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

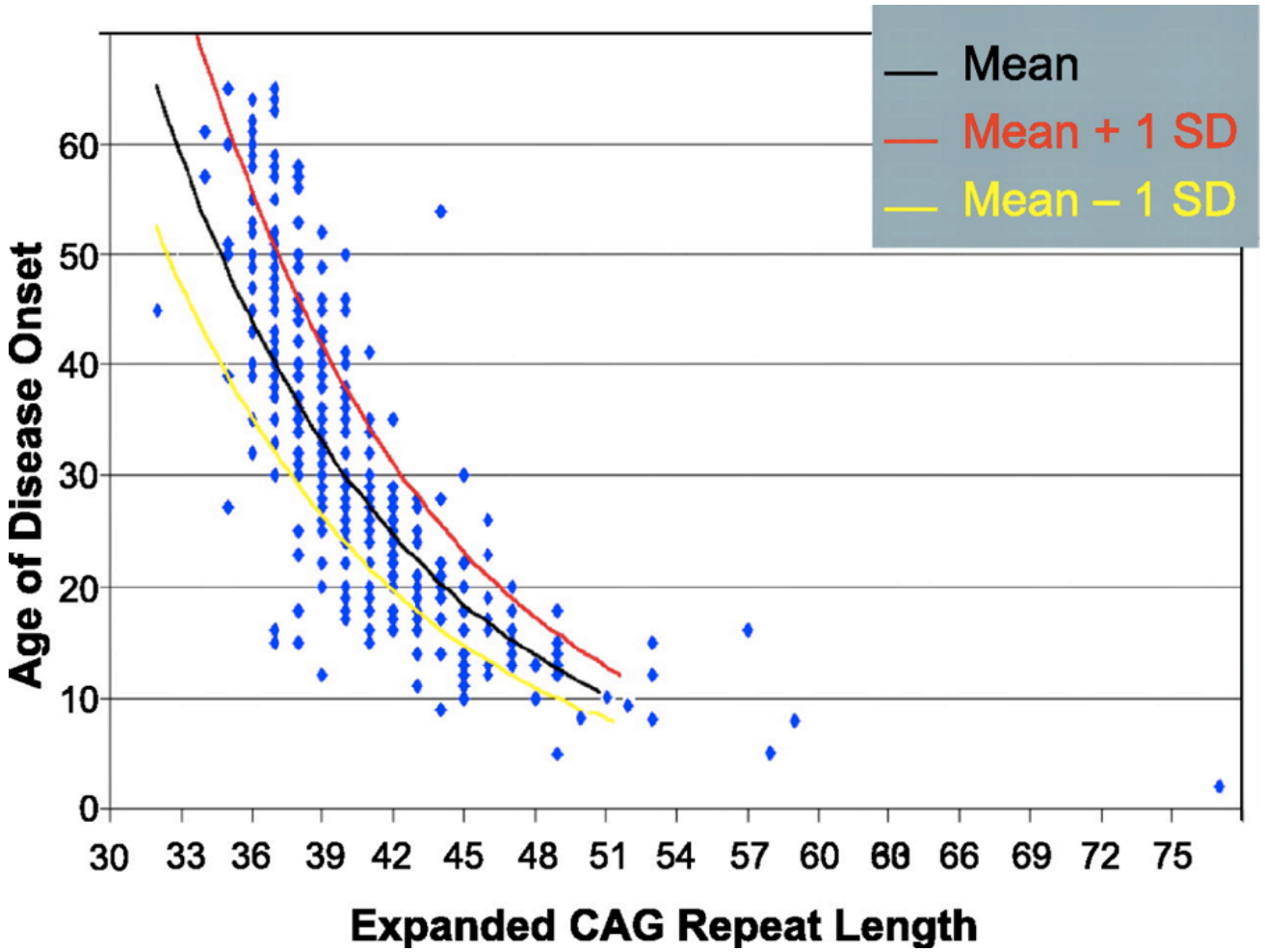
Daniel R. Scoles

THE UNIVERSITY OF UTAH

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).

Anticipation



Pulst et al. Brain 2005

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

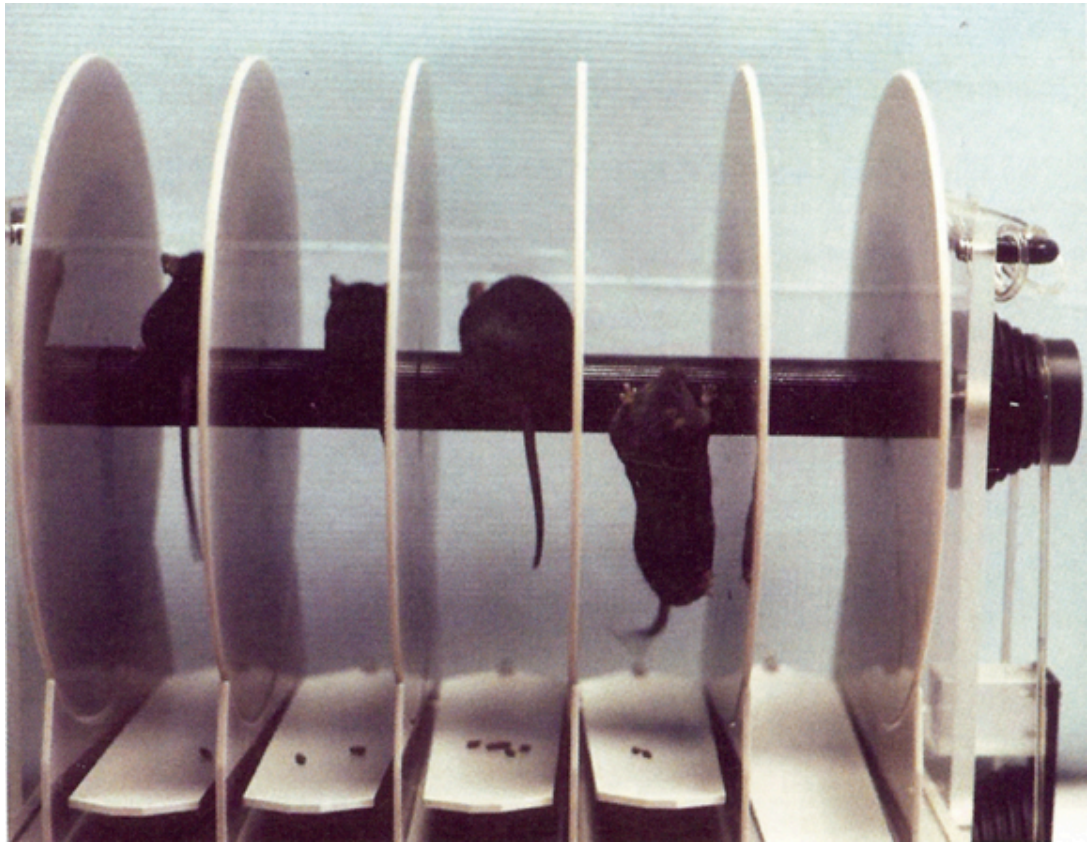
Calcium Movement

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

Hypothesis

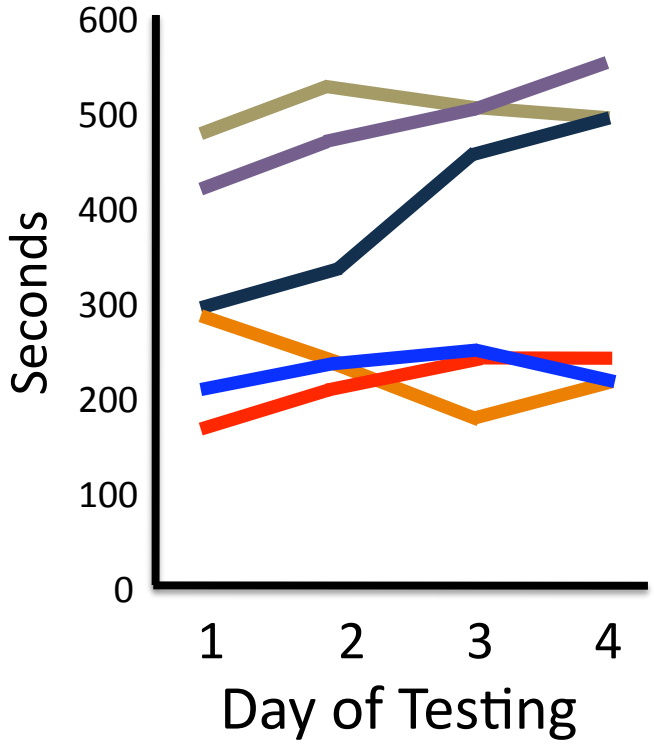
Reduction of ataxin-2 expression or mRNA stability provides a therapeutic avenue for SCA2.

- 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).



Mouse Models

Rotorod performance for SCA2 Q58 transgenic mice

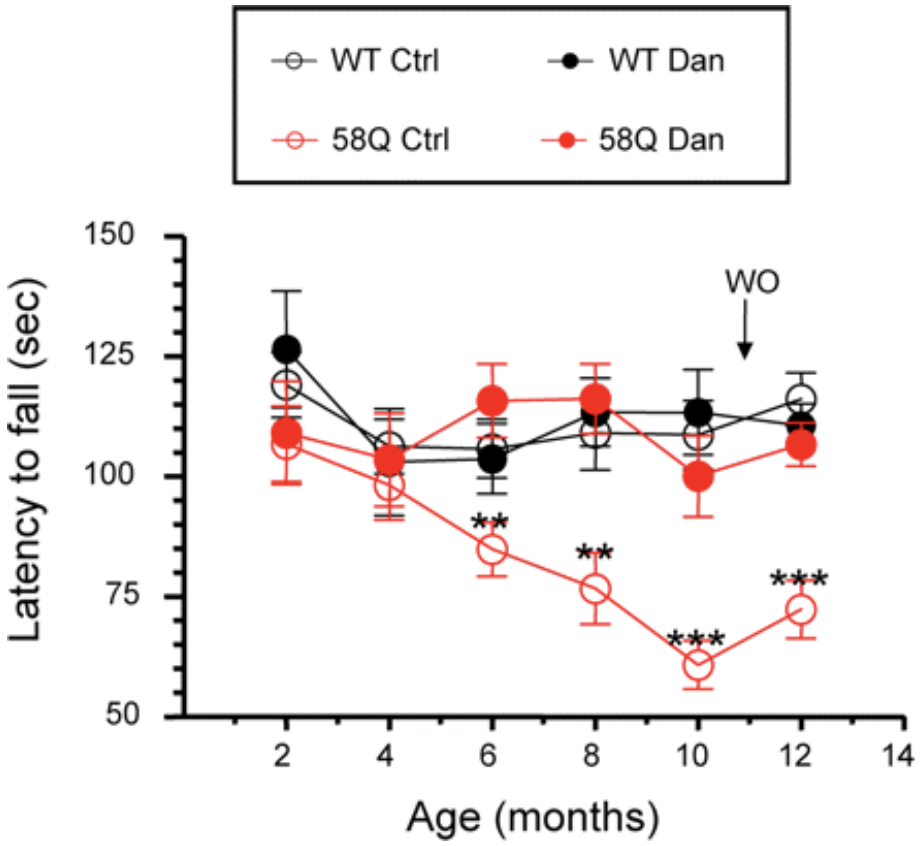


+/+	14 wks (n=10)
+/+	26 wks (n=8)
+/Q58	14 wks (n=8)
+/Q58	16 wks (n=12)
Q58/Q58	26 wks (n=9)
Q58/Q58	26 wks (n=9)

4 to 40 rpm for 10 min

Huynh et al., *Nature Genetics 26, 44 - 50 (2000)*

Rotorod analysis used to measure dantroline effect on SCA2 Q58 transgenic mice



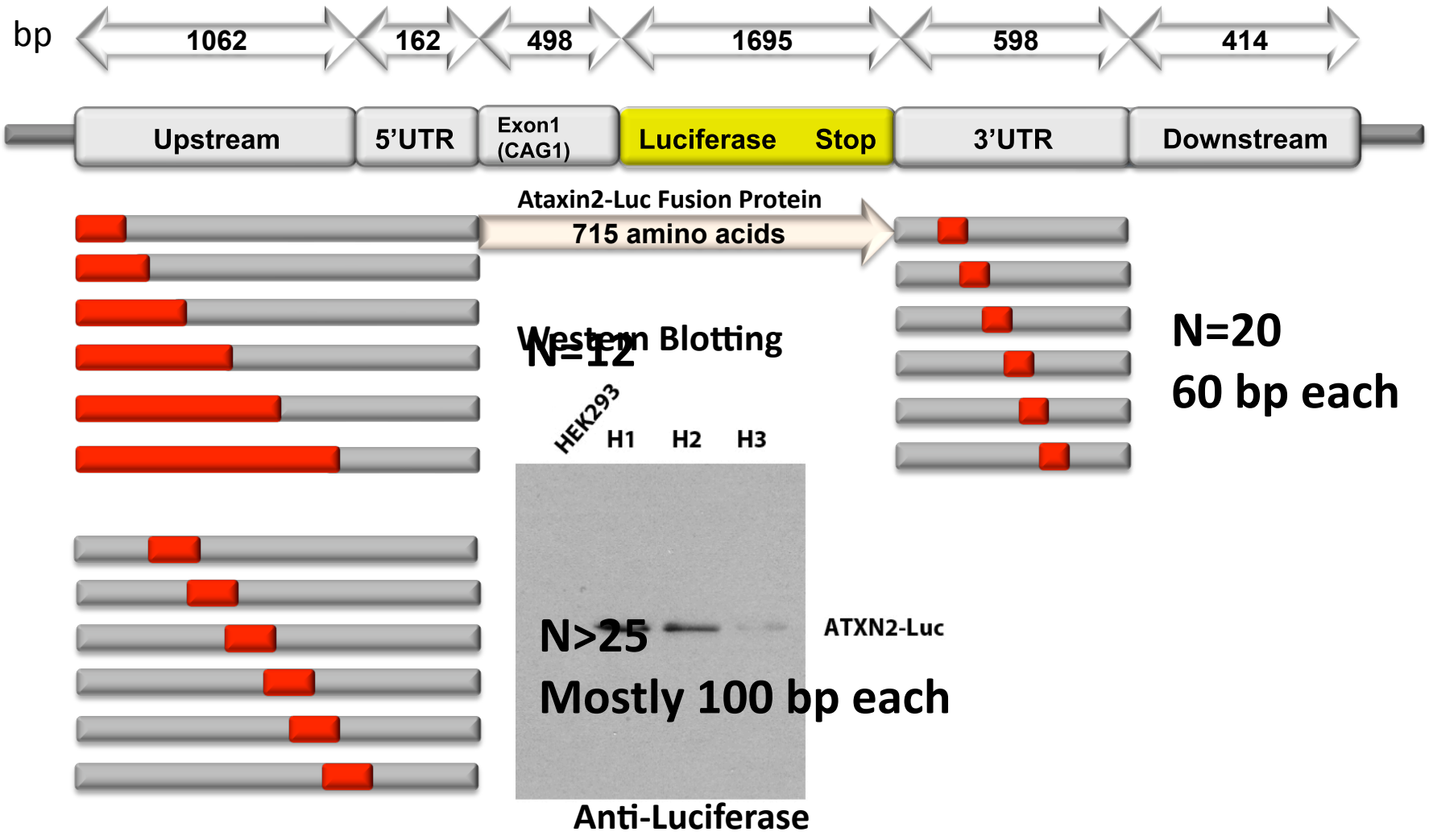
PCP2 promoter

N=9-12 mice per group
0 to 40 rpm over 200 s
Fed 5 mg/kg dantrolene orally twice/wk

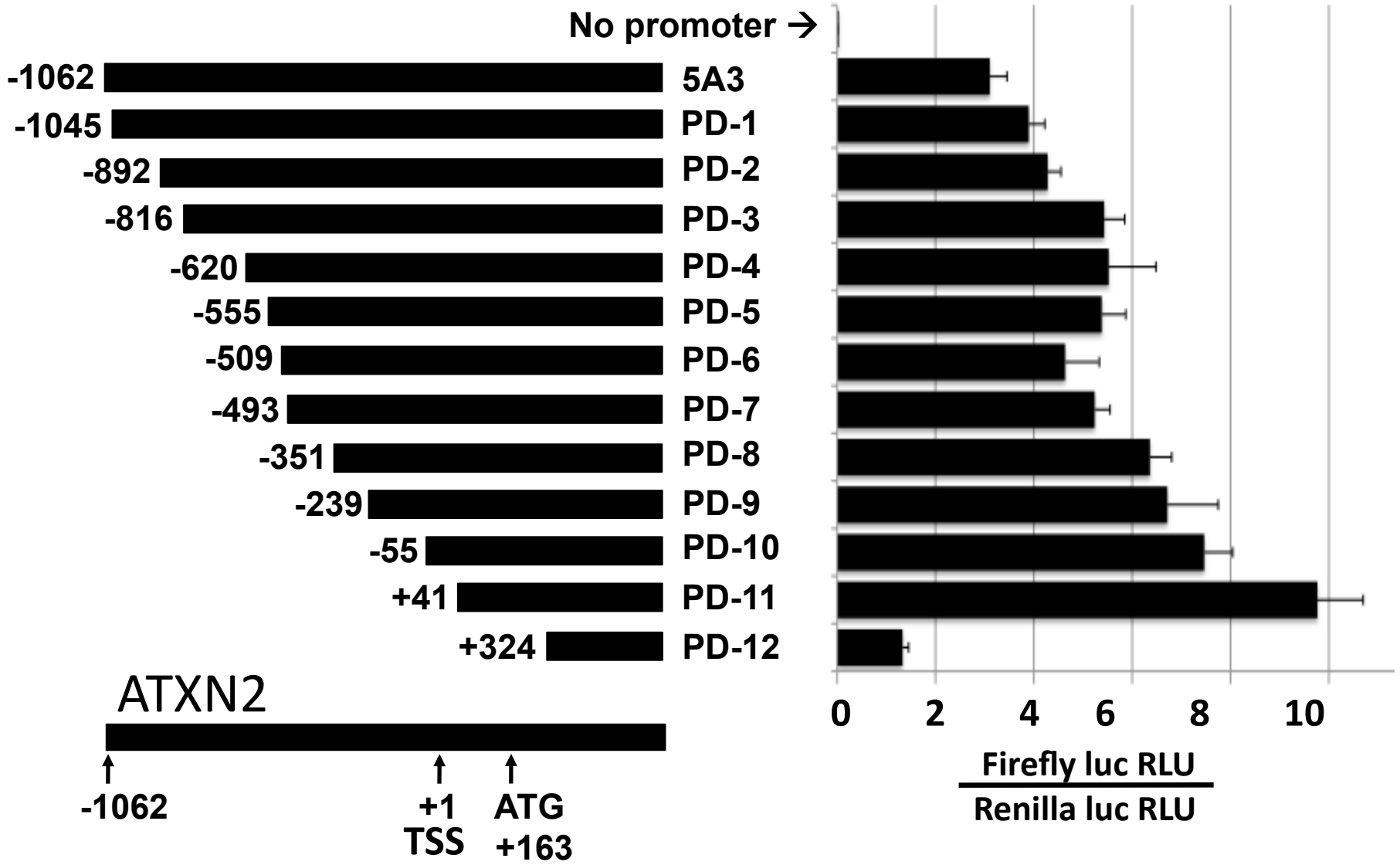
Liu et al., J. Neurosci 29,2009.
Ilya Bezprozvanny

pGL2-ATXN2-Luc

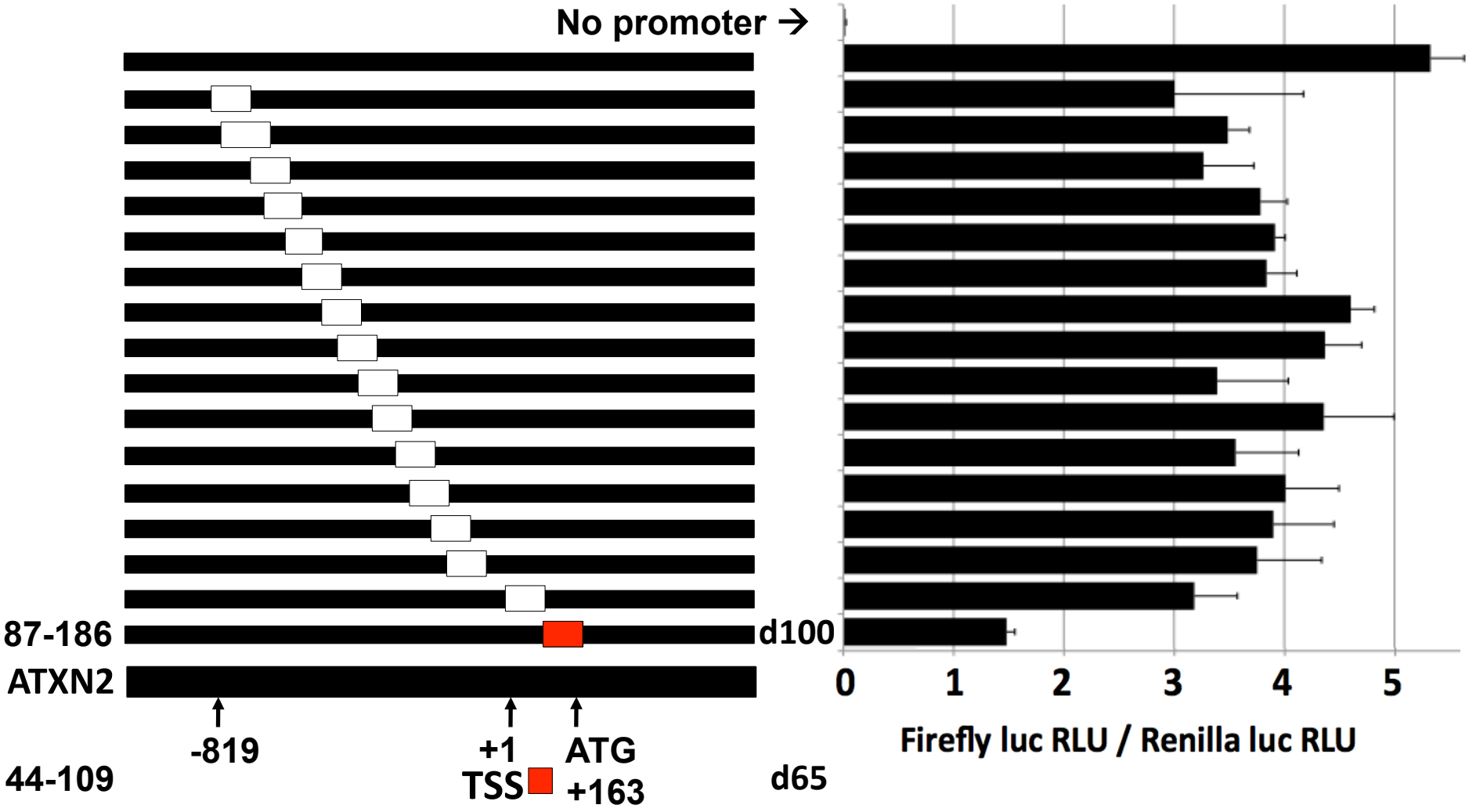
ATXN2-Luciferase Expression Construct



ATXN2 promoter deletions



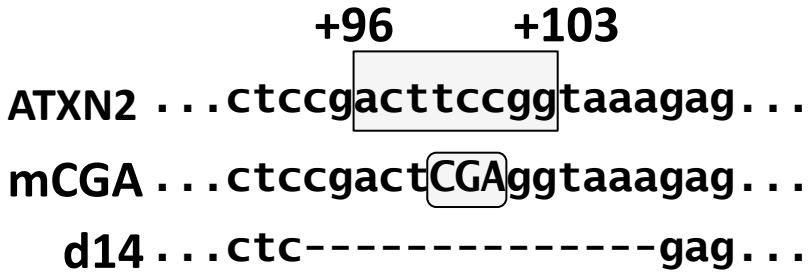
ATXN2 Interstitial Promoter Deletions

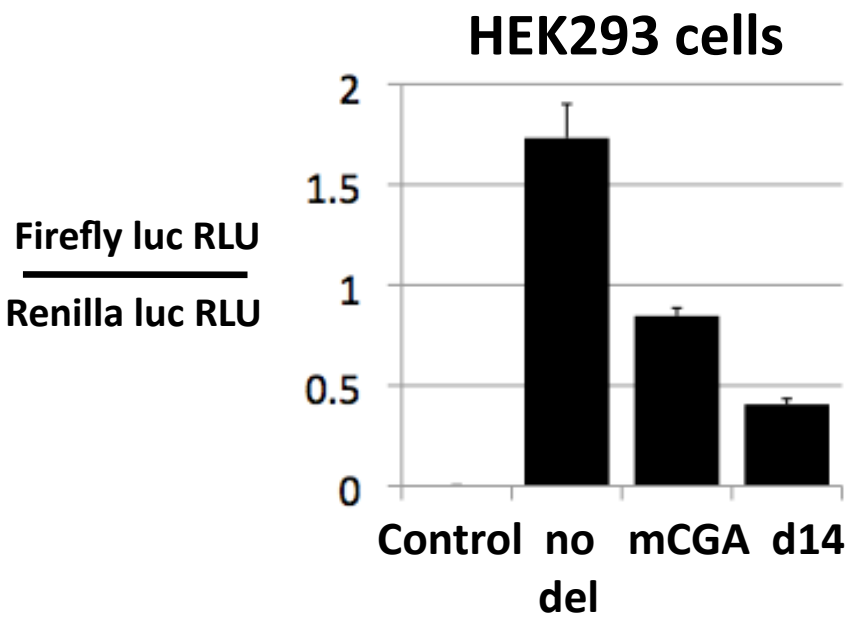


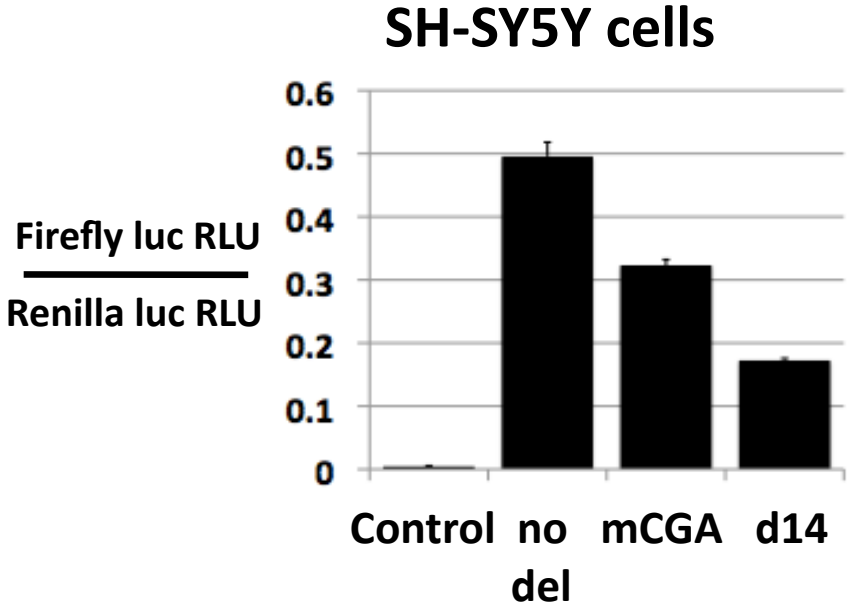
Deletion of an ETS family transcription factor binding site reduced ATXN2-luc expression

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

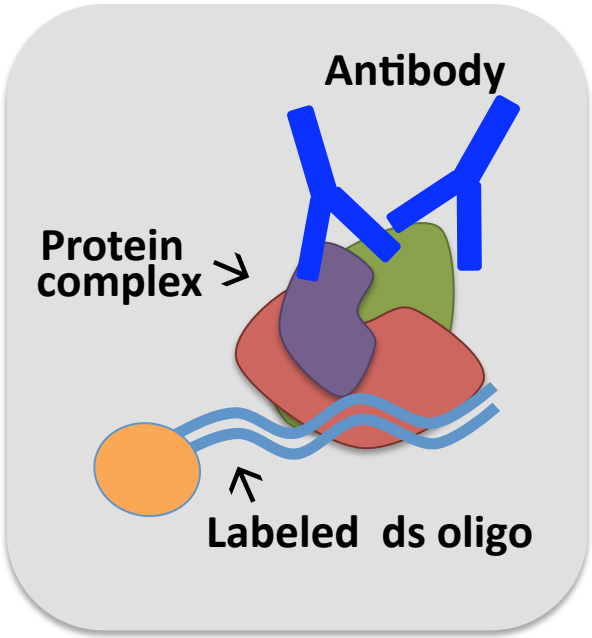
5'-CCGGAAGT-3'

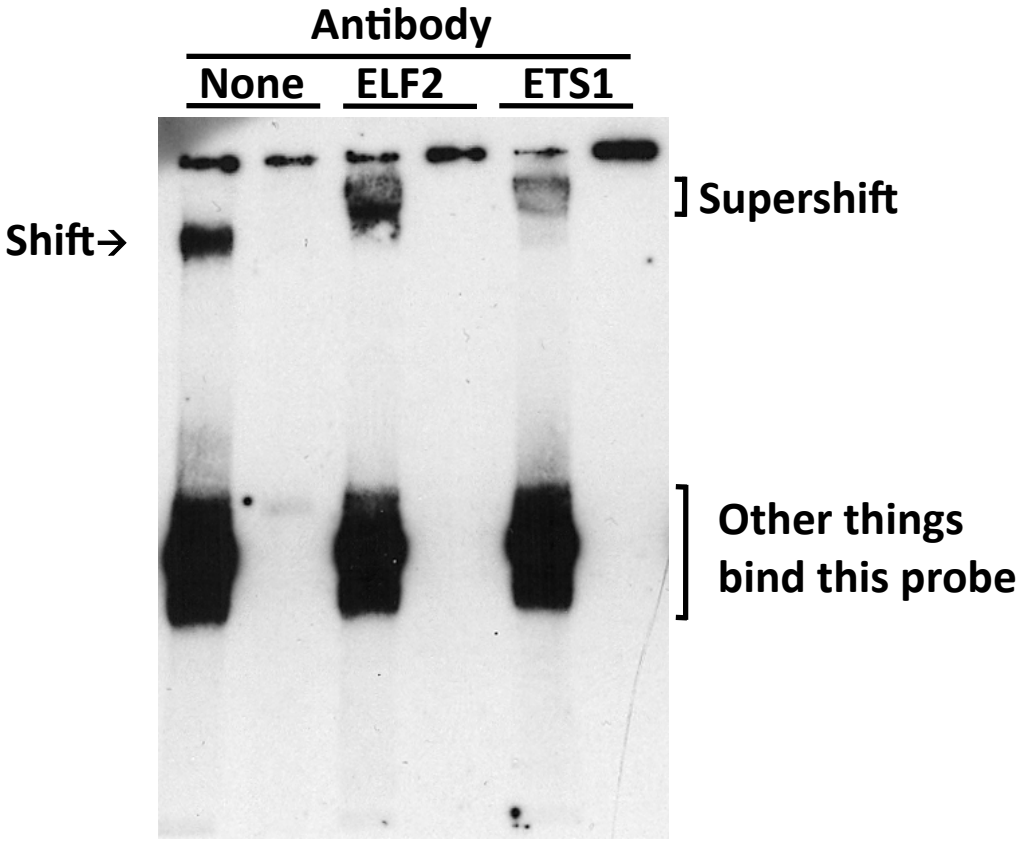






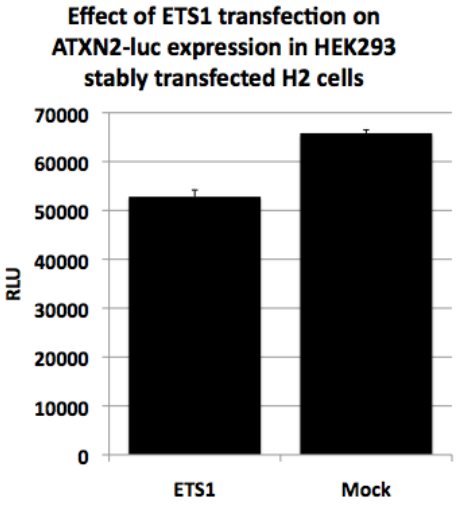
EMSA supershift assays verified interactions with ETS family transcription factors ELF-2 and ETS-1

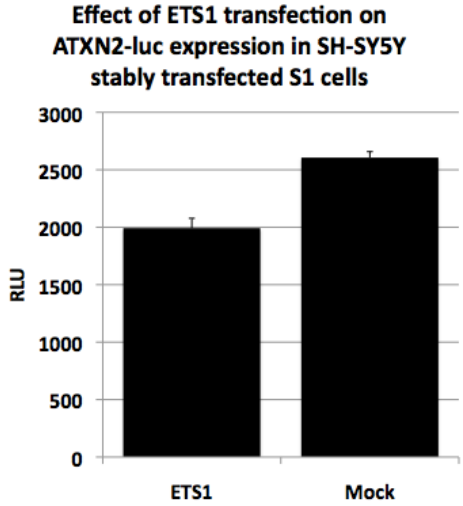


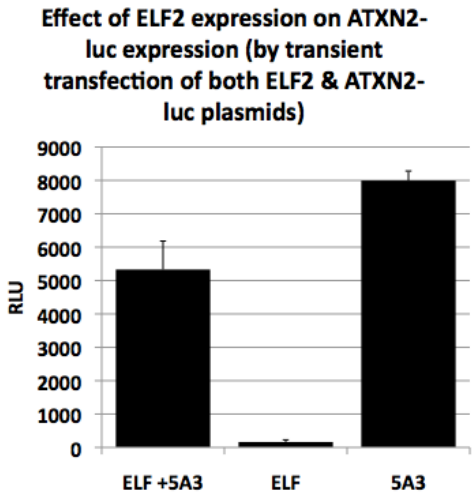


6% Native gel
SH-SY5Y protein lysate

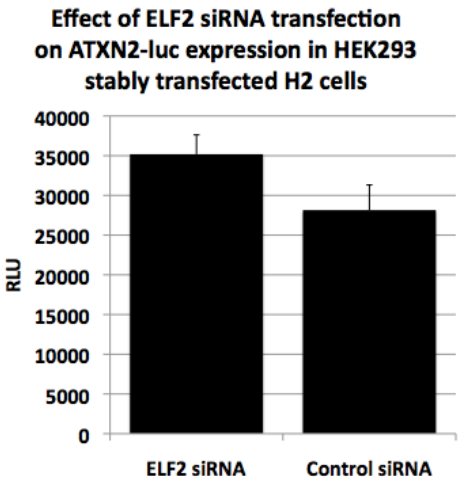
OVER-ESPRESSION OF ETS1 OR ELF2 REDUCES ATXN2-LUC EXPRESSION

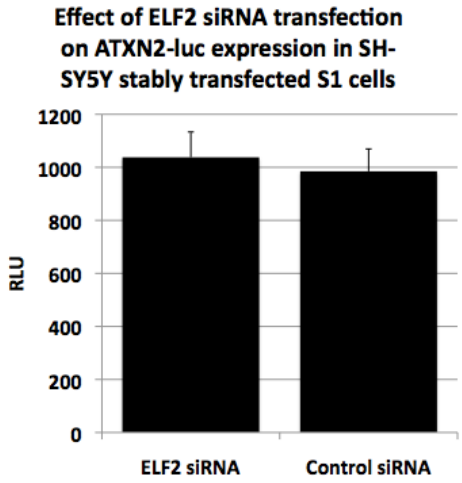






UNDER-EXPRESSION OF ELF2 INCREASES ATXN2-LUC EXPRESSION

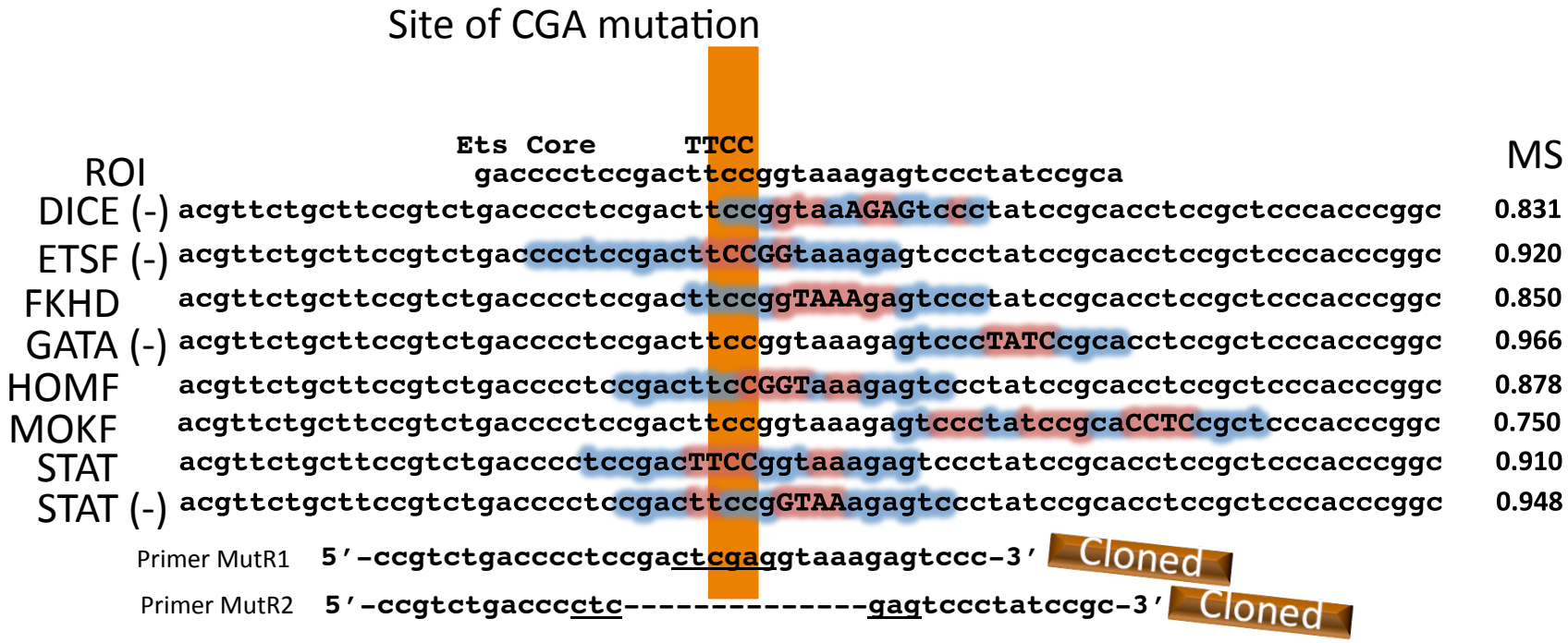




HYPOTHESIS:

ETS factors inhibit ATXN2 expression by preventing other factors from binding this very same element to turn up ATXN2-luc expression.

Matinspector Analysis



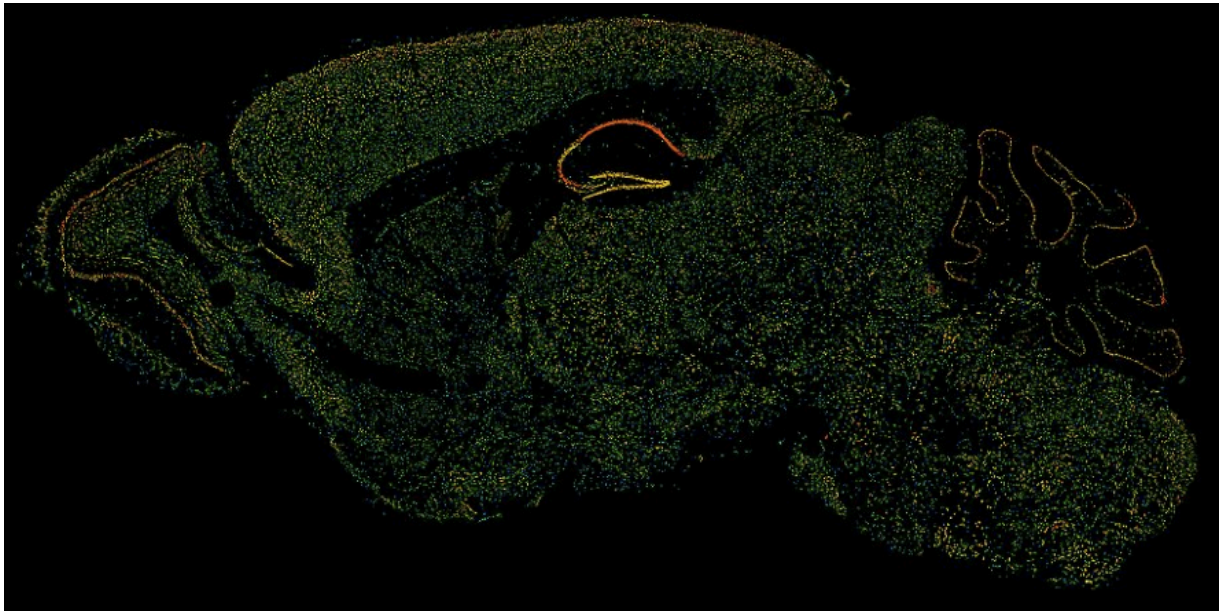
Caps = Core Sequence, the four highest conserved consecutive positions
Red = *Ci* vector (consensus index vector) > 60, ranges from 0-100
MS = Matrix similarity. Good match > 0.8, perfect match = 1.0

GATA-3 Has Dual Regulatory Functions in Human Interleukin-5 Transcription*

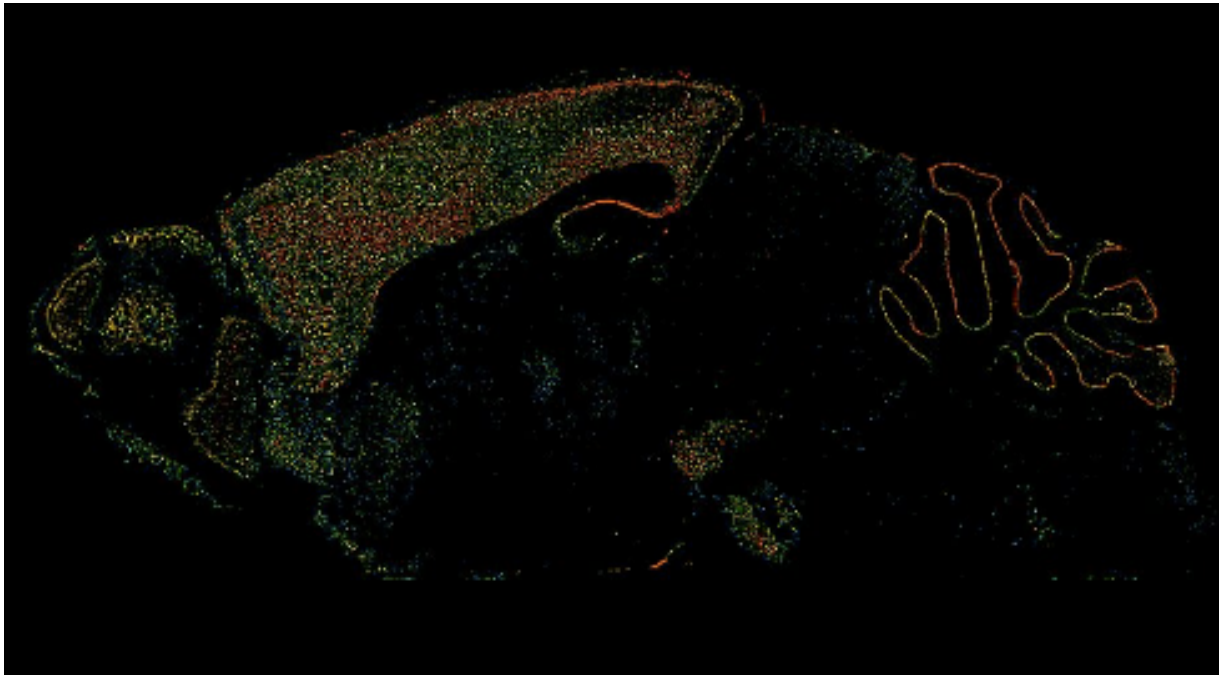
Gretchen T. F. Schwenger‡§, Régis Fournier¶, Chee Choy Kok‡, Viatcheslav A. Mordvinov‖, Deborah Yeoman‡ and Colin J. Sanderson‡

December 21, 2001 The Journal of Biological Chemistry, 276, 48502-48509.

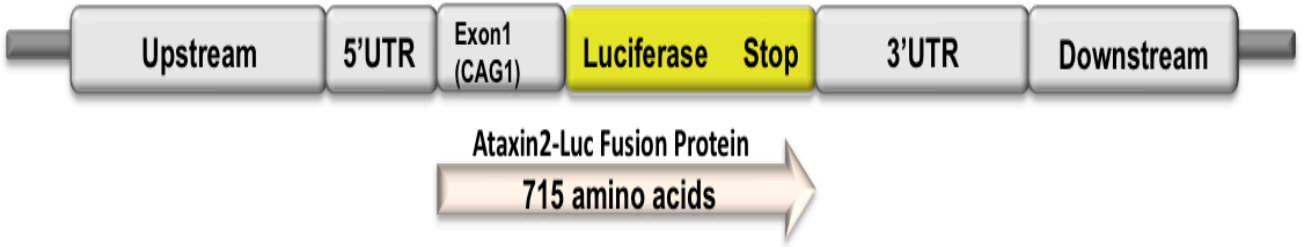
ATXN2

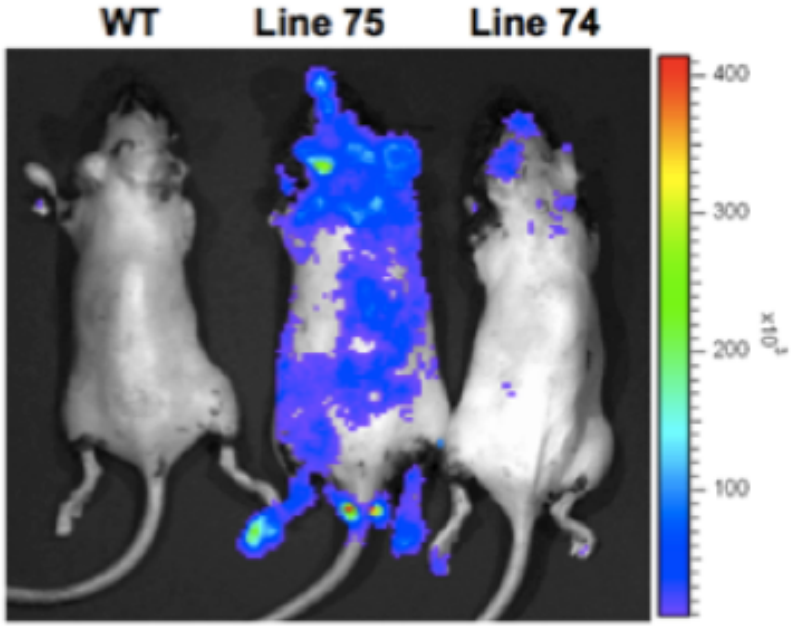


ETV5

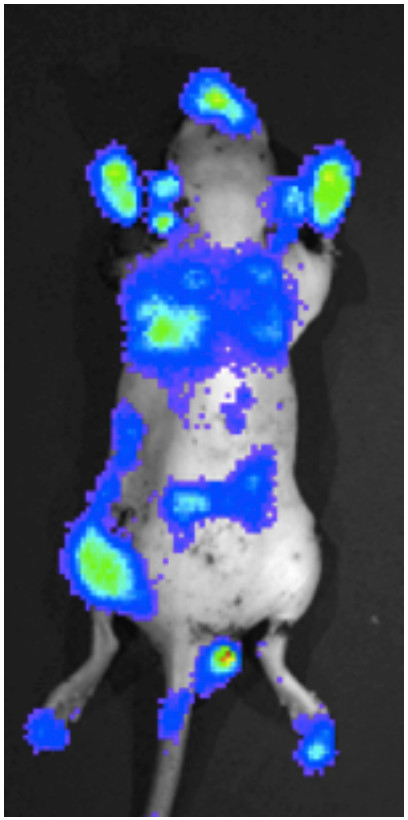


ATXN2-luc expression in mice





Line 75



ATXN2 expression in sensory – like tissues

Line 75

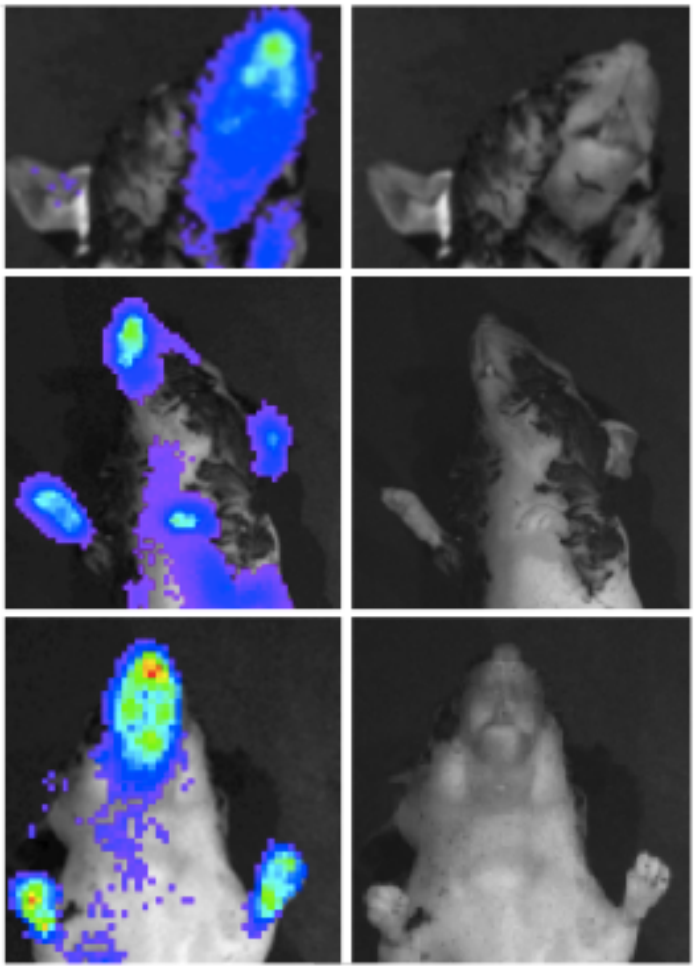
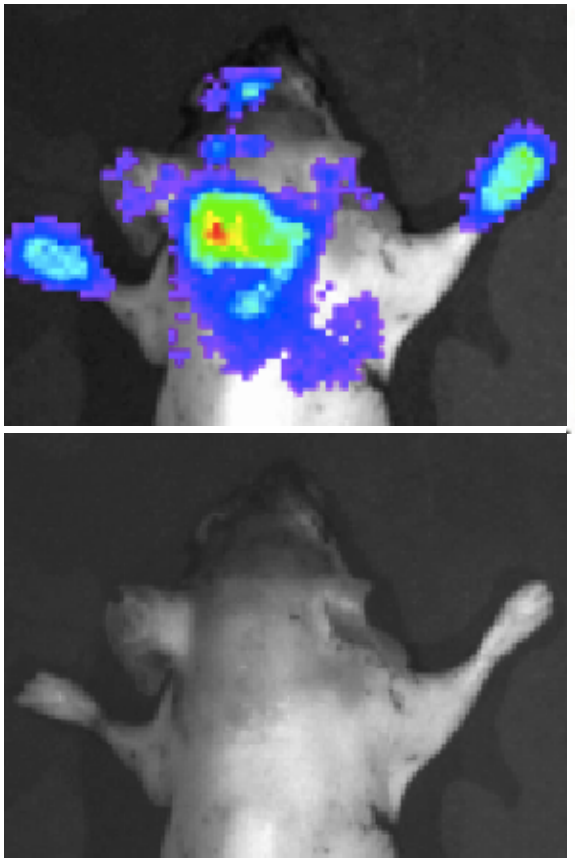
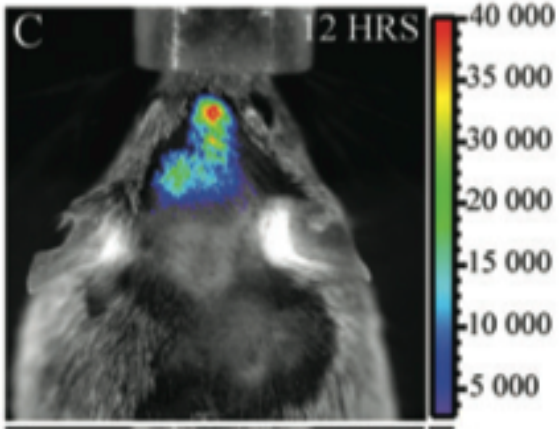


Photo with overlay Photo only

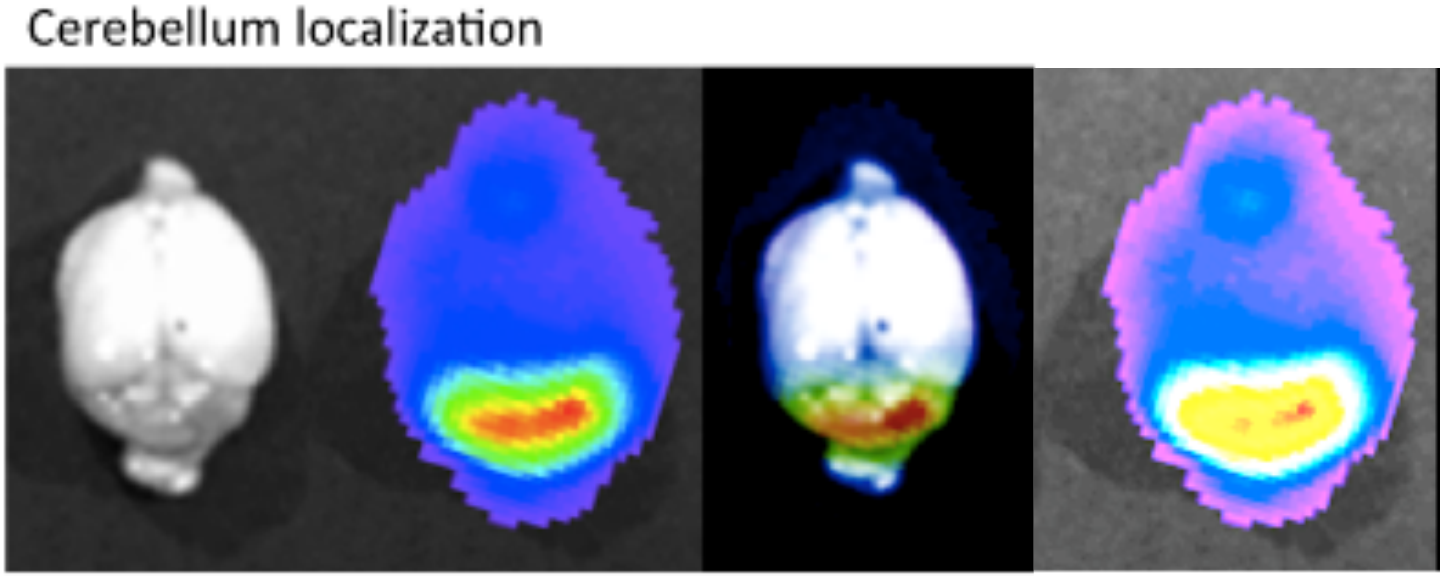
Line 75





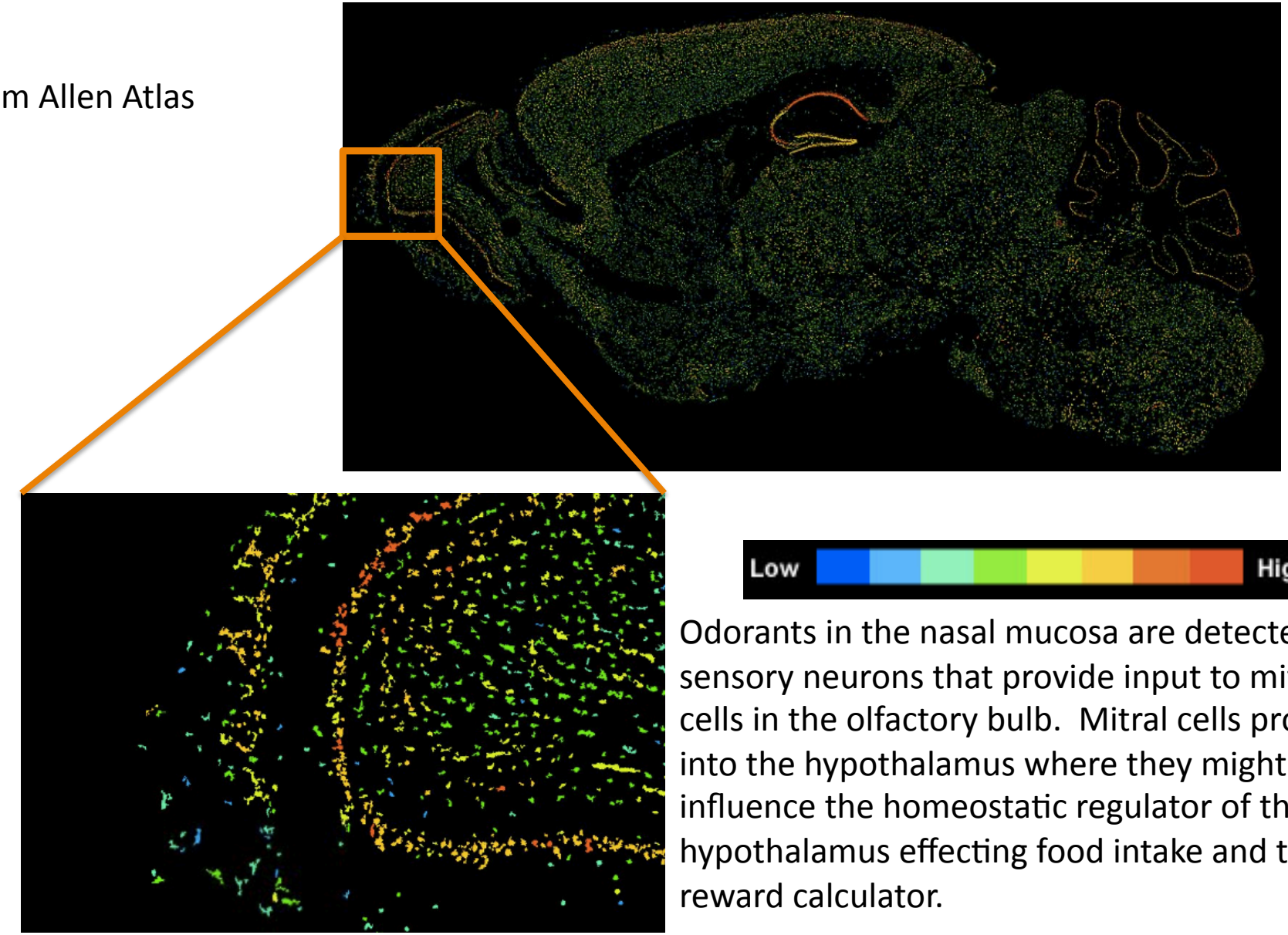
TLR4 in olfactory bulb
Brain 2009 132:940

ATXN2-luc expression in mice



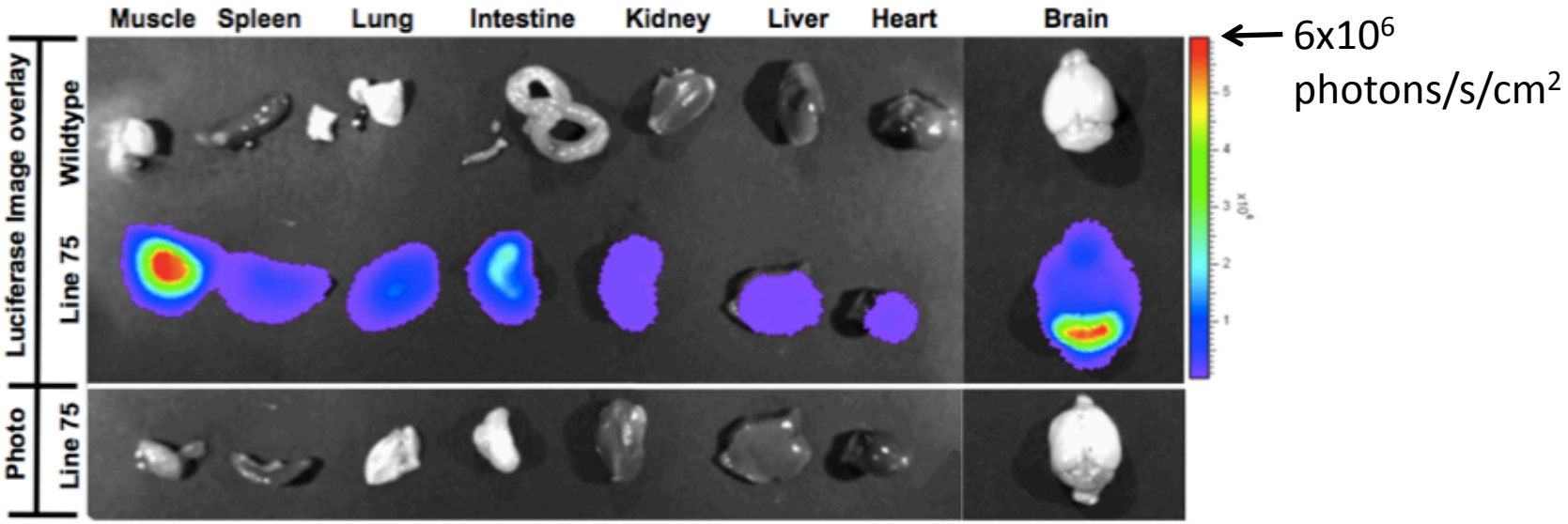
ATXN2 in olfactory bulb

From Allen Atlas



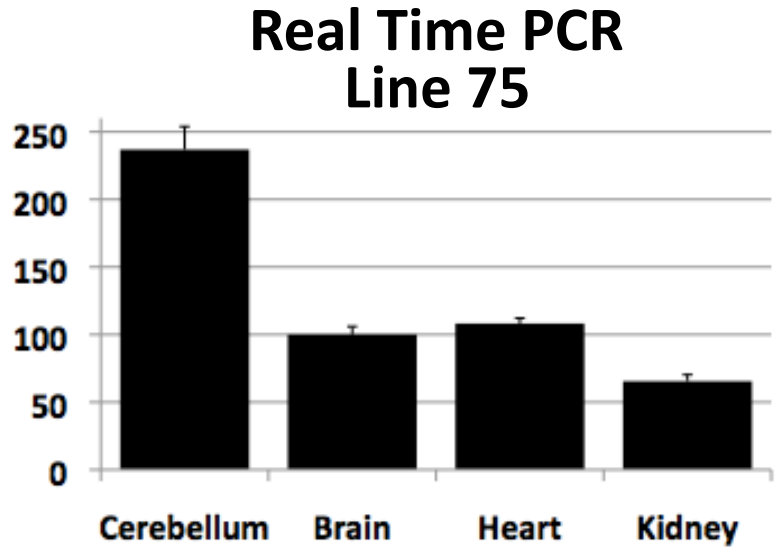
Odorants in the nasal mucosa are detected by sensory neurons that provide input to mitral cells in the olfactory bulb. Mitral cells project into the hypothalamus where they might influence the homeostatic regulator of the hypothalamus effecting food intake and the reward calculator.

ATXN2-luc expression in excised tissues



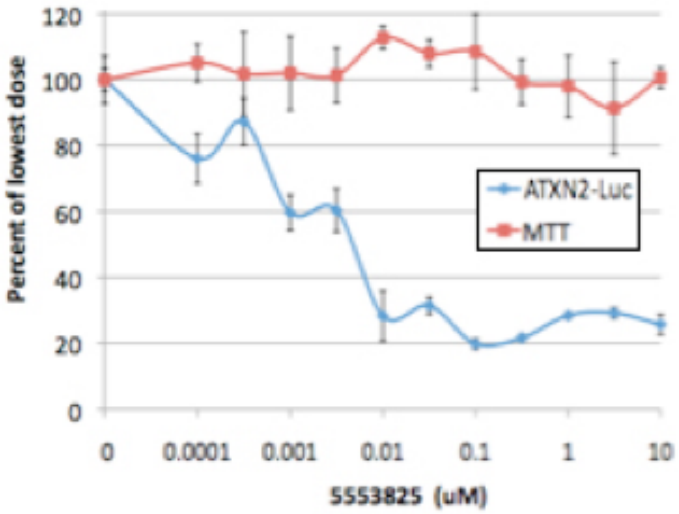
Luciferase / Mouse *Atxn2*

(% relative to brain)



Compound Screen

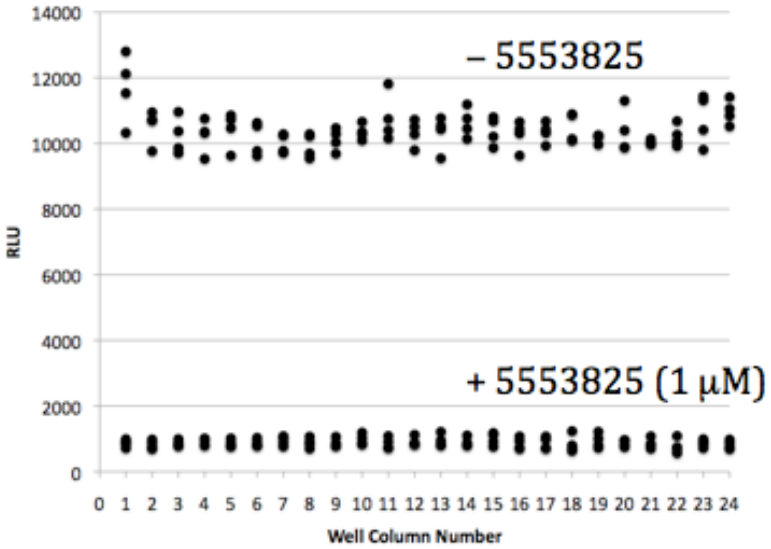
Quality of signal computations



$$\frac{S}{N} = \frac{10{,}321 - 388}{464 - 149} = 31.5$$

$$\frac{S}{B} = \frac{10{,}321}{388} = 26.6$$

$$Cv = \frac{\delta}{\mu} = \frac{1}{S/N} = \frac{1}{31.5} = 3.2\%$$



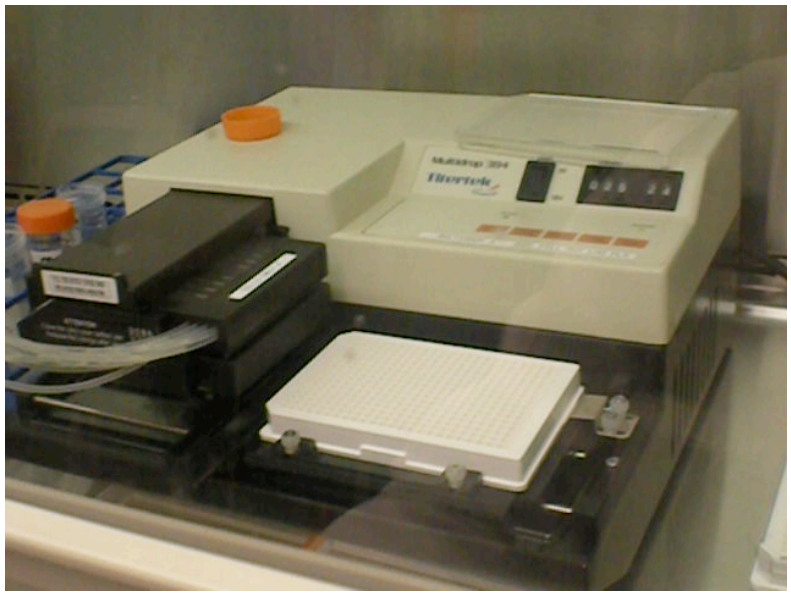
384 well plate

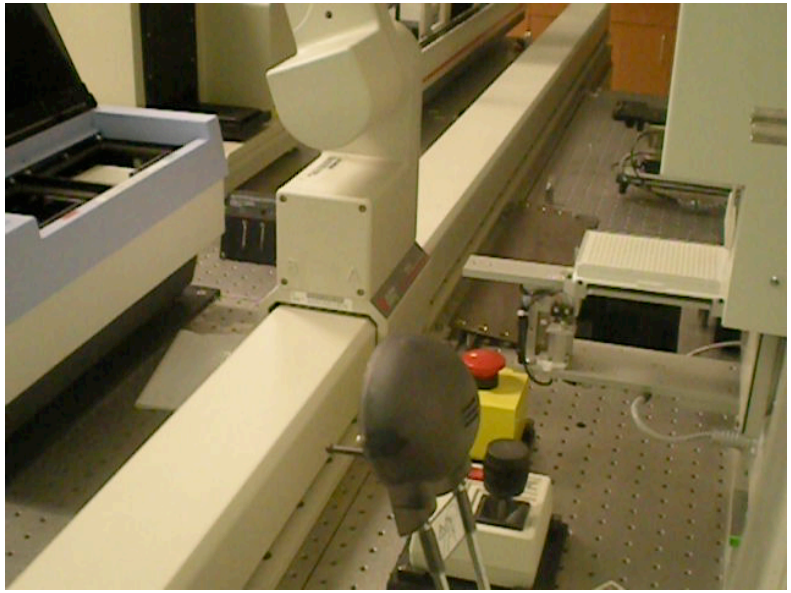
Measure of dynamic range

Z'-Factor = $1-[(3(\sigma_{exp}+\sigma_{cont}))/|\mu_{exp}-\mu_{cont}|]$

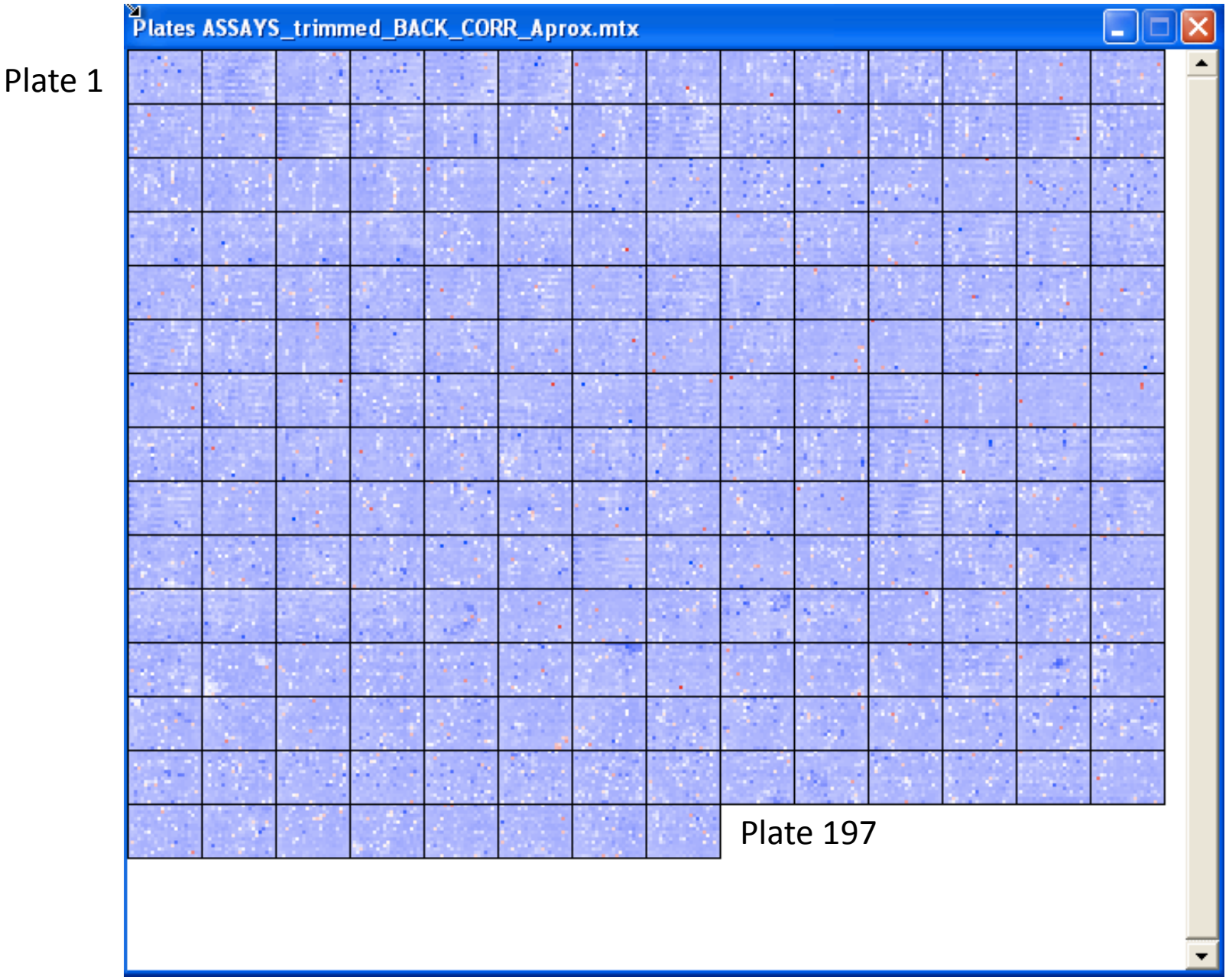
Z'-Factor = 0.8

Conducting the screen

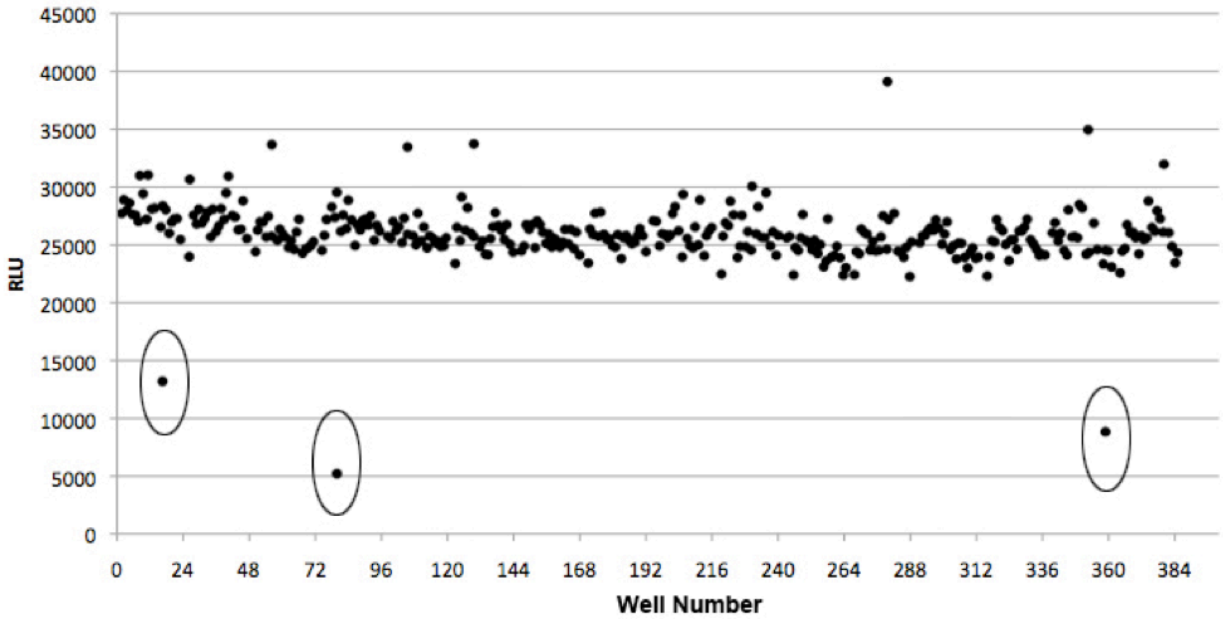




Corrected RLU Scores

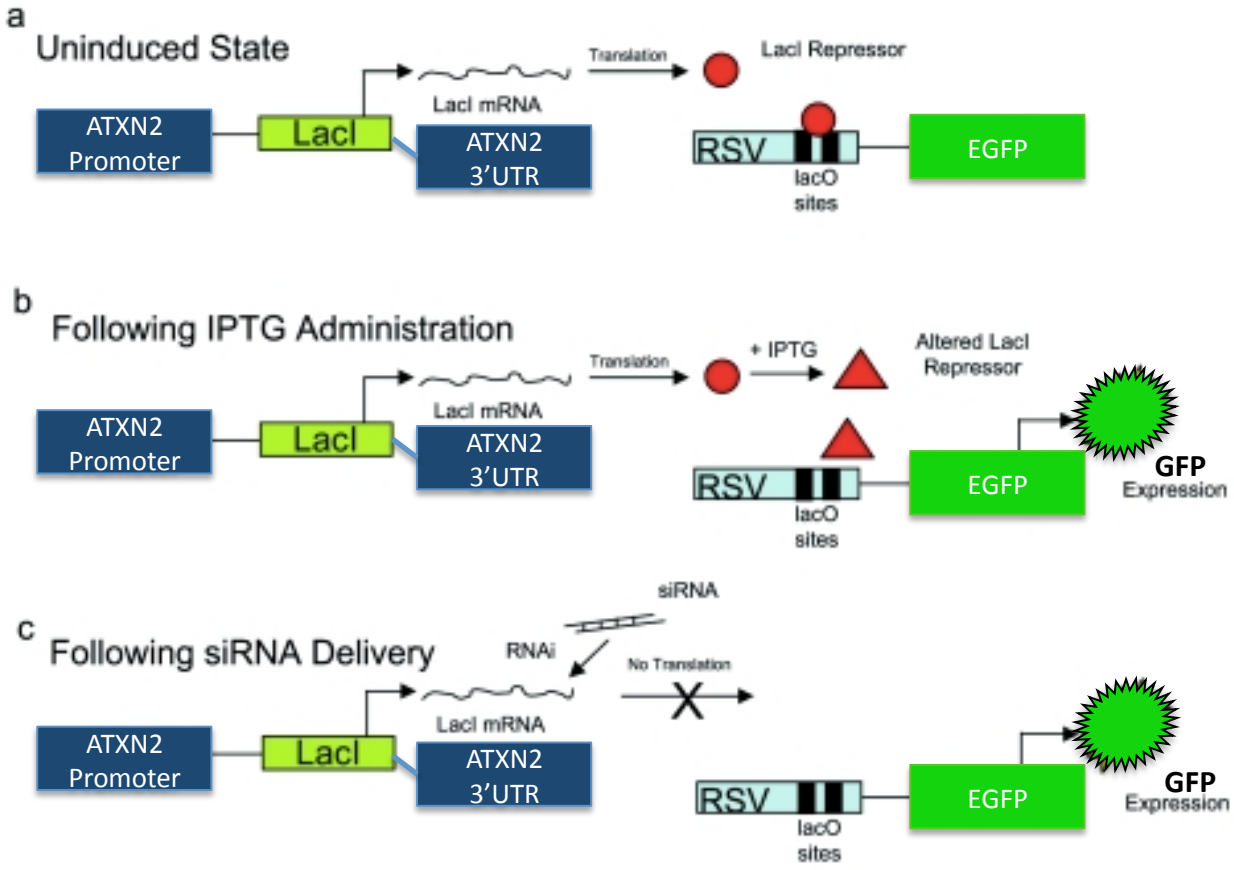


Compound Screen



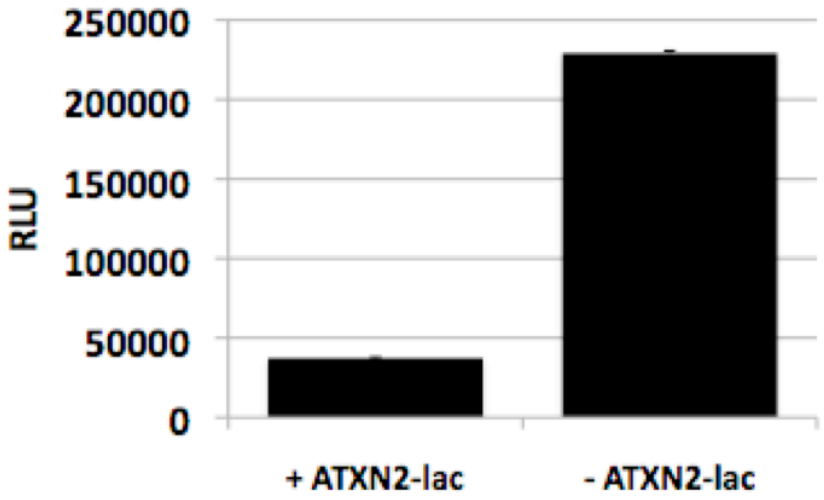
3’-UTR

Positive readout system



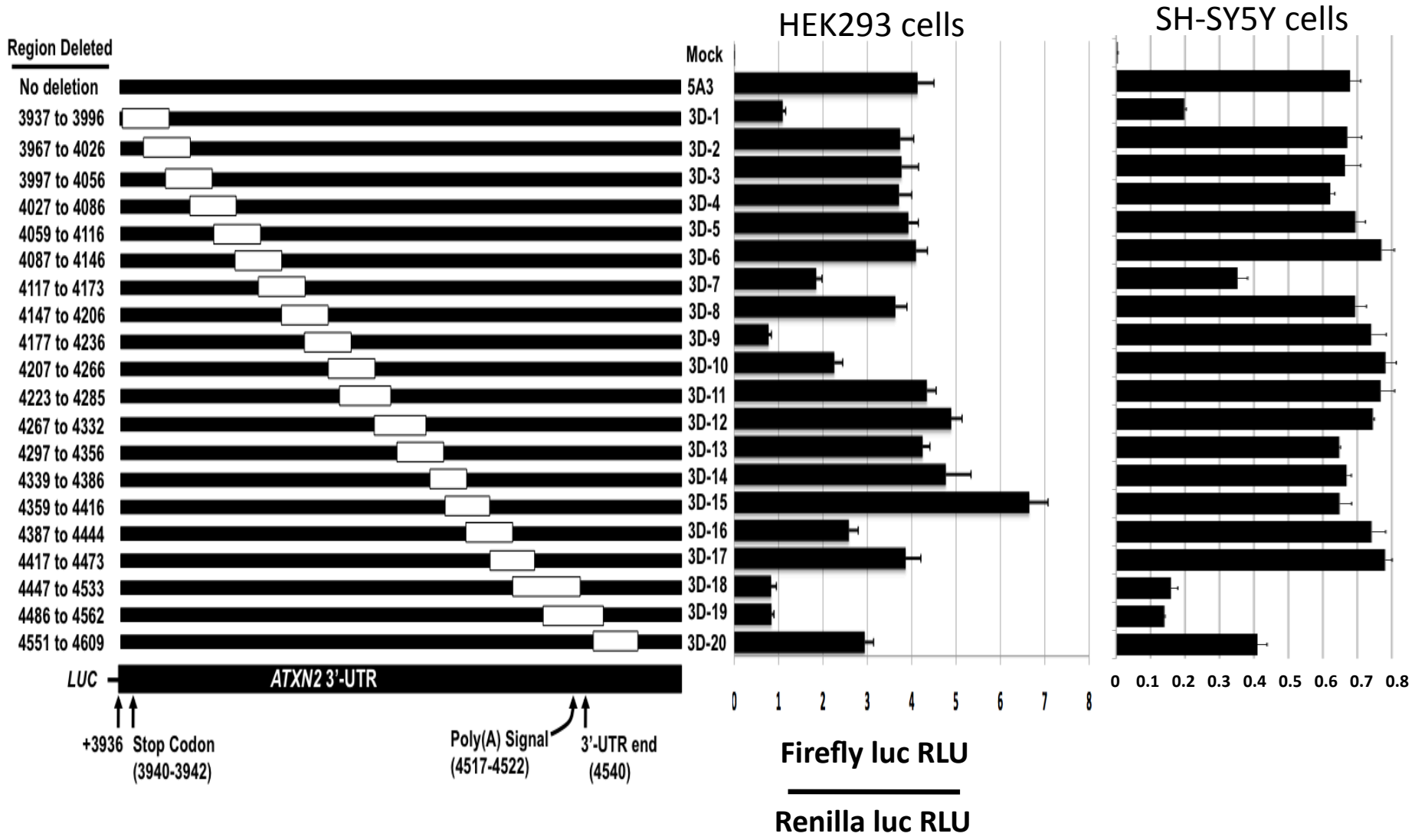
Mark Stevenson Oxford J RNAi Gene Silencing. 2009 Jun 12;5(1):331-8.

ATXN2 Positive readout system



HEK293 cells transiently transfected with pOPRSVI-luc+

ATXN2 3’-UTR Deletions



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.**

Acknowledgments

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THE UNIVERSITY OF UTAH

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"Dr. Duong Huynh is one of the few neuroscientists who is wheelchair-bound, and he is unrestricted, even unperturbed, by it. He has used his disability as inspiration and motivation to research the molecular biology of Parkinson's disease. In addition, he has been a model and mentor for Vietnamese students interested in biology and neuroscience."

— STEFAN PULST, MD, PROFESSOR AND CHAIR OF