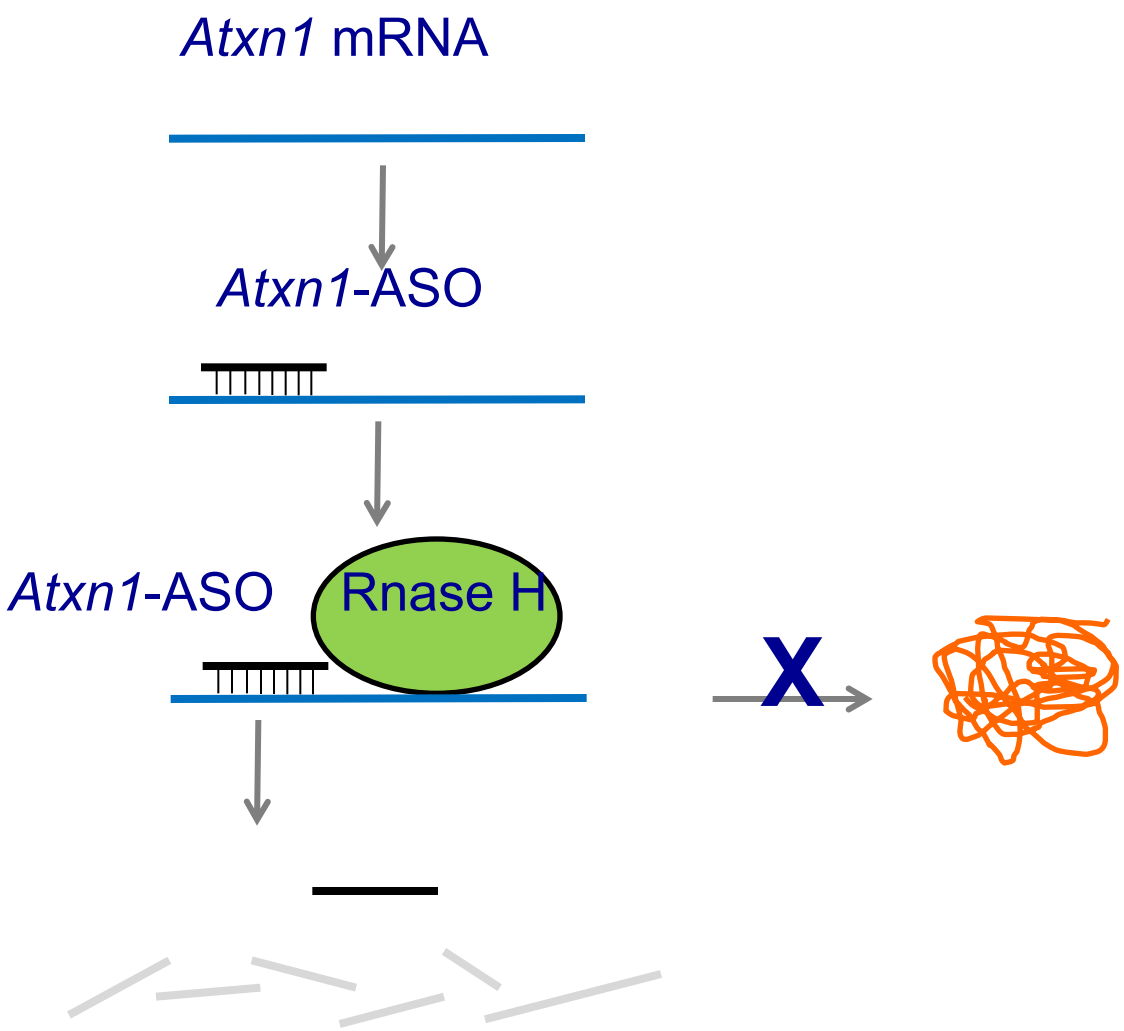


Would lowering ATXN1 levels using a strategy that directly target ATXN1 mRNA (Anti Sense Oligonucleotide—ASO) alleviate symptoms?

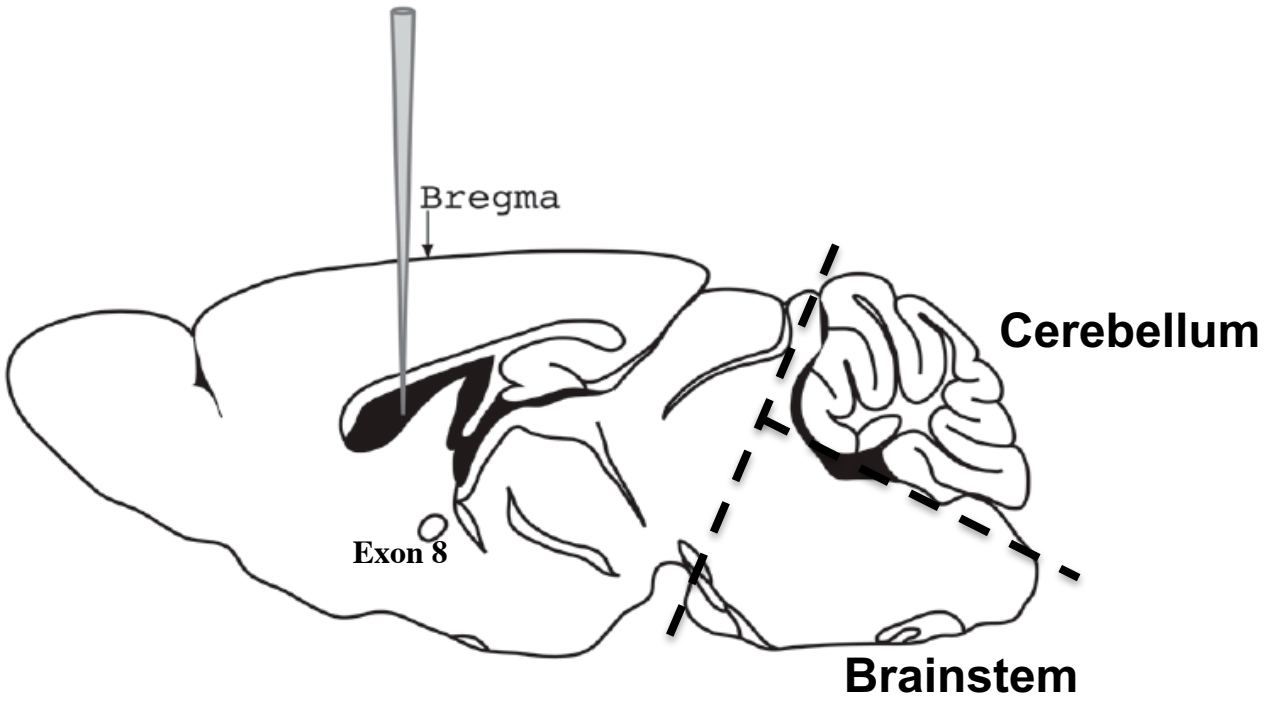
Orr lab in collaboration with Holly Kordasiewicz

IONIS PHARMACEUTICALS

Molecular mechanism of ASO-mediated gene silencing

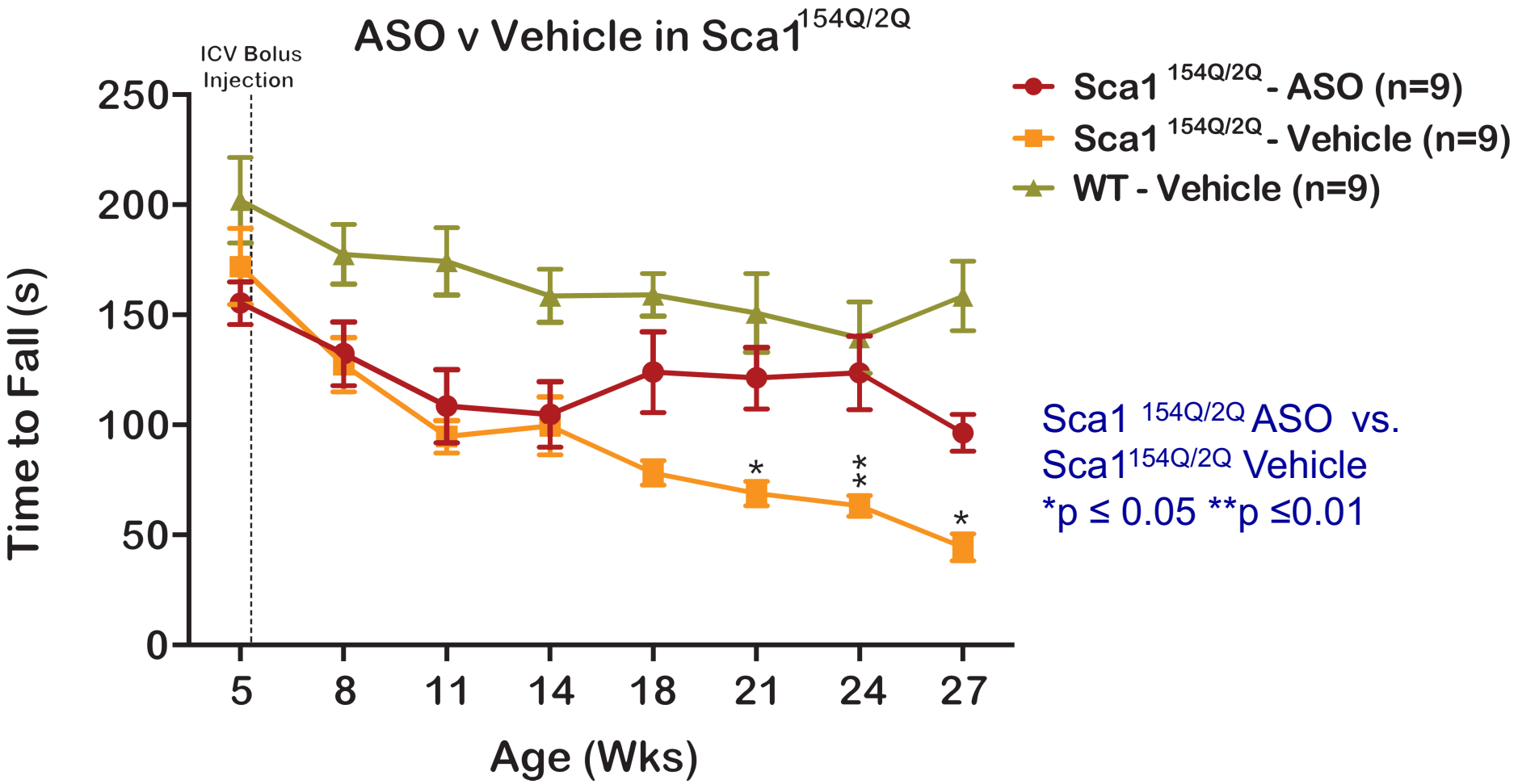


Intraventricular delivery of 500μg Atxn1-ASO to five-week old *Sca1*$^{154Q/+}$ *mice*



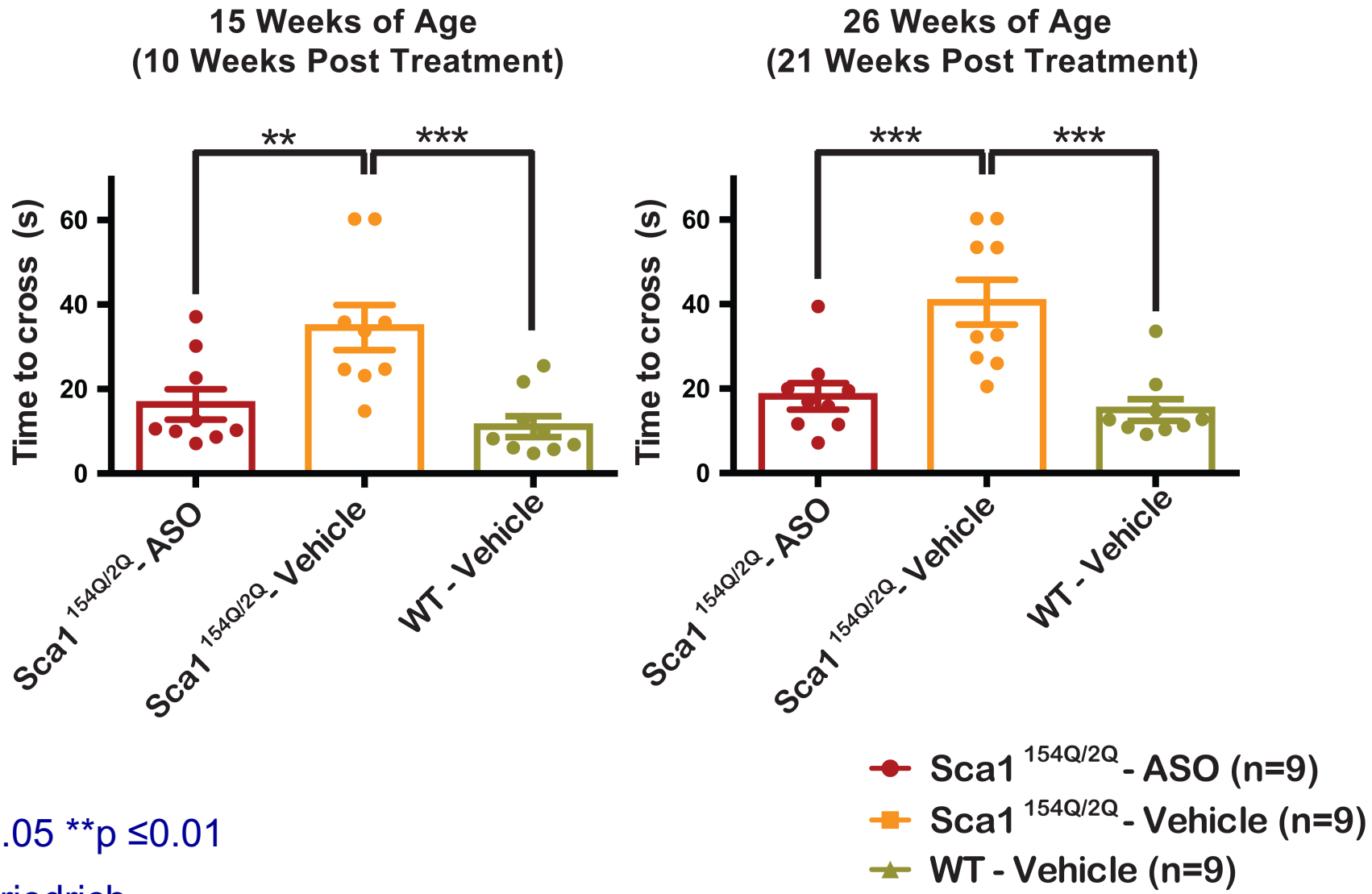
Orr lab

Atxn1-ASO improves rotating rod performance in *Sca1*$^{154Q/+}$ *mice*



Sca1 $^{154Q/2Q}$ ASO vs.
Sca1$^{154Q/2Q}$ Vehicle
*p ≤ 0.05 **p ≤0.01

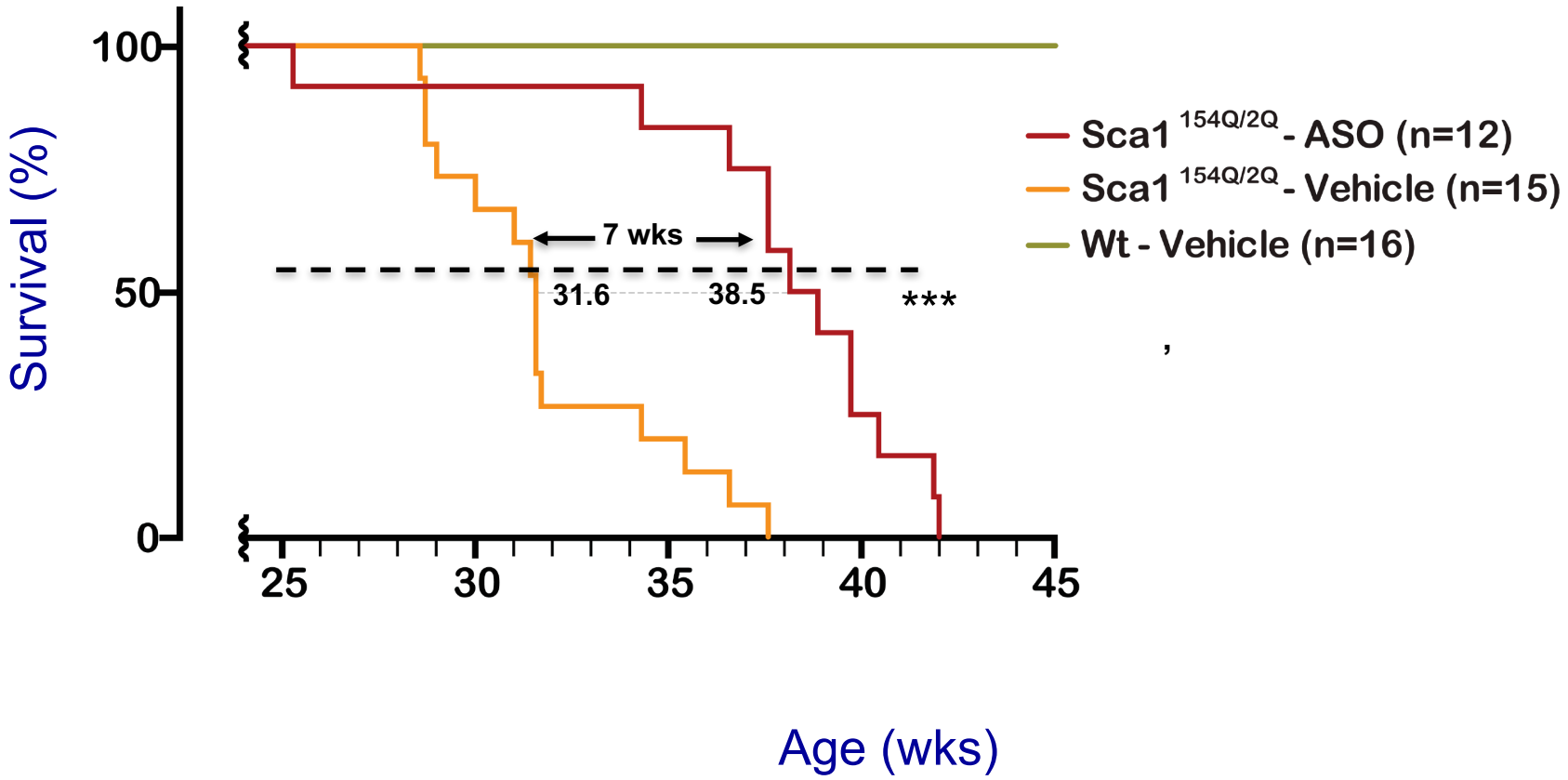
Atxn1-ASO improves beam corssing in *Sca1*$^{154Q/+}$ *mice*



*p ≤ 0.05 **p ≤0.01

Jill Friedrich

Atxn1-ASO improves survival of *Sca1*$^{154Q/+}$ *mice*



***p=0.0001

Jill Friedrich

ASO therapy advantages and limitations

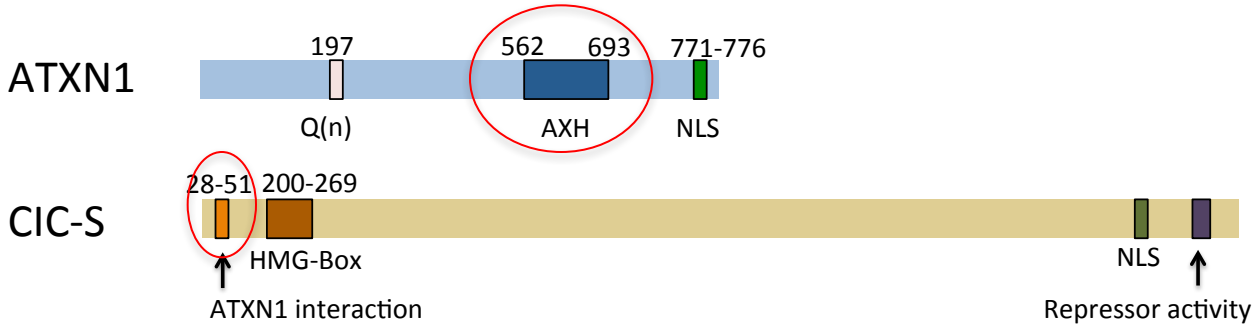
Advantages:

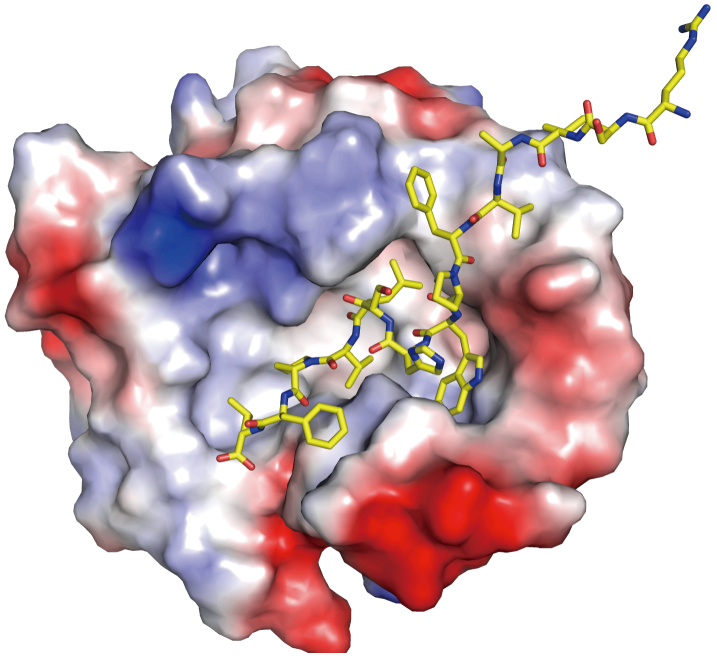
- It is specific and targets the disease-driving protein
- It can be rapidly advanced into clinical trials given precedent for other neurological disorders (SMA)

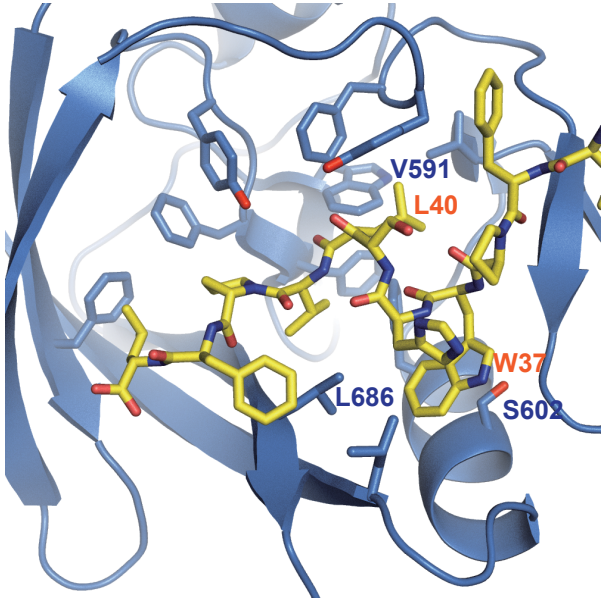
Limitations:

- It requires monitoring of the target protein (ATXN1) levels to insure that the reduction is mild to moderate (20-40%) given the important role of ATXN1 with Capicua
- It is invasive requiring repeated intrathecal injections

ATXN1-CIC interact and function together for normal brain function and to suppress tumors

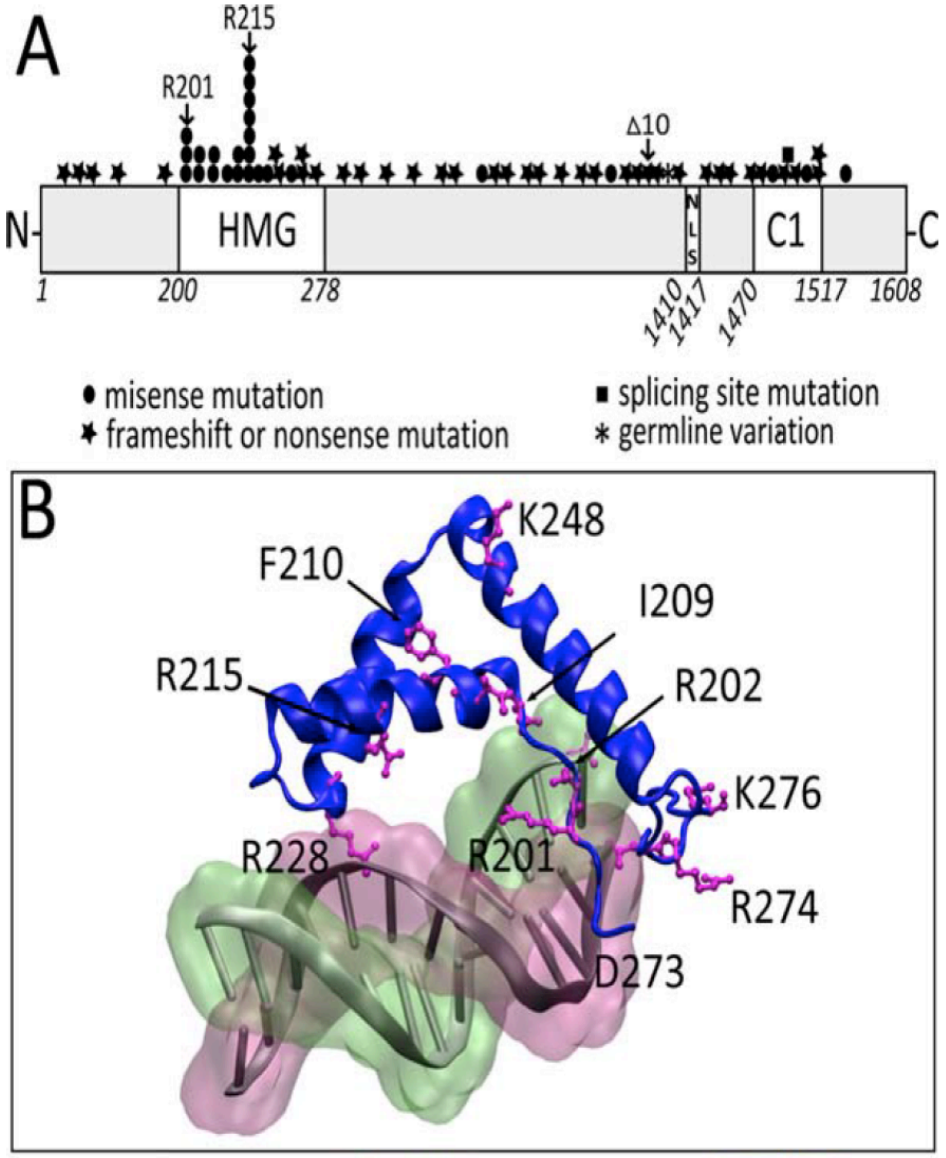






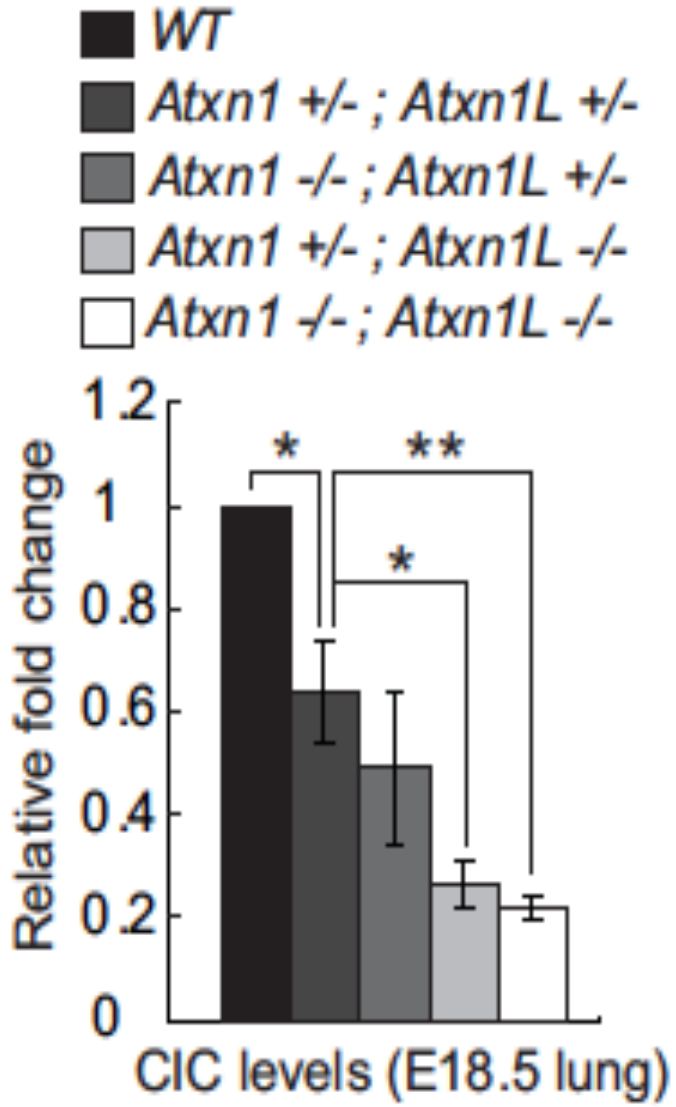
(Kim E, *et al*., *Genes and Development* 2013)

CIC mutations were observed in 60 patients with oligodendroglioma



Gleize, 2015

Capicua stability depends on ATXN1 and ATXN1Like levels

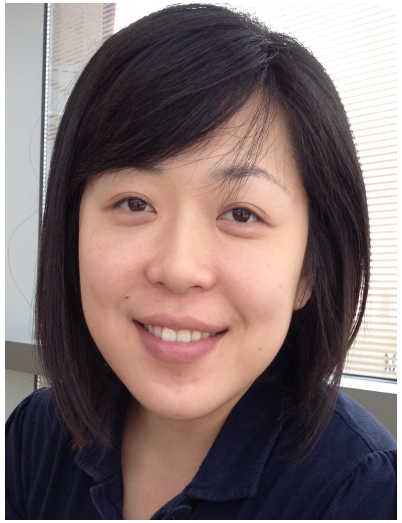


Ongoing studies to optimize feasibility of translational ASO therapy

- Establish the extent of the effects of ASO therapy on ATXN1 levels and CIC levels
- Determine the ideal dose that partially lowers ATXN1 without impacting CIC levels and that is effective
- Determine the distribution of ASO after intrathecal injection in non-human primates and its effects on ATXN1 and CIC levels

Can we develop a complementary strategy that barely lowers ATXN1 and has the potential of being used chronically

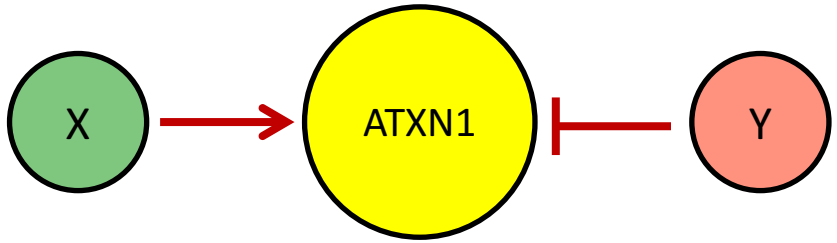
There should be targets that, when inhibited, reduce ATAXIN1 levels and could provide therapeutic entry points for SCA1



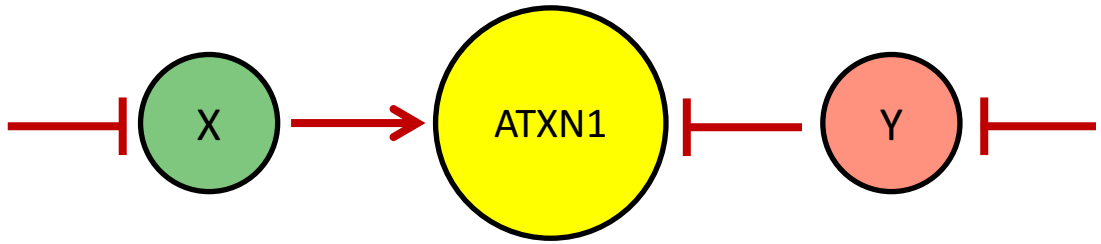
Jeehye Park, PhD

Ismael Al-Ramahi, PhD

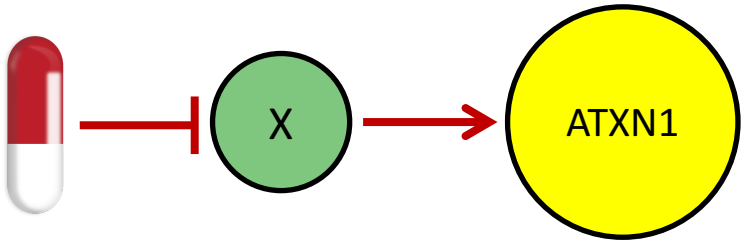
Find genes that regulate ATXN1 levels



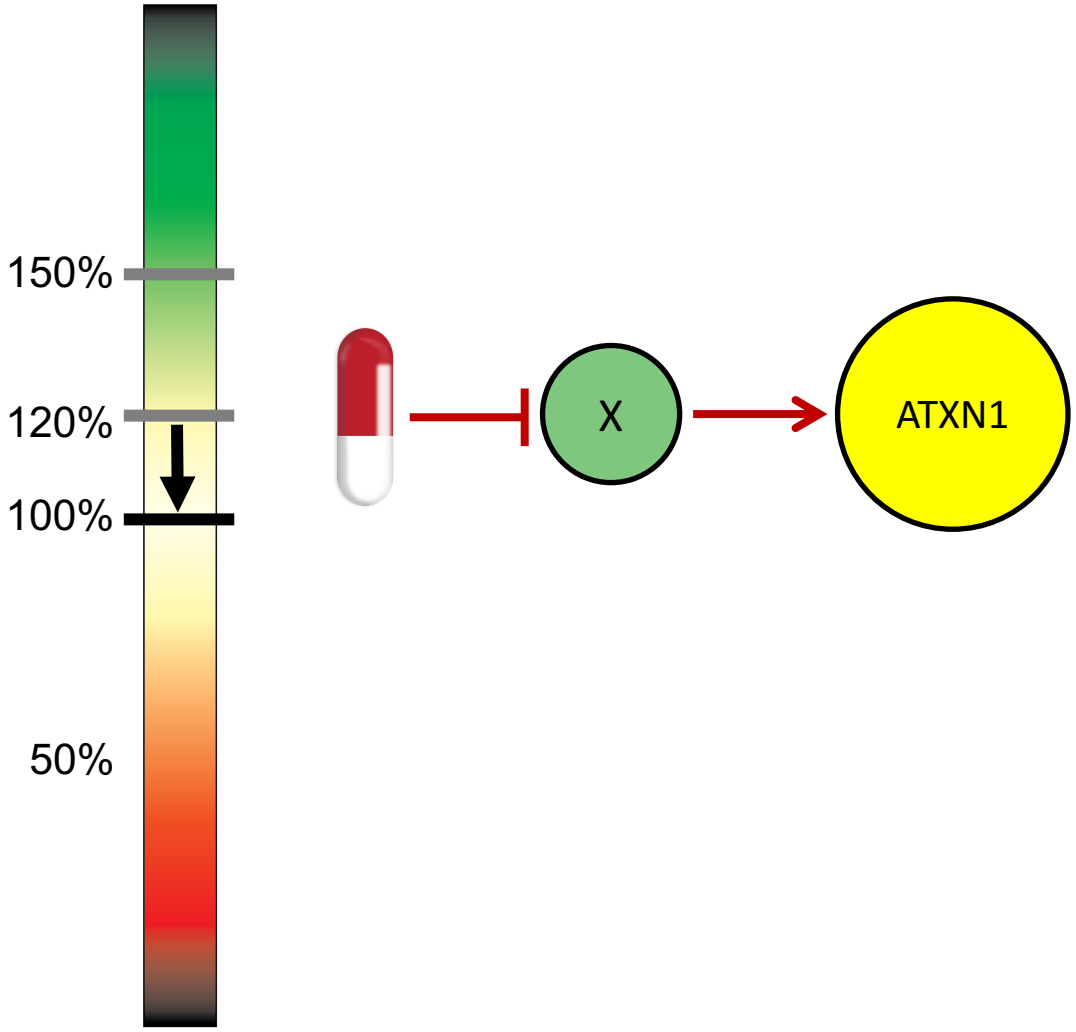
Find genes that regulate ATXN1 levels by systematically inhibiting them using genetics



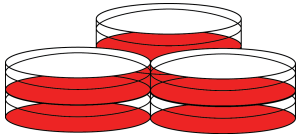
Identify regulators of ATXN1 levels & develop a pharmaceutical to inhibit them

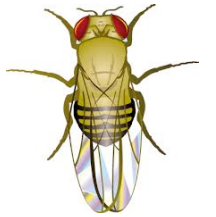


Aim to lower ATXN1 protein levels by ~20%



The strategy to identify ATXN1 regulators

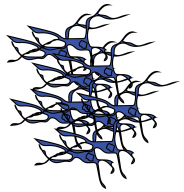




Primary screens in cells & flies

Single-target, cell validation

Human neuron validation

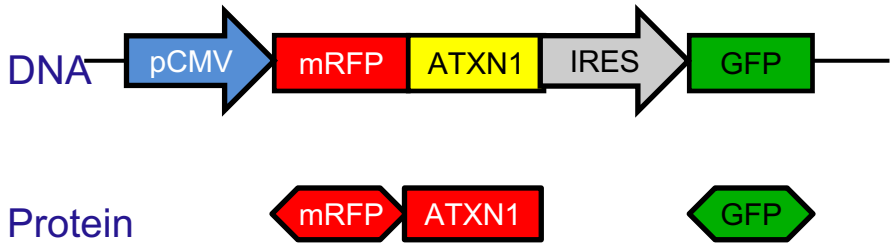


Mouse validation

Mechanism

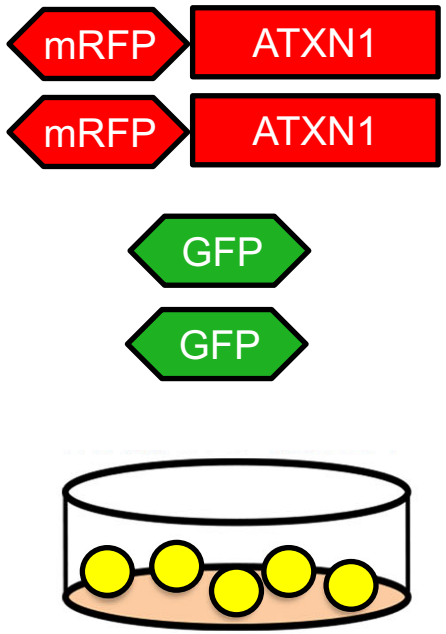
Candidate drug targets

Transgenic cell line that allows monitoring ATXN1 protein levels



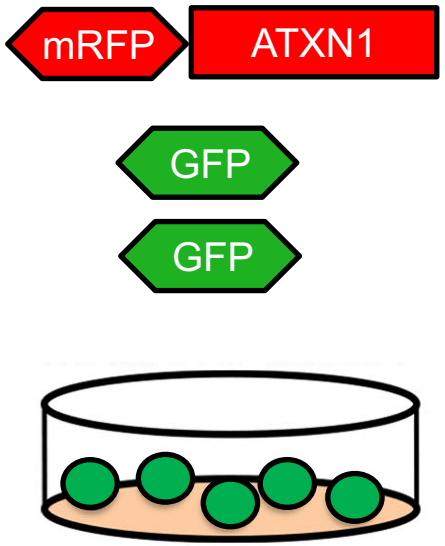
Red:green ratio helps identify regulators

No treatment

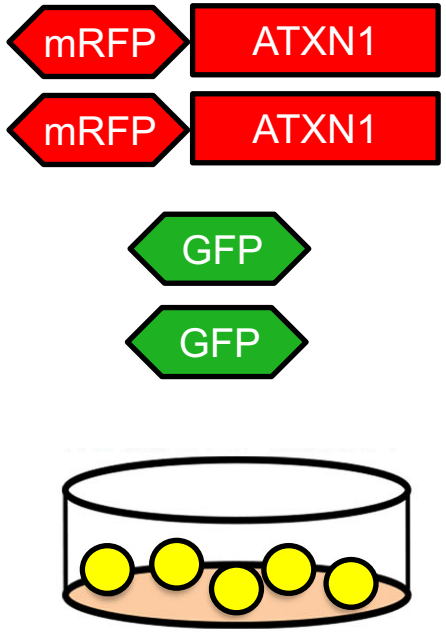


Red:green ratio helps identify regulators

ATXN1 decrease



No treatment



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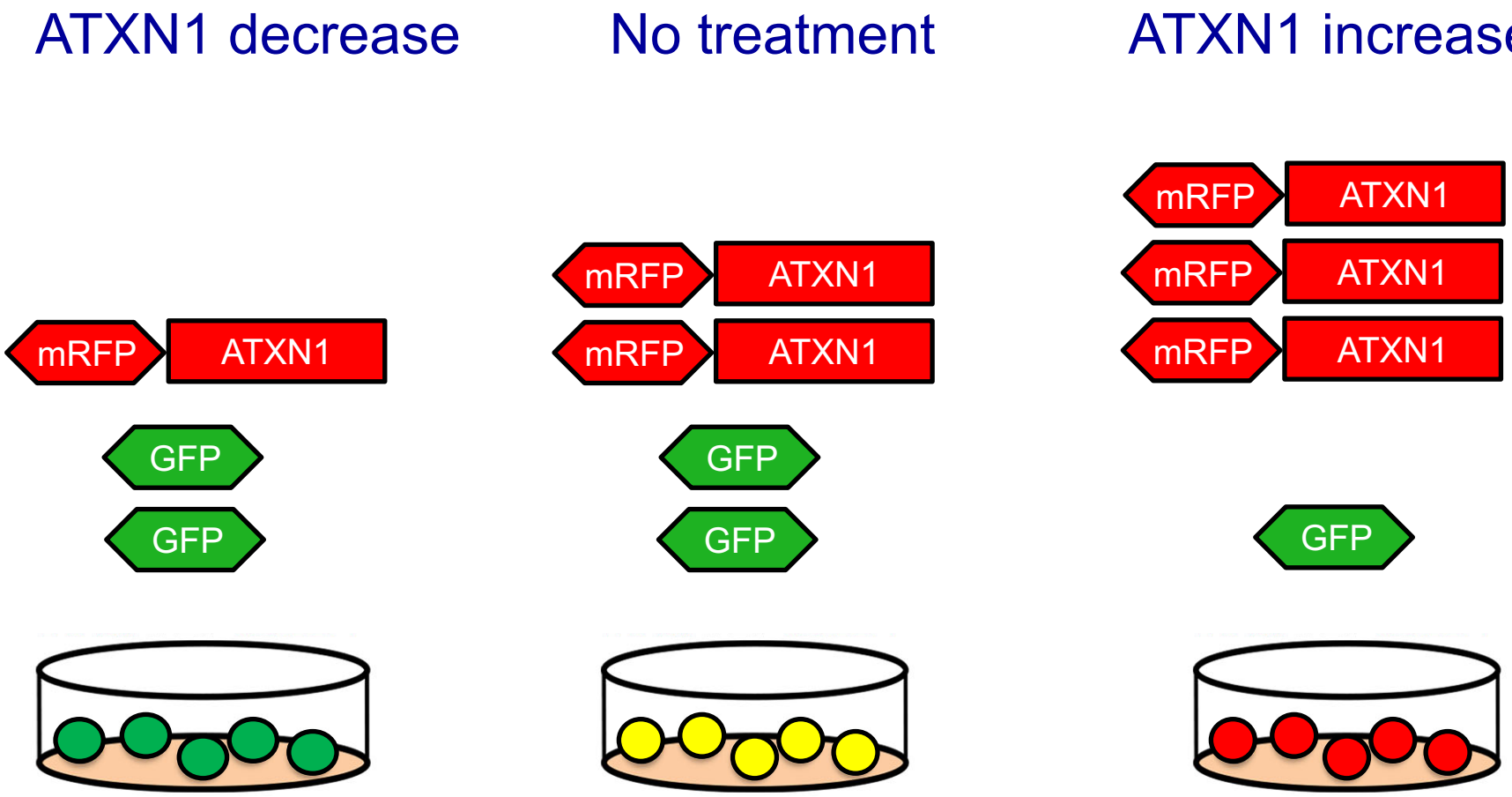
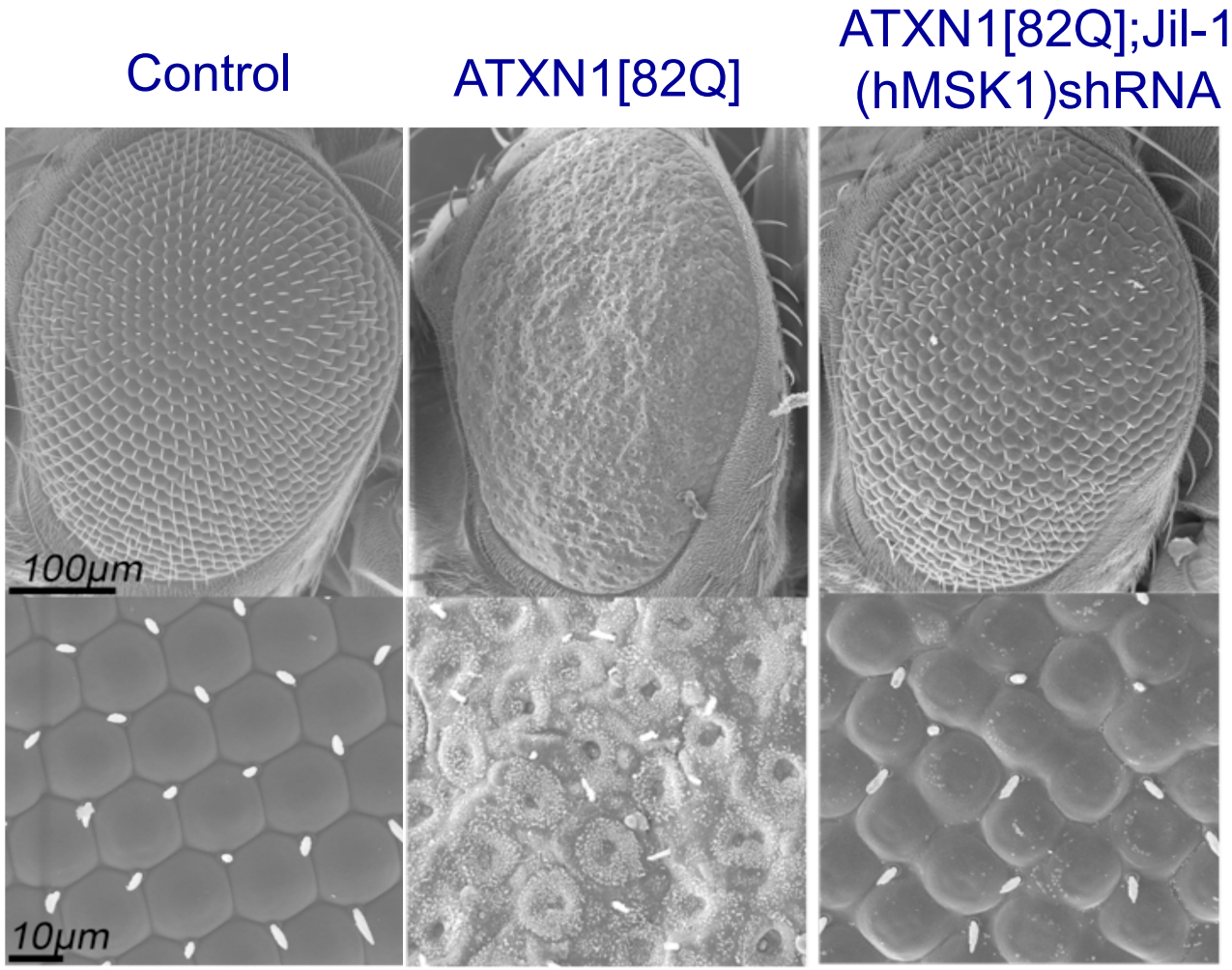
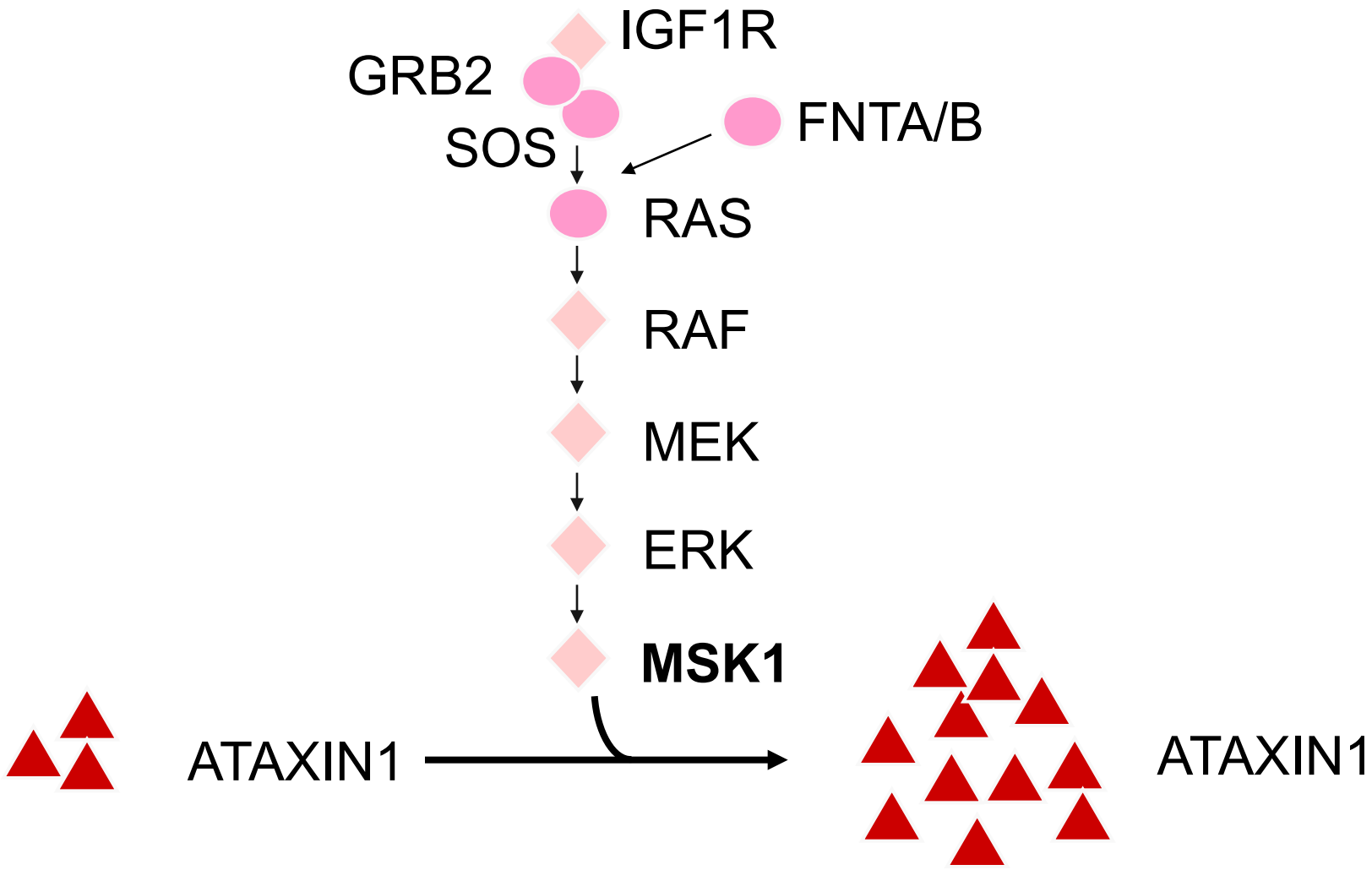


Reduction of the *Drosophila* homolog of MSK1 rescues neurodegeneration

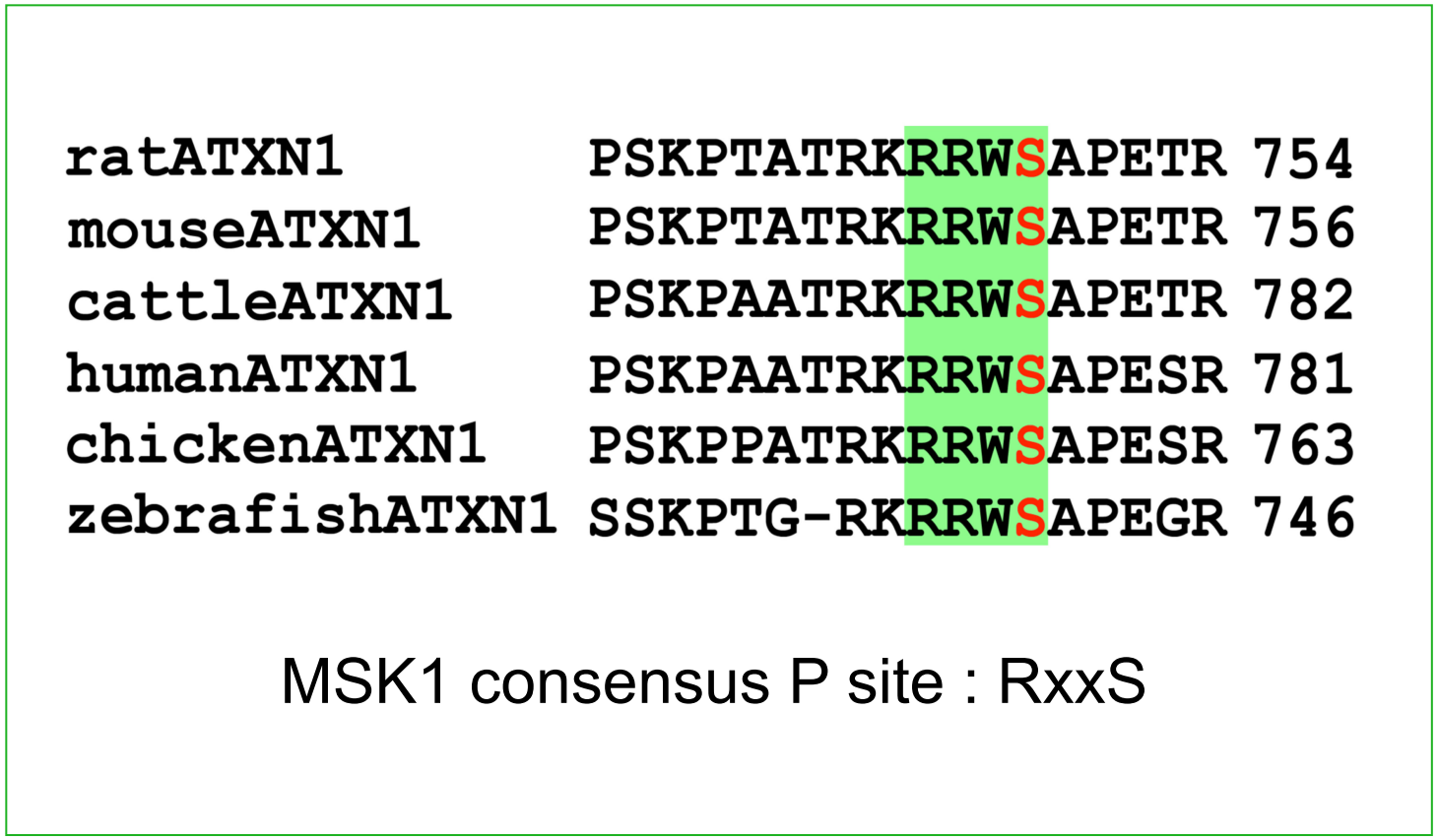


Park, Al-Ramahi et al., Nature 2013

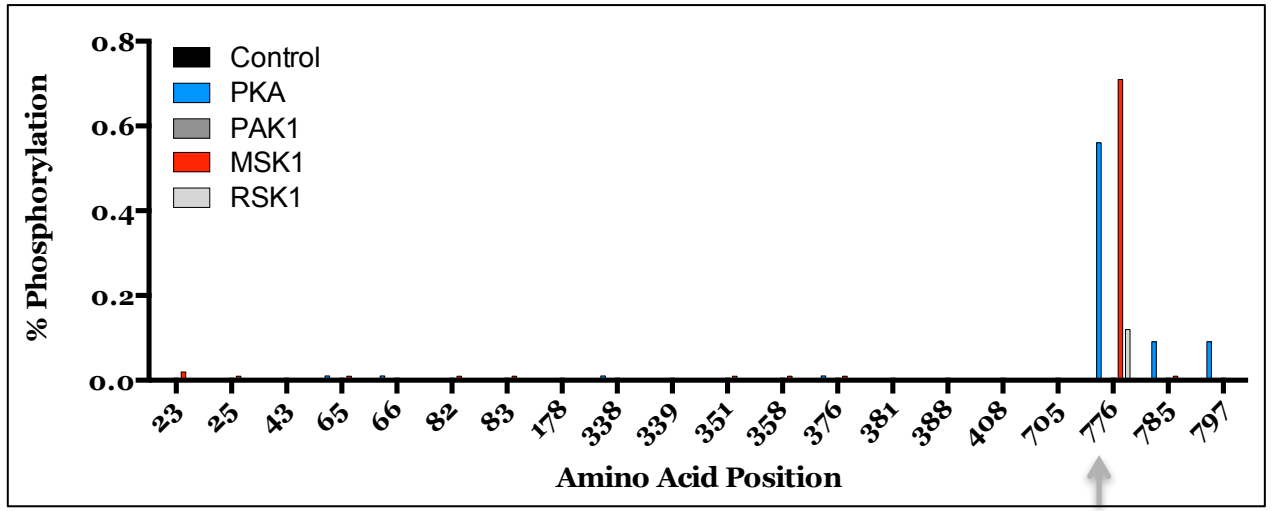
MAPK pathway modulates ATAXIN1 level & toxicity

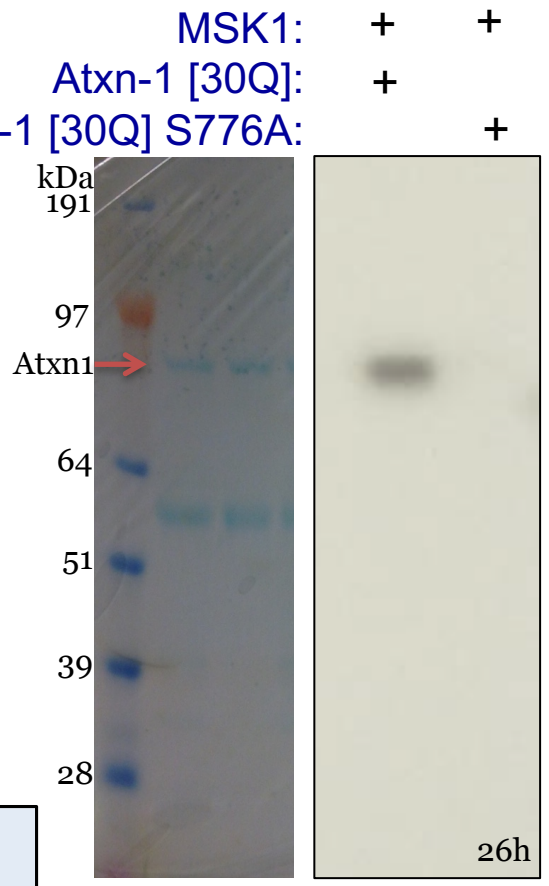


S776 falls within MSK1 consensus site



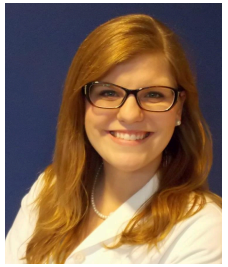
MSK1 phosphorylate Ataxin-1 S776





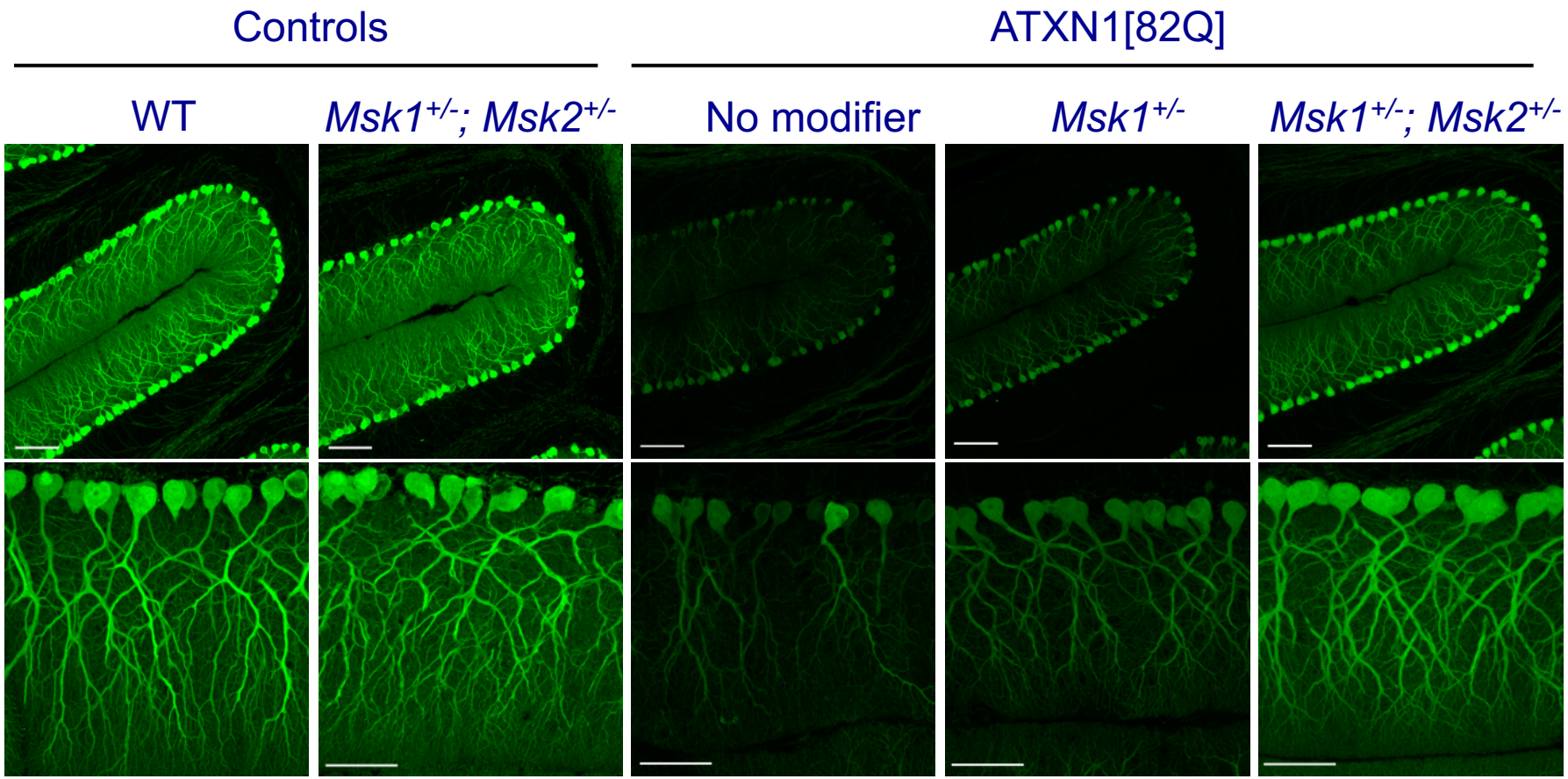
Mass Spec Results on Atxn-1[30Q] substrate

% Phosphorylation Ser776				
Control	**PKA**	**PAK1**	**MSK1**	**RSK1**
0	56	0	**71**	12



Carolyn Adamski, PhD

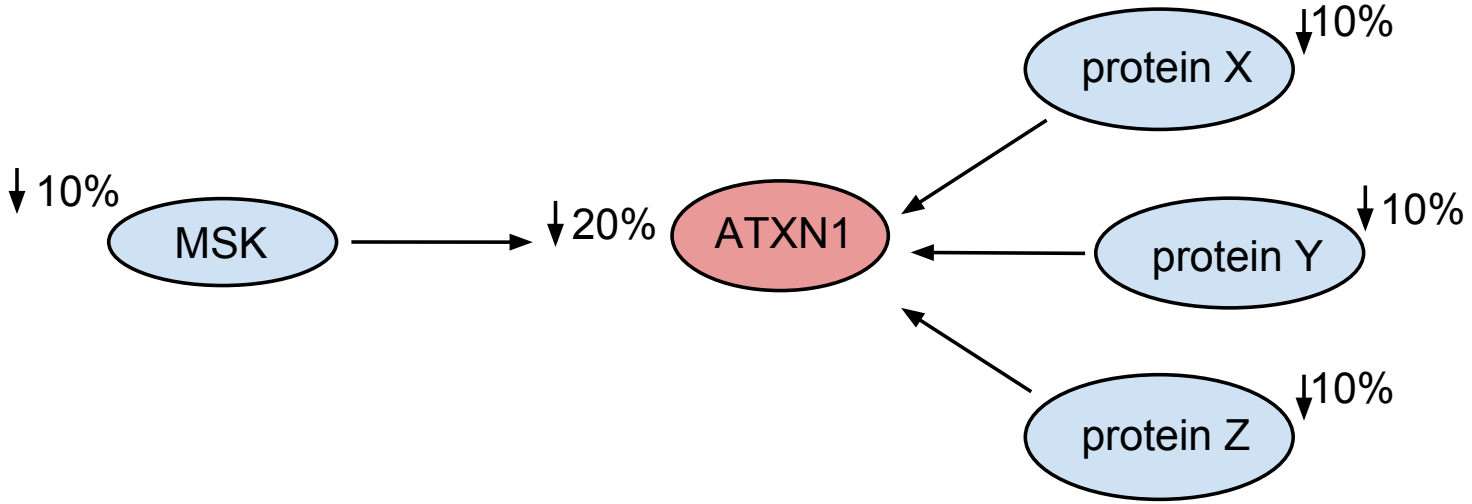
Reducing Msk1/2 rescues neurodegeneration in SCA1 mice



Conclusions from SCA1 studies

- Cross-species kinome screen identified modifiers that regulate ATXN1 stability and toxicity
- MSK1 increases ATXN1 stability by phosphorylating S776 and its reduction rescues degeneration in SCA1 mice
- Pharmacological inhibition of MSK1 decreases ATXN1 levels and warrants preclinical studies

Combination therapy is more effective and likely less toxic for a chronic disease like SCA1



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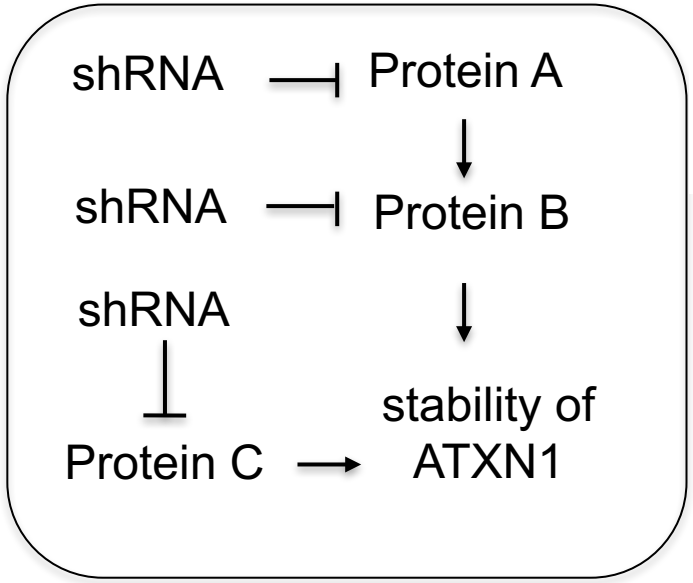
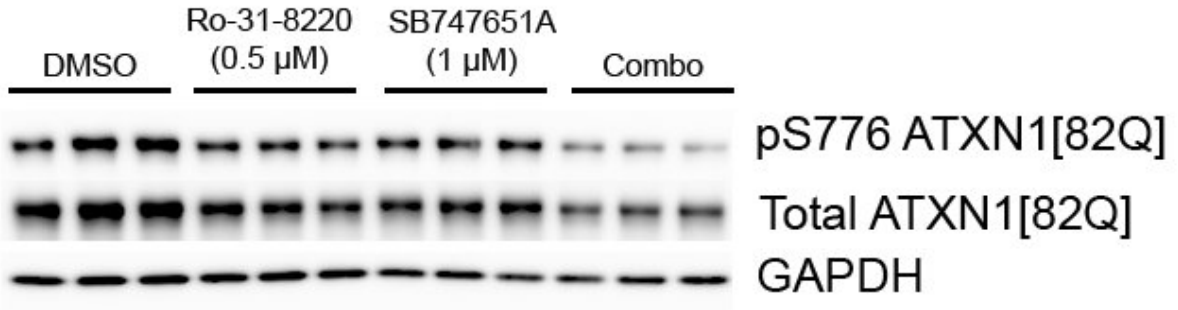
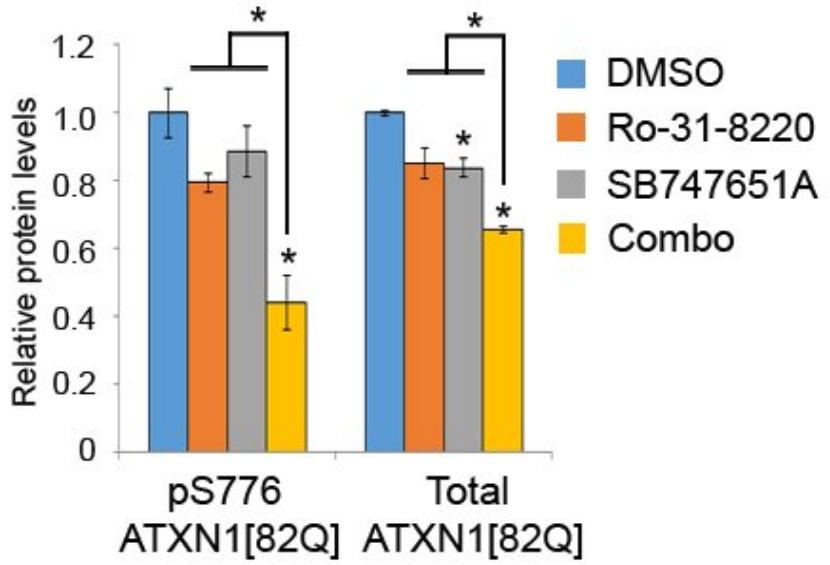
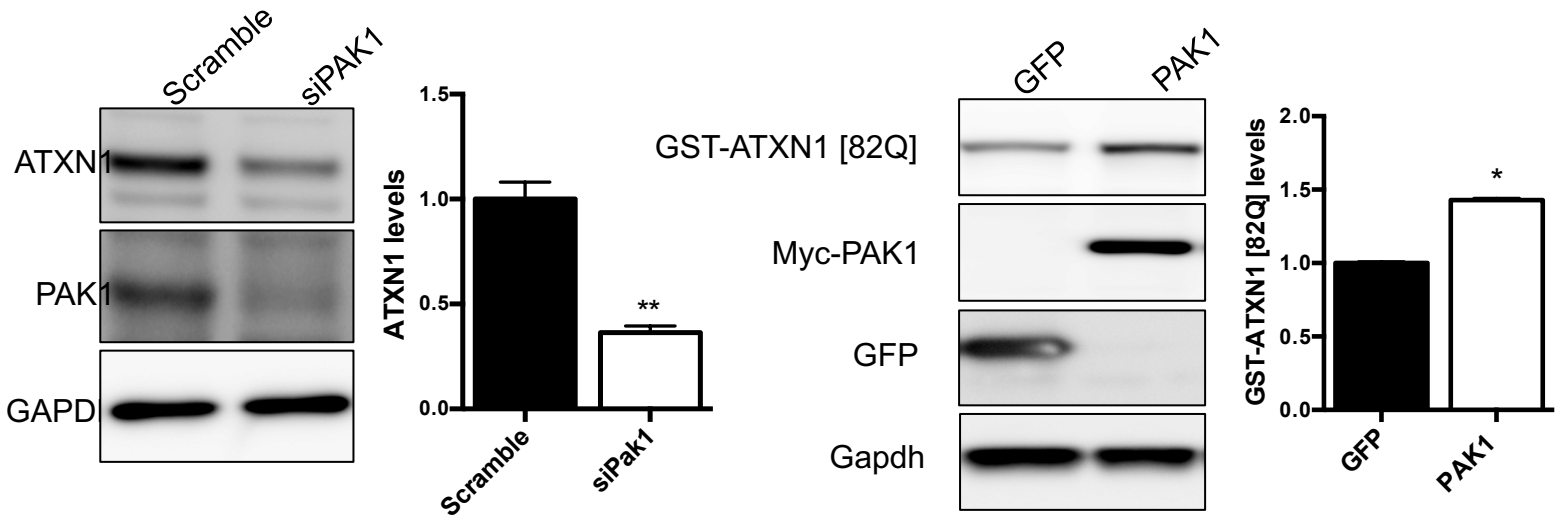






PAK1 genetic perturbation modulates ATXN1 levels

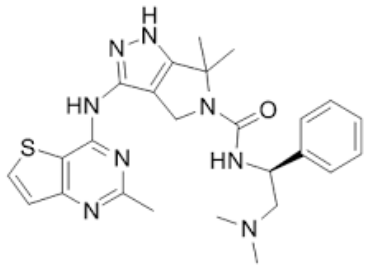


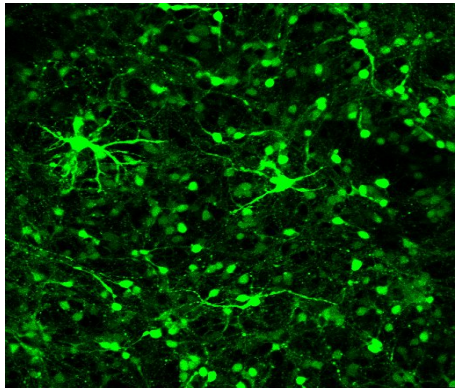
*p<0.05

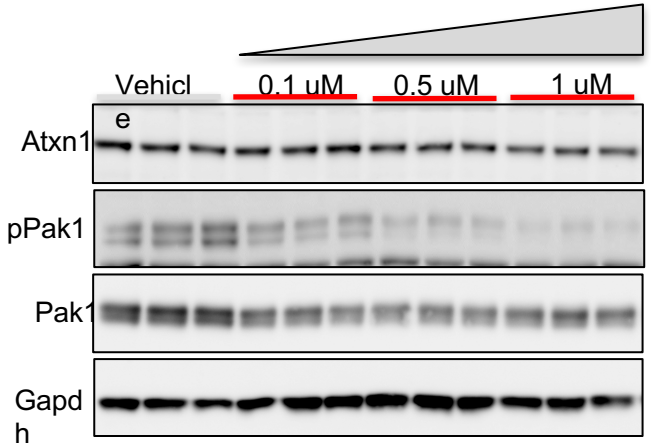
Vitaliy Bondar

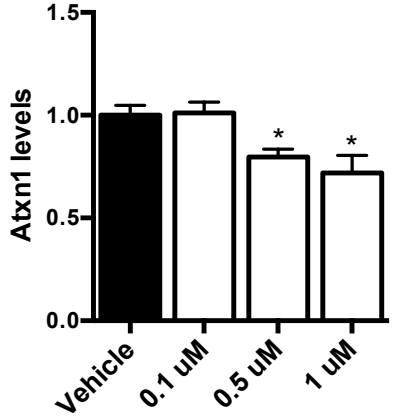
Pharmacological inhibition of PAKs decreases ATXN1 in mouse cerebellar granule neurons

panPAK inhibitor:
PF3758309





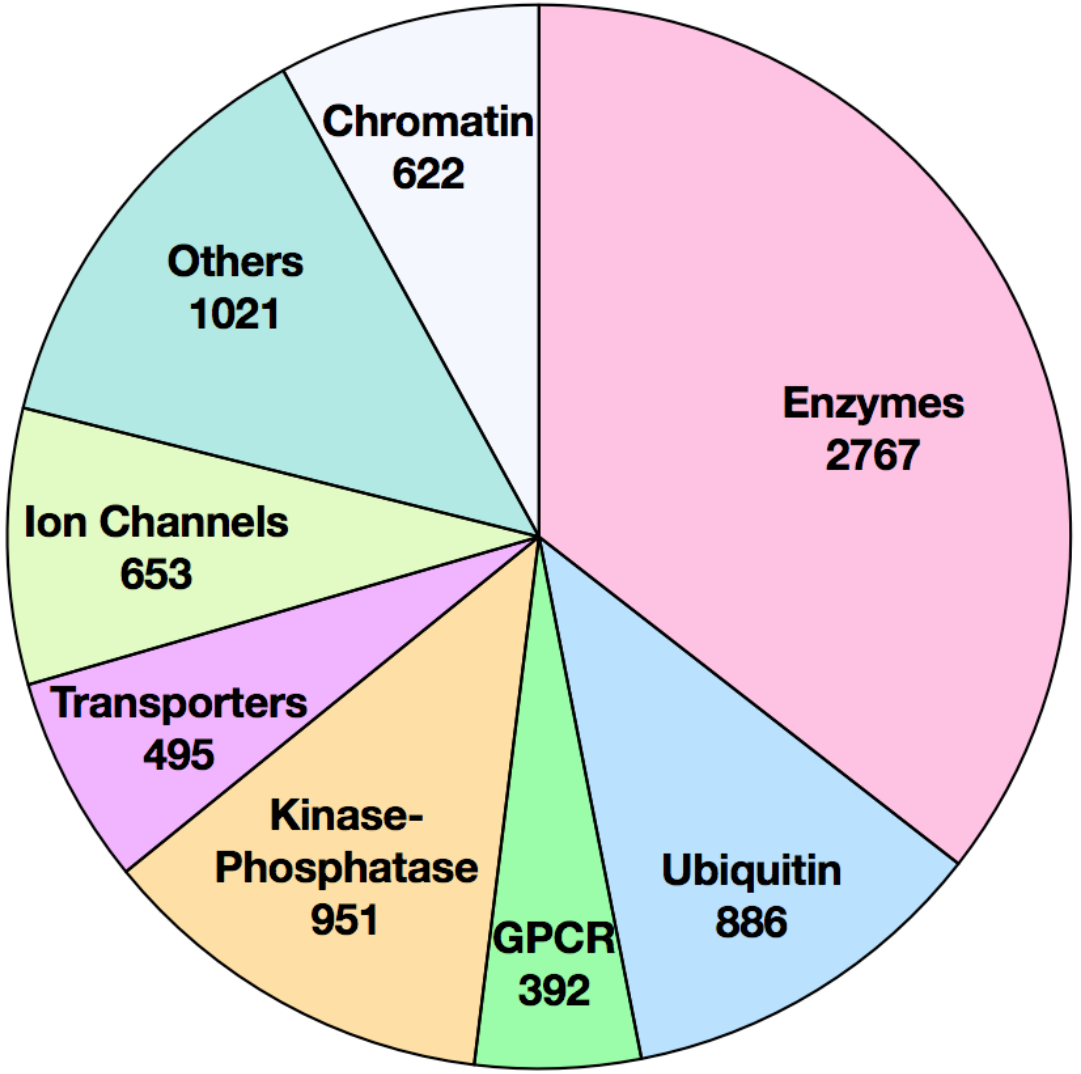




*p<0.05

Vitaliy Bondar

The druggable genome is composed of eight sub-libraries



7,787 genes x 10 shRNAs/gene= 77,870 shRNAs

In Collaboration with Stephen Elledge

Elevated protein levels are a common theme among spinocerebellar ataxias

Less protein

More protein

Conclusions

- Understanding disease mechanism and function of disease-driving proteins are crucial for designing safe interventions
- ASOs and modulators of ATXN1 provide a viable path to develop interventions for SCA1
- Key to any therapeutic intervention is long-term safety as therapy will be required for decades

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