Overview

Obj: To show ETS overexpression alters ATXN2-luc expression

Method: Co-transfect ATXN2-firefly luc with an ETS expression plasmid
Also do same with ATXN2-firefly luc with ETS site mutated.
Transfections are normalized by including a renilla luciferase plasmid.
Measure firefly luc RLU relative to Renilla luc RLU.

Result: First we look at just the renilla luciferase plasmid… the result showed ETS reduced expression from SV40-renilla luc but slightly increased TK-renilla luc expression, so we used the TK-renilla luc plamid in our expreiments.

Ultimately the data suggest ETS factors inhibit ATXN2-luciferase expression.

The slideshow ends with a “working model” for how ETS factors binding at multiple sites in ATXN2 might alter ATXN2 expression, based on the data we have so far.

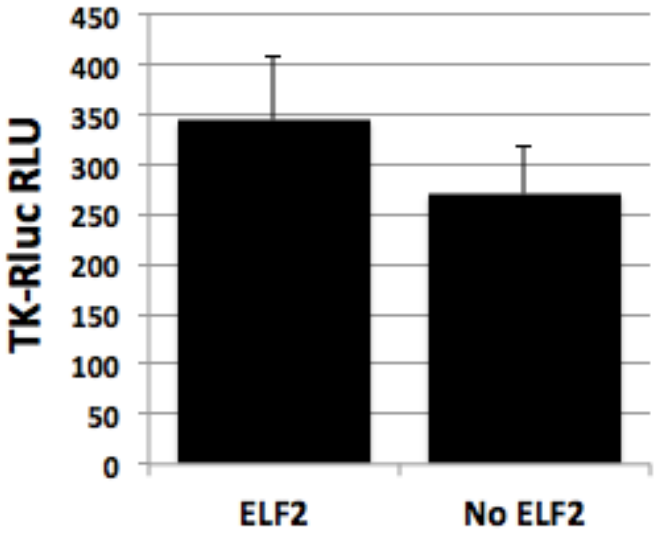
We found that ETS factors altered the expression of our Renilla luciferase reporter plasmids used for transfection normalizaiton

ETS factors slightly increase TK-Rluc expression
ETS factors highly reduce SV40-Rluc expression

The next 6 slides present this:

Cotransfection of ELF2 plasmid with TK-Renilla luc plasmid increased TK-Renilla luc expression

P<0.05 (t-test) N=6 transfections



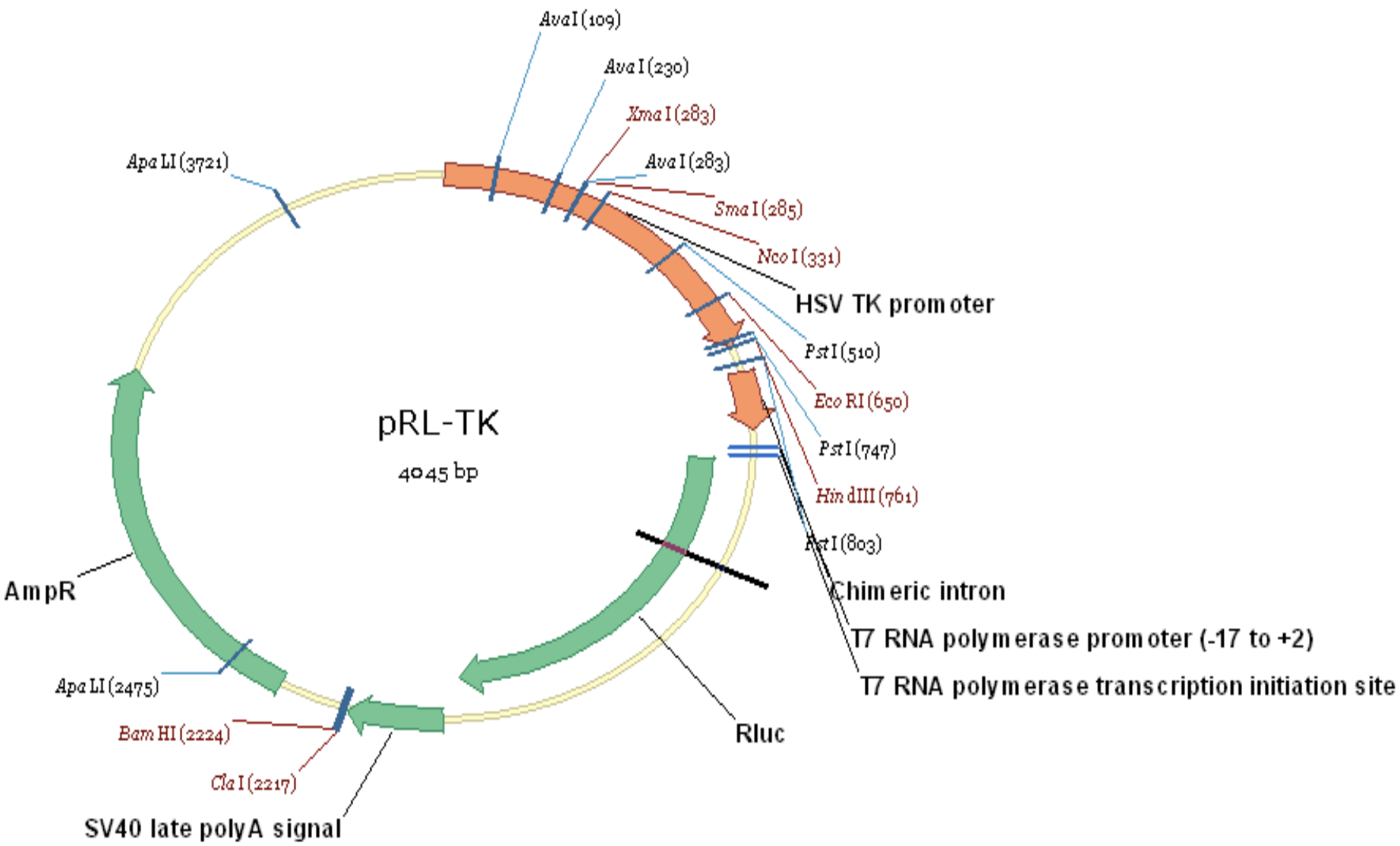
This test was repeated with the same result

HEK293 cells

We hadn't completed this with ETS1 yet

The TK-renilla plasmid map and sequence are in the next two slides…

TK-Renilla plasmid:



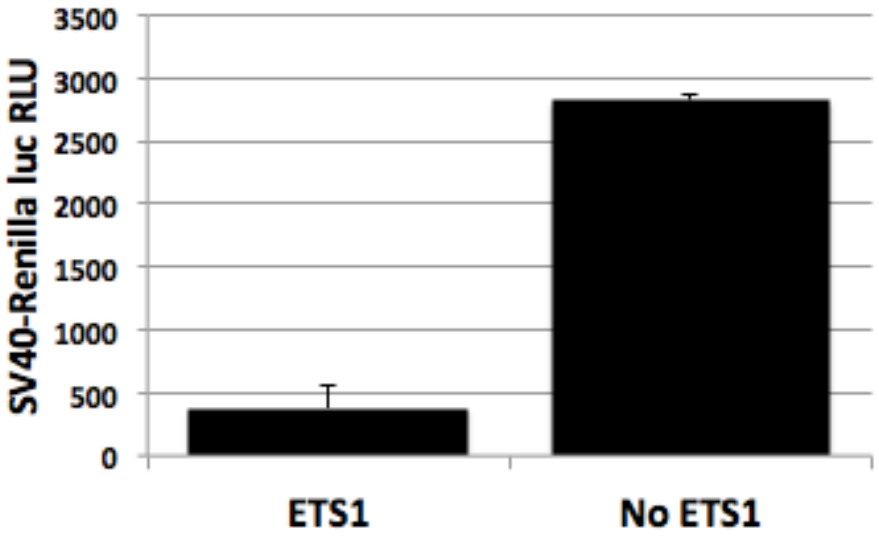
TK promoter (including intron up to Rluc)

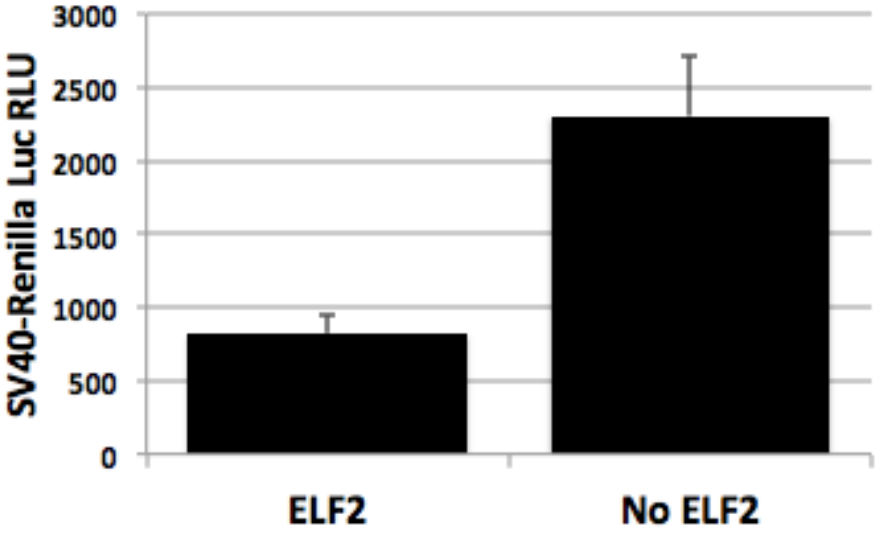
GGAA sequences highlighted No TTCC sequences are present

aaatgagtcttcggacctcgcgggggccgcttaagcggtggttagggtttgtctg
acgcgggggagggggaaggaacgaaacactctcattcggaggcggctcggggtt
tggtcttggtggccacgggcacgcagaagagcgccgcgatcctcttaagcacccc
cccgccctccgtggaggcgggggtttggtcggcgggtggtaactggcgggccgct
gactcgggcgggtcgcgcgccccagagtgtgacctttcggtctgctcgcagacc
cccgggcggcgccgccgcggcggcgacgggctcgctgggtcctaggctccatggg
gaccgtatacgtggacaggctctggagcatccgcacgactgcggtgatattaccg
gagaccttctgcgggacgagccgggtcacgcggctgacgcggagcgtccgttggg
cgacaaacaccaggacggggcacaggtacactatcttgtcacccggaggcgcgag
ggactgcaggagcttcagggagtggcgcagctgcttcatccccgtggcccgttgc
tcgcgtttgctggcggtgtccccggaagaaatatatttgcatgtctttagttcta
tgatgacacaaaccccgcccagcgtcttgtcattggcgaattcgaacacgcagat
gcagtcggggcggcgcggtcccaggtccacttcgcatattaaggtgacgcgtgtg
gcctcgaacaccgagcgaccctgcagcgacccgcttaaaagcttgattcttctga
cacaacagtctcgaacttaagctgcagaagttggtcgtgaggcactgggcaggta
agtatcaaggttacaagacaggtttaaggagaccaatagaaactgggcttgtcga
gacagagaagactcttgcgtttctgataggcacctattggtcttactgacatcca
ctttgcctttctctccacaggtgtccactcccagttcaattacagctcttaaggc
tagagtacttaatacgactcactataggctagccaccATG-luciferase

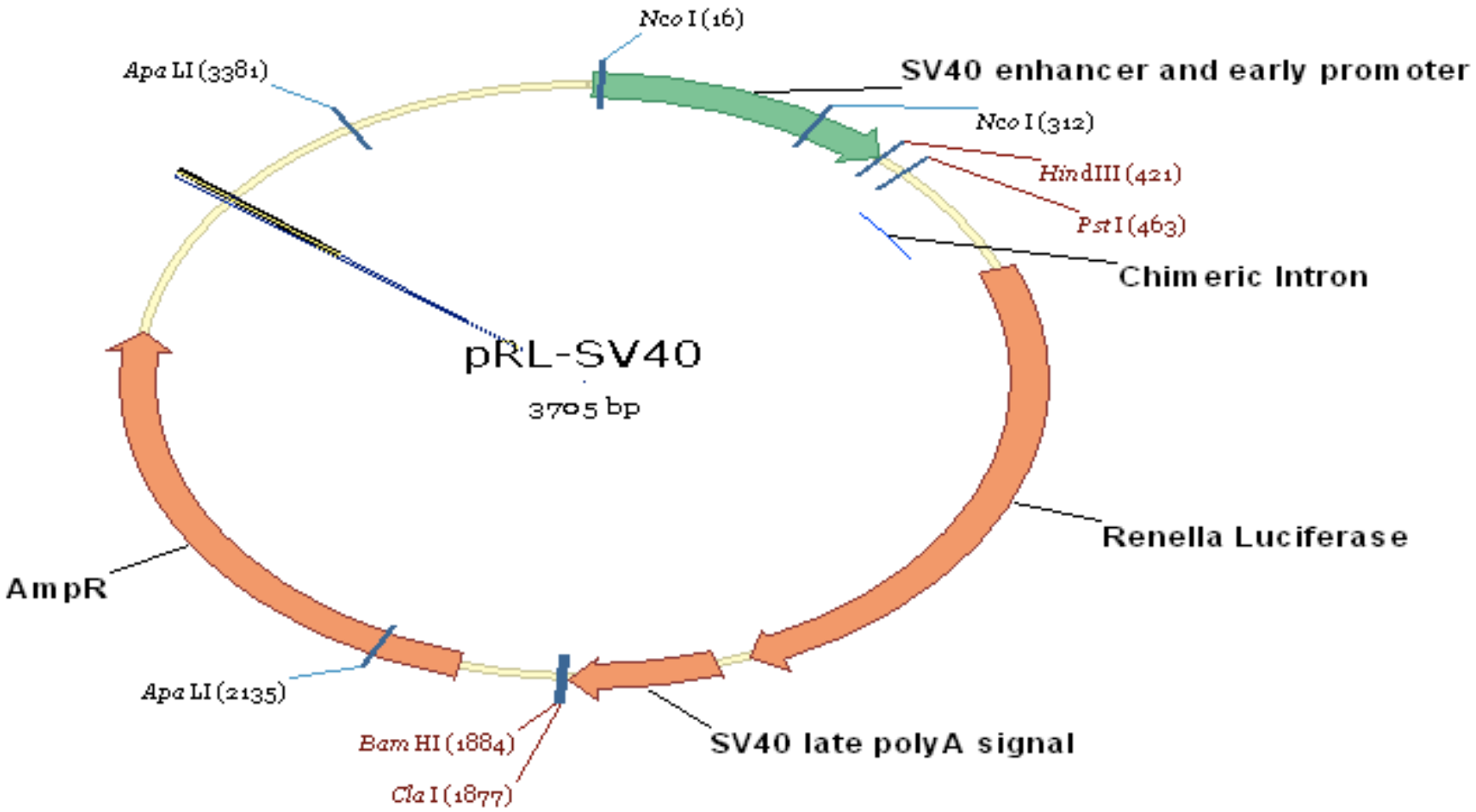
Cotransfection of ELF2 or ETS1 with SV40-Renilla luc reduced SV40-Renilla luc expression

N=2 transfections HEK293T cells





SV40-Renilla plasmid:



SV40 promoter (including intron up to Rluc)
GGAA sequences highlighted in red
TTCC sequences highlighted in yellow

gcgcagcaccatggcctgaaataacctctgaaagaggaacttggttaggtacctt
ctgaggcggaaagaaccagctggaatgtgtgtcagttagggtgtggaagtcc
ccaggctccccagcaggcagaagtatgcaaagcatgcatctcaattagtcagcaa
ccaggtgtggaagtccccaggctccccagcaggcagaagtatgcaaagcatgca
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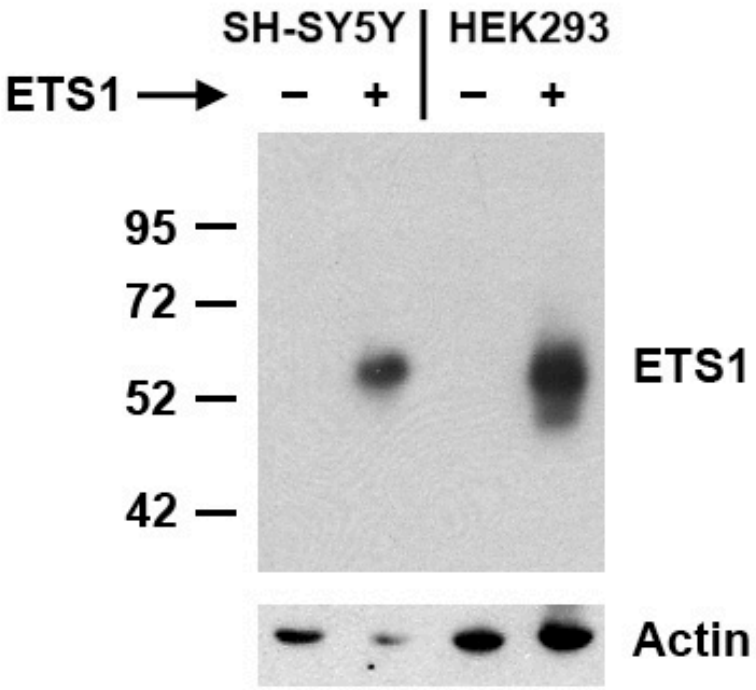
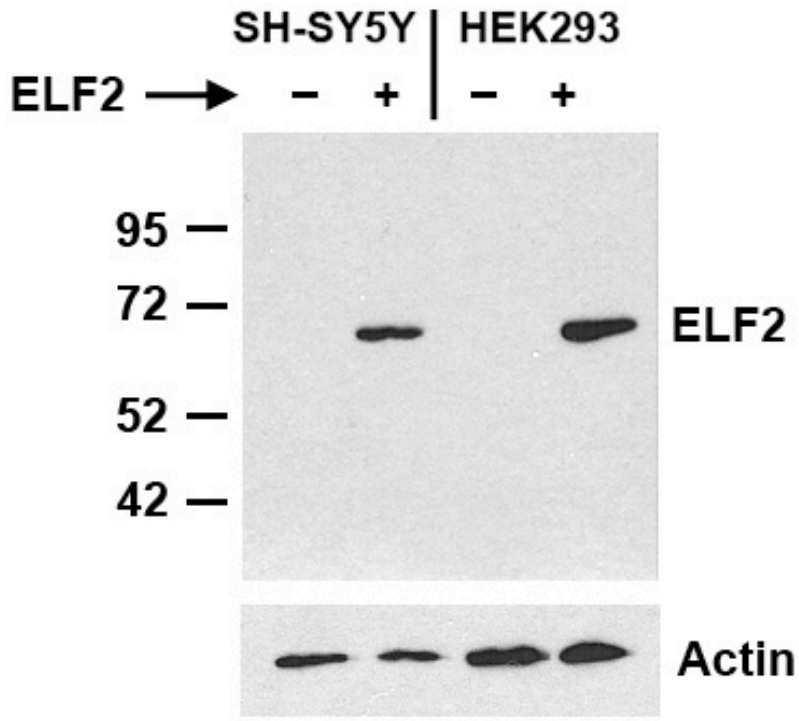
tcgaacttaagctgcagaagttggtcgtgaggcactgggcaggtaagtatcaagg
ttacaagacaggtttaaggagaccaatagaaactgggcttgtcgagacagagaag
actcttgcgtttctgataggcacctattggtcttactgacatccactttgccttt
ctctccacaggtgtccactcccagttcaattacagctcttaaggctagagtactt
aatacgactcactataggctagccaccATG-luciferase

Note there are good predictions for ETS sites on the negative strand

Westerns showing ETS1 and ELF2 transgene expression



Santa Cruz Ets-1 (C-20) sc-350
Predicted 55 kDa



Santa Cruz NERF (C-20) sc-6828
Predicted 64 kDa

Mutation of an ETS family transcription factor binding site in the ATXN2 5'-UTR reduced *ATXN2-luc* expression. Note the 14 bp deletion resulted in further reduced expression suggesting some other transcription factor (TFX) binds here to support ATXN2 expression.

100% match
ETS consensus sequence:

5'-CCGGAAGT-3'

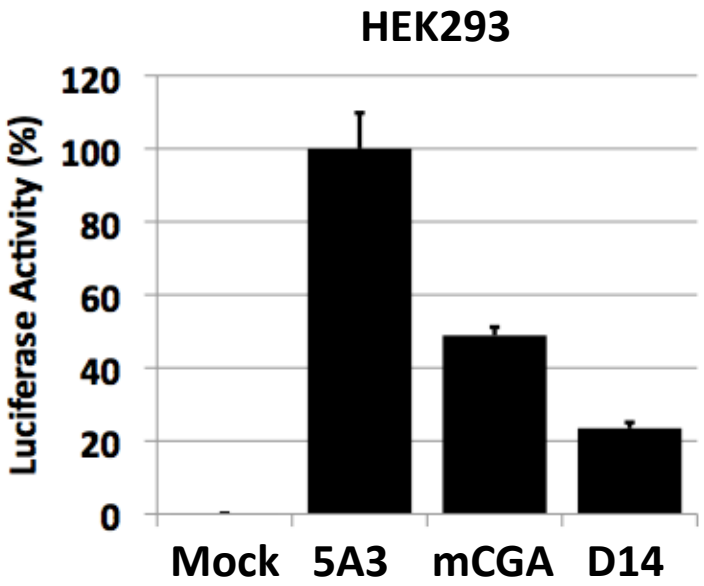
(numbering relative to TSS)

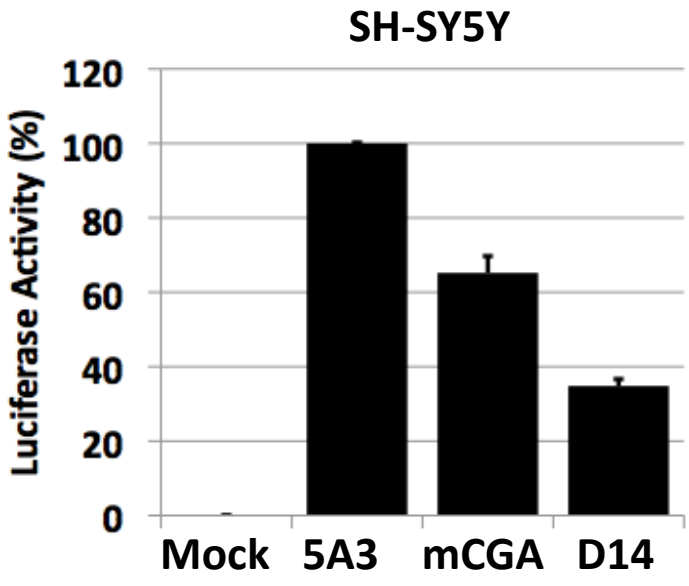
+87 +96 +103 +109

ATXN2
5'-ACCCCTCCGACTTCCGGTAAAGA-3'
3'-TGGGGAGGCTGAAGGCCATTTCT-5'

mCGA
5'-ACCCCTCCGACTCGAGGTAAAGA-3'
3'-TGGGGAGGCTGAGCTCCATTTCT-5'

D14
5'-ACCCCTC--------------GA-3'
3'-TGGGGAG--------------CT-5'

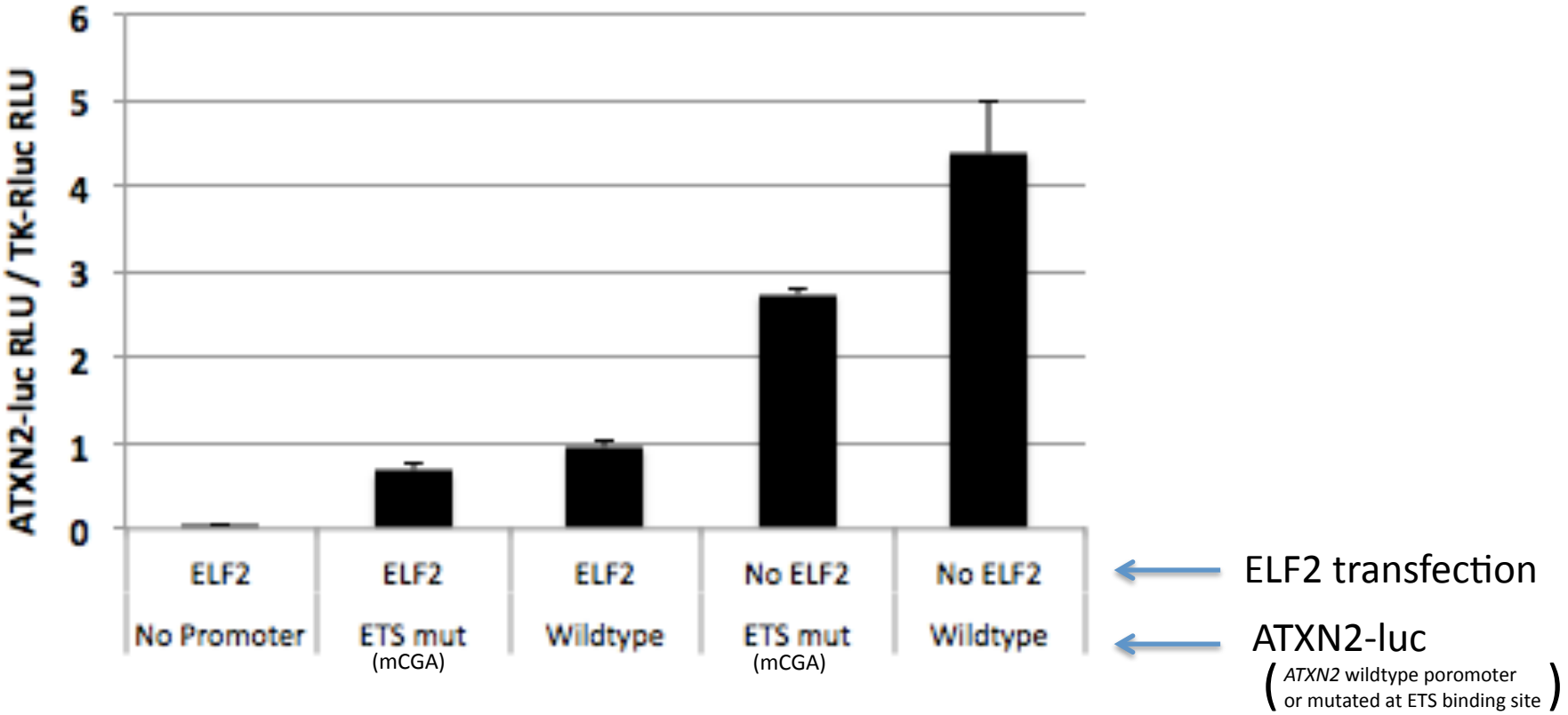




Next we cotransfected ELF2 with ATXN2-luc (using TK-luc for normalizing transfection).
Observations:

ELF2 expression reduced ATXN2-luc expression

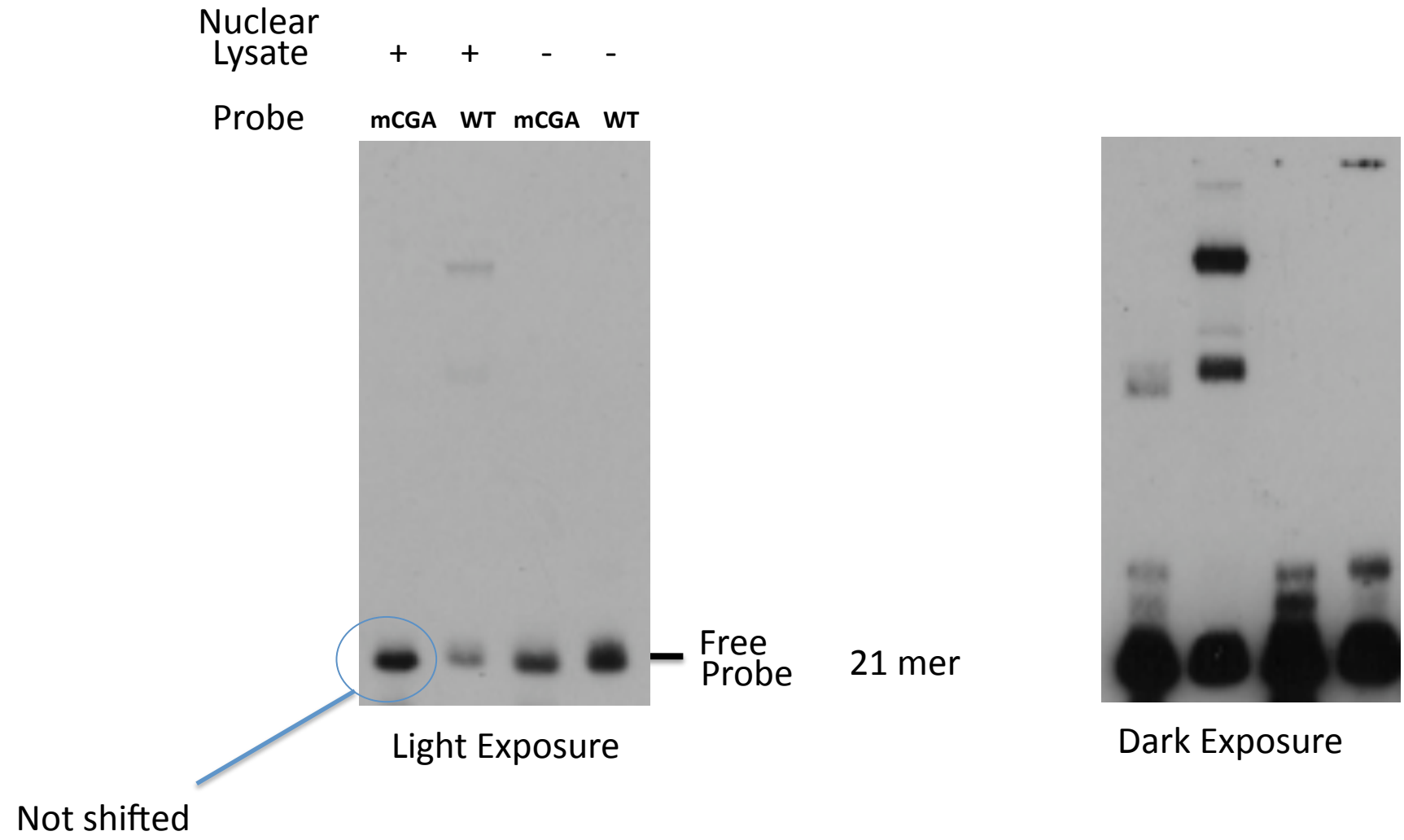
→ Mutation in ATXN2 further reduced expression regardless of ELF2 overexpression



The same for ETS1 is not yet completed using the TK-luc for controlling transfection. Trials using SV40-luc are convincing that the pattern for ETS1 will be the same as for ELF2.

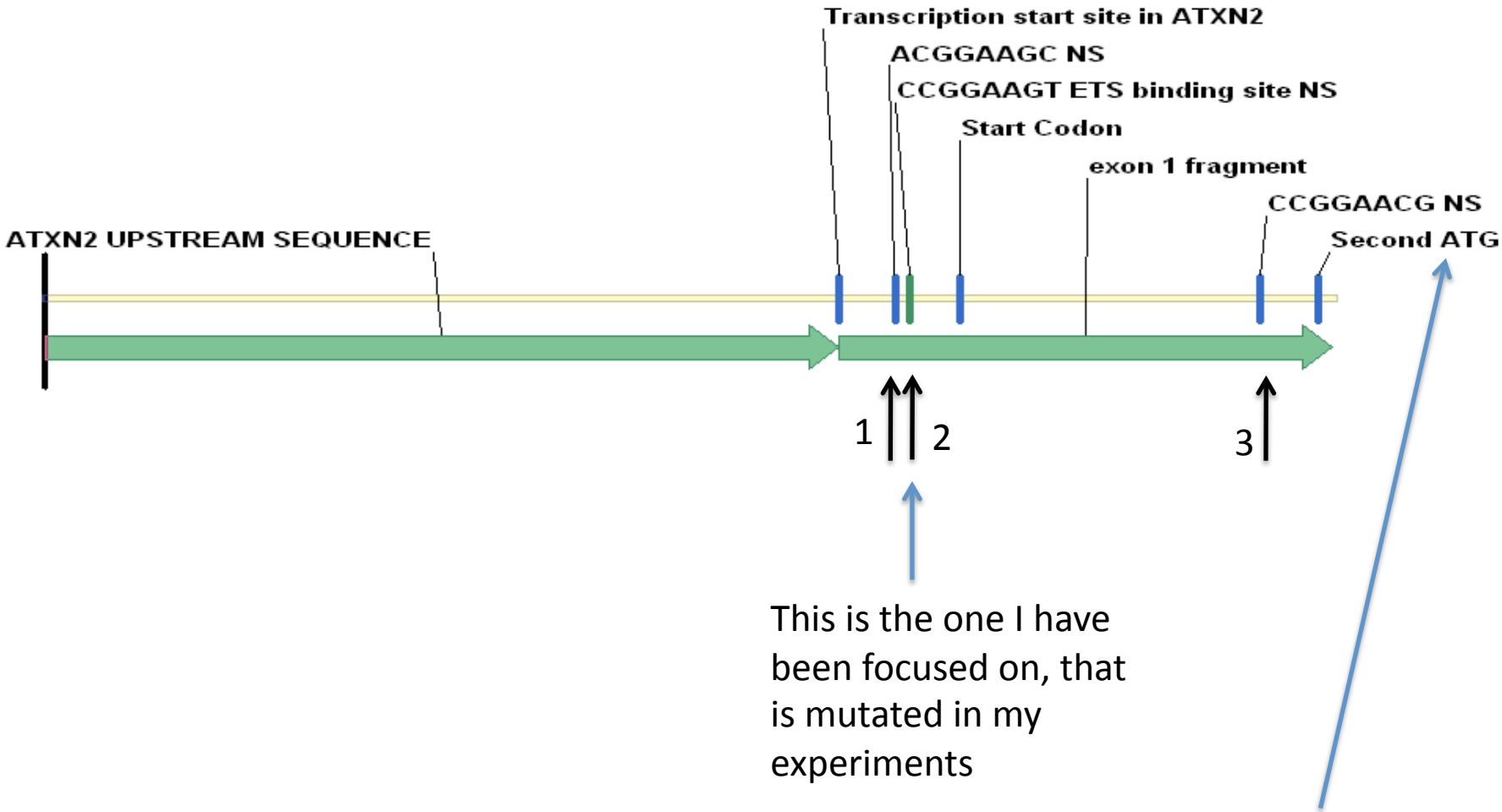
EMSA SHOWING THAT THE mCGA MUTATION IN THE ETS BINDING SITE PREVENTS NEARLY ALL DETECTABLE PROTEIN BINDING

The WT probe is shifted but not the mutant probe
Short run gel to view the free probe



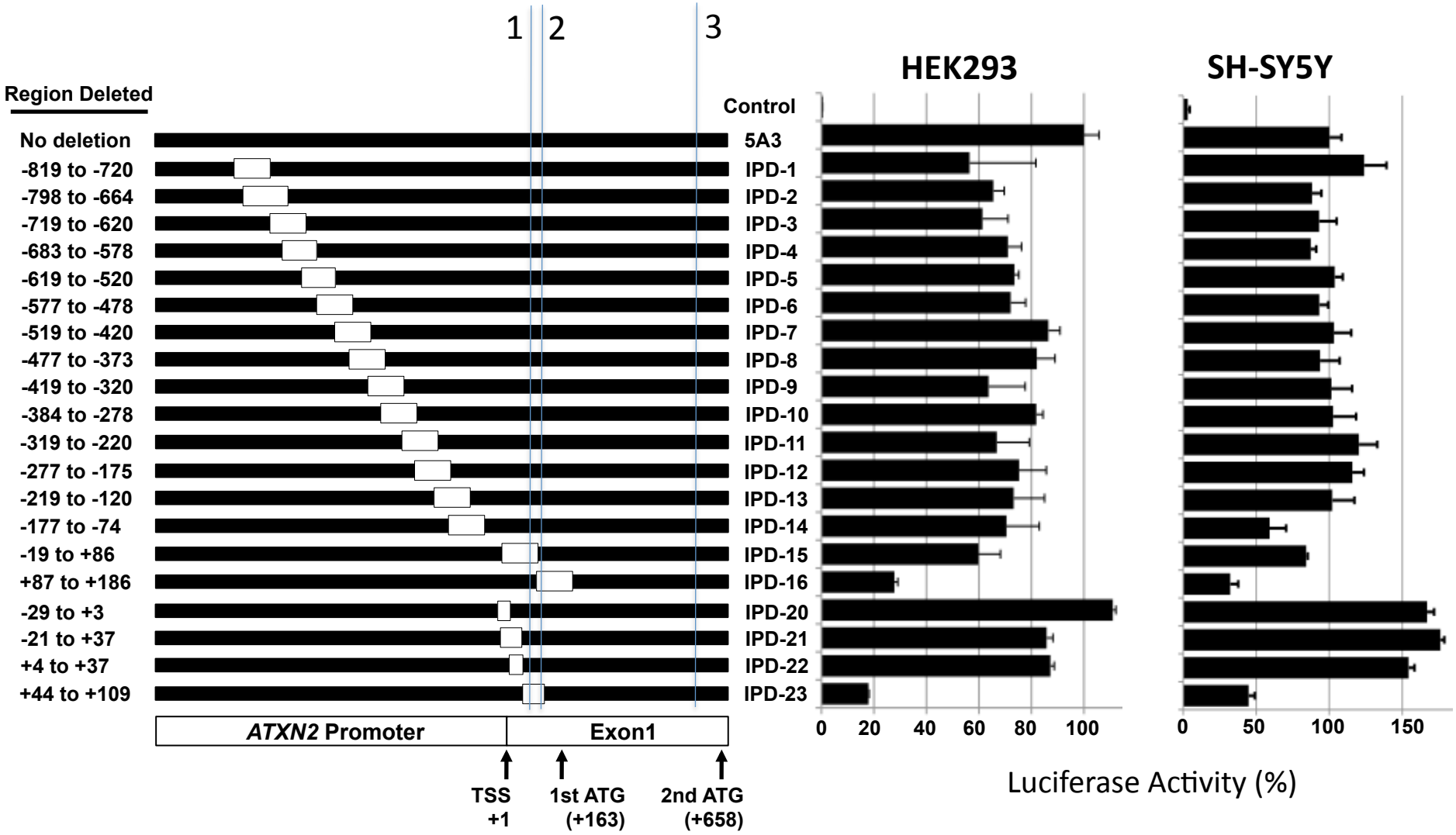
SH-SY5Y nuclear lysate

There are three ETS predictions in the 5'-UTR of ATXN2 that are on the negative strand (NS)



NOTE ALSO I HAVE NEW DATA SHOWING ONLY START CODON 2 IS USED

Deletion of 2 reduced expression but not 1. We have never surveyed 3



Conclusions:

Overexpression of ETS factors decreased ATXN2 expression

There are multiple other ETS sites in the ATXN2 promoter but the only one that when deleted alters ATXN2 expression is #2 on the negative strand (see map 2 slide #13) (note we haven't surveyed #3).

ETS expression also inhibited SV40-Rluc expression. We noted ETS sites on the SV40 negative strand, and wonder if ETS sites on the negative strand can inhibit expression??

Since 14 bp deletion including the ETS #2 site reduces ATXN2 expression (see slide #10), it seems some other transcription factor must bind the promoter there to support ATXN2 expression (although the 3bp mutation eliminated most protein binding in our EMSAs)

Then to explain the data shown in slide 11 (you might need to look back), we came up with the model on the next slide.

The conclusion is the ETS #2 site inhibits ATXN2 expression and we need to find out what other transcription factors bind there to support ATXN2 expression.

Model for the action of ETS and an unknown transcription factor on ATXN2 expression