Regulatory Features of the Spinocerebellar Ataxia Type 2 Gene *ATXN2* Promoter and 3'-UTR

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Spinocerebellar Ataxia Type 2 (SCA2)

- SCA2 is a polyglutamine disorder caused by *ATXN2* mutation.
- Gait ataxia, frontal executive dysfunction, slow saccades, and parkinsonism.
- Age of onset is characterized by anticipation where CAG22-23 is normal and CAG>32 causes disease.
- Characterized by Purkinje cell death.
- Gain of normal function (Duvick et al., Neuron 2010).

Ataxin 2 regulates mRNA, Ca^{2+} movement and endocytosis

RNA Binding Proteins

A2BP1/Fox 1	Shibata et al., HMG 9:1303-13; 2000
PABP1	Nonhoff et al., PNAS 98:4409-13; 2001
DDX6	Nonhoff et al., MBC 18:1385-96; 2007
TDP-43	Elden et al., Nature 466:1069-75; 2010

Endocytosis and EGFR Function

Endophilins	Nonis et al., Cell Signal 20:1725-39; 2009

Calcium Movement

IP3R	Liu et al., J Neurosci 29:9148-62;2009

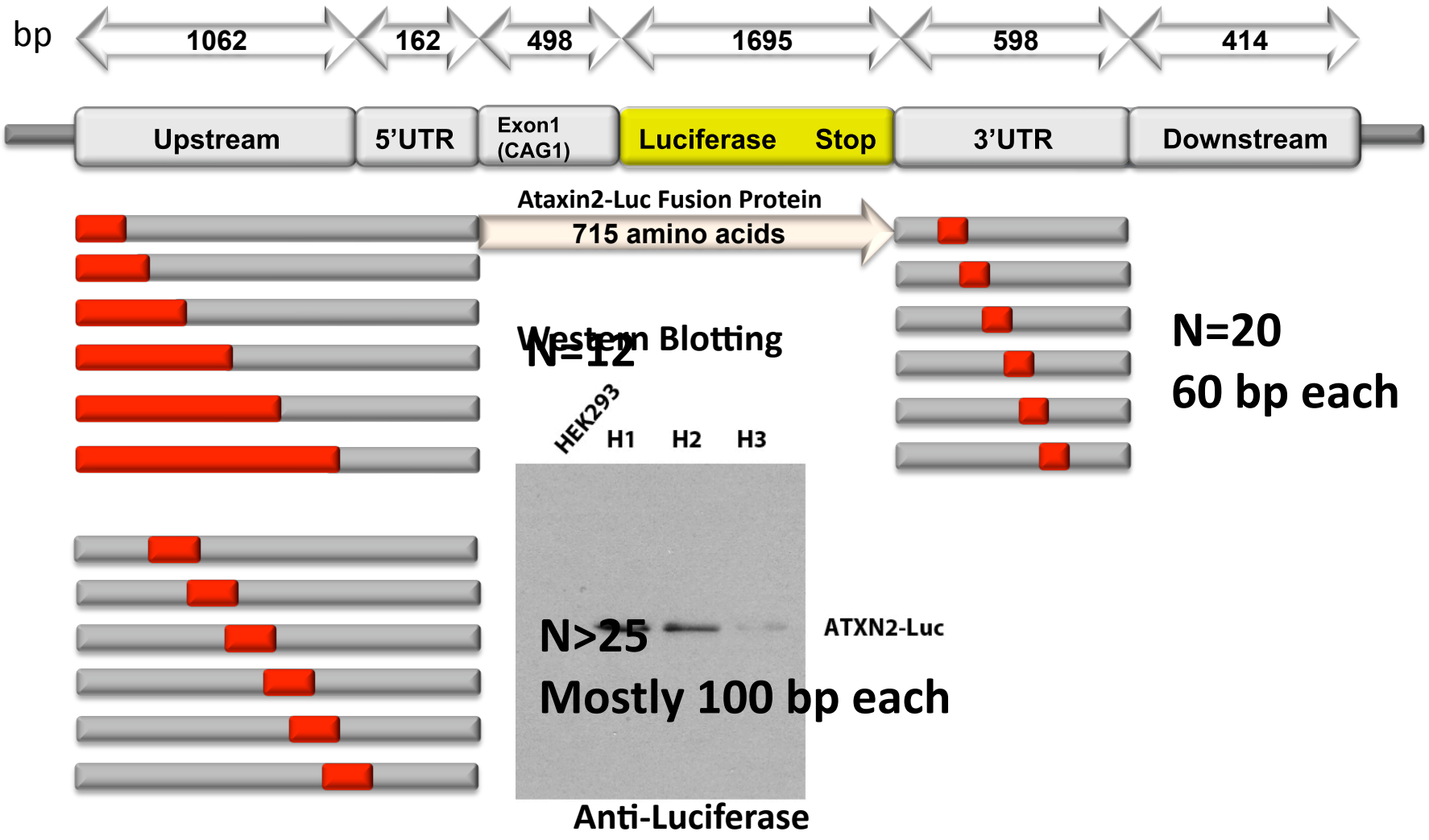
Hypothesis

Reduction of ataxin-2 expression or mRNA stability provides a therapeutic avenue for SCA2.

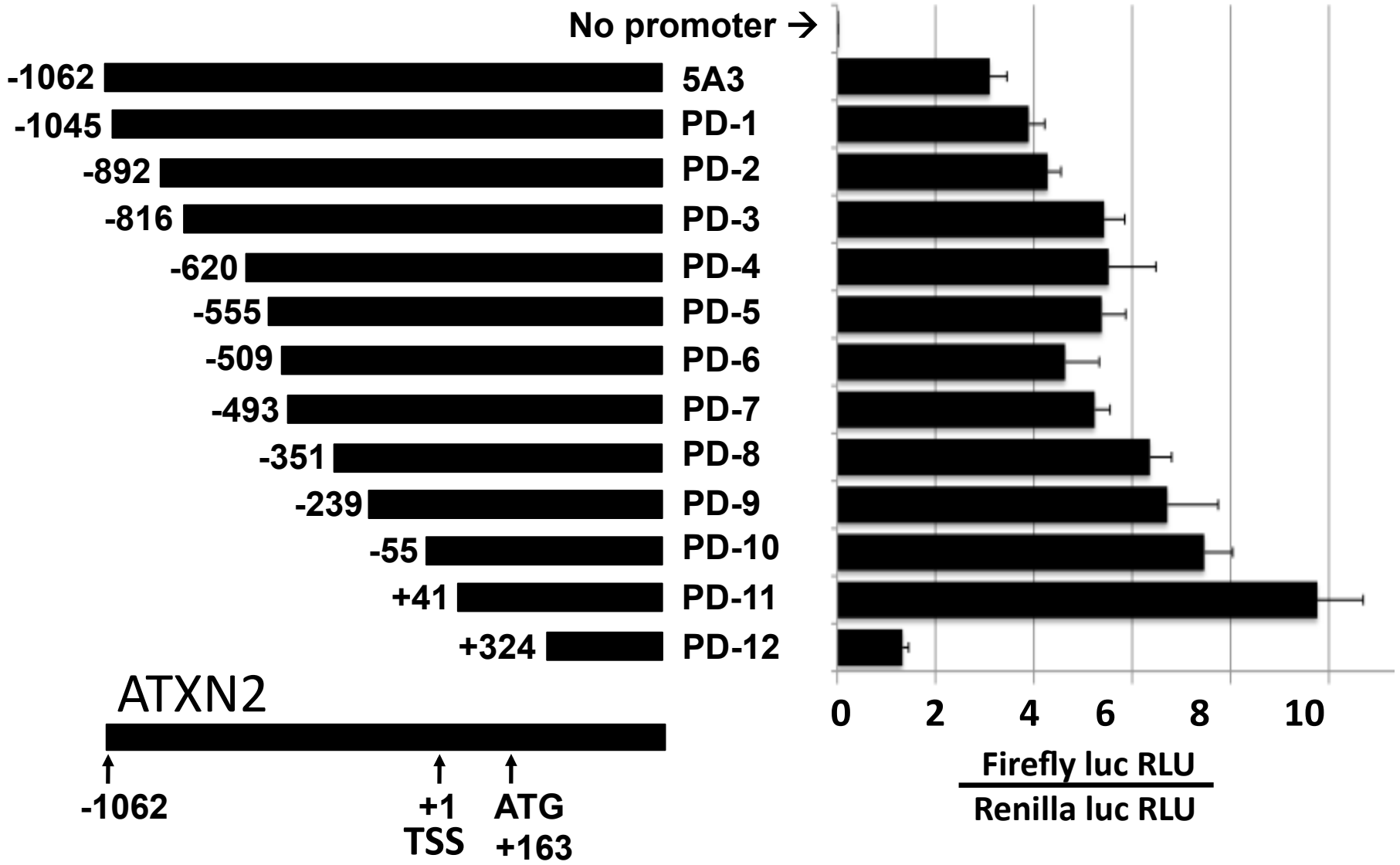
- SCA2 phenotype is worse in patients homozygous for the disease allele (Ragothaman and Muthane, 2008).
- SCA2 phenotype is worse in homozygous vs heterozygous *ATXN2* transgenic mice (Huynh et al., 2000).
- *ATXN2* knockout mice are obese (Kiehl et al., 2006; Huynh et al., 2009).
- Reversibility of SCA1&3 transgenic mouse phenotype (Zu et al., 2004; Boy et al., 2009).
- shRNA injection in brains of *ATXN1* mouse improved phenotype (Xia et al., 2004).

pGL2-ATXN2-Luc

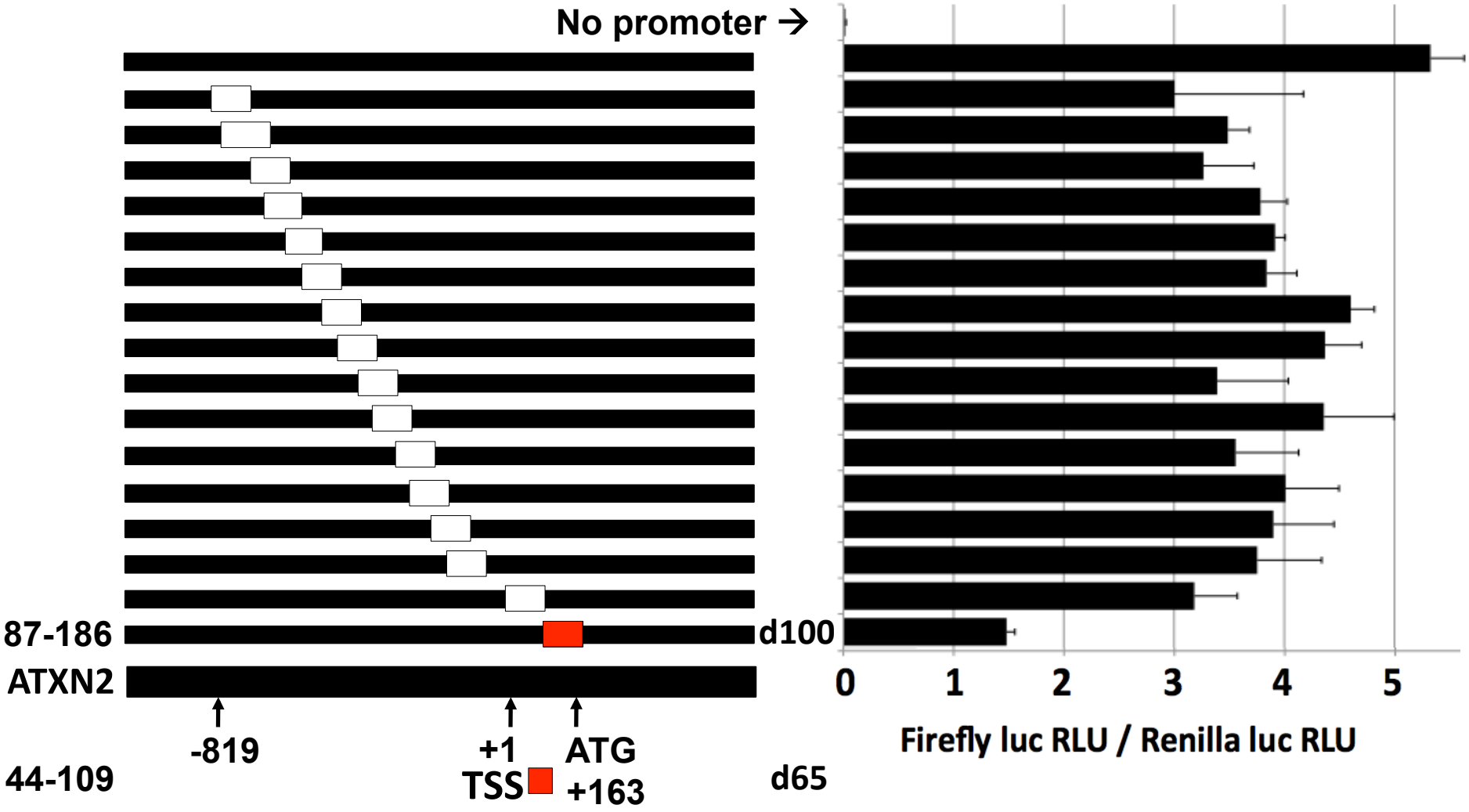
ATXN2-Luciferase Expression Construct



ATXN2 promoter deletions



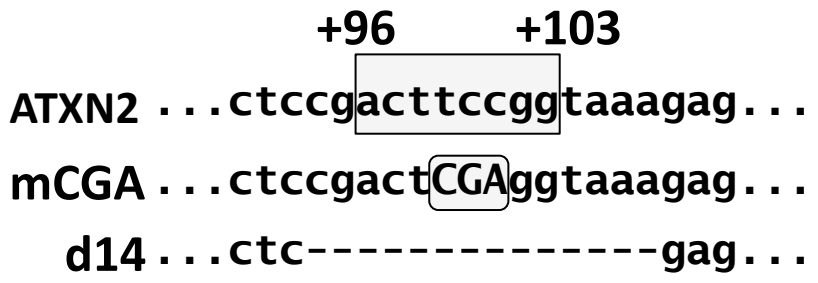
ATXN2 Interstitial Promoter Deletions

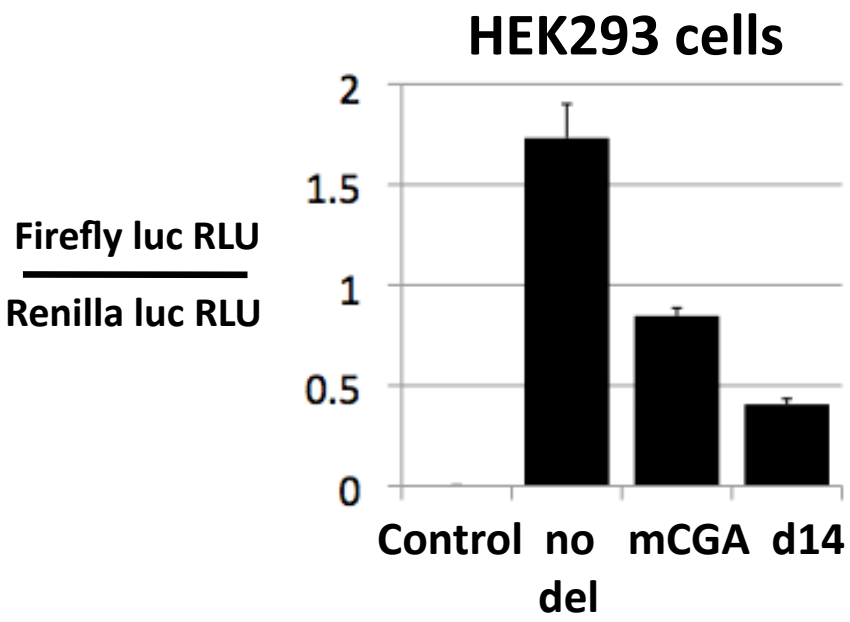


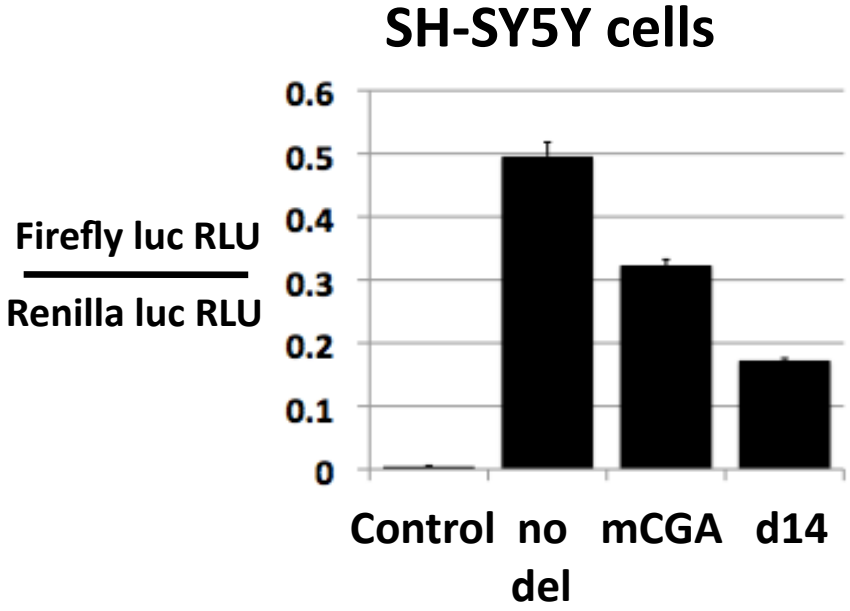
Deletion of an ETS family transcription factor binding site reduced ATXN2-luc expression

100% match for an ETS family transcription factor element located on the *ATXN2* negative strand:

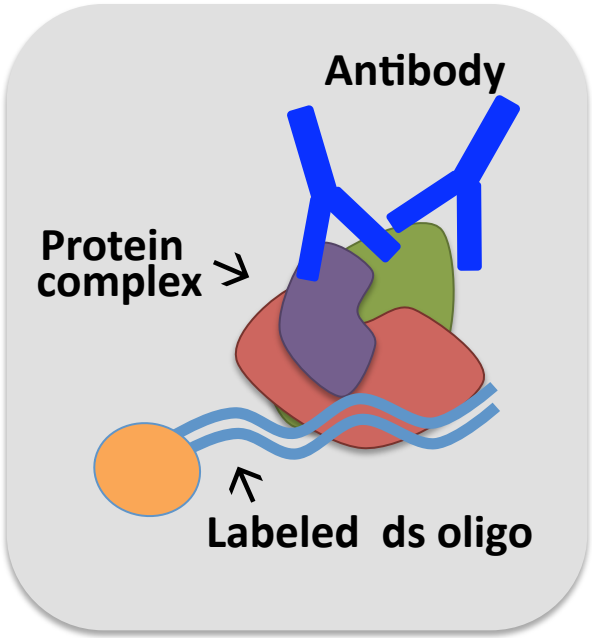
5'-CCGGAAGT-3'

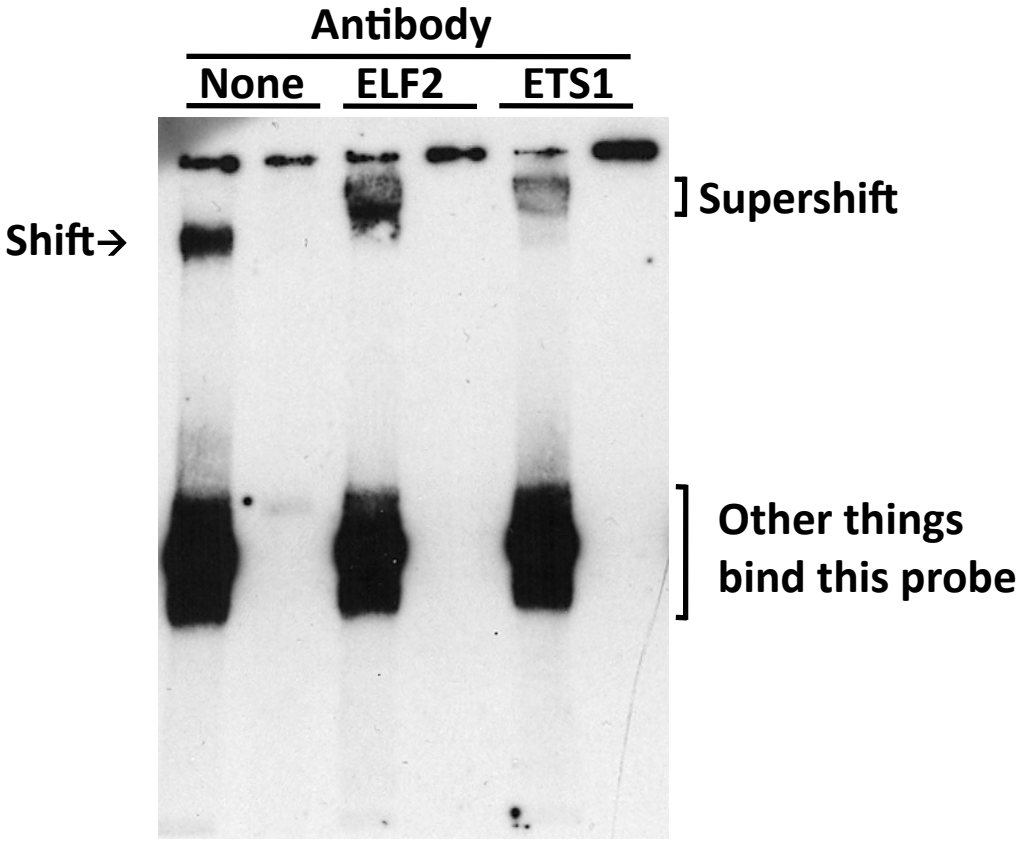






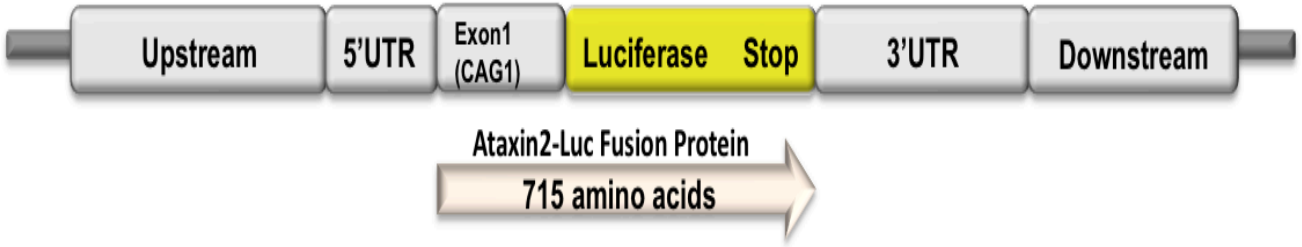
EMSA supershift assays verified interactions with ETS family transcription factors ELF-2 and ETS-1

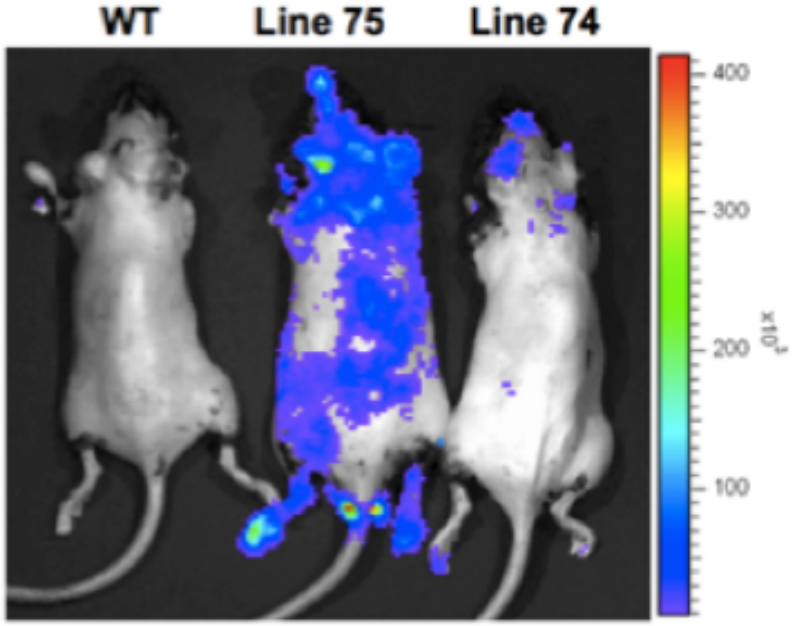




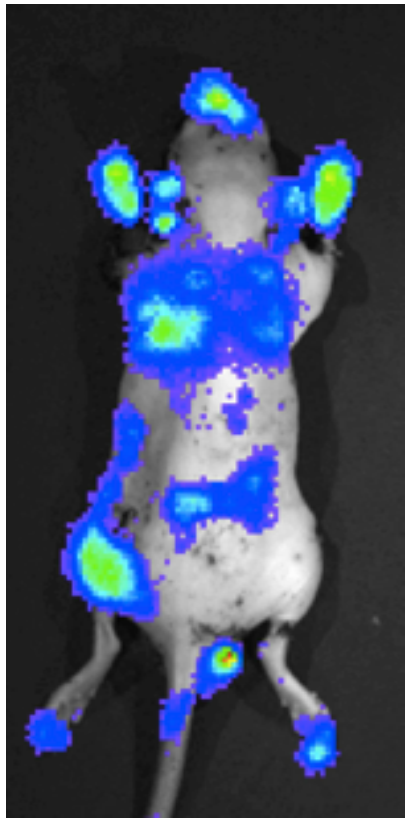
6% Native gel
SH-SY5Y protein lysate

ATXN2-luc expression in mice

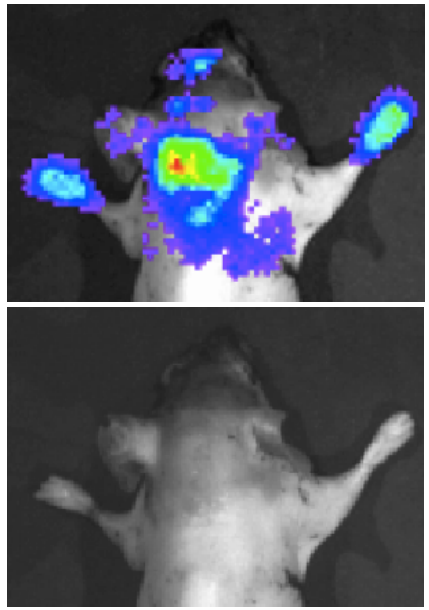




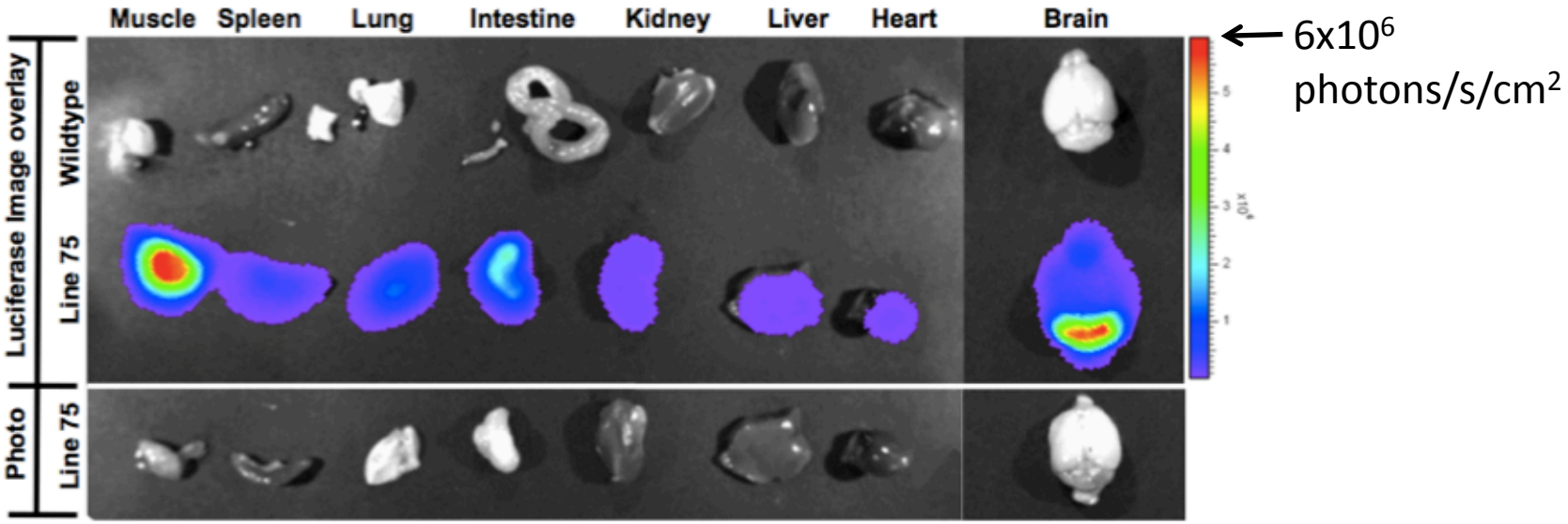
Line 75



Line 75

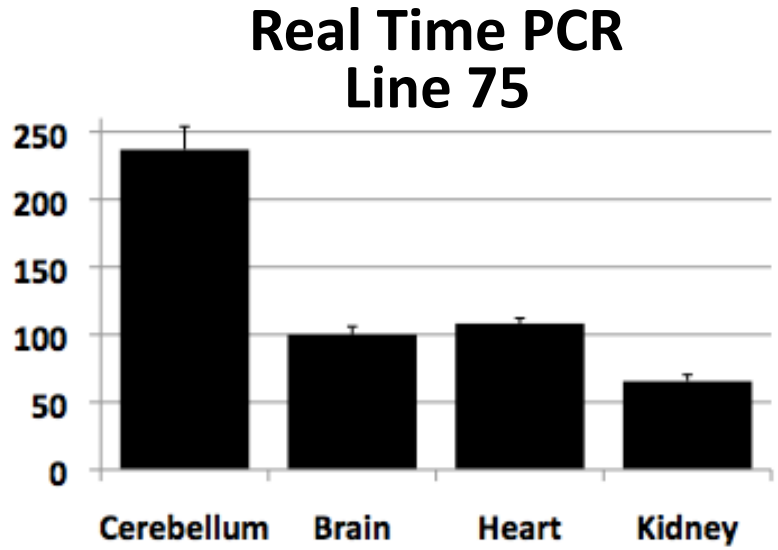


ATXN2-luc expression in excised tissues



Luciferase / Mouse *Atxn2*

(% relative to brain)



Summary & Conclusions

- **New reporter system for studying *ATXN2* expression control.**
- **Inhibitory elements likely exist to control *ATXN2* expression.**
- **We identified a critical region in *ATXN2* where ETS factors act.**
- ***ATXN2-luc* was highly expressed in cerebellum.**

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ATXN2 3’-UTR Deletions