

Lab Meeting
Dan

August 19, 2010

55 slides

Overview

1. ATXN2 promoter paper overview & update

Includes some positive progress on EMSA studies at the end of this part

2. Other ongoing projects

3. Cameleon fluorescent calcium reporter progress

**Regulatory features of the spinocerebellar ataxia
type 2 gene (*ATXN2*).**

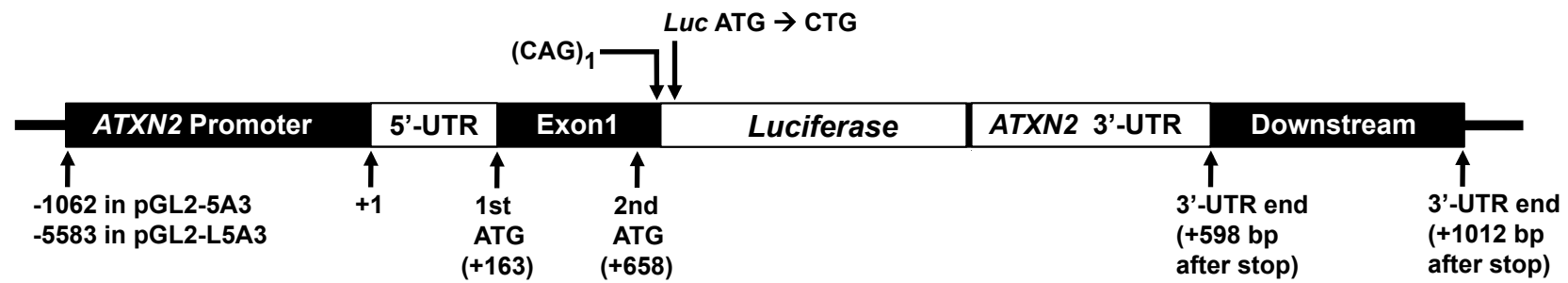


FIG 1

A

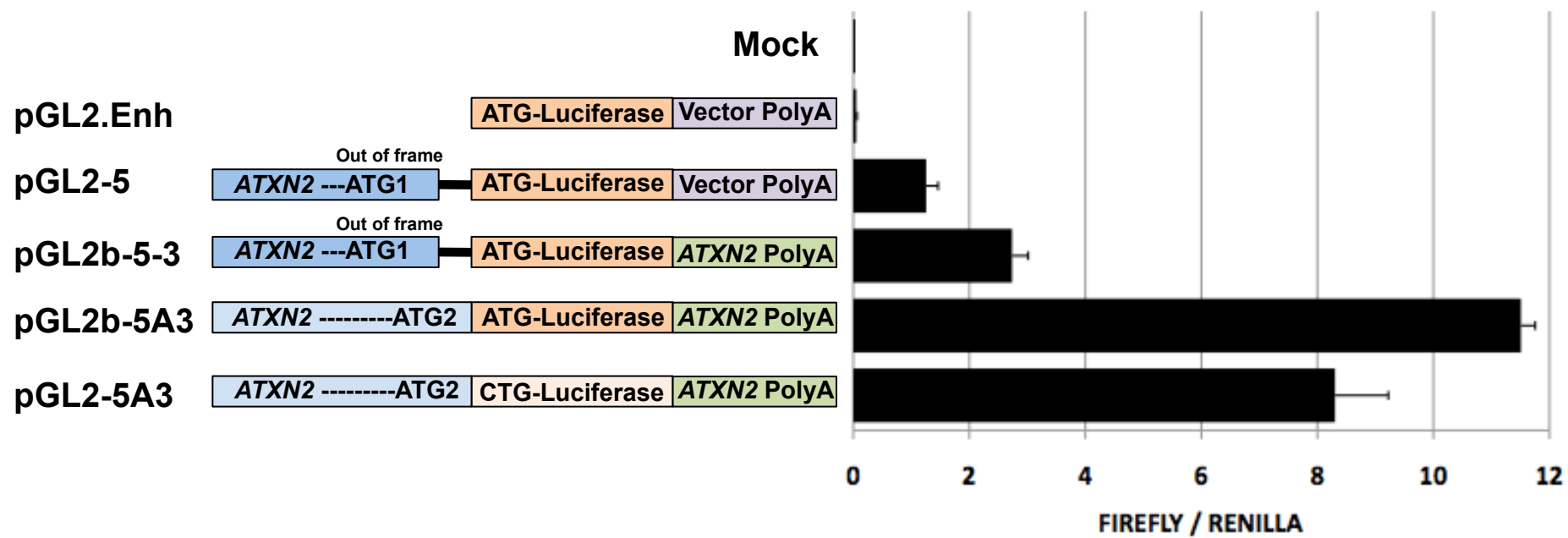
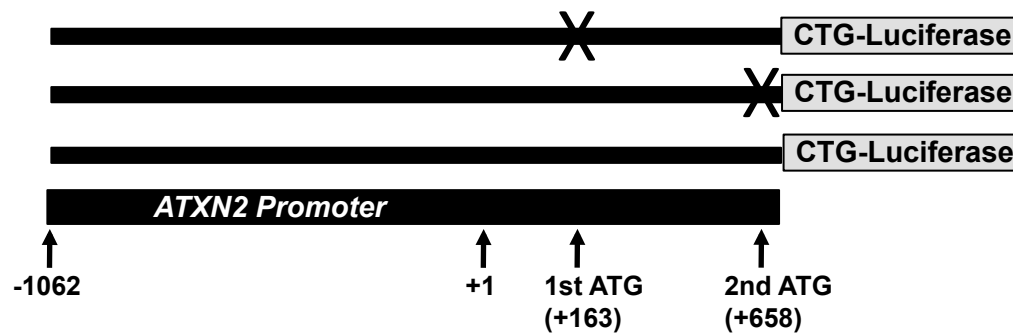
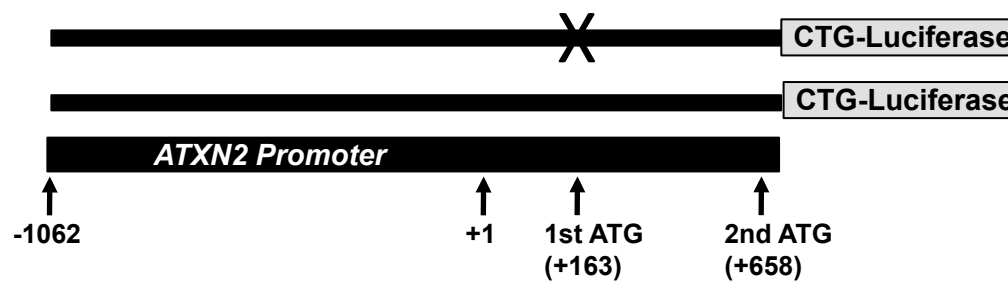


Fig 2A

B



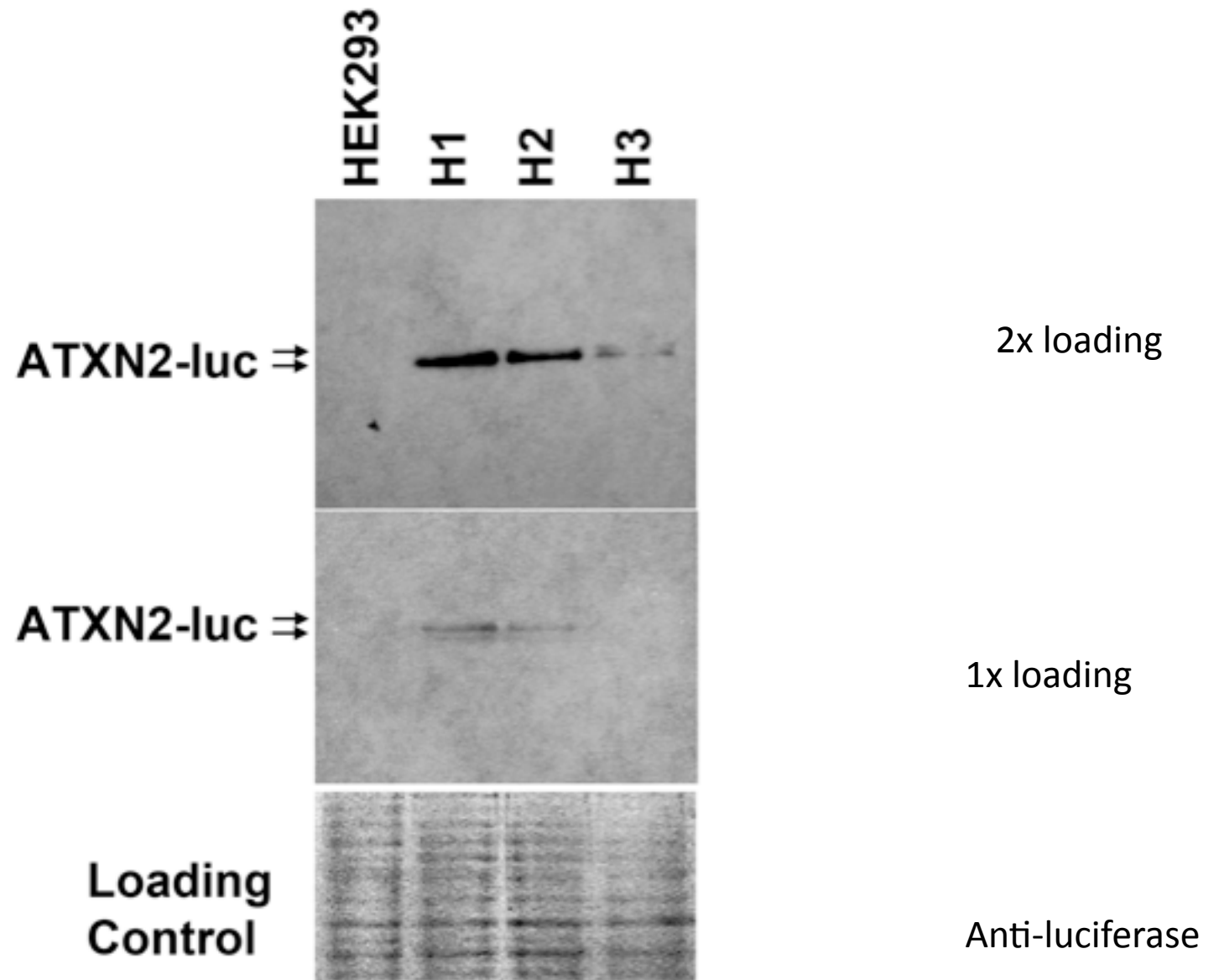
ADD CHART



ADD CHART

Fig 2B

C



A



ADD CHART

Fig 3A

B

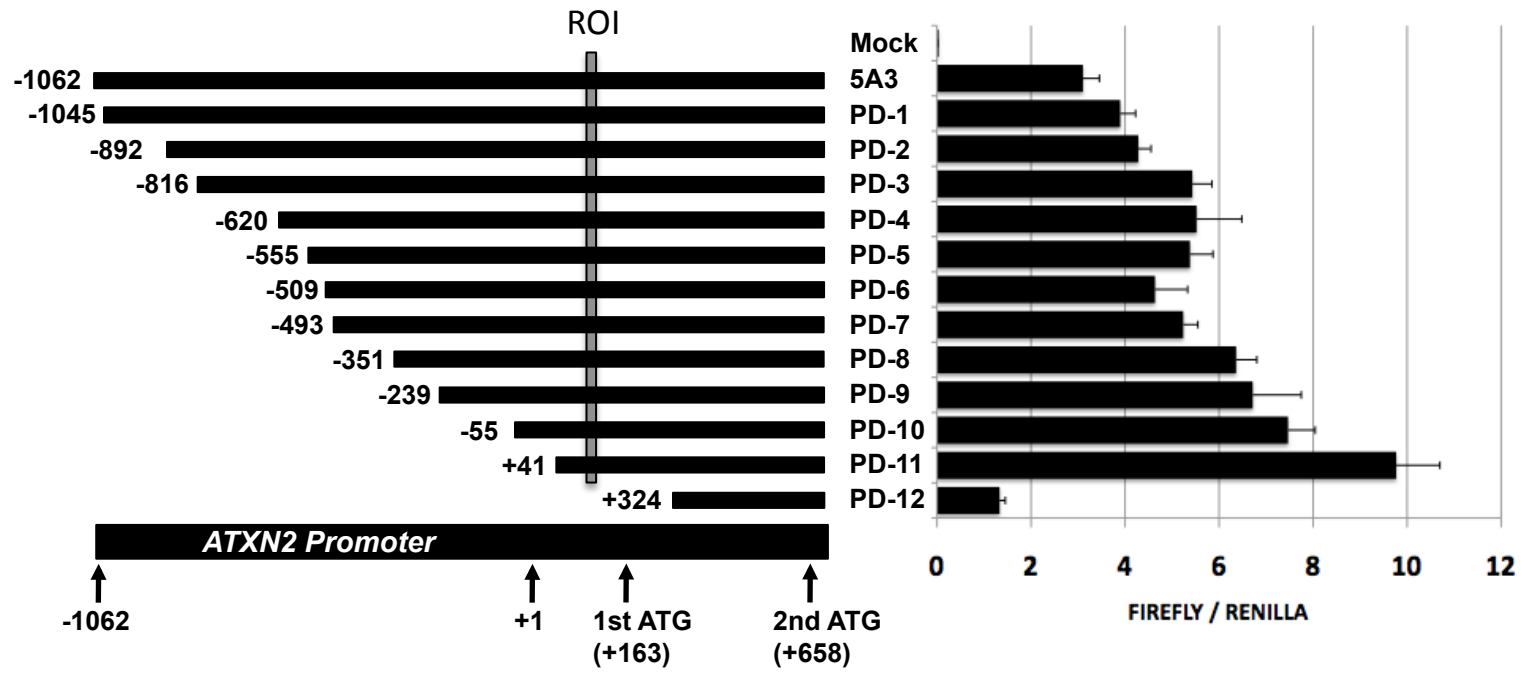


Fig 3B

C

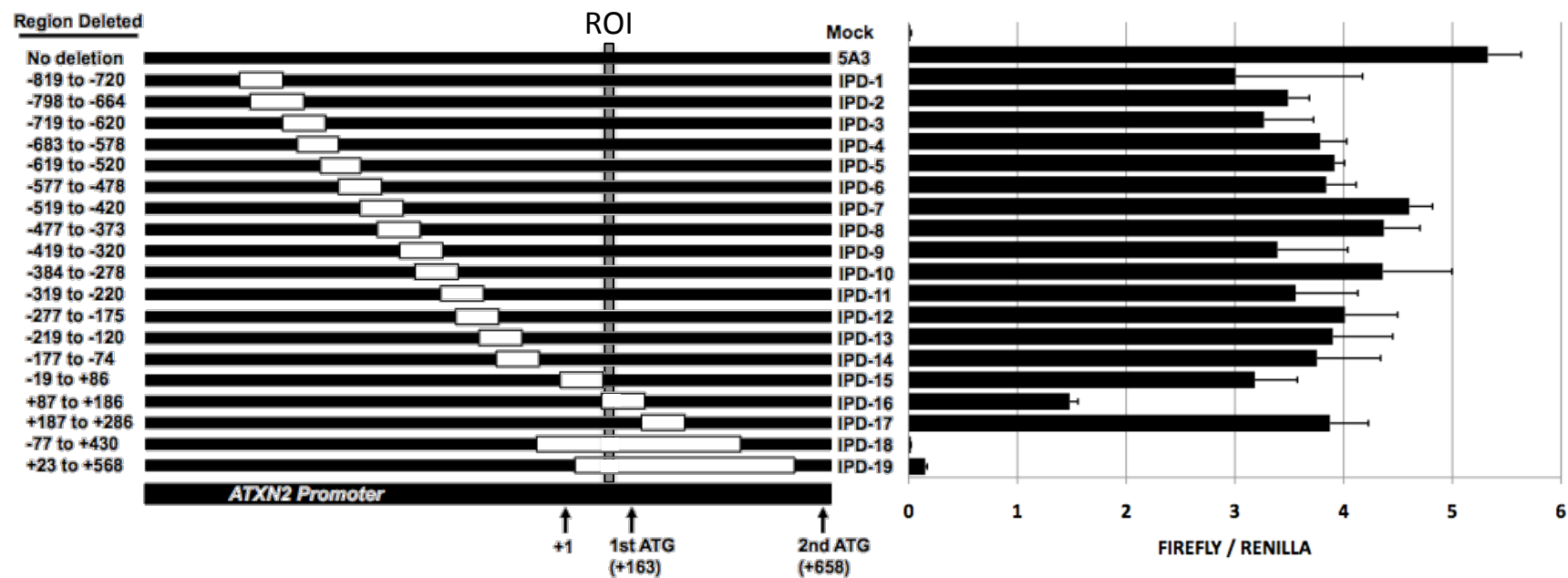
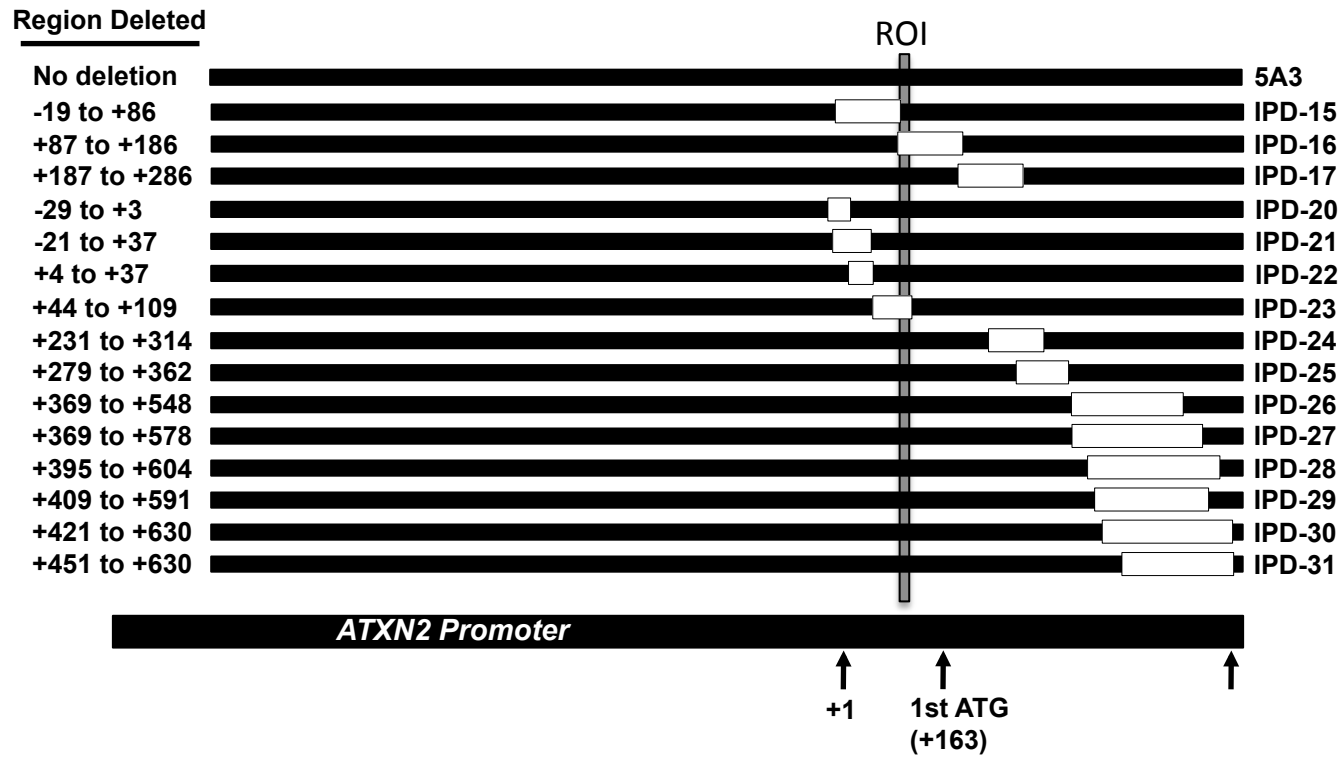


Fig 3C

D



ADD Chart

Fig 3D

A

↙ +69 ↙ +86, start of IPD-16 +140 ↘
 DICE (-) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 ETSF (-) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 FKHD (+) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 HOMF (+) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 STAT (+) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 STAT (-) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 Primer MutR1 5' -ccgtctgacccctccgactcgaggtaaagagtccc-3'
 Primer MutR2 5' -ccgtctgacccctc-----gagtcctatccgc-3'

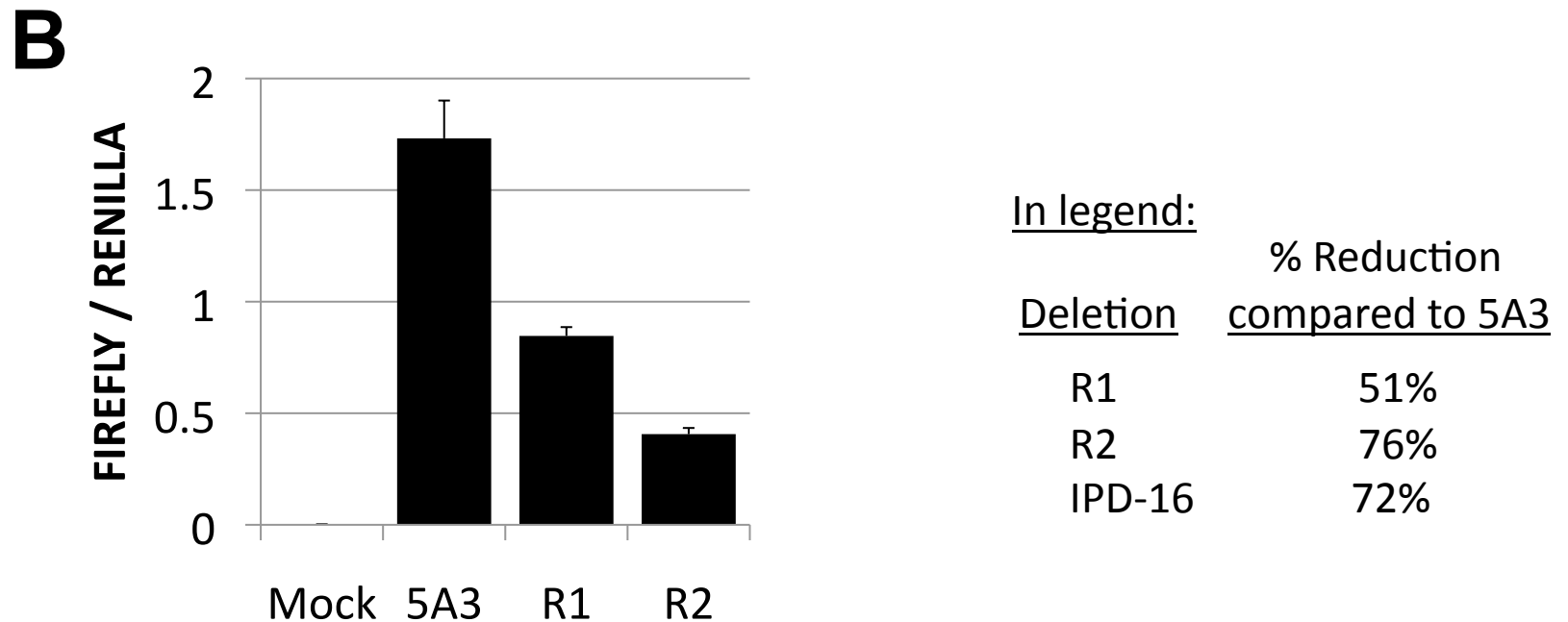
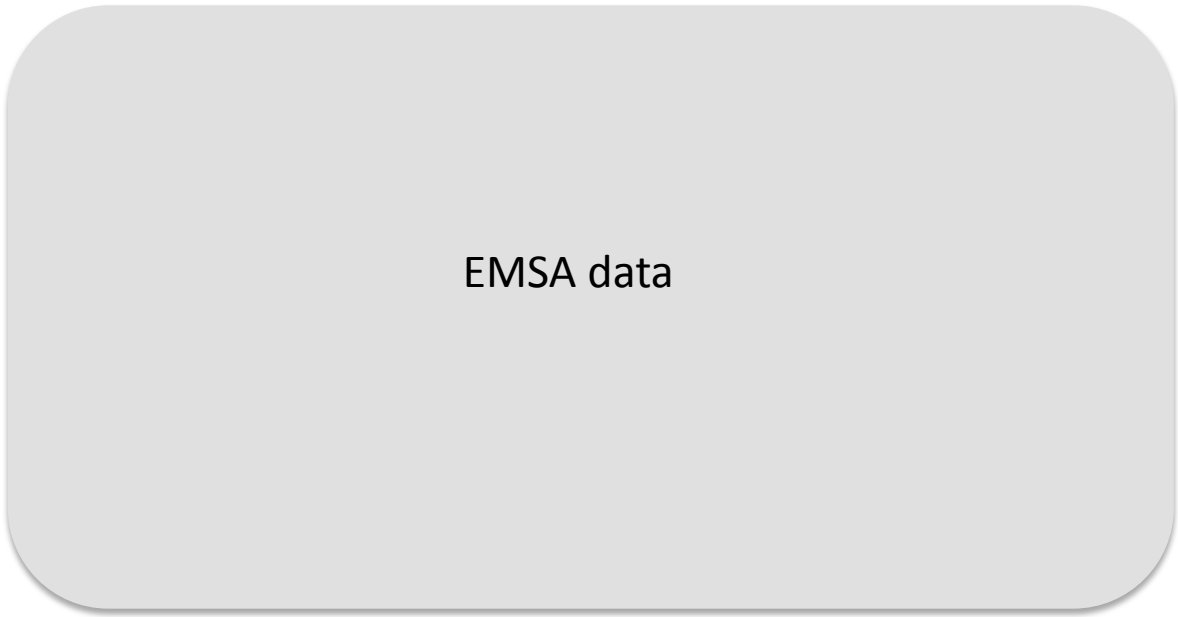


Fig 4

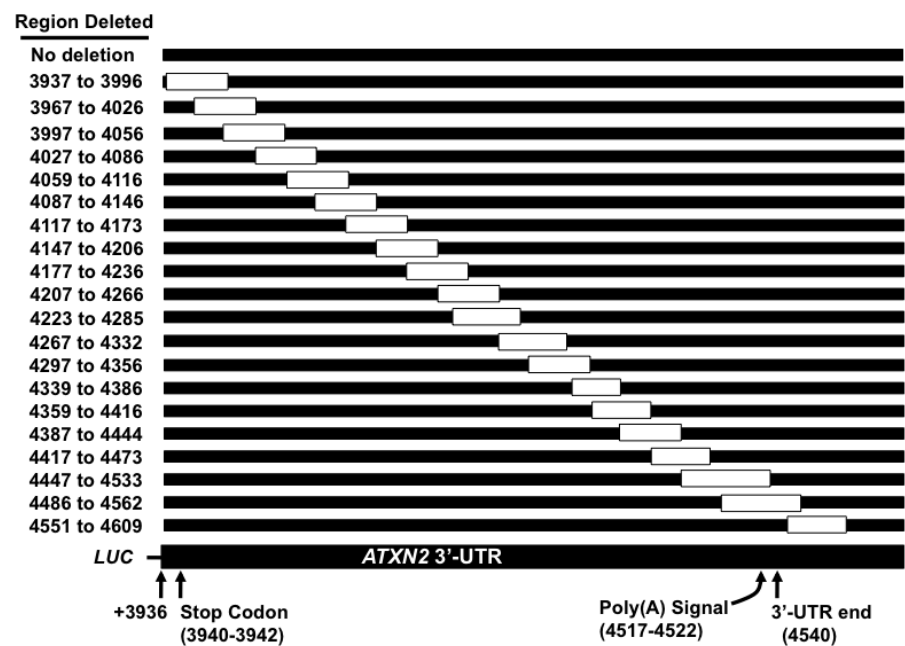


EMSA data

SEE BELOW

Fig 4C

A



B

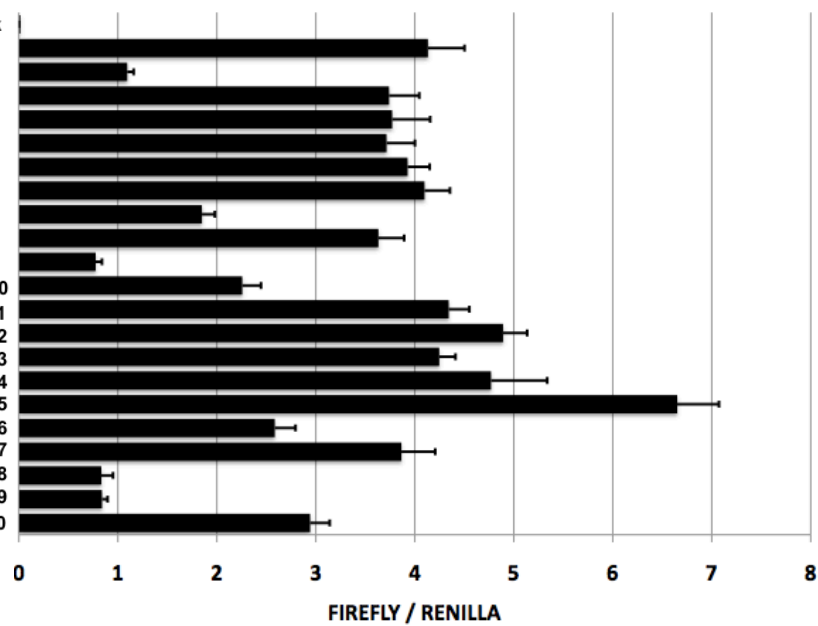


Fig 5

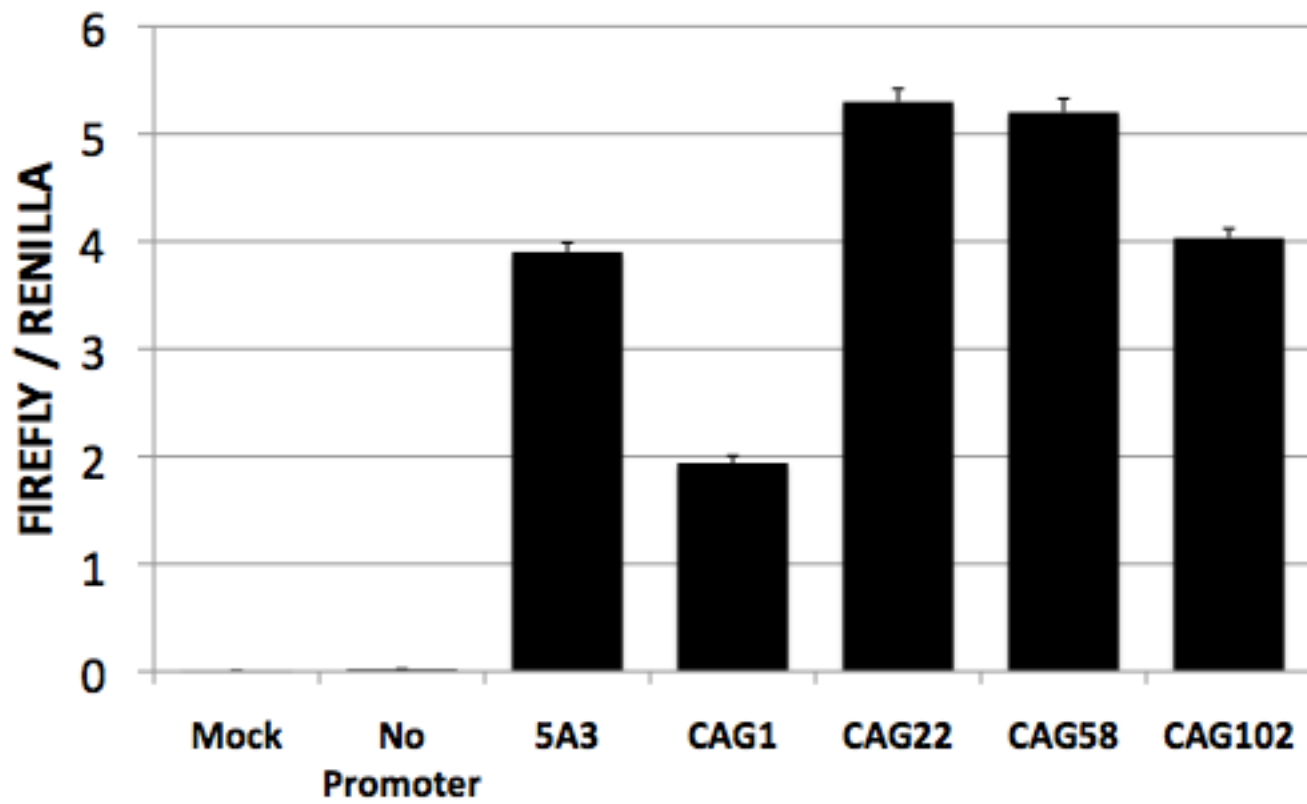


Fig 6

38 aa of ATXN2 following CAG repeat

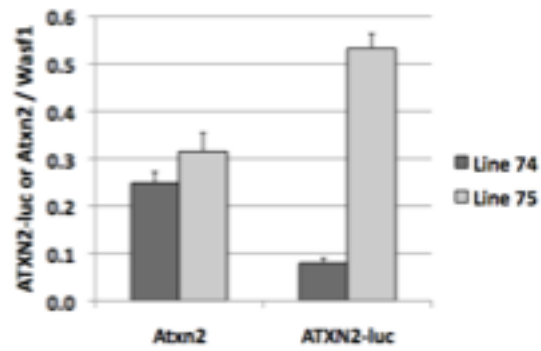
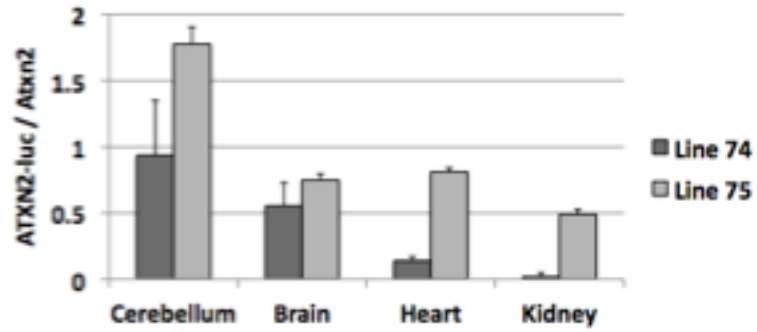
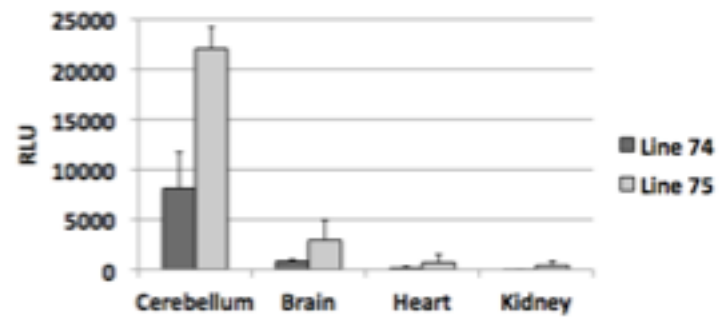
A**B****C**

Fig 7

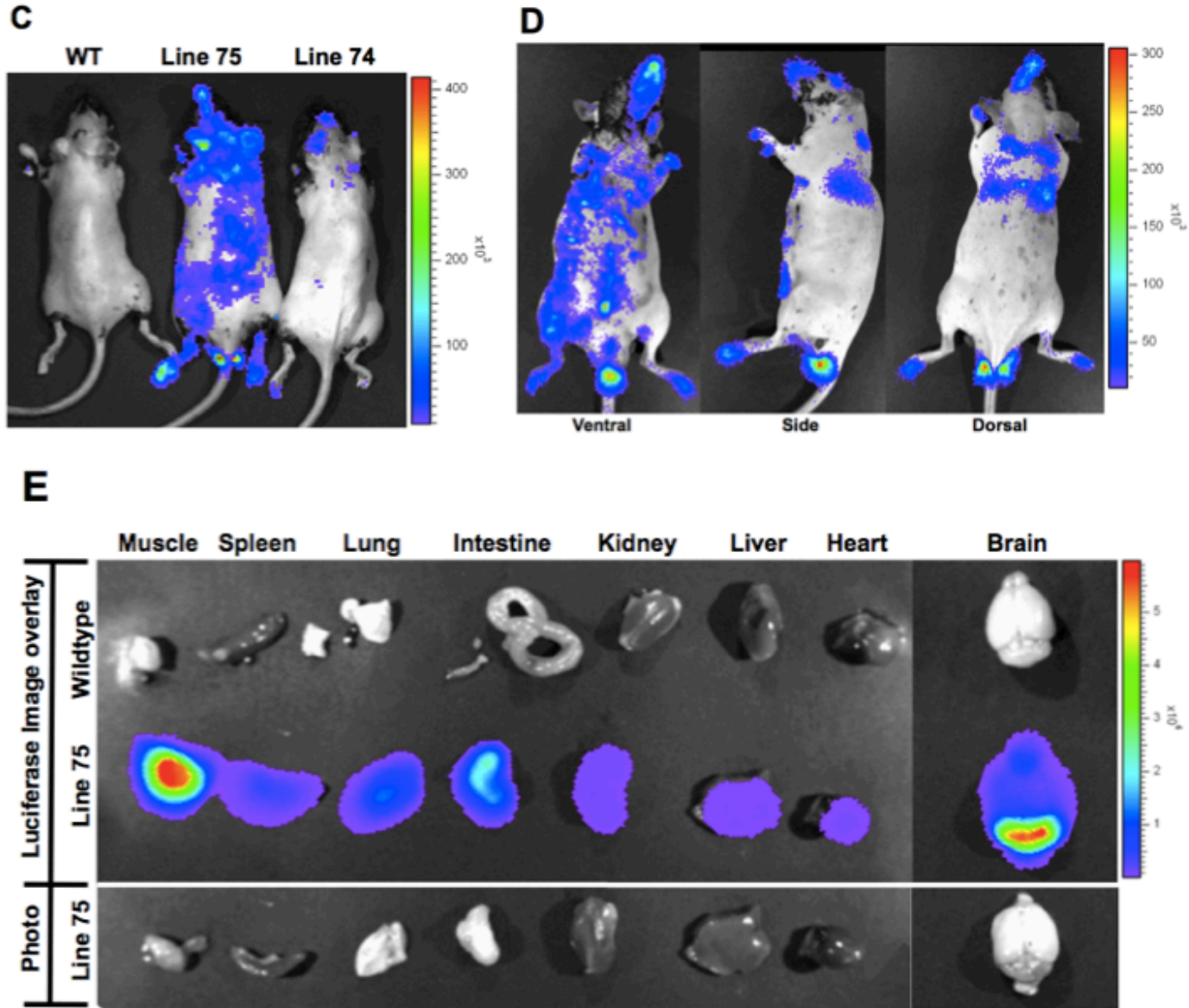


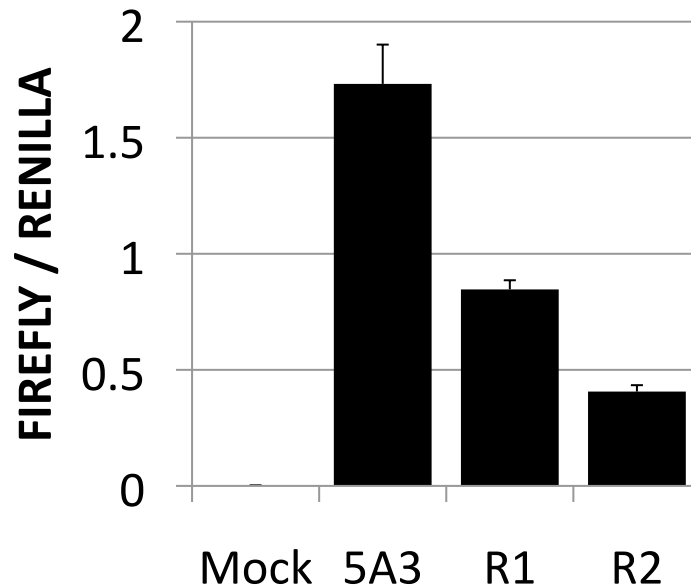
Fig 7

EMSA and Pull-Down

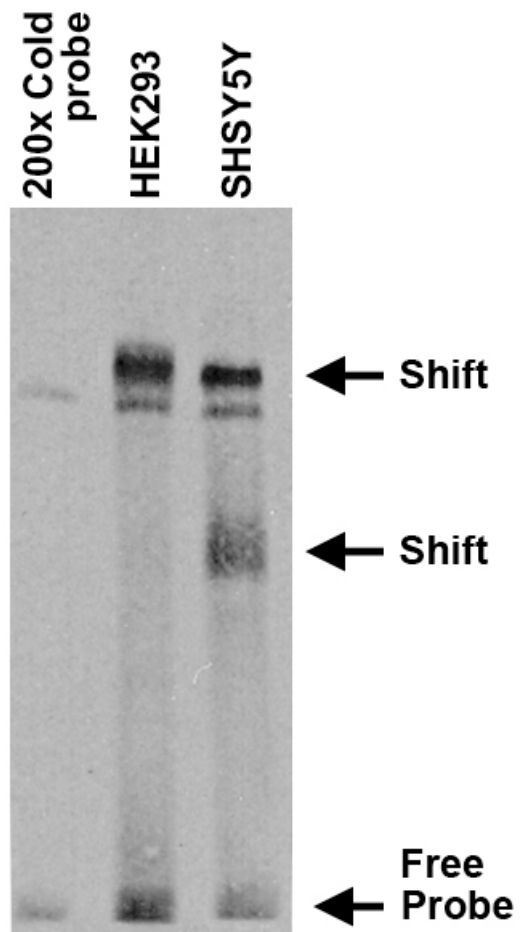
A

↙ +69 ↙ +86, start of IPD-16 +140 ↘
 DICE (-) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 ETSF (-) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 FKHD (+) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 HOMF (+) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 STAT (+) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 STAT (-) acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 "SO7" acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc
 "WT" acgttctgcttccgtctgacccctccgacttccggtaaagagtccctatccgcacctccgctcccaccggc

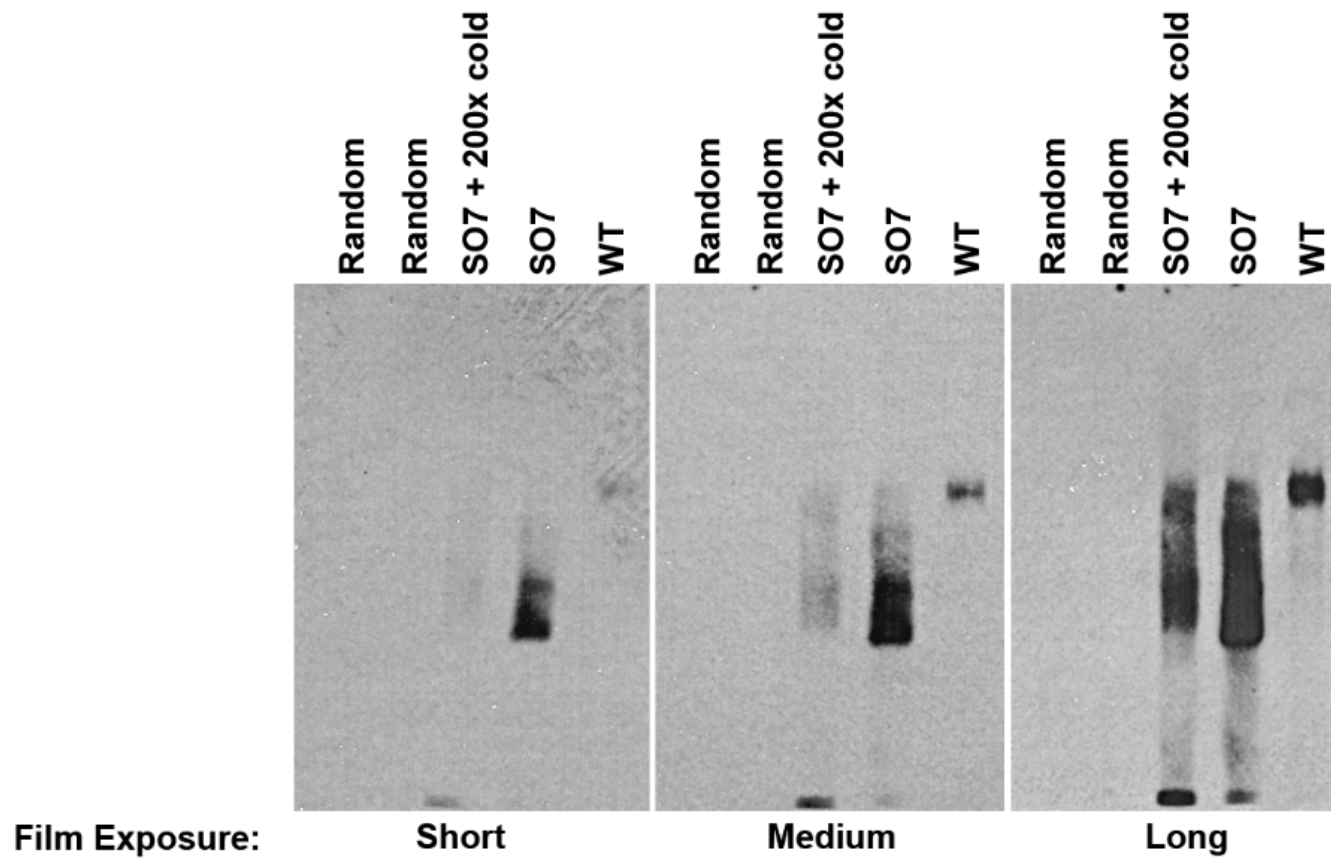
Primer MutR1 5'-ccgtctgacccctccgactcgaggtaaagagtccc-3' → ETSF & FKHD remain
 Primer MutR2 5'-ccgtctgacccctc-----gagtcctatccgc-3' → All eliminated

B

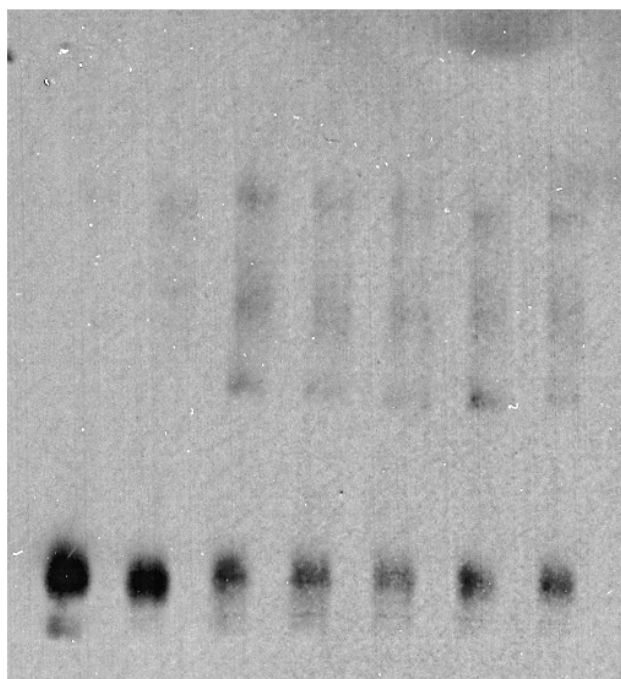
<u>Deletion</u>	<u>% Reduction compared to 5A3</u>
R1	51%
R2	76%
IPD-16	72%



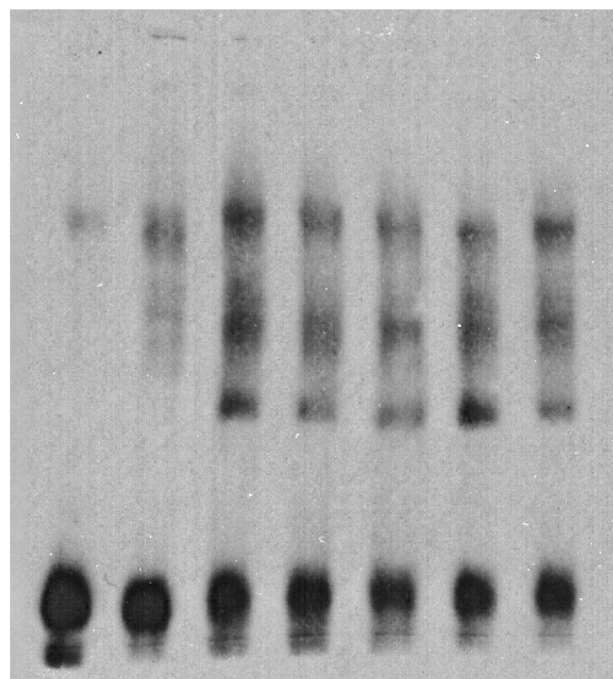
Probe = "WT"



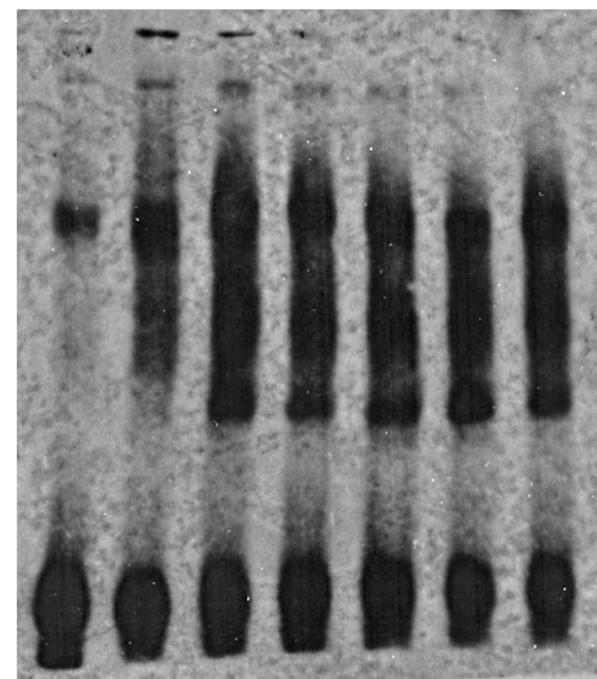
Lysate: Hek293



10 sec



2 min

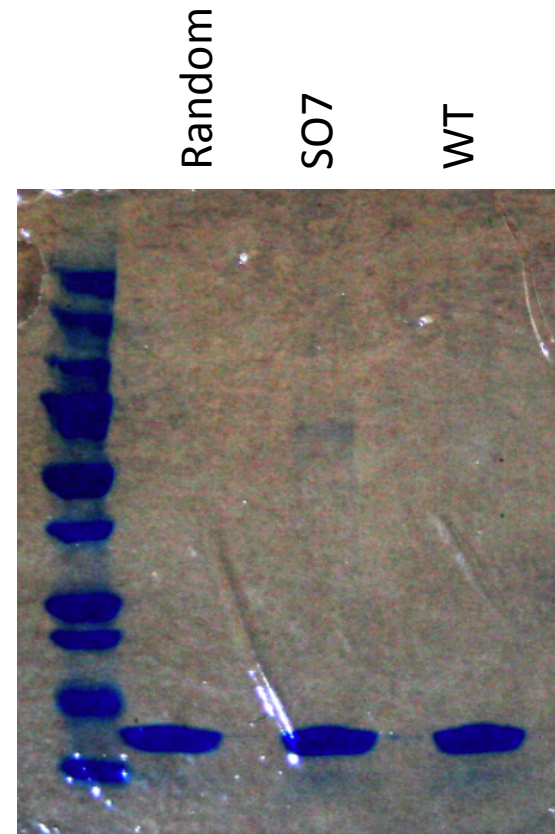
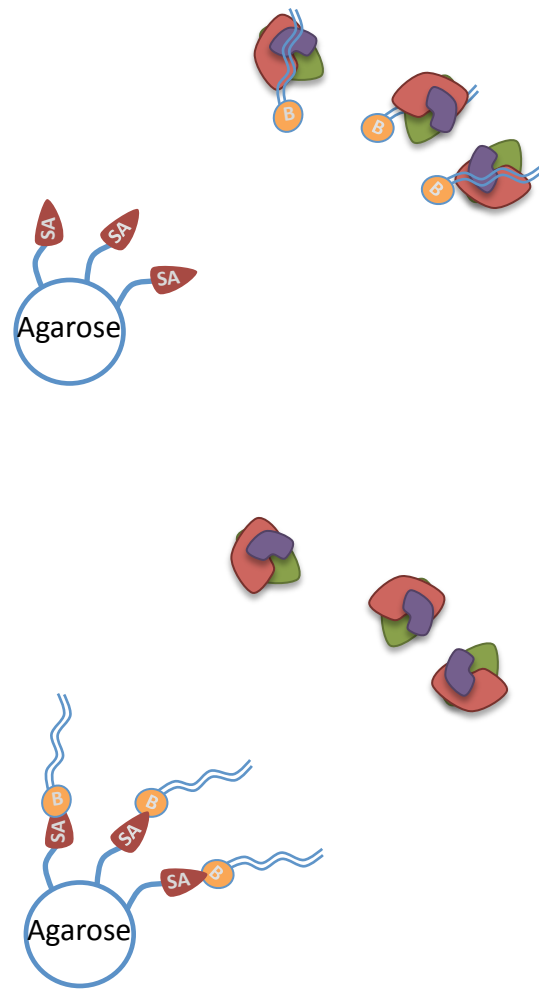


Overnight

RND2
 SO7 + 200x Cold Probe
 SO7
 SO7 + STAT1 antibody
 SO7 + STAT2 antibody
 SO7 + STAT3 antibody
 SO7 + STAT5 antibody

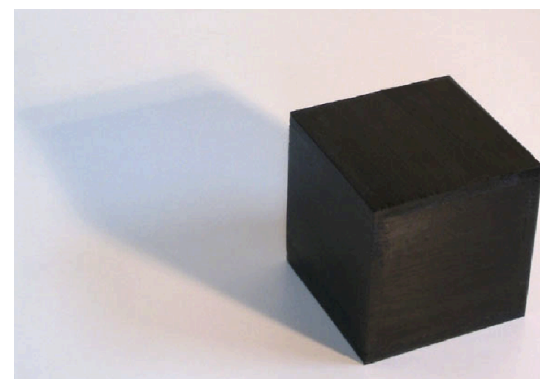
Lysate: Hek293

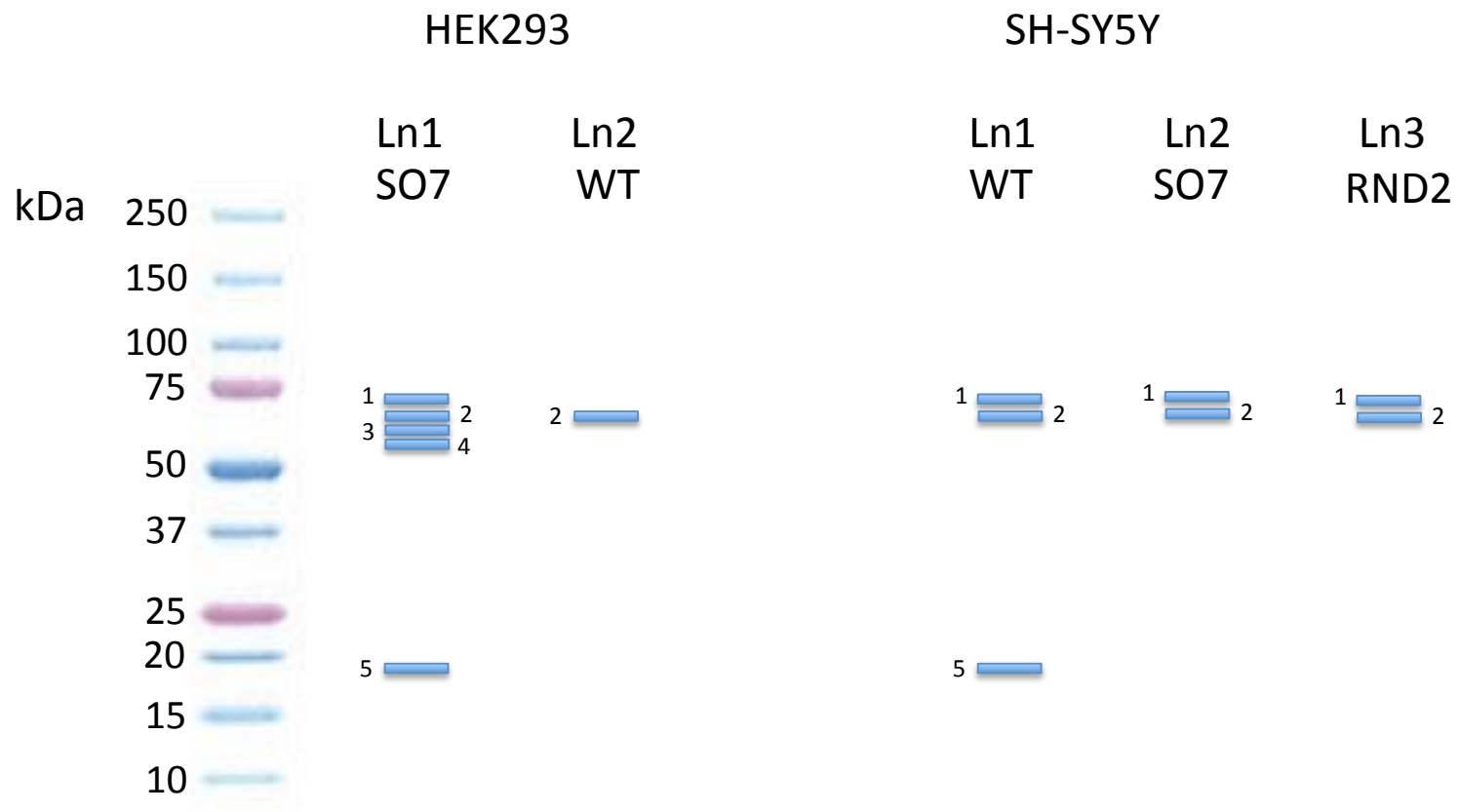
Pull-Down



FTICR ANALYSIS

(Fourier transform ion cyclotron resonance mass spectrometry)





MOWSE = molecular weight search

SERC Daresbury Laboratory has what they call a MOWSE peptide-mass database
fragment database that is derived from over 50,000 proteins

So, if you know the mass of a peptide generated by trypsin you can determine its amino acid composition from the database.

The probability of a positive hit is calculated from the number of times a peptide is observed and number of times other peptides from the same protein are observed in your sample.

The MOWSE factor M is calculated by a matrix approach of database information, where each row is an interval of 100 Da peptide and each column an interval of 10,000 Da in intact protein mass.

As each peptide is processed, f_{ij} are incremented accumulating statistics on size distribution of peptide masses as a function of protein mass.

$$m_{i,j} = \frac{f_{i,j}}{|f_{i,j}|_{\max \text{ in column } j}}$$

After searching the experimental mass values against a calculated peptide mass database, the score for each entry is calculated according to:

$$\text{Score} = \frac{50,000}{M_{\text{Prot}} \times \prod_n m_{i,j}}$$

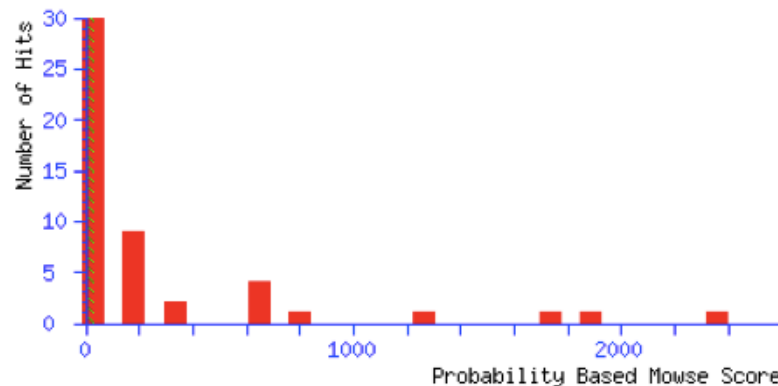
The total score is the absolute probability that the observed match is a random event. With that one can calculate a significance based.

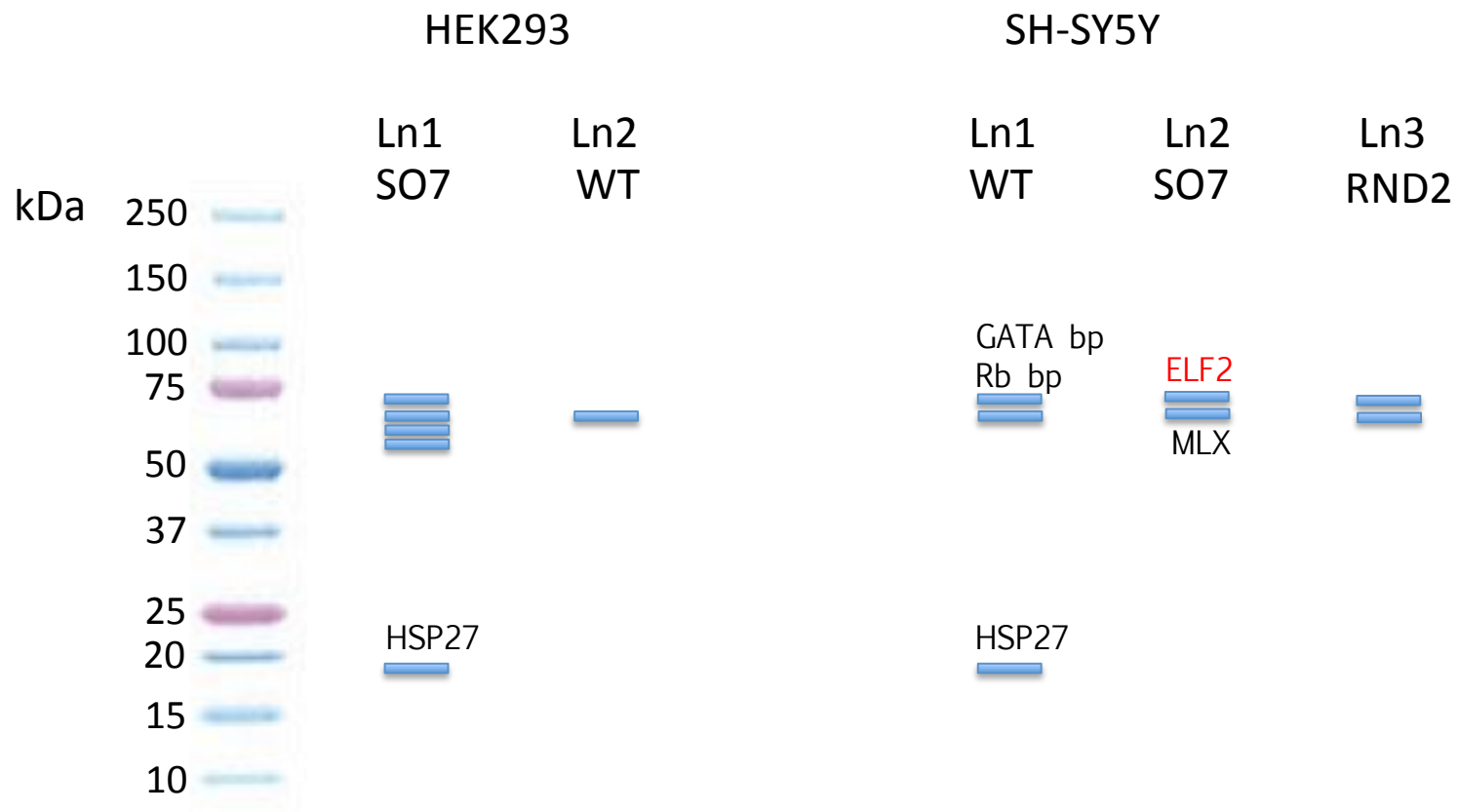
Each run outputs a cut-off score, everything $\geq$ is significant, and probability is calculated from the scores from

Score = $-10 \cdot \log_{10}(P)$ and a chart is provided:

Probability Based Mowse Score

Ions score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event. Individual ions scores > 27 indicate identity or extensive homology ($p < 0.05$). Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.





e-box: CANNTG

27. [gi|6857816](#) Mass: 57353 Score: 45 Queries matched: 1 emPAI: 0.06

E74-like factor 2 (ets domain transcription factor) isoform 2 [Homo sapiens]

☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Peptide
☒ 1426	523.8083	1045.6020	1045.6019	0.06	0	45	0.00054	1	R.VSLSAESLLK.A

Proteins matching the same set of peptides:

[gi|40674085](#) Mass: 49234 Score: 45 Queries matched: 1

ELF2 protein [Homo sapiens]

[gi|42544172](#) Mass: 62672 Score: 45 Queries matched: 1

E74-like factor 2 (ets domain transcription factor) isoform 1 [Homo sapiens]

[gi|114596085](#) Mass: 63927 Score: 45 Queries matched: 1

PREDICTED: E74-like factor 2 (ets domain transcription factor) isoform 9 [Pan troglodytes]

[gi|114596093](#) Mass: 56098 Score: 45 Queries matched: 1

PREDICTED: E74-like factor 2 (ets domain transcription factor) isoform 8 [Pan troglodytes]

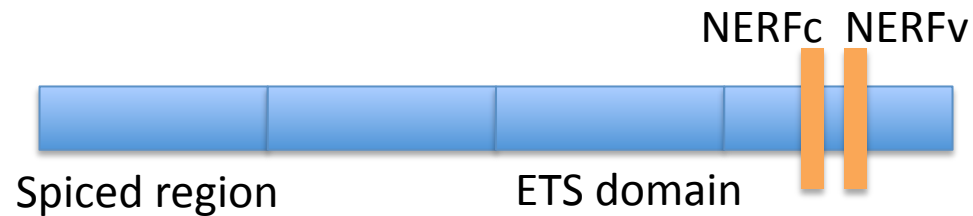
ELF2

E74-like transcription factor isoform 2

NERF = New ETS related factor

NERF= NERF1a NERF1b NERF2
splicing in the N term

Member of the ETS family



ETSF (-) acgttctgcttccgtctgacccctccgacttccggtaaagagtcctatccgcacctccgctcccaccggc
ETS core acgttctgcttccgtctgacccctccgacttccggtaaagagtcctatccgcacctccgctcccaccggc

The ETSF binding site is on the “-” strand... the other strand at the predicted binding site looks like this:

TCTTTACCGGAAGTCGGAGGG
ETS core

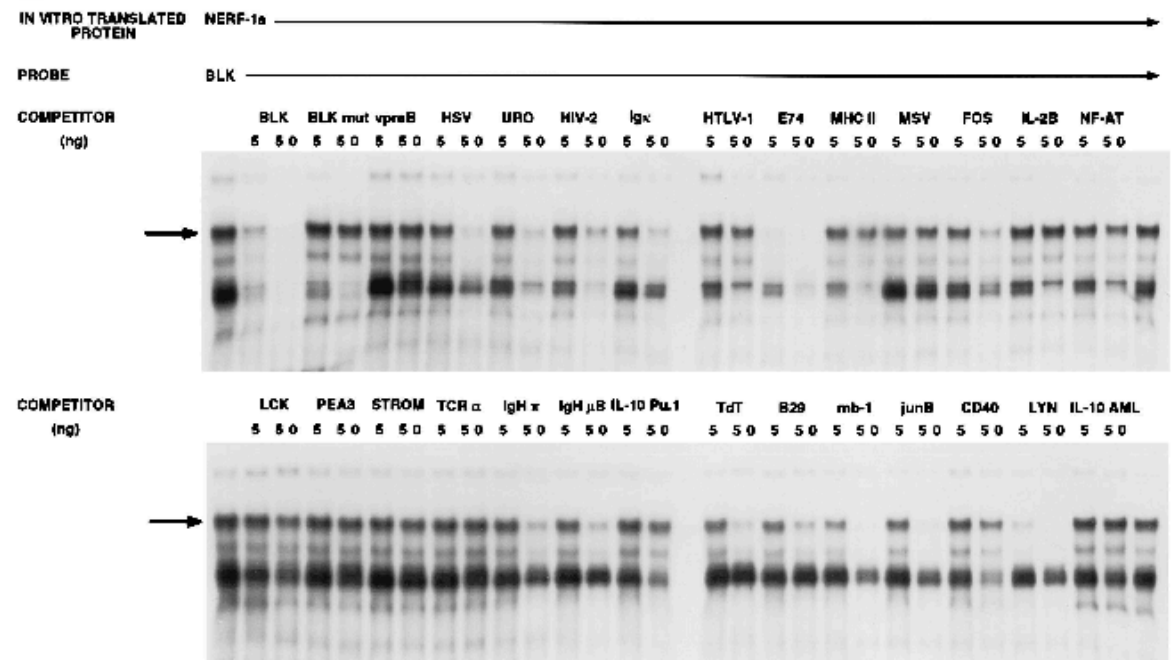
From Oettgen et al., 1996, characterization of NERF...

MutR1 TACCTCGAGTCG
ATXN2 TACCGGAAGTCG

a

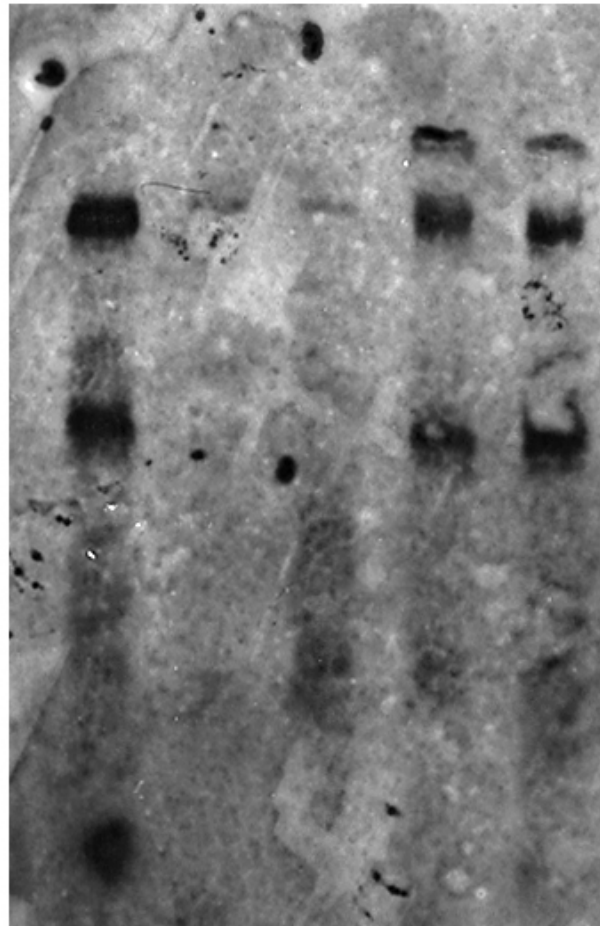
ENHANCER	BINDING SITE	NERF-1a
blk	TCCAGGAAGTAT	++++
lyn	ACCAGGAAGTAG	++++
E74	AACCGGAAGTAA	++++
HSV ICP4	GAACGGAAGCGG	++
IgH Enh. π site	GGCAGGAAGCAG	++
IgH Enh. μ B site	TTGGGGGAAGGGA	++
TdT	GGCAGGAAGCTG	++
mb-1	AACAGGAAGTGA	++
junB	GTCAGGAAGCGC	++
UROKINASE	AGGAGGAAATGA	++
B29	GGCAGGAAGGGG	++
HIV-2 LTR CD3R	GACAGGAACAGC	++
Igk 3' enh.	TTGAGGAACTGA	+
LCK Prom.	GGCAGGAAGCTT	+
c-FOS SRE	CACAGGATGTC	+
IL-10 Pu.1	ATGCAGGAAGTTC	+
IL-2 NF-AT	AGGAGGAAAAAC	-
HTLV-1 LTR	GGGAGGAAATGG	-
STROMELYSIN Prom.	AGCAGGAAGCAT	-
MSV LTR	GAGCGGAAGCGC	-
CD40	CCCAGGAAGAGG	-
MHC class II Prom.	GTGAGGAATCAA	-
Polyoma PEA3	AGCAGGAAGTGA	-
IL-2 IL-2B	AAGAGGAAATAAT	-
TCR α Enh. T α 2	CAGAGGATGTGG	-
vpreB Prom.	GGGAGGAAGCAC	-
CONSENSUS	TCCAGGAAGTAN AA C	

b



No antibody
200x excess
Mutant oligo
NERFc antibody
NERFv antibody

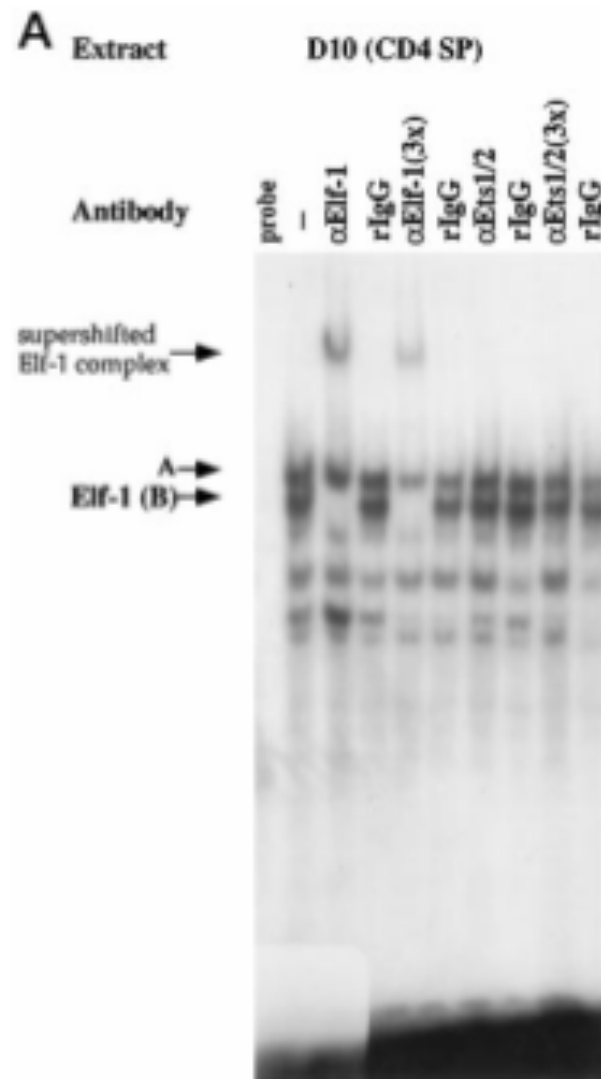
SH-SY5Y



□ Supershift bands

6% gel run 125V until
the position of ½-way
between the two dye
fronts running off
(medium length run).

Last Friday



4% gel run at 250V for four hours (super long run).

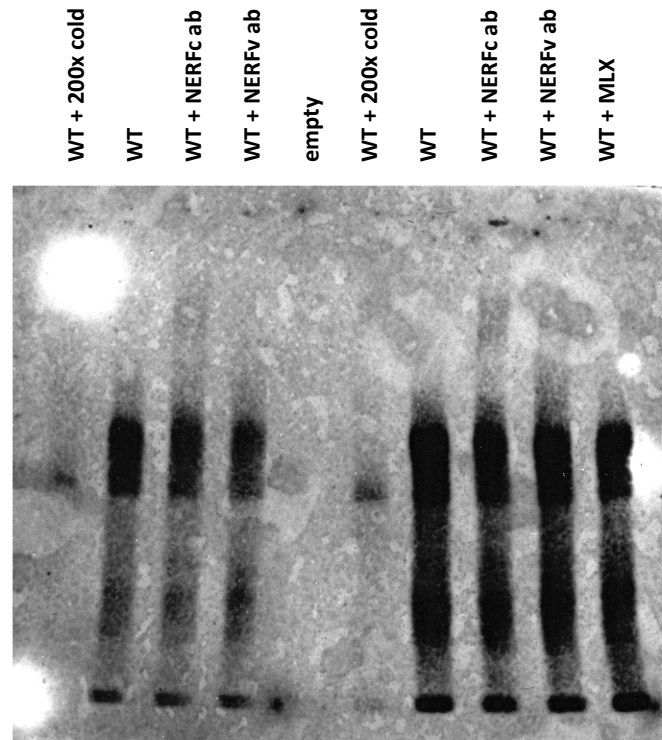
A Potential Role for Elf-1 in *CD4* Promoter Function*

(Received for publication, December 3, 1998, and in revised form, March 10, 1999)

Sophia Sarafova[‡] and Gerald Siu^{§¶}

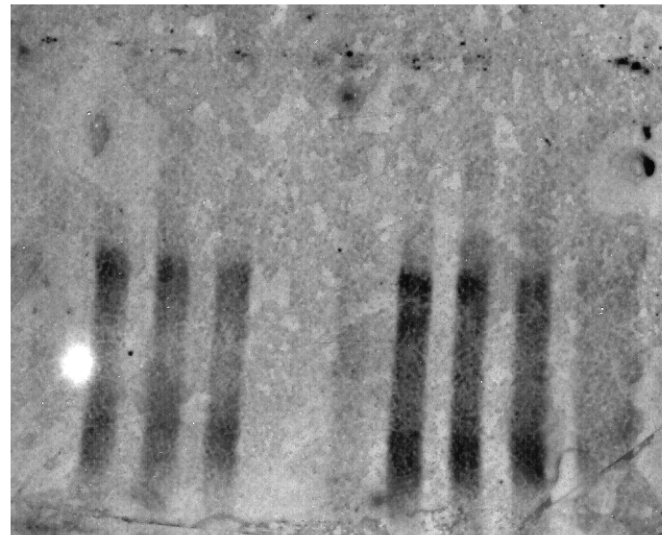
From the [§]Department of Microbiology and the [‡]Integrated Program in Cellular, Molecular, and Biophysical Studies, Columbia University, College of Physicians and Surgeons, New York, New York 10032

SH-SY5Y



□ Supershift bands

HEK293



5% gel run at 150V until
15 minutes after the
second dye front ran off
(long run).

Monday

EMSA-Western

4416–4417 *Nucleic Acids Research*, 1993, Vol. 21, No. 18

Antibody detection of protein complexes bound to DNA

Anastasia M.Khoury Christianson* and Fotis C.Kafatos

Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138, USA

Received June 21, 1993; Accepted July 30, 1993

In this technique, the signal depends on the concentrations and affinities of the antibody, antigen, and DNA. Typically, EMSA reactions are performed at equilibrium conditions with femtomolar to nanomolar radiolabeled DNA concentrations, but at such conditions the resulting complexes have insufficient protein for detection with antibody. To shift the equilibrium (protein + DNA $\rightleftharpoons$ complex) to the right, either protein or DNA concentration must be increased. Large protein excess is

Completed Wednesday

ELF2 Function

ETS family transcription factors are responsible for spatial and temporal expression of genes supporting angiogenesis

ELF2 can support angiogenesis

ELF2 can bind and activate the promoter of the TIE receptor (angiopoietin receptor)

ELF2 can support TIE expression under hypoxic conditions (think of tumors)

A role for ELF2 in angiogenesis and vascular development

Mostly endothelial

ELF2 controls regulation of T-cell and B-cell specific genes

ELF2 binds and activates *lyn* and *blk* gene promoters in B-cells

Lyn is Src-type tyrosine kinase, activator of MEKK and downregulator of stem cell factor KIT

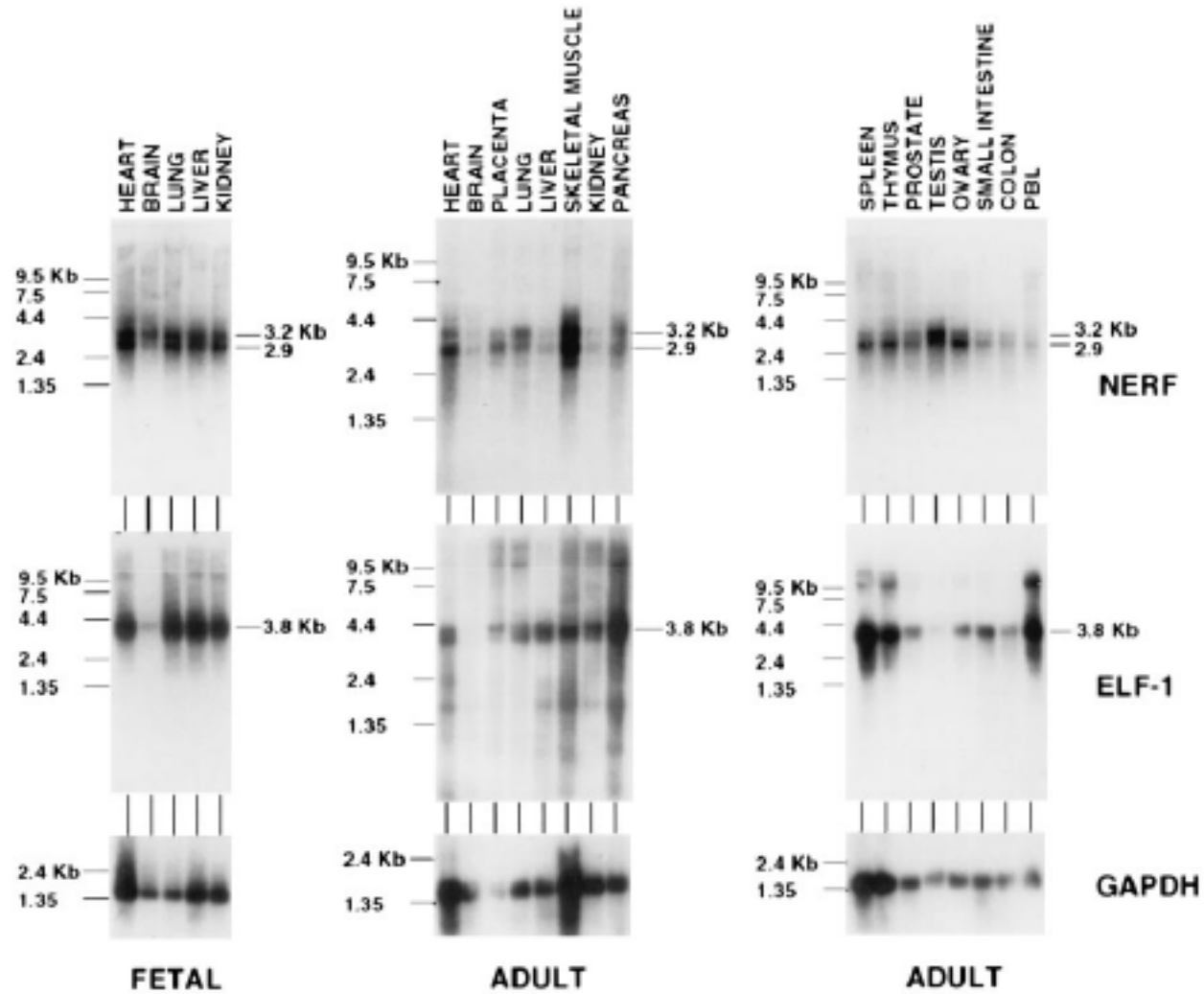
Blk is a B-cell specific Src-type tyrosine kinase

ELF2 is expressed in brain (next slide)

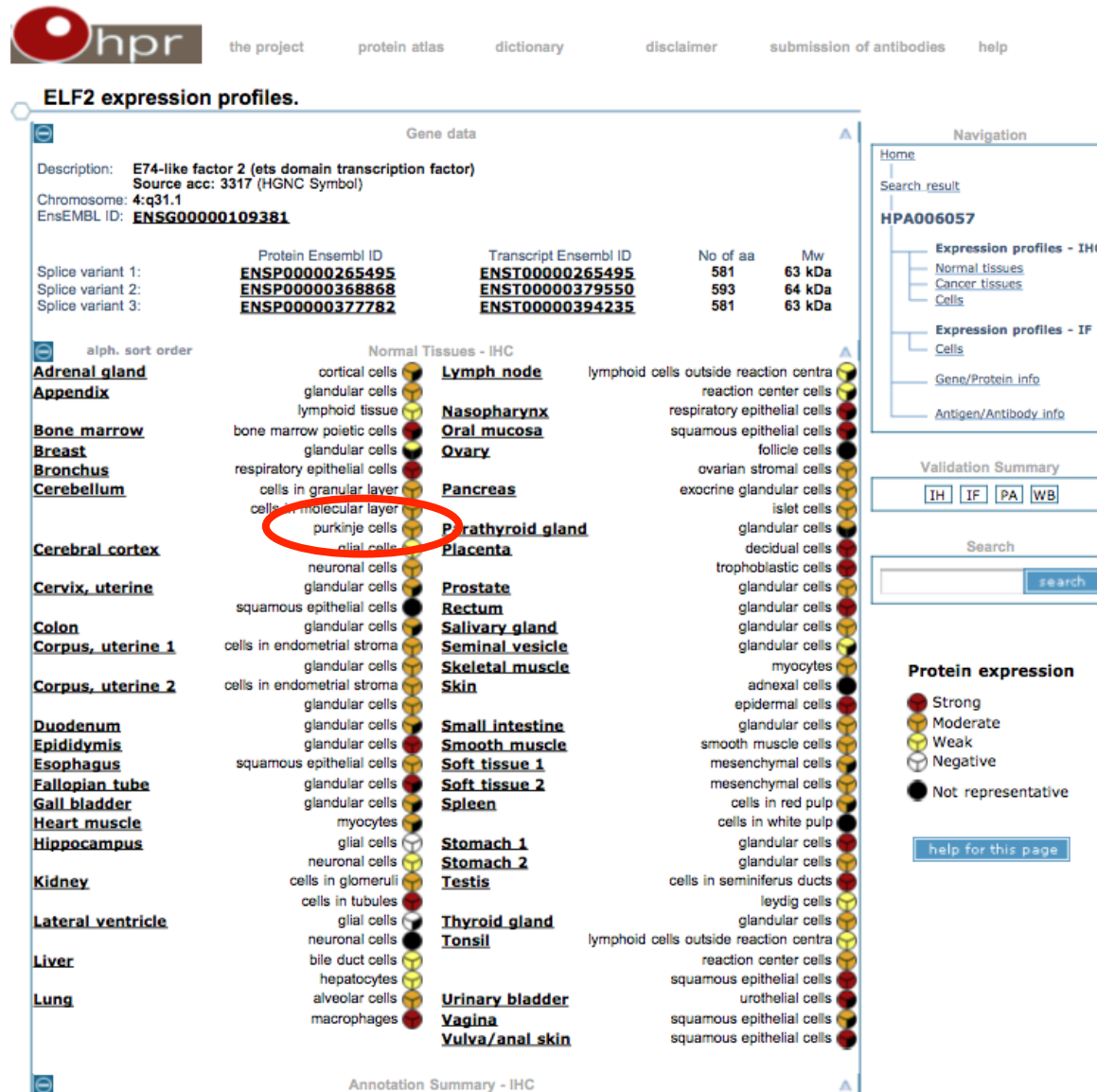
	PC	SH-SY5Y	Hek293
NERF	++	+++	++
Blk	+	++	++
Lyn	-	++	+
Ang1	-	+ / ++	++

-absent, + weak, ++ moderate, +++ strong

Northern



Expression based on information in the Human Protein Atlas www.proteinatlas.org/



Cerebellum [ELF2]

Cell Type	Intensity	Quantity	Localization
Cells in granular layer	moderate	75%-25%	cytoplasmic and/or membranous
Cells in molecular layer	moderate	75%-25%	cytoplasmic and/or membranous
Purkinje cells	moderate	>75%	cytoplasmic and/or membranous

Male, age 45 Cerebellum (T-X6000) Normal tissue, NOS (M-00100) Patient id: 2521	Female, age 19 Cerebellum (T-X6000) Normal tissue, NOS (M-00100) Patient id: 2524	Female, age 54 Cerebellum (T-X6000) Normal tissue, NOS (M-00100) Patient id: 2523

Comments: Purkinje cells in C negative.

Brown color indicates presence of protein, blue color shows cell nuclei. [Image Usage Policy](#)



Arbor Vitae

Nuclear Layer

Molecular Layer

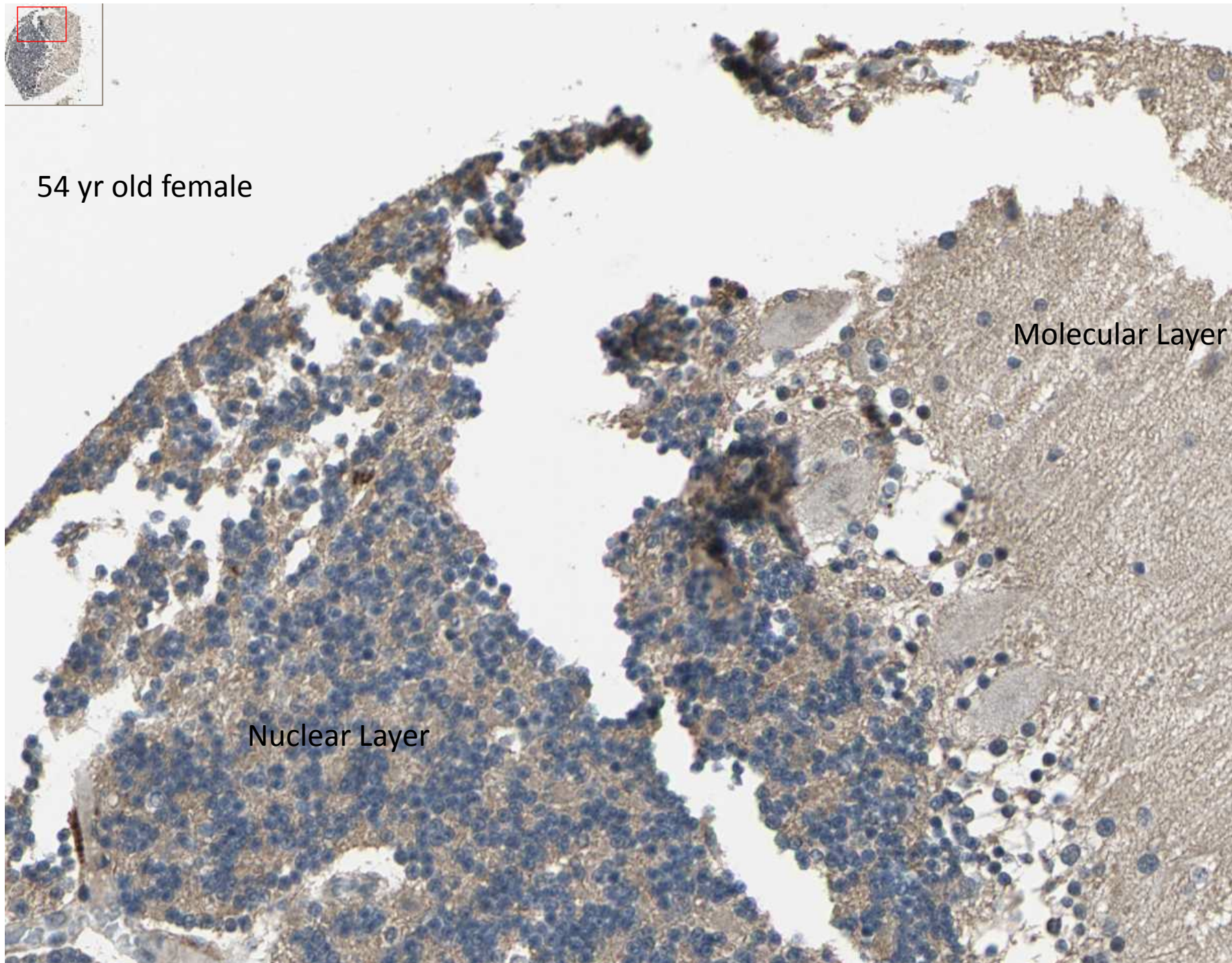
19 yr old female



54 yr old female

Molecular Layer

Nuclear Layer





45 yr old male

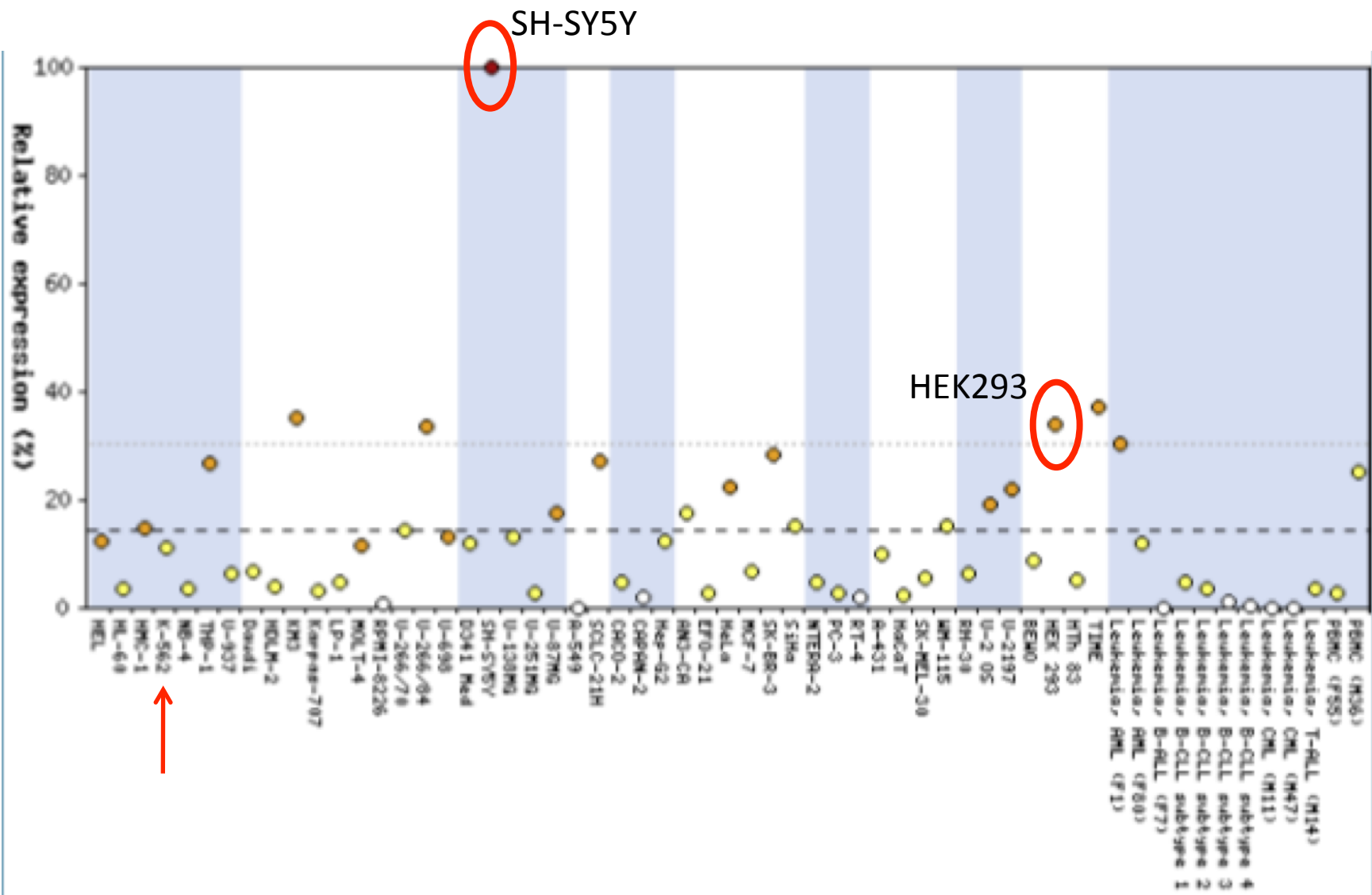
Molecular Layer

> close <

Cell lines - IHC	
Myeloid	Abdominal
<u>HEL</u>	<u>CACO-2</u>
<u>HL-60</u>	<u>CAPAN-2</u>
<u>HMC-1</u>	<u>Hep-G2</u>
<u>K-562</u>	Breast, female reproductive system
<u>NB-4</u>	<u>AN3-CA</u>
<u>THP-1</u>	<u>EFO-21</u>
<u>U-937</u>	<u>HeLa</u>
Lymphoid	<u>MCF-7</u>
<u>Daudi</u>	<u>SiHa</u>
<u>HDLM-2</u>	<u>SK-BR-3</u>
<u>Karpas-707</u>	Urinary, male reproductive system
<u>KM3</u>	<u>NTERA-2</u>
<u>LP-1</u>	<u>PC-3</u>
<u>MOLT-4</u>	<u>RT-4</u>
<u>RPMI-8226</u>	Skin
<u>U-266/70</u>	<u>A-431</u>
<u>U-266/84</u>	<u>HaCaT</u>
<u>U-698</u>	<u>SK-MEL-30</u>
Brain	<u>WM-115</u>
<u>D241 Med</u>	Sarcoma
<u>SH-SY5Y</u>	<u>RH-30</u>
<u>U-138MG</u>	<u>U-2 OS</u>
<u>U-251MG</u>	<u>U-2197</u>
<u>U-87MG</u>	Miscellaneous
Lung	<u>BEWO</u>
<u>A-549</u>	<u>HEK 293</u>
<u>SCLC-21H</u>	<u>H1h-83</u>
	<u>TIME</u>

Protein expression

- Strong
- Moderate
- Weak
- Negative



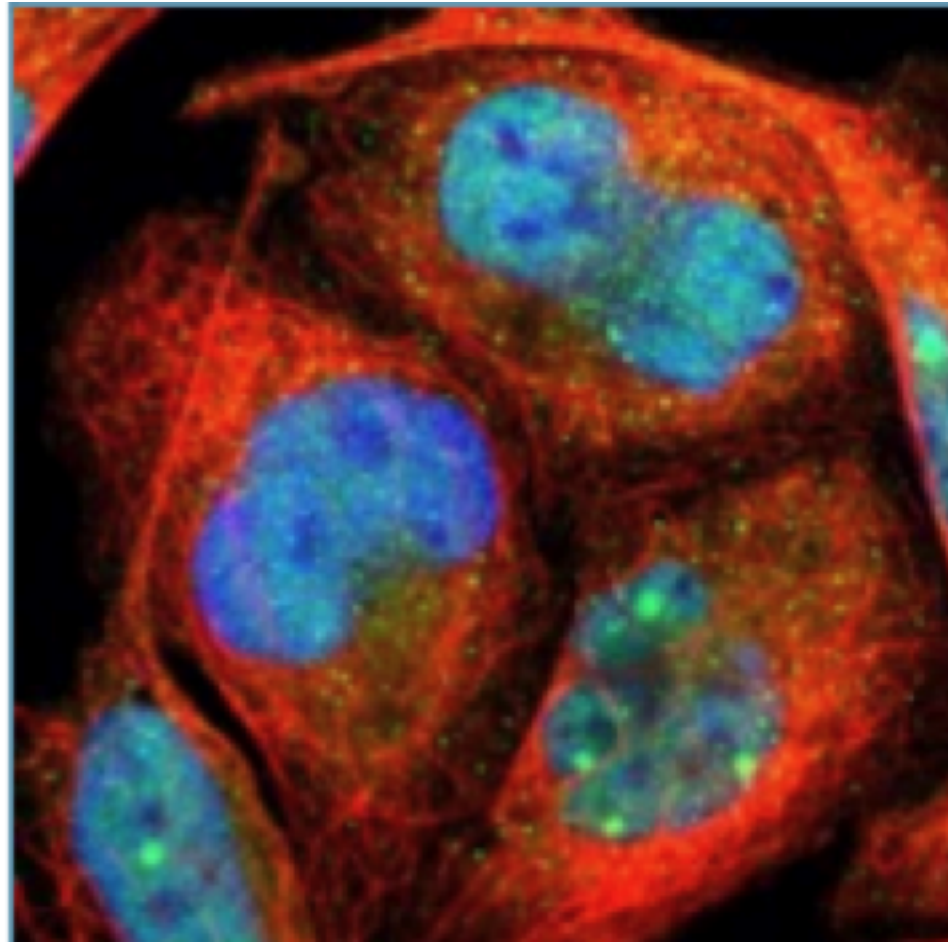
ELF2

A-431
U-2 OS
U-251MG

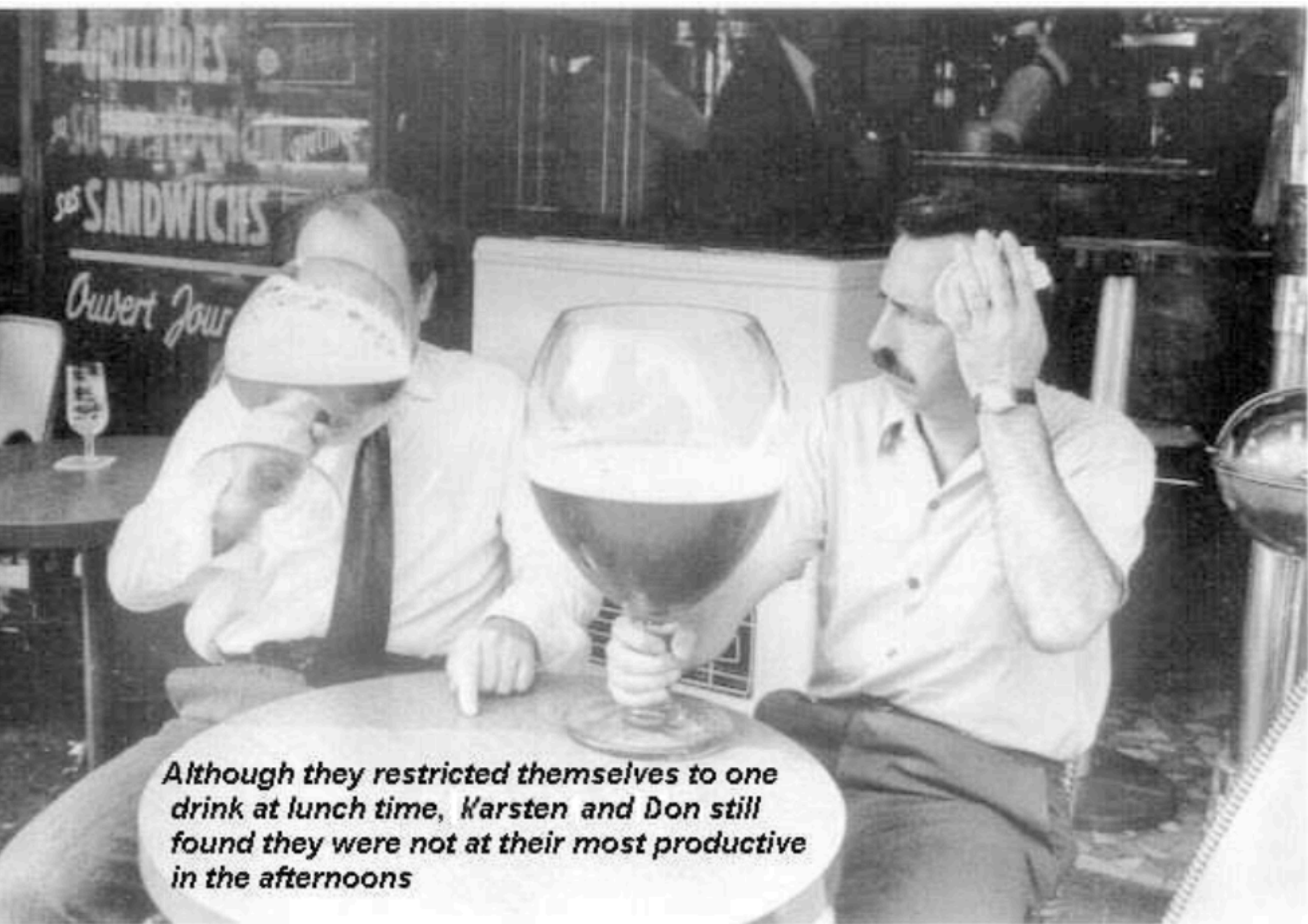
Cell lines - IF


Annotation summary:
Staining of nuclei but not nucleoli in all three cell lines. With additional staining of nuclear bodies and cytoplasm.

A431= Epithelial carcinoma
U2OS= Osteosarcoma
U251MG= Glioblastoma multiforme



Three controls are used, dapi, tubulin, calreticulin



Although they restricted themselves to one drink at lunch time, Karsten and Don still found they were not at their most productive in the afternoons

Other odds n ends

CAG lengths and RT PCR

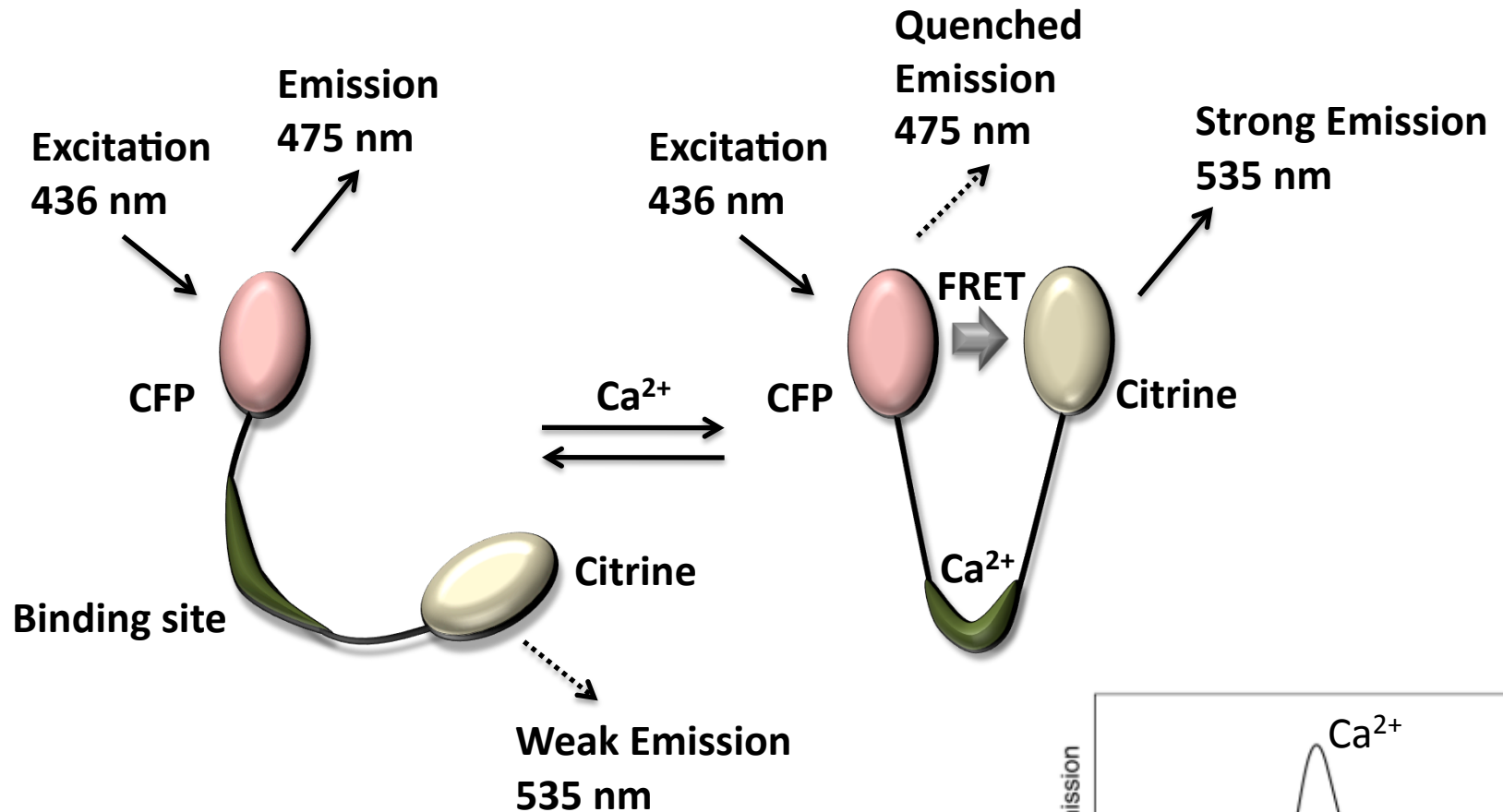


Compounds and RT PCR

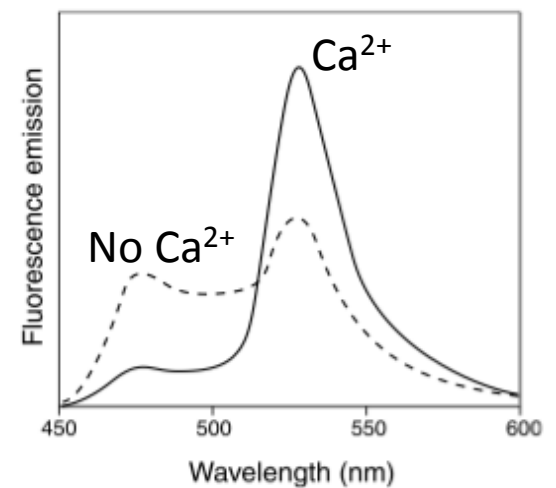
Positive readout system

Cameleon cells

D1ER Cameleon



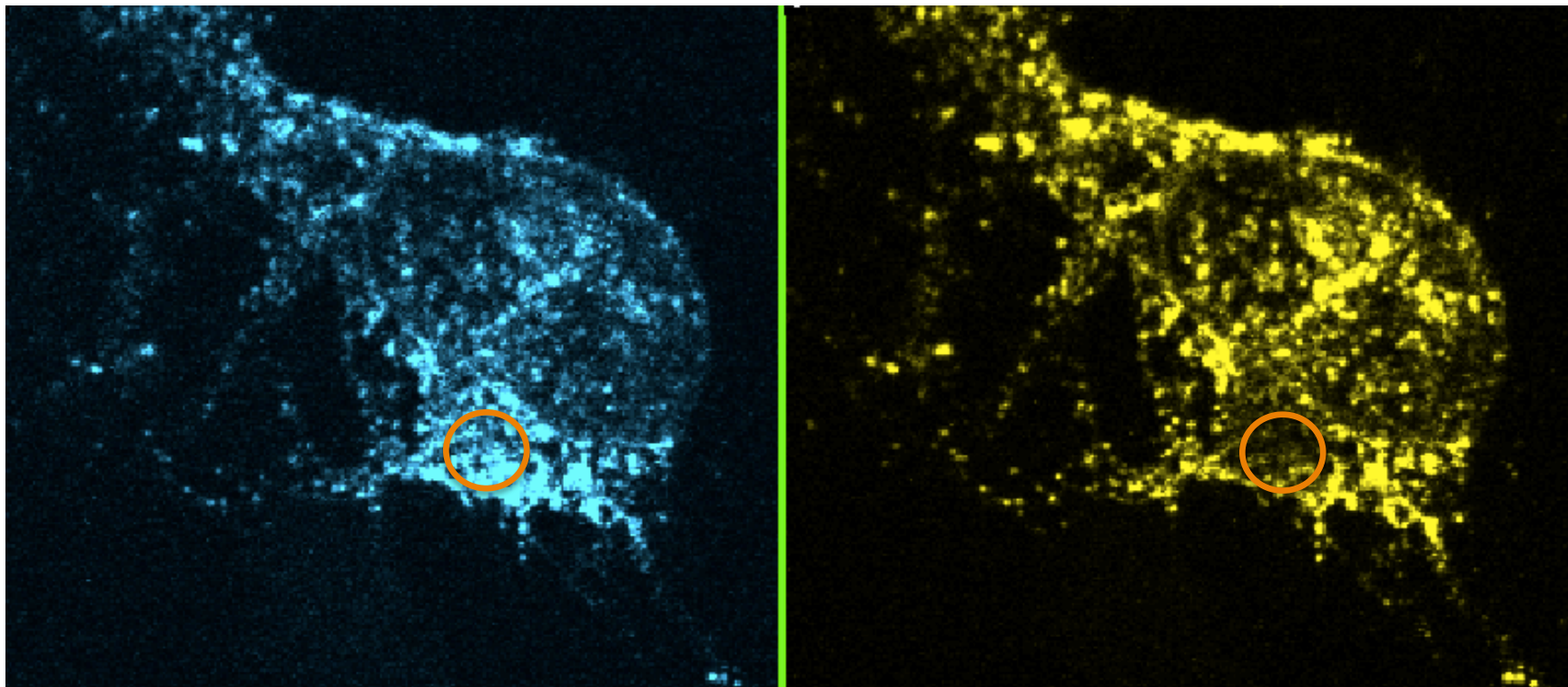
Targeted to the endoplasmic reticulum
From Roger Tsien's lab



D1ER cells “old”

CFP

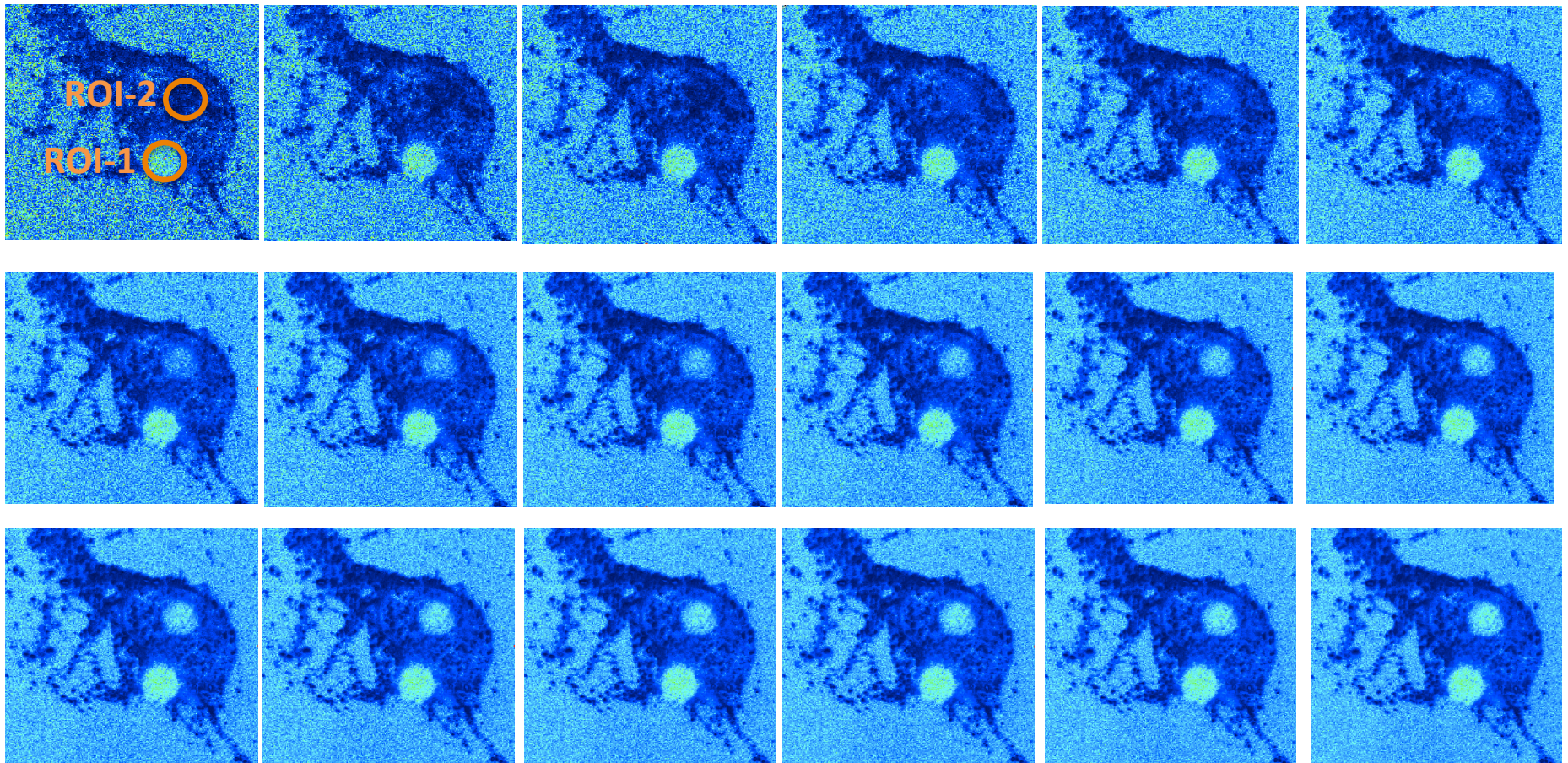
YFP



(after photobleaching)

Excitation at CFP, Emission at CFP and YFP emission peaks

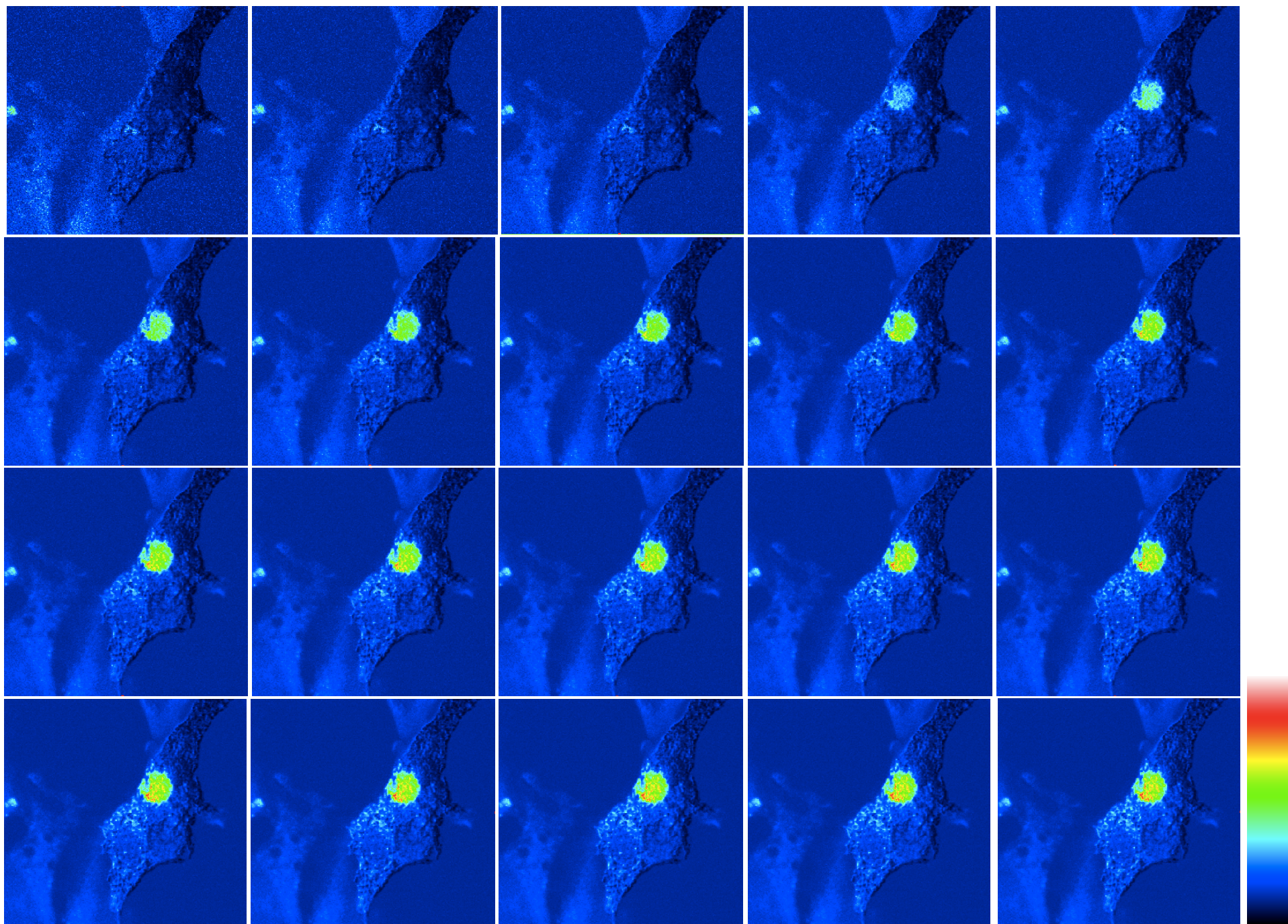
Before starting photobleaching series at ROI-2, we took one image and photobleached at ROI-1 then took another image and divided. Then we photobleached, took image, photobleached, took image...18 times. All images are ratio of pixel intensity after/before photobleaching.



D1ER cells made in the HEK293 cell line

“D1ER old”

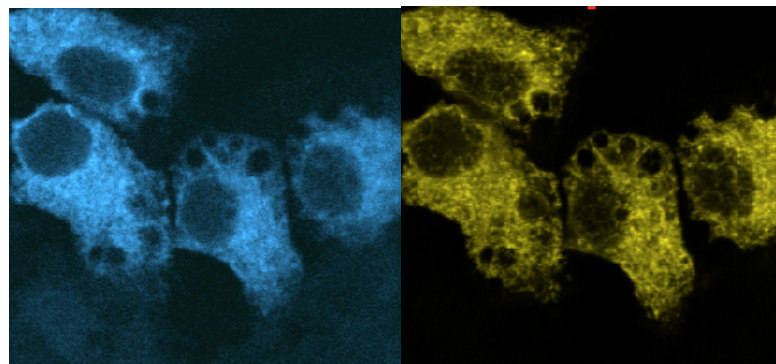
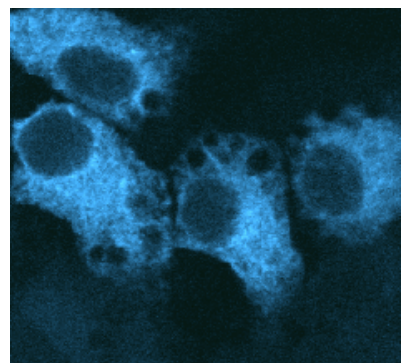
D1ER “new”



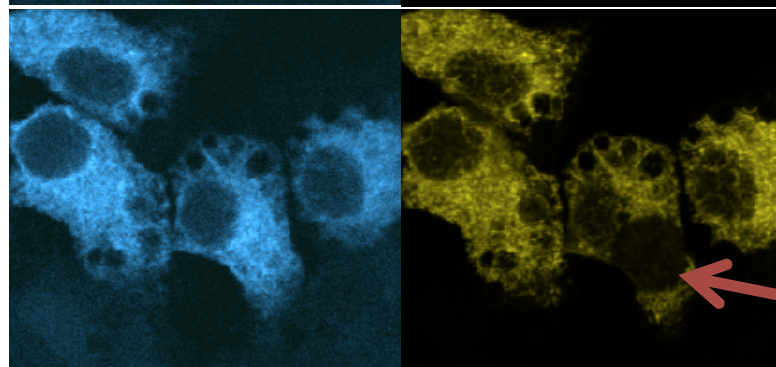
D1ER "new"

CFP

YFP



Before photobleaching



After photobleaching

ROI

2

4

15

Image
division

