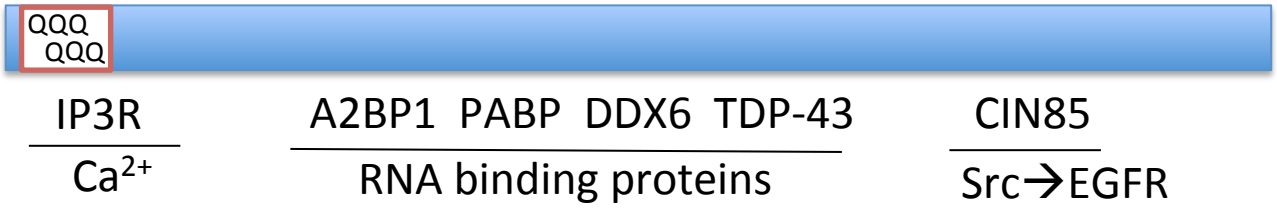
Developing a therapy for spinocerebellar ataxia type 2 (SCA2)

Spinocerebellar Ataxia Type 2 (SCA2)

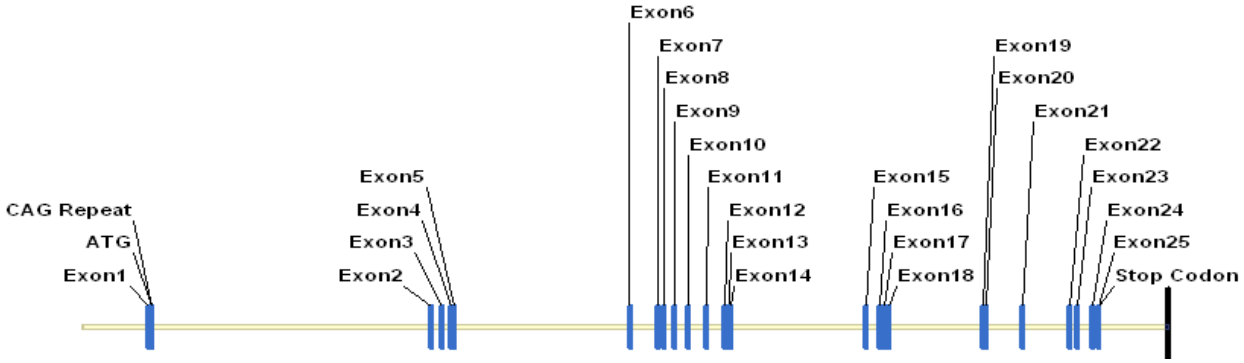
- Caused by CAG expansion → expanded polyQ
- CAG22 is common, CAG >35 causes disease

- Protein: ataxin-2



126.8 kDa
1152 aa

- Gene: *ATXN2*



12q24.12
147.5 kb
25 exons
5'-UTR 642 bp
3'-UTR 601 bp
Transcript 4702 bp

Hypothesis

Reduction of ataxin-2 dose is therapeutic for SCA2.

- SCA2 phenotype is worse in patients homozygous for the disease allele.
- SCA2 phenotype is worse in homozygous vs heterozygous *ATXN2* transgenic mice.
- *ATXN2* knockout mice are obese but have no neurodegeneration, while SCA2 patients are lean.
- SCA1 & SCA3 mouse phenotypes are reversible.
- *ATXN1* shRNA injection improves *ATXN1* mouse phenotype.

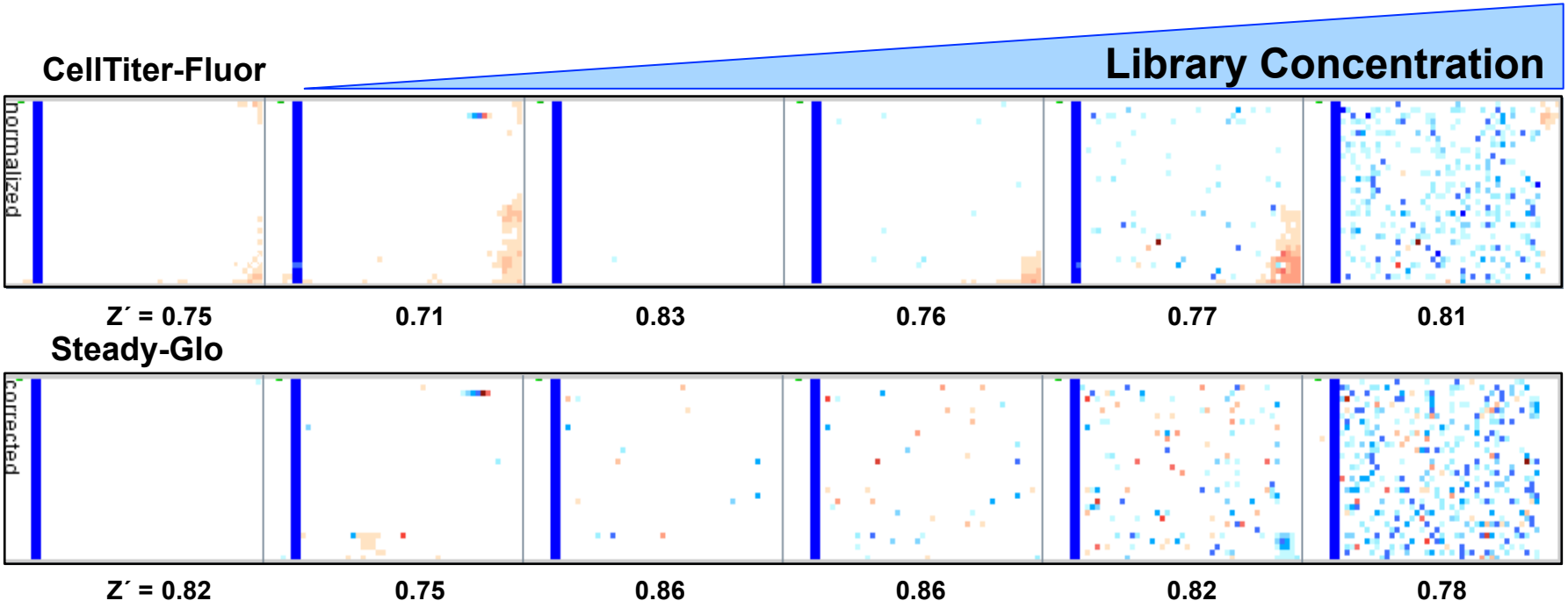
Compound Screening

Screen 350,000+ compounds for inhibitors of *ATXN2-luc* expression

NCGC	Primary assay:	qHTS for ATXN2-luc inhibition
	Secondary assay 1:	Recombinant FFLuc counter-screen
	Secondary assay 2:	SH-SY5Y toxicity test
UTAH	Secondary Assay 3:	ATXN2-lac repressor / lac operator luc
	Secondary Assay 4:	CMV-luc
	Tertiary assay 1:	qRT-PCR for endogenous *ATXN2*
	Tertiary assay 2:	Western blots for ataxin-2

LOPAC Screen

1280 compounds in 1536-well plates

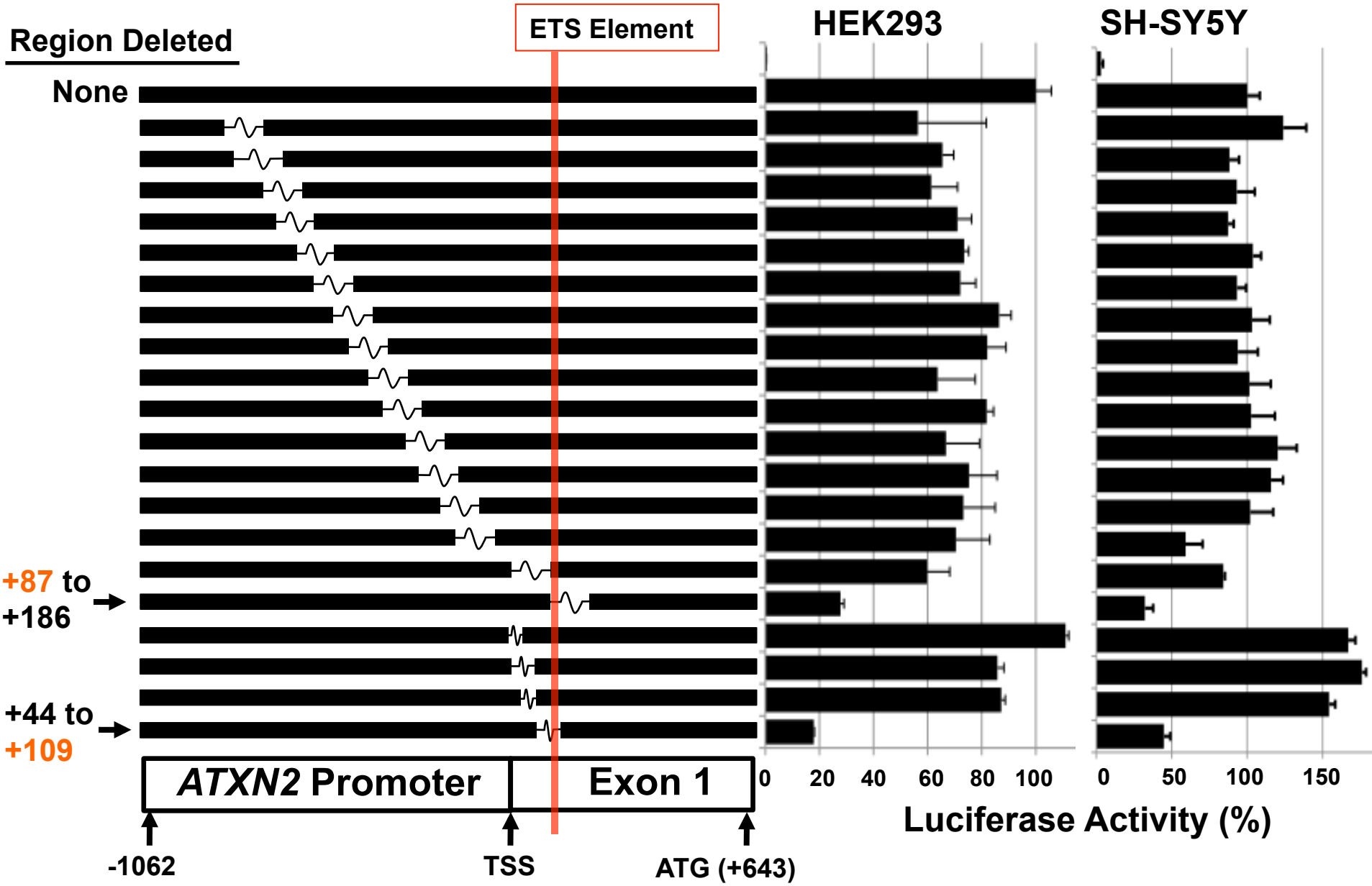


Promoter analysis

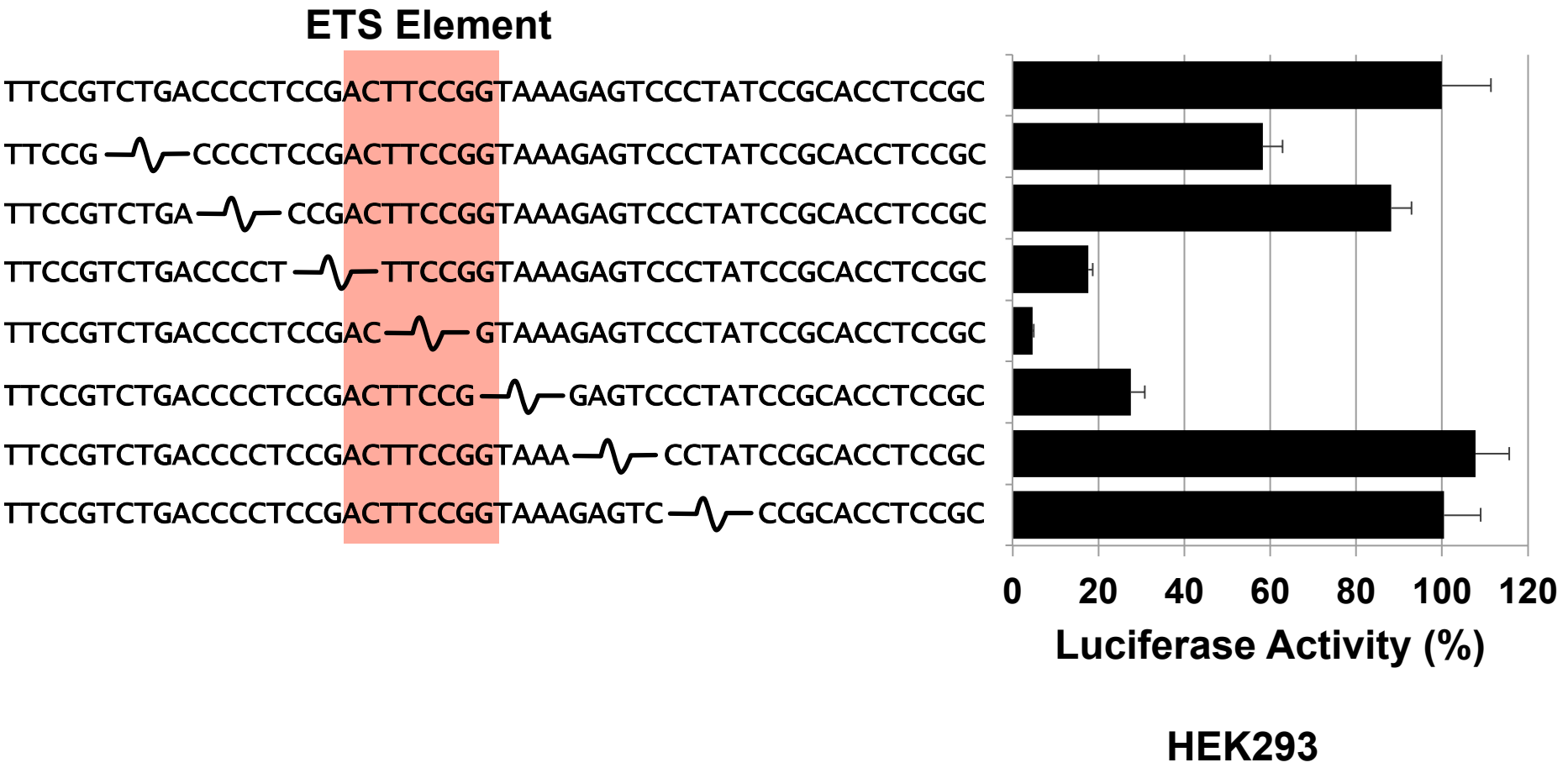
- Characterize the expression control regions of *ATXN2* and see if anything could be exploited therapeutically

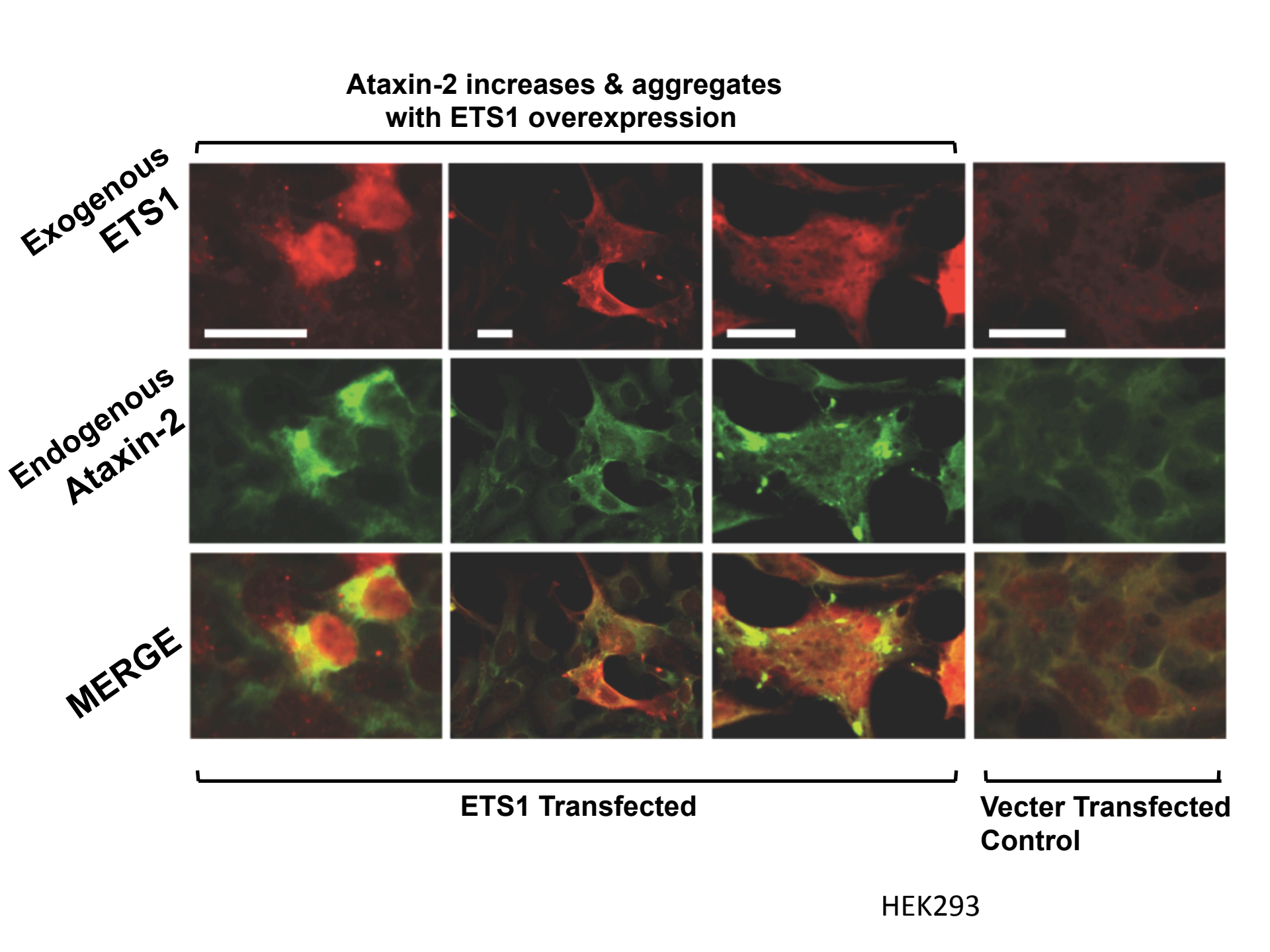
- *ATXN2* promoter and 3'-UTR deletions useful for determining promoter regions required for compound action

ATXN2 promoter deletions containing an ETS element reduce luciferase expression

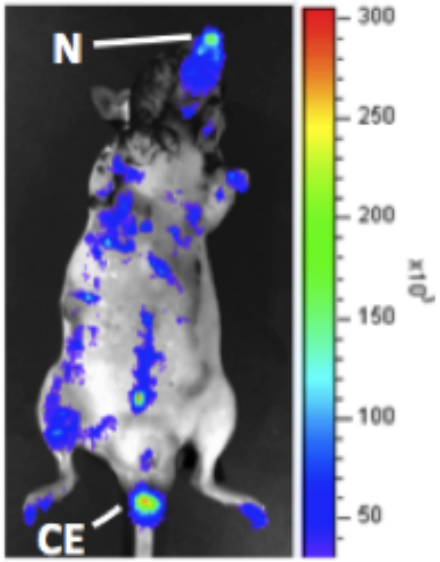


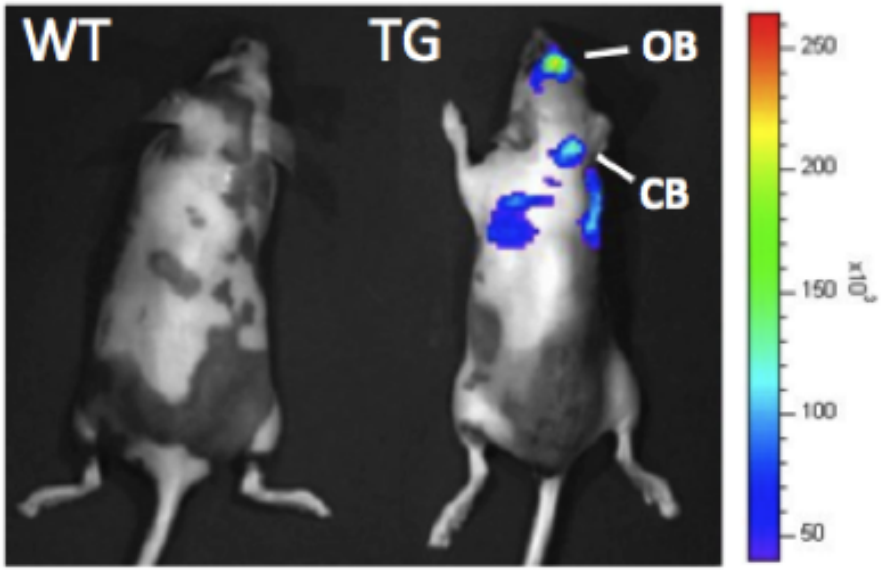
Like for regions required for ETS transcription factor action, *ATXN2* deletions may be useful for identifing regions required for compound action

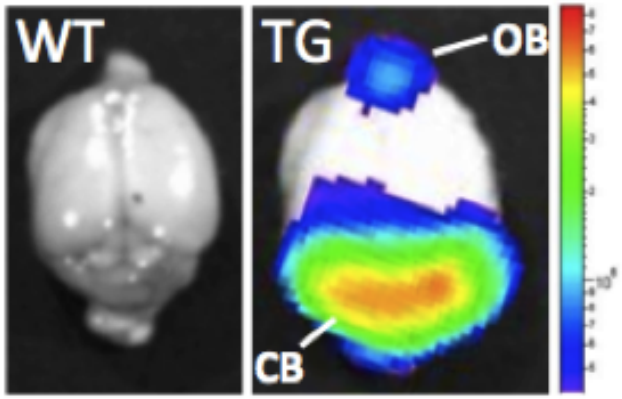


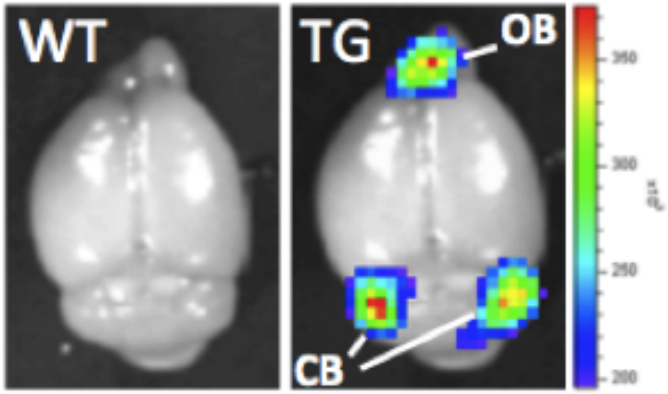

Ataxin-2 increases & aggregates
with ETS1 overexpression
Exogenous ETS1
Endogenous Ataxin-2
MERGE
ETS1 Transfected
Vecter Transfected Control
HEK293

ATXN2-luc Transgenic Mouse

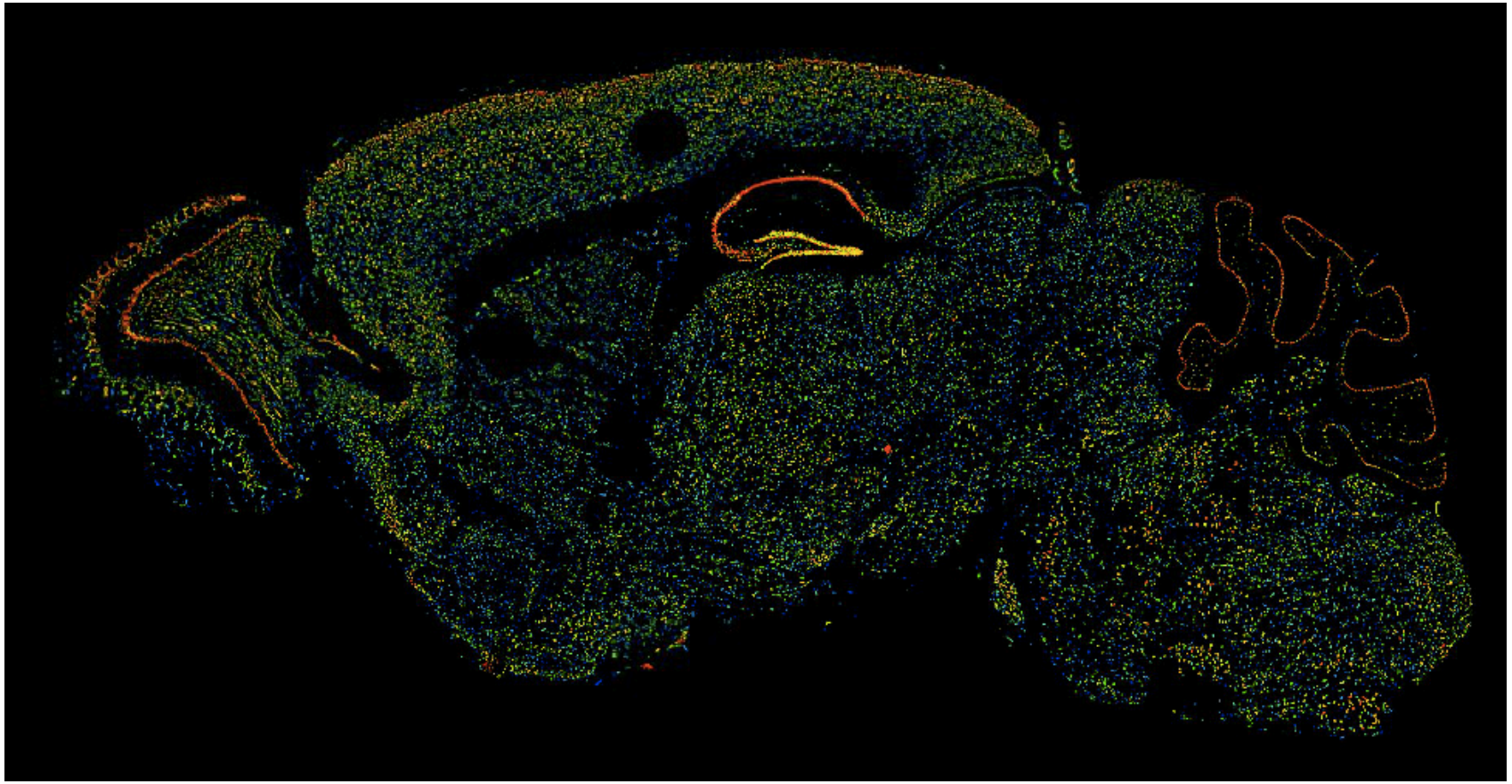




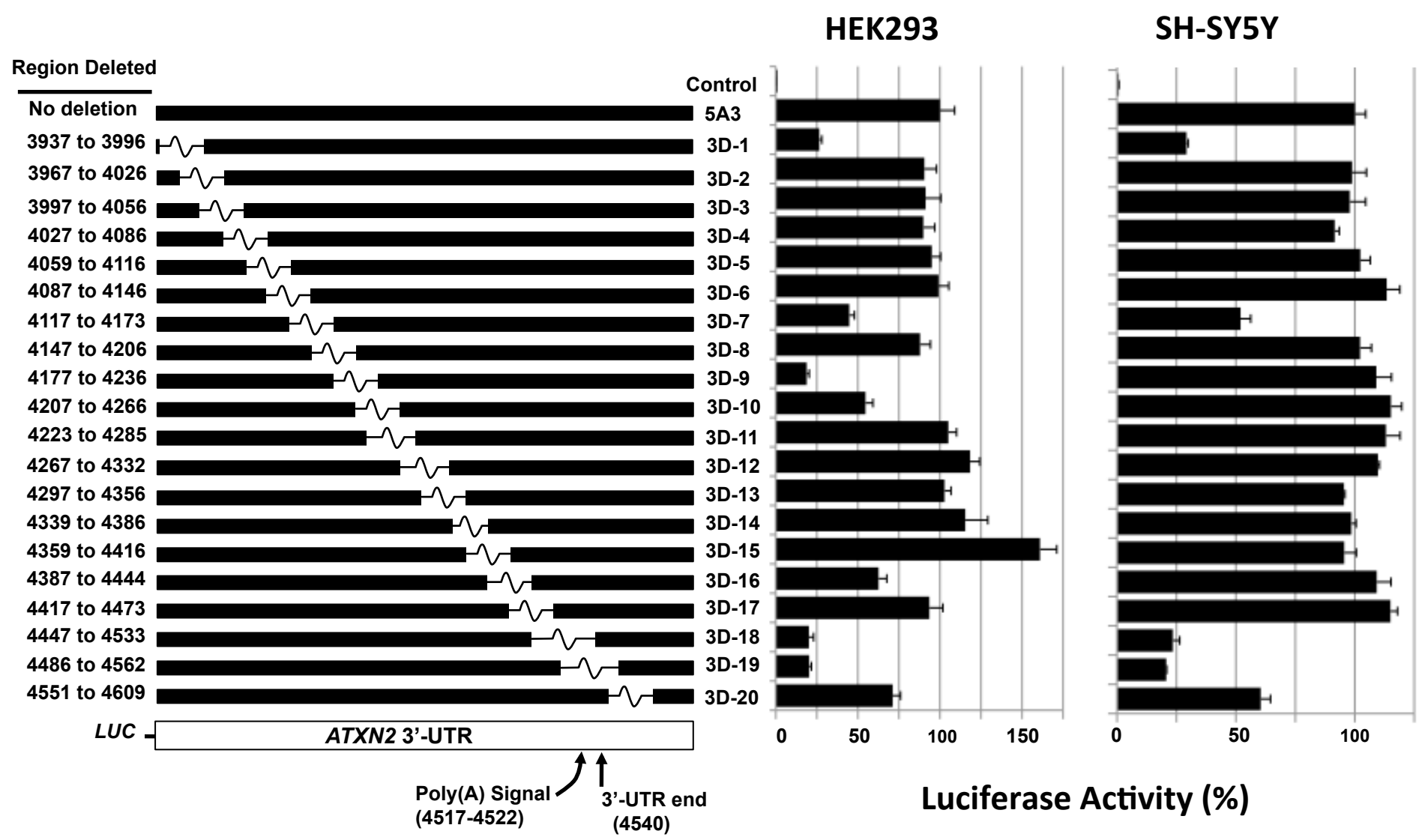




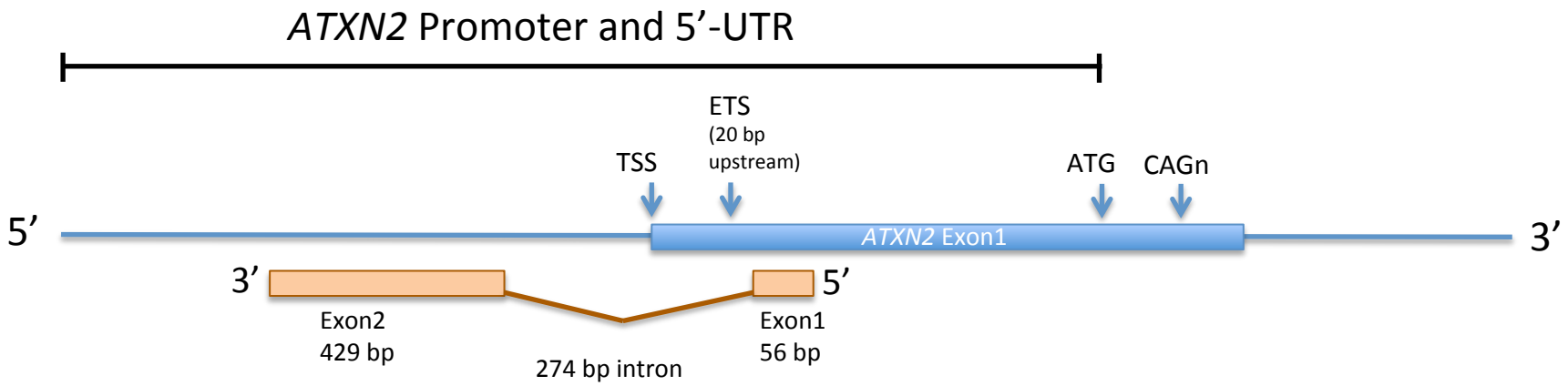
ATXN2 expression
Allen Brain Atlas



ATXN2 3’-UTR Deletions



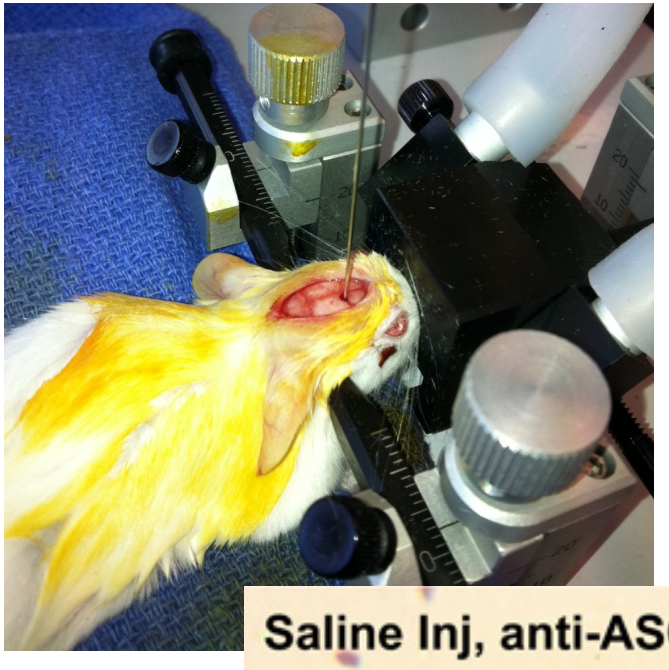
Expression of *ATXN2* antisense ncRNA may regulate *ATXN2*

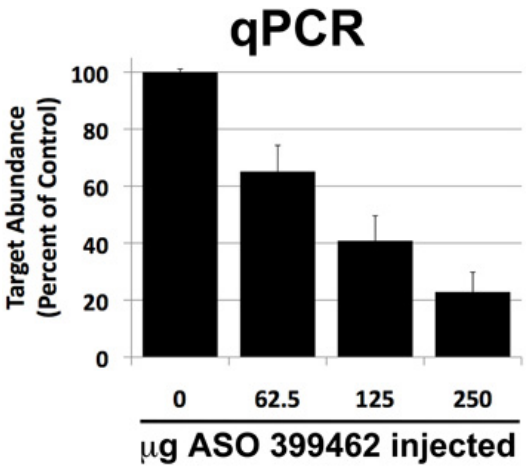


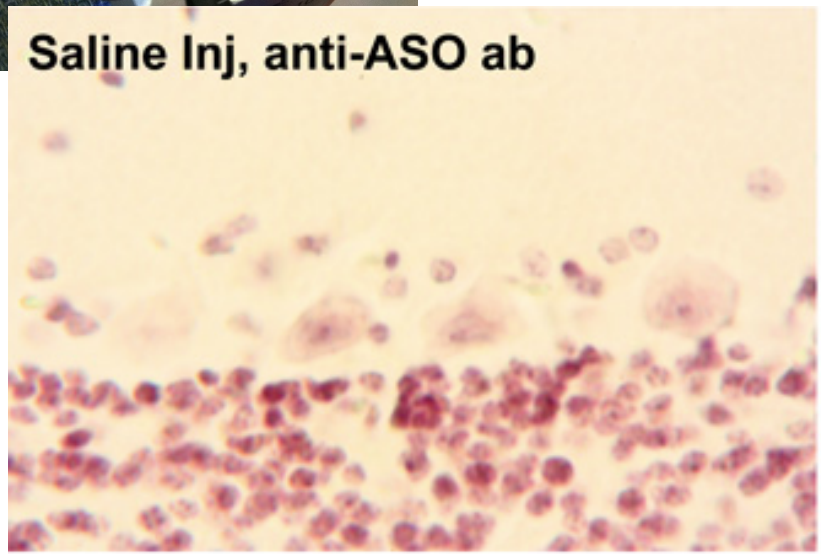
- Compounds downregulating *ATXN2* may up- or down-regulate *ATXN2* ncRNA
- A similar type of regulatory mechanism has been observed for SCA7:

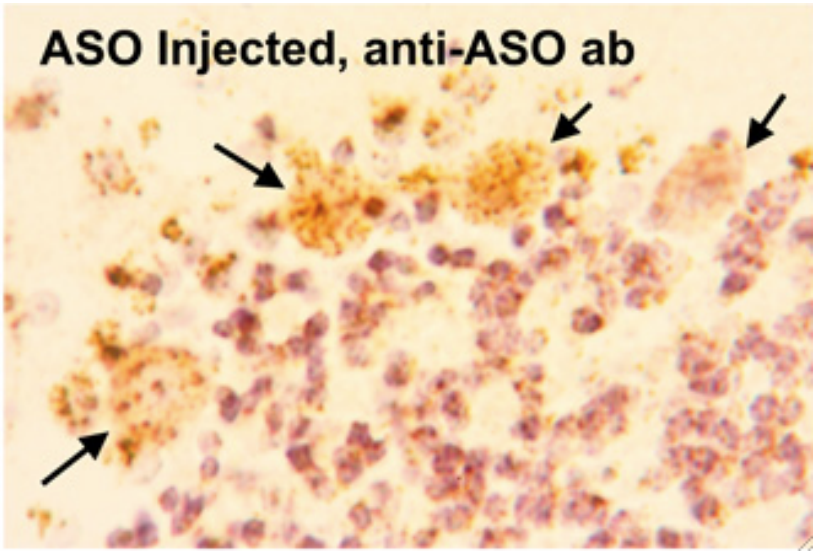
  Sopher et al (La Spada lab) CTCF regulates ataxin-7 expression through promotion of a convergently transcribed, antisense noncoding RNA. Neuron. 2011 Jun 23;70(6):1071-84.

ATXN2 Antisense ODNs









Citations supporting the hypothesis

Alves, S., Nascimento-Ferreira, I., Dufour, N., Hassig, R., Auregan, G., Nobrega, C., Brouillet, E., Hantraye, P., Pedroso de Lima, M.C., Deglon, N.*, et al.* (2010) Silencing ataxin-3 mitigates degeneration in a rat model of Machado-Joseph disease: no role for wild-type ataxin-3? *Hum Mol Genet,* 19:2380-2394.

Boy, J., Schmidt, T., Wolburg, H., Mack, A., Nuber, S., Bottcher, M., Schmitt, I., Holzmann, C., Zimmermann, F., Servadio, A.*, et al.* (2009) Reversibility of symptoms in a conditional mouse model of spinocerebellar ataxia type 3*. Hum Mol Genet,* 18:4282-4295.

Huynh, D.P., Figueroa, K., Hoang, N. and Pulst, S.M. (2000) Nuclear localization or inclusion body formation of ataxin-2 are not necessary for SCA2 pathogenesis in mouse or human. *Nat Genet,* 26:44-50.

Ragothaman, M. and U. Muthane. (2008) Homozygous SCA 2 mutations changes phenotype and hastens progression. *Mov Disord* 23:770-1.

Yamamoto, A., Lucas, J.J., and Hen, R. (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease*. Cell,* 101:57-66.

Zu, T., Duvick, L.A., Kaytor, M.D., Berlinger, M.S., Zoghbi, H.Y., Clark, H.B., and Orr, H.T. (2004) Recovery from polyglutamine-induced neurodegeneration in conditional SCA1 transgenic mice*. J Neurosci,* 24:8853-8861.