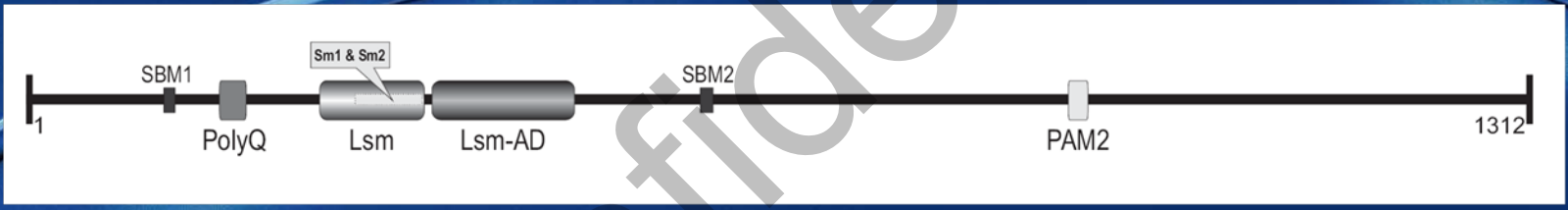
SCA2 pathogenesis:
Altered splicing caused by gain of normal ataxin-2 function

Introduction

- Disease pattern: Loss of balance and motor coordination
- Pathogenesis: Dysfunction/Degeneration of the cerebellum and adjacent tissues/connections
- Age of onset: Normally between the 3rd and 5th decade



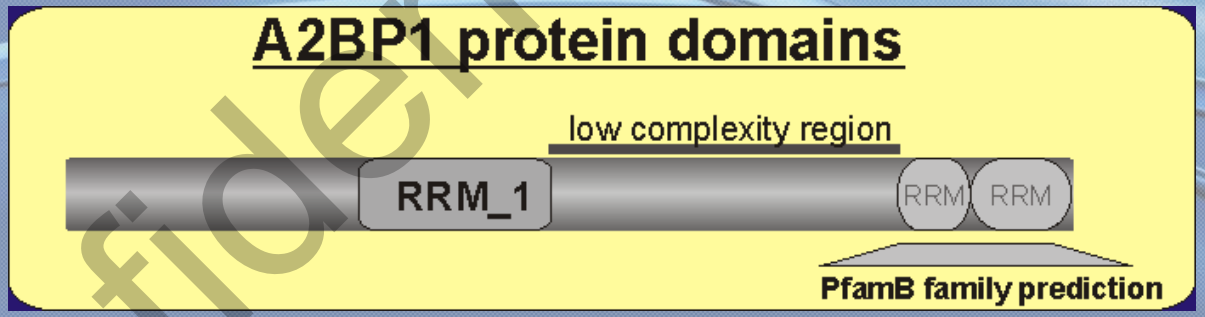
SBM	-	SH3-binding motif (protein-protein interactions)
Lsm	-	like-SM domain (RNA binding)
Sm	-	Splicing motif
Lsm-AD	-	SM associated domain with trans-Golgi-signal and ER export signal
PAM2	-	*PolyA binding protein* interacting motif

➔ RNA interaction

Interaction with A2BP1

- First identified interaction partner: A2BP1 (fox-1)
- Nuclear as well as cytoplasmic localization
- RNA binding motifs
- tissue-specific splicing
- mRNA splicing triggered by a specific recognition sequence: UGCAUGU
- Disease releated links:

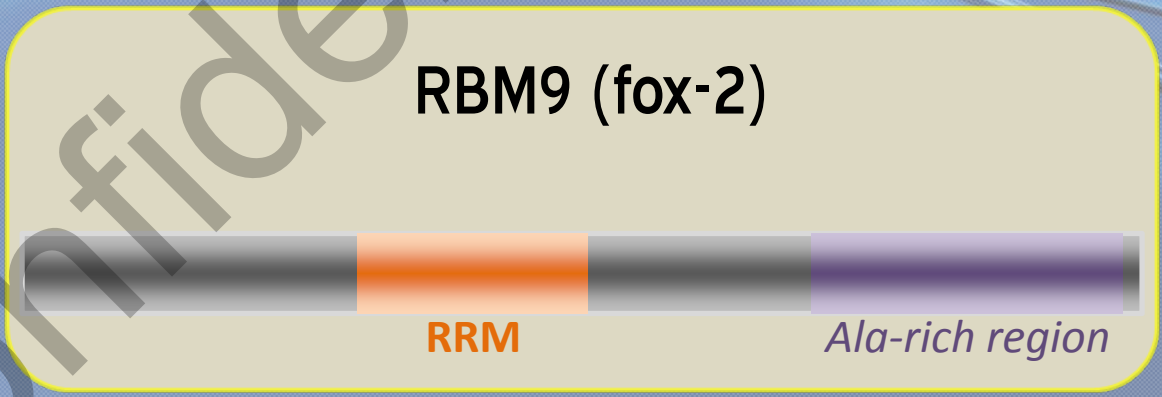
  - A2BP1 gene maps to an locus for autism
  - Chromosome 16 translocation in two cases of epilepsy and mental retardation disrupt A2BP1 gene



RRM: Eucaryotic RNA Recognition Motif

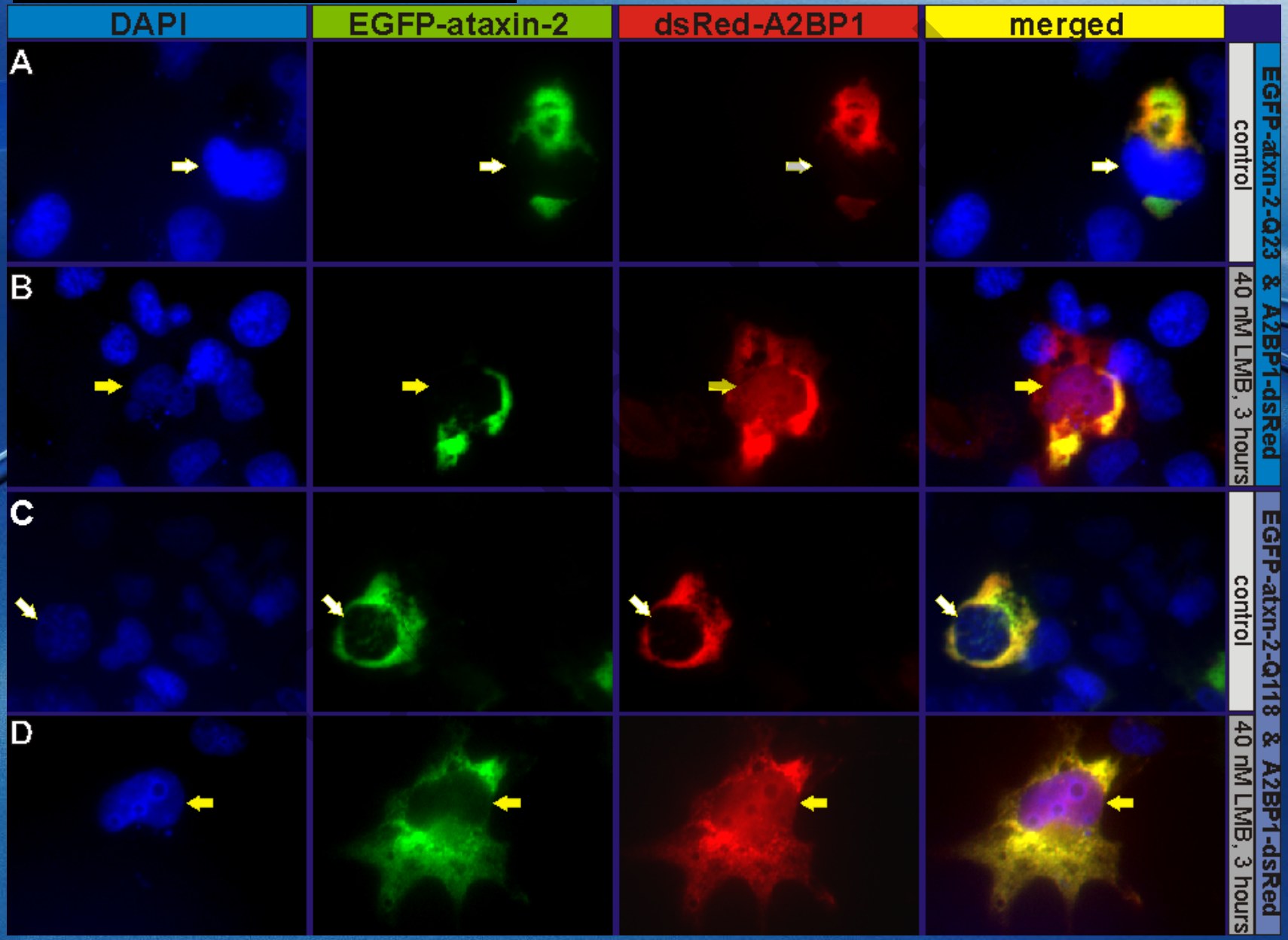
Interaction with A2BP1

- Another protein RBM9 has the identical recognition sequence as A2BP1
- Nuclear as well as cytoplasmic localization (predicted)
- RNA binding motifs
- two isoforms in human
- mRNA splicing triggered by a specific recognition sequence: UGCAUGU
- expression pattern like a house-keeping gene



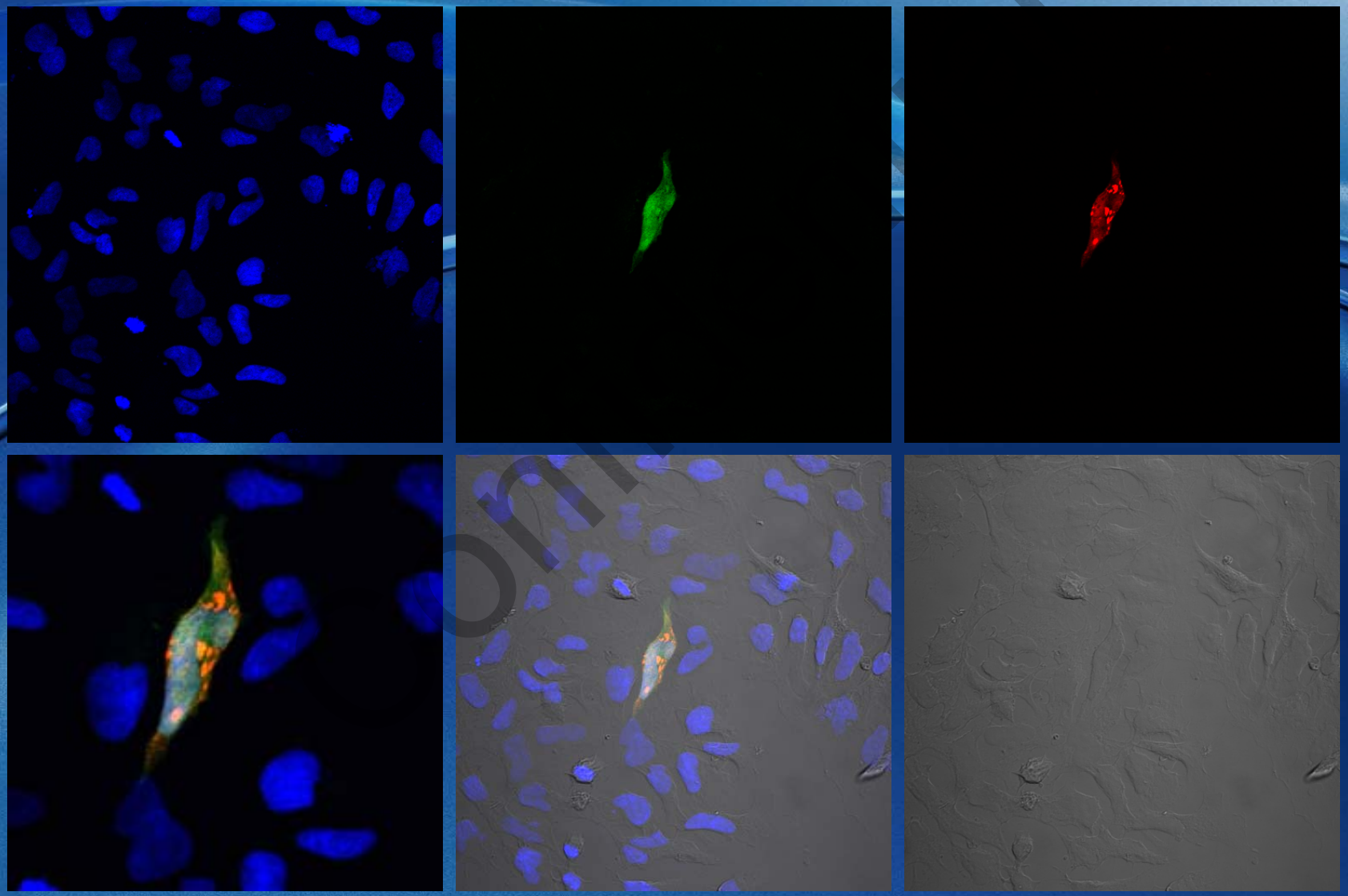
Introduction

Recruitment of A2BP1 by ataxin-2:



Confocal Microscopy of over-expressing HEK293T cell lines

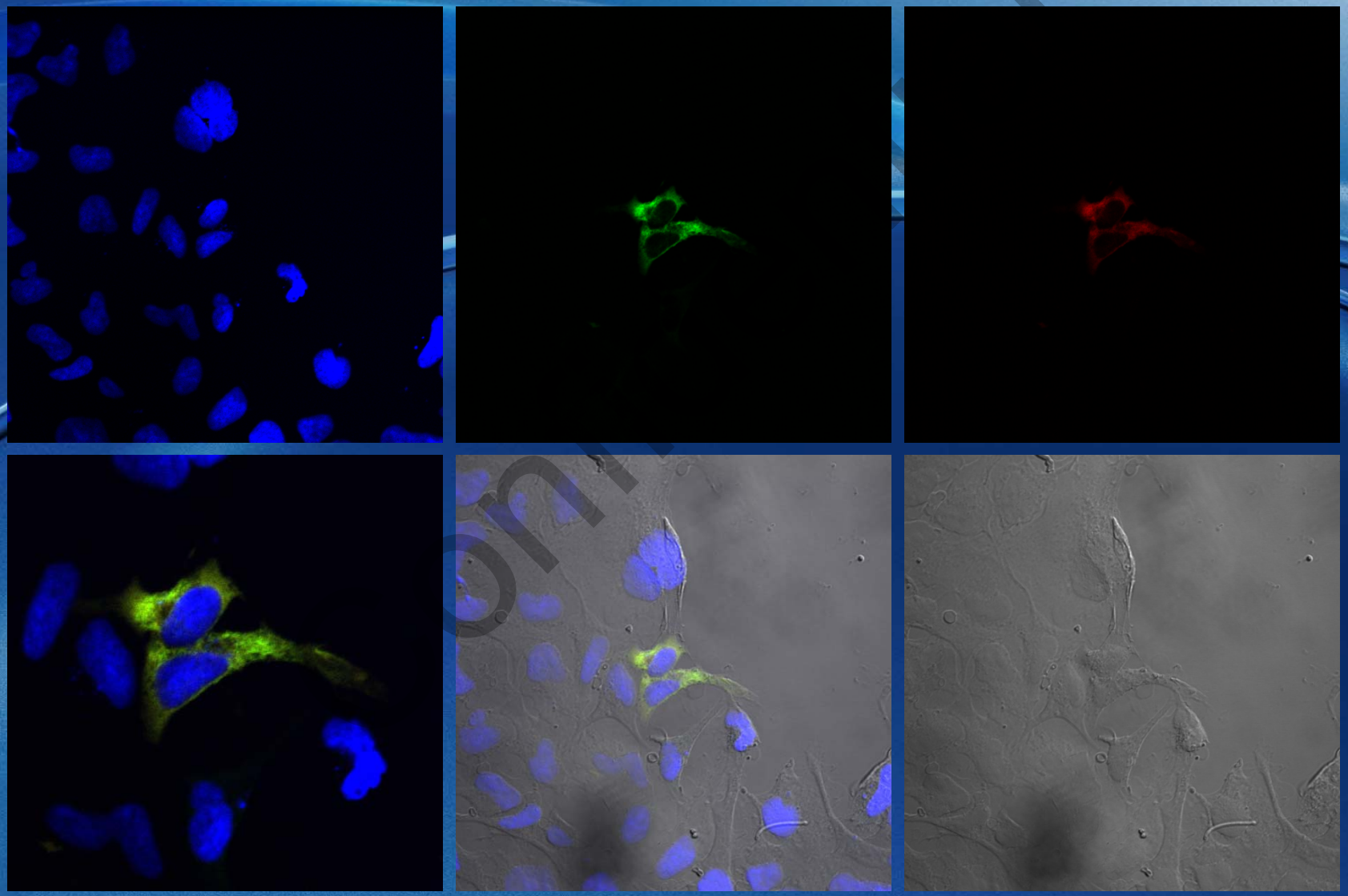
pEGFP-N1 | A2BP1-dsRED



pEGFP-A2BP1 control Stack1.ics

Confocal Microscopy of over-expressing HEK293T cell lines

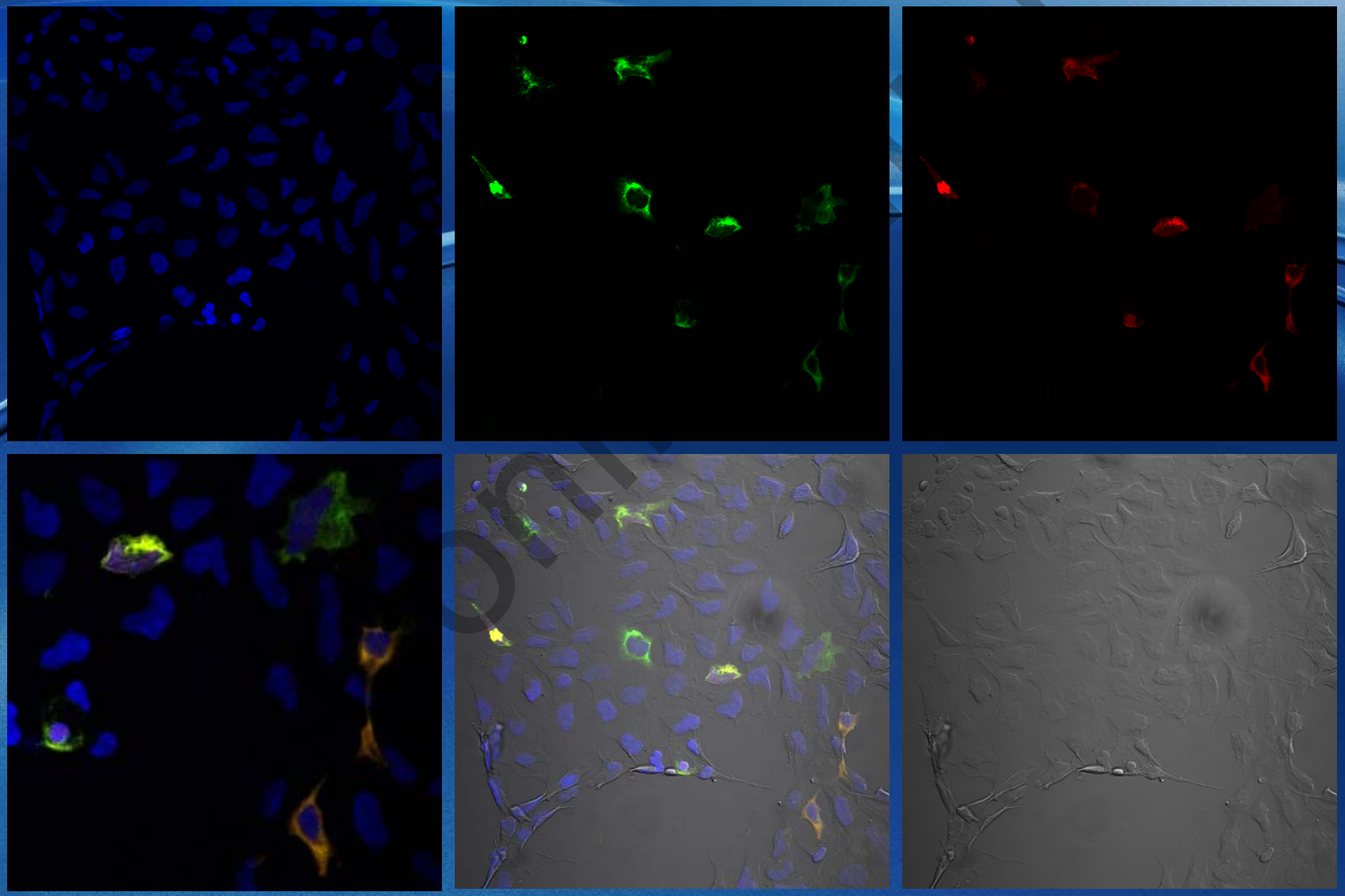
Atxn2-Q22-pEGFP | A2BP1-dsRED



V001-A2BP1 control Stack3.ics

Confocal Microscopy of over-expressing HEK293T cell lines

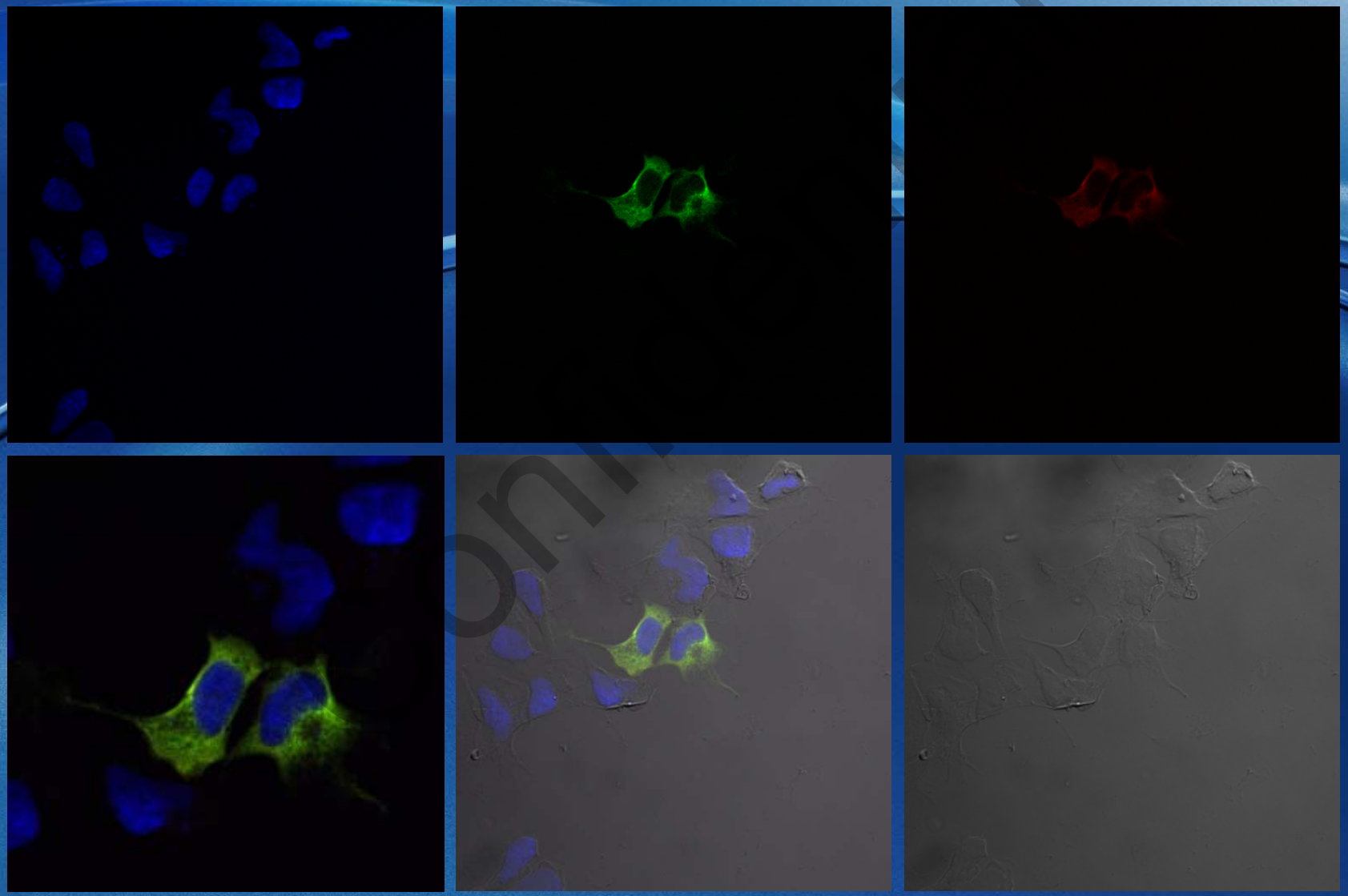
Atxn2-Q58-pEGFP | A2BP1-dsRED



V002-A2BP1 control Stack4.ics

Confocal Microscopy of over-expressing HEK293T cell lines

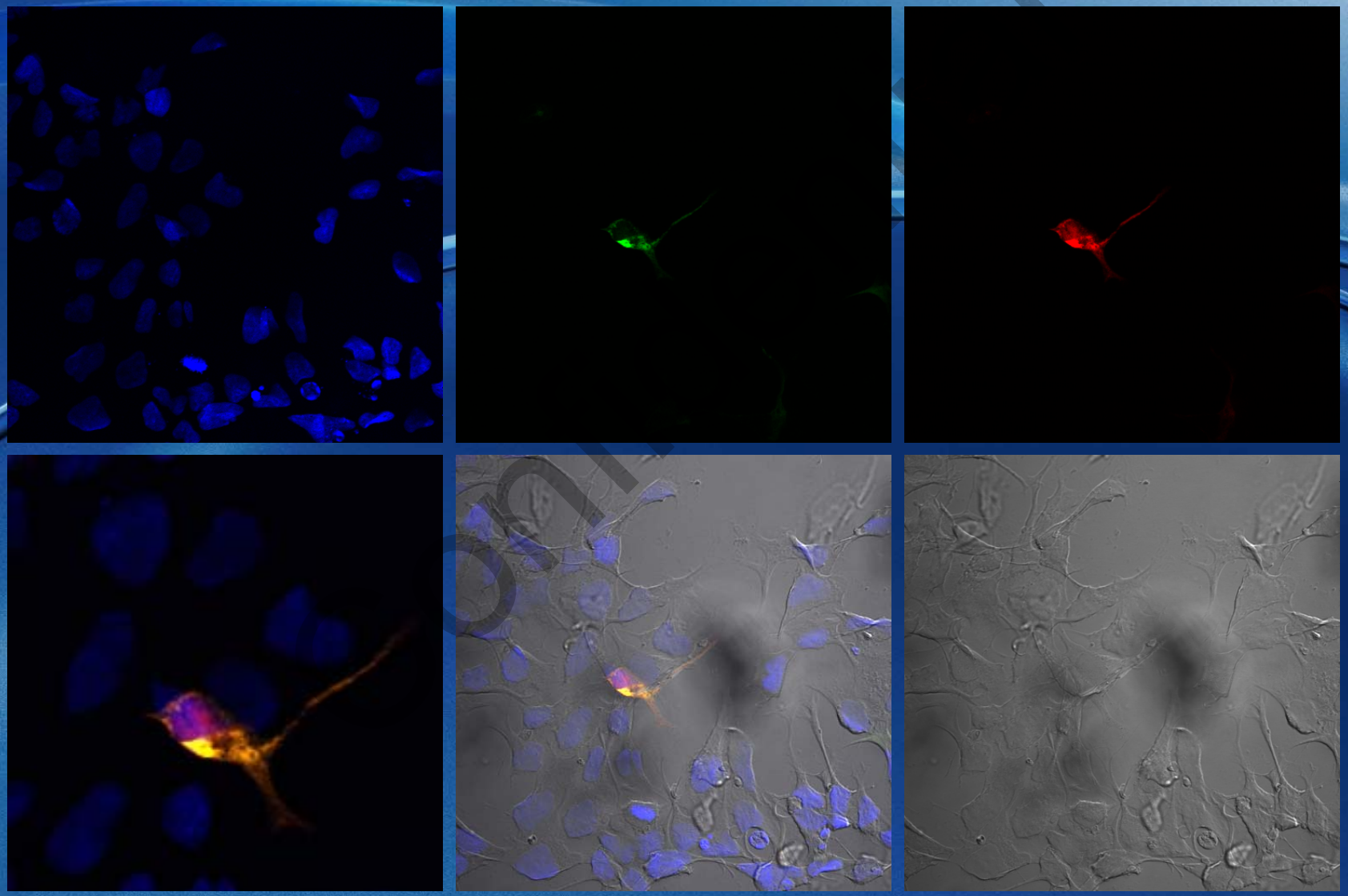
Atxn2-Q127-pEGFP | A2BP1-dsRED



V003-A2BP1 control Stack2.ics

Confocal Microscopy of over-expressing HEK293T cell lines

Atxn2-Q22-pEGFP | A2BP1-dsRED LMB



V001-A2BP1 LMB Stack3.ics

Conclusions of A2BP1 Recruitment by Atxn2

- Elevated levels of atxn2 lead to an altered localization of A2BP1
- No remarkable differences between expanded polyQ forms and wt atxn2
- A2BP1 shuttling occurs still on a very low level >> LMB treatment
-

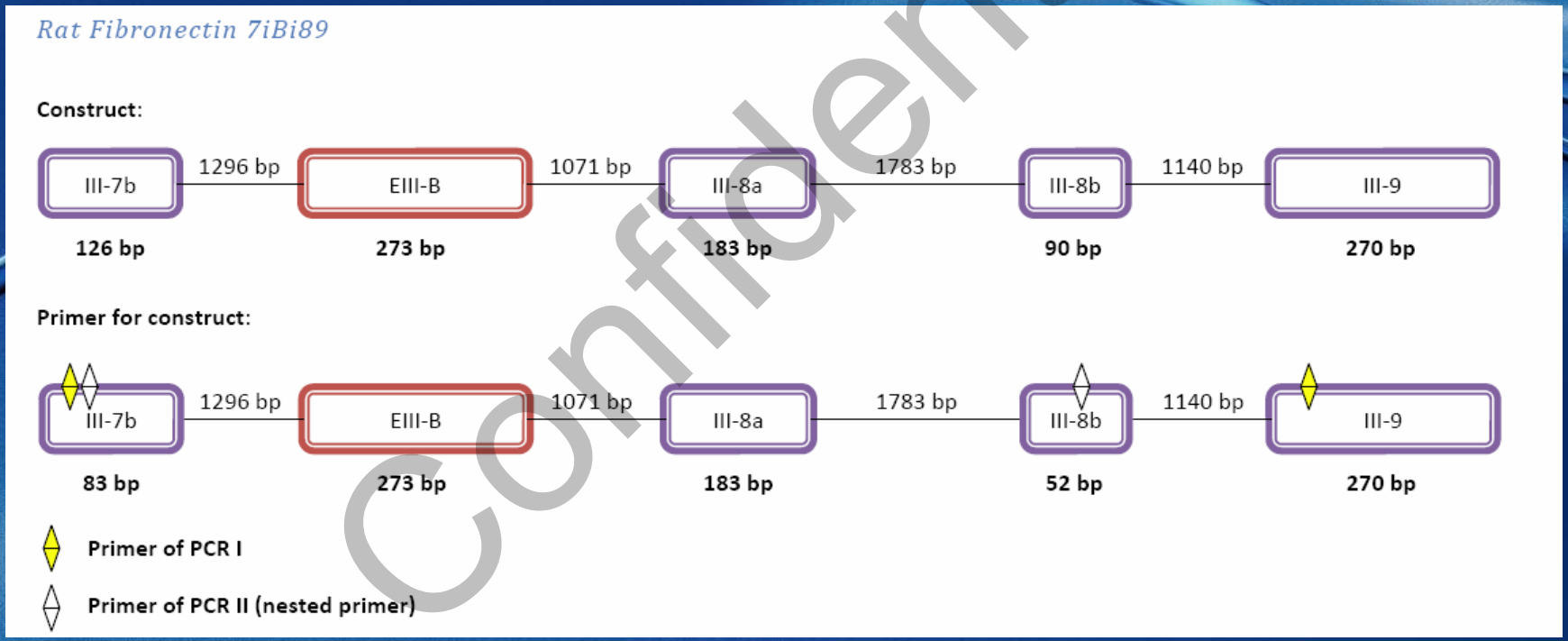
- Can elevated concentrations of atxn2 alter the splicing function of A2BP1?
- Is RBM9 able to rescue for the loss of A2BP1 function
- Disease related link between polyQ repeat expansion and loss of A2BP1 function
-

Atxn2 Mediated Splicing Alterations of A2BP1

Artifical splicing construct: Minigene *7iBi89 rat fibronectin*

Transfected into the cells > no effects on cellular pathways

One exon is spliced due to A2BP1 recognition sequence

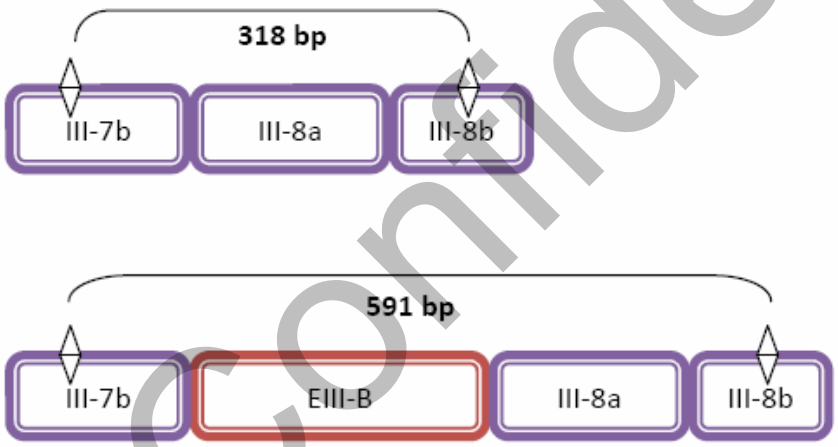


Atxn2 Mediated Splicing Alterations of A2BP1

Artifical splicing construct: Minigene *7iBi89 rat fibronectin*

A2BP1 mediates an exon inclusion in the minigene

cDNA amplicons:



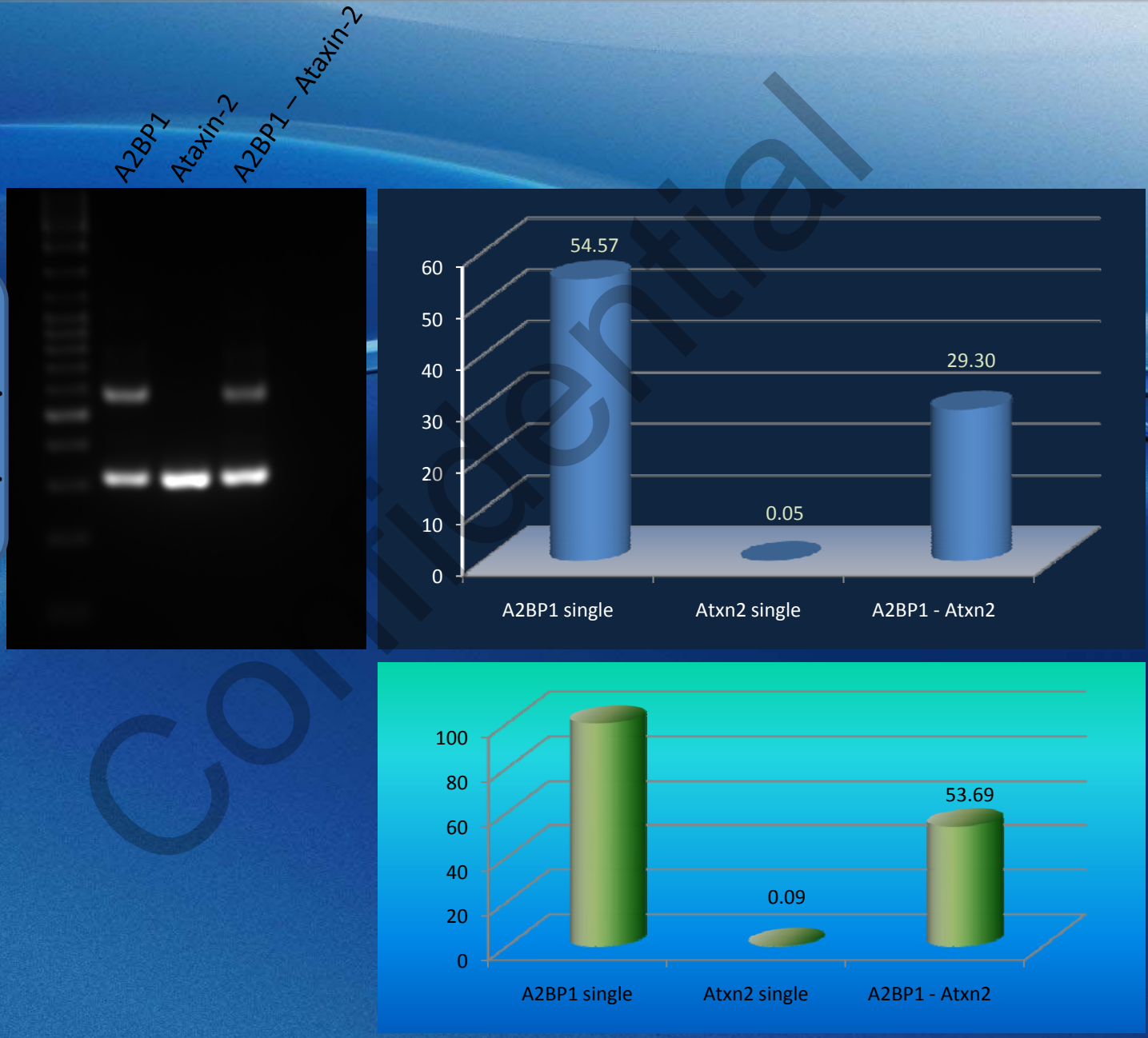
A2BP1 - OFF

Exon exclusion

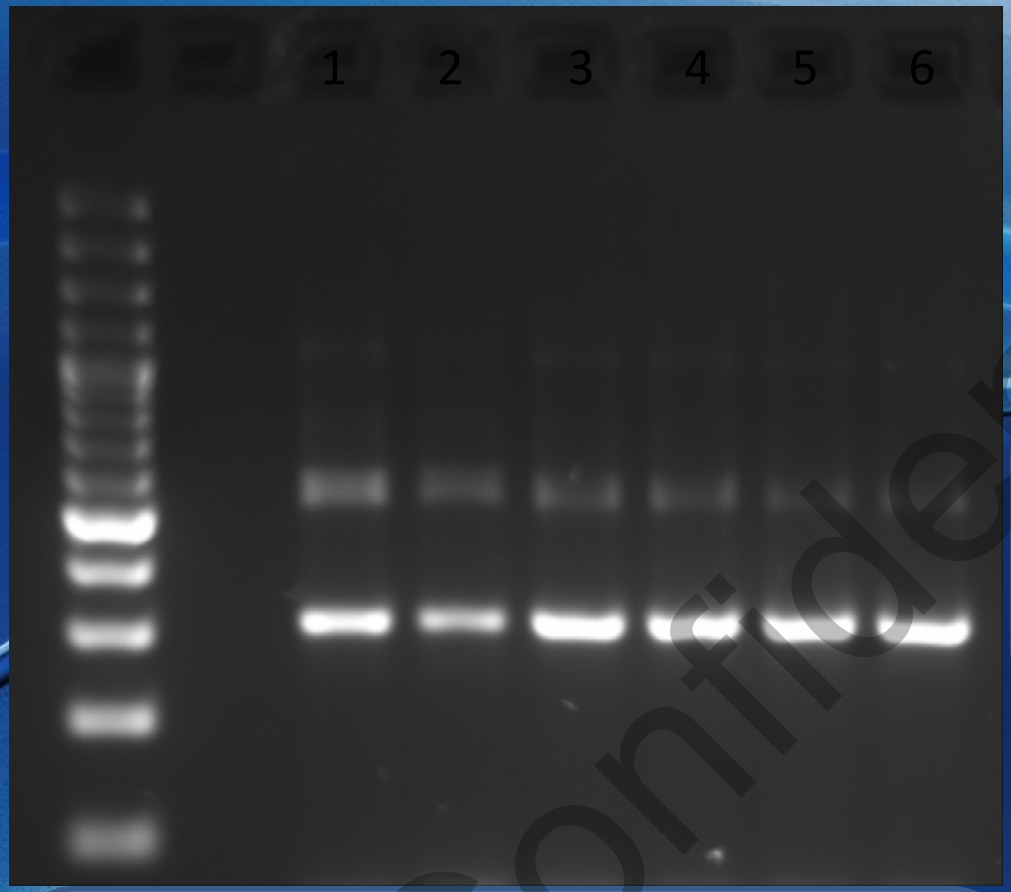
A2BP1 - ON

Exon inclusion

Atxn2 Mediated Splicing Alterations of A2BP1



Atxn2 Mediated Splicing Alterations of A2BP1



Artifical splicing construct:
Minigene *7iBi89 rat fibronectin*

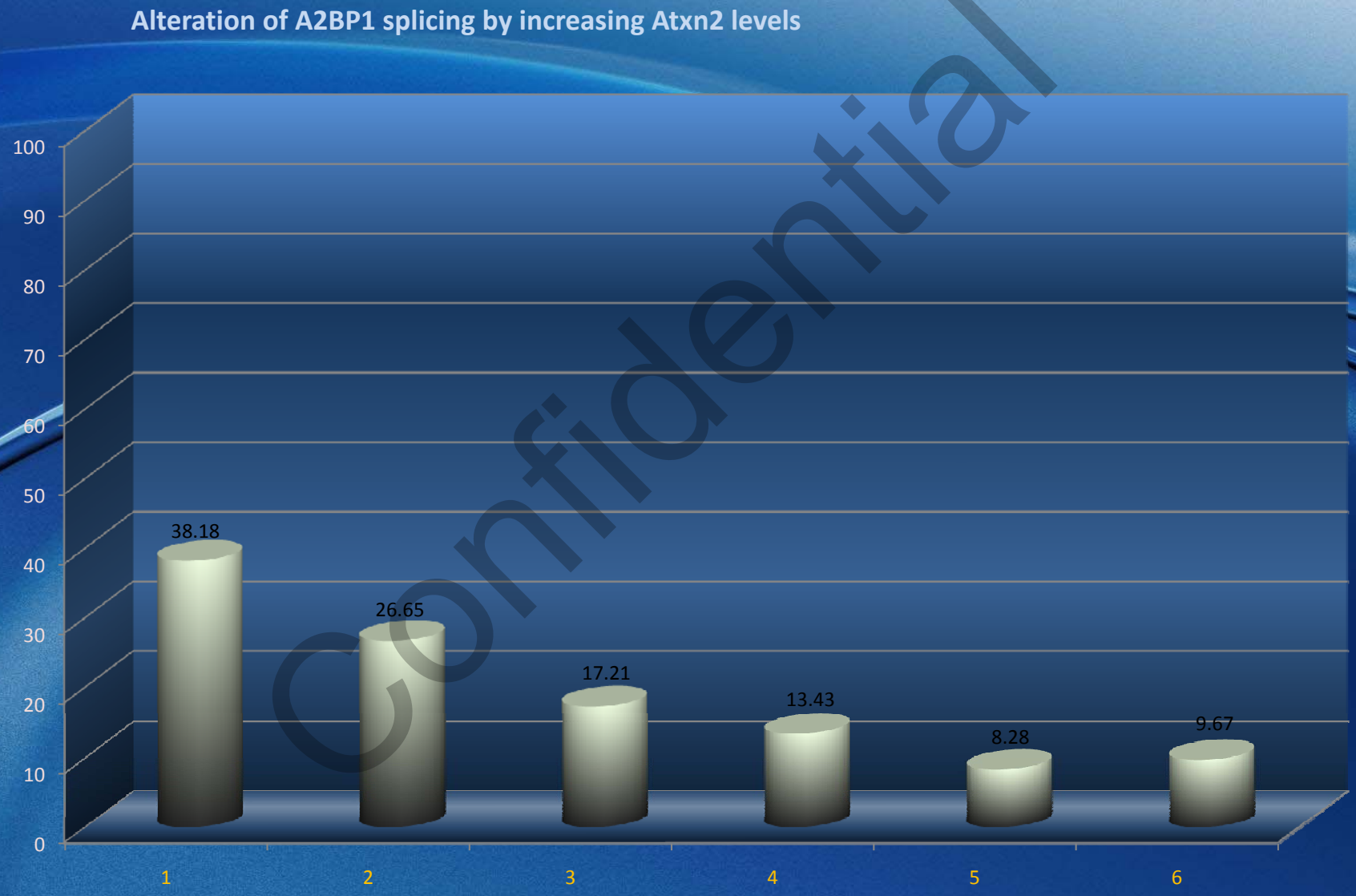
< A2BP1 mediated exon inclusion

< exon exclusion

Atxn2

A2BP1	500	500	500	500	500	500
Atxn2	-	383	765	1147	1530	2295
GFP	2295	1530	1147	765	383	-
factor	-	0.5x	1x	1.5x	2x	3x

Atxn2 Mediated Splicing Alterations of A2BP1



Atxn2 Mediated Splicing Alterations of A2BP1

Which reason could lead to higher ataxin-2 levels in the PK cells of SCA2 patients

- Expanded polyQ-forms are more stable than wt form and degrade slower

  ➔ accumulation of ataxin-2 over time

- Expanded polyQ-forms decrease proteasomal degradation

  ➔ all cellular proteins have longer half-life but control of expression might regulate toxic effects

- Expanded polyQ lead to formation of protofibrils

  ➔ Increase in misfolded proteins in the cell over time as protofibrils are hard to degrade in the UPS

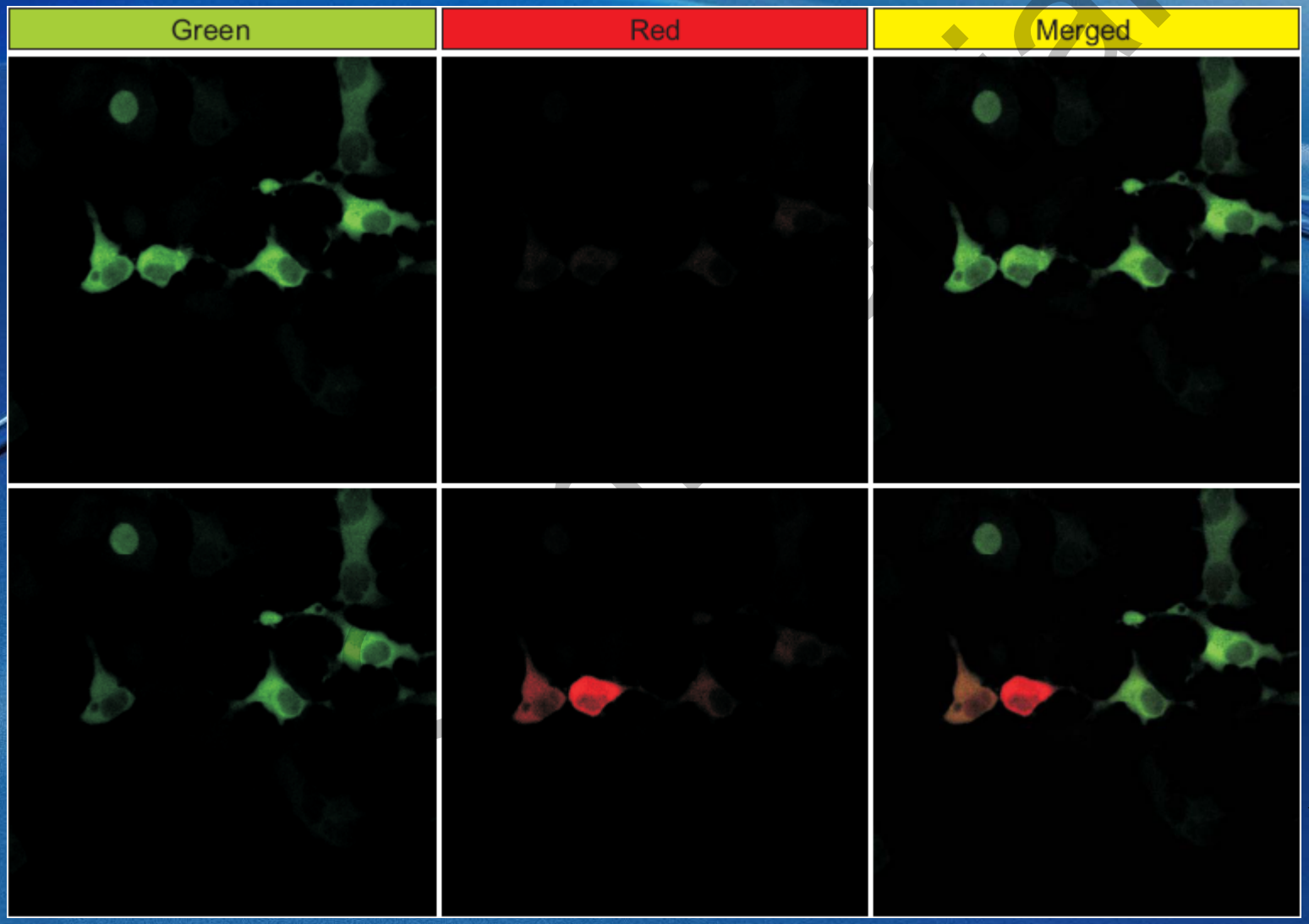
  ➔ protein accumulation >> increase in cellular stress like in PD

  ➔ *has almost nothing to do with splicing differences*

Ataxin-2 degradation: Kikume vector

Degradation of different polyQ forms

➔ Photoconversion of Kikume-green to Kikume-red



Transfection

Incubation
24-48 hours

Photoconversion

Measurement

Other ideas

Inhibition of proteasome by expanded polyQ forms:

• Proteasomal degradation assay points out if proteasomal function is decreased due to inhibition of ataxin-2 polyQ forms

• Increase of ubiquitinated proteins in the cell

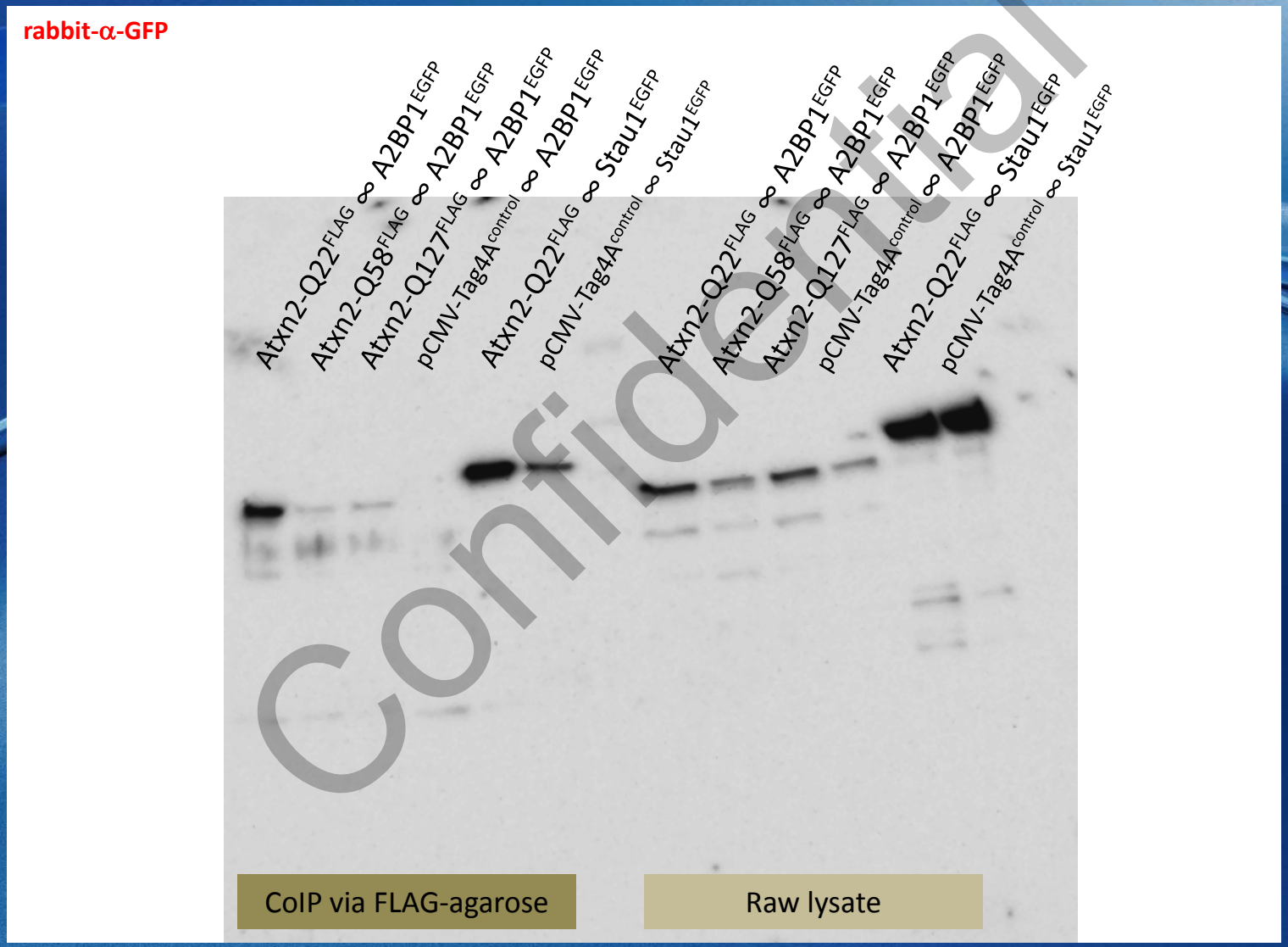
➔ Stable expressing ataxin-2 cells are needed

Formation of protofibrils by expanded polyQ's:

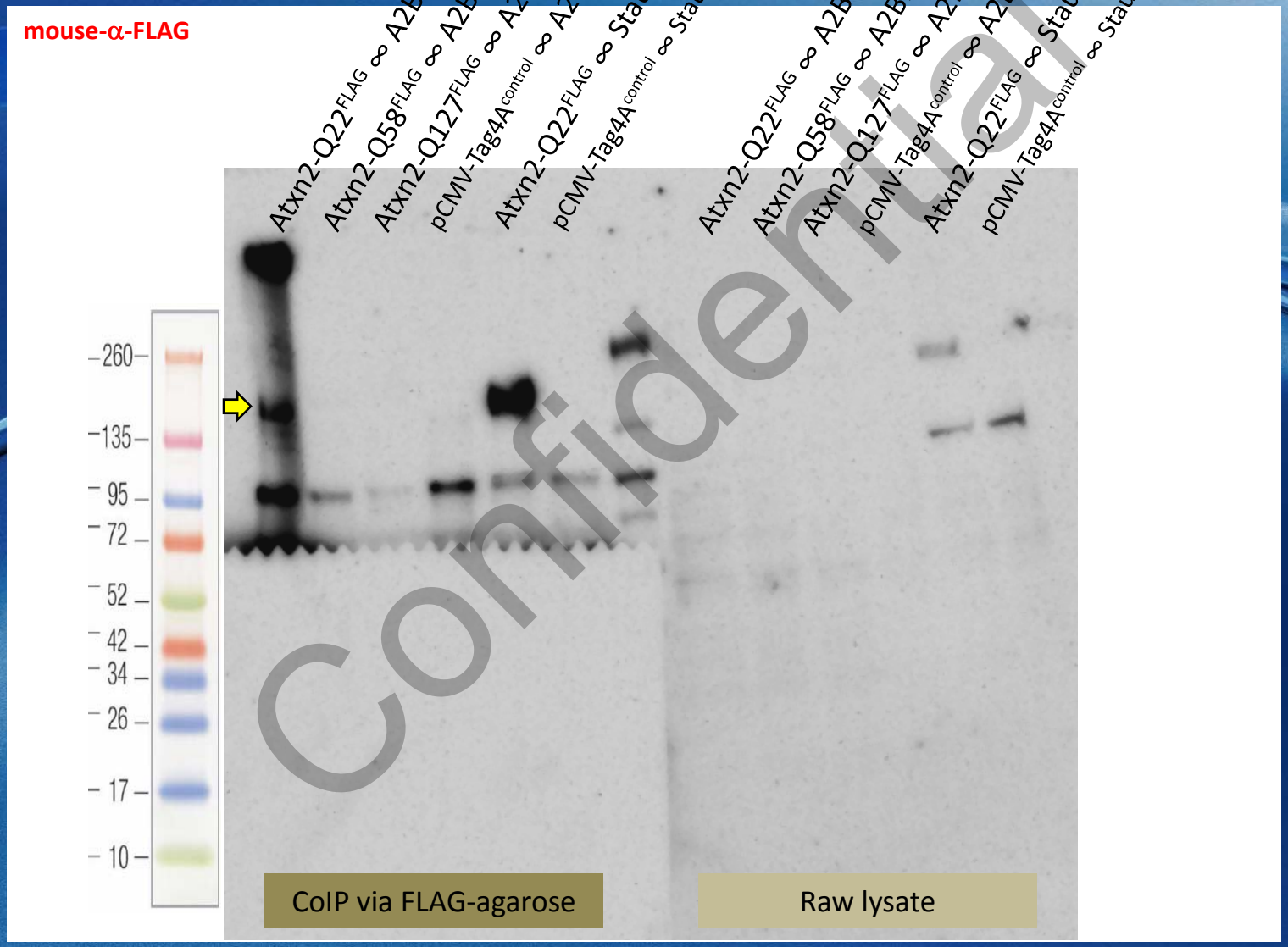
• Fluorescence correlation spectroscopy between wt and expanded polyQ repeats in stable expressing cell lines

➔ Has to be done in cooperation with an experienced lab

Atxn2 Interaction with A2BP1



Atxn2 Interaction with A2BP1

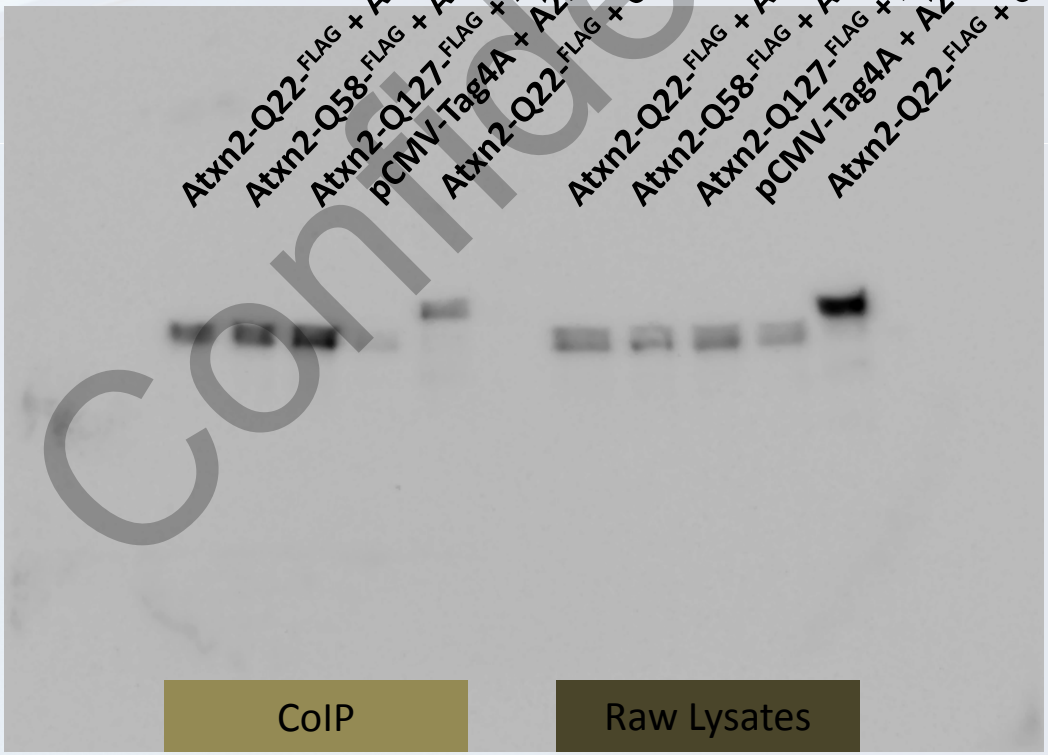


More Atxn2 Interactions

Interaction of different polyQ forms with A2BP1
Interaction of wt Atxn2 with CPEB

Pulldown of atxn2 with FLAG agarose

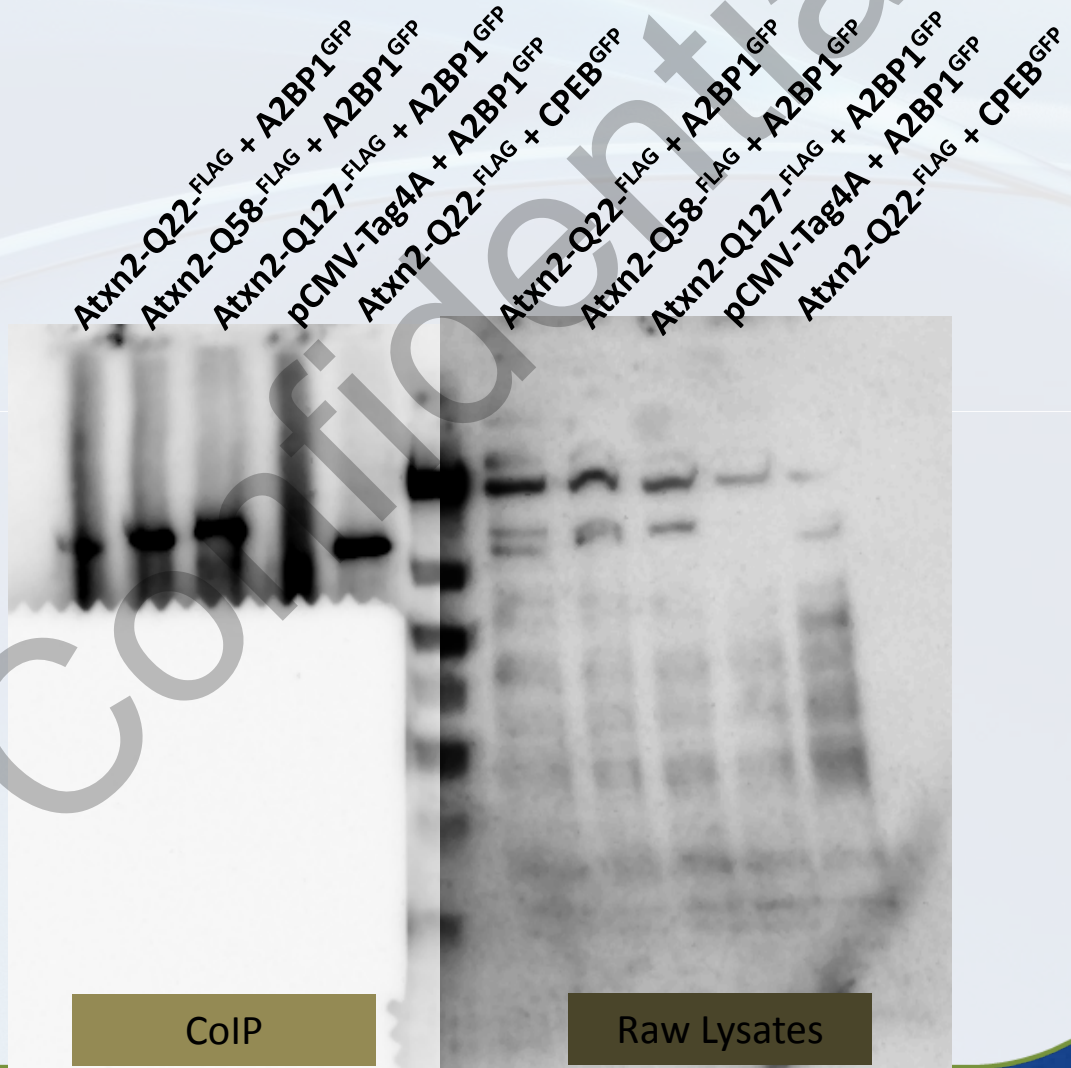
Detection with α-GFP antibody



More Atxn2 Interactions

Pulldown of atxn2 with FLAG agarose

Detection with α-FLAG antibody after stripping

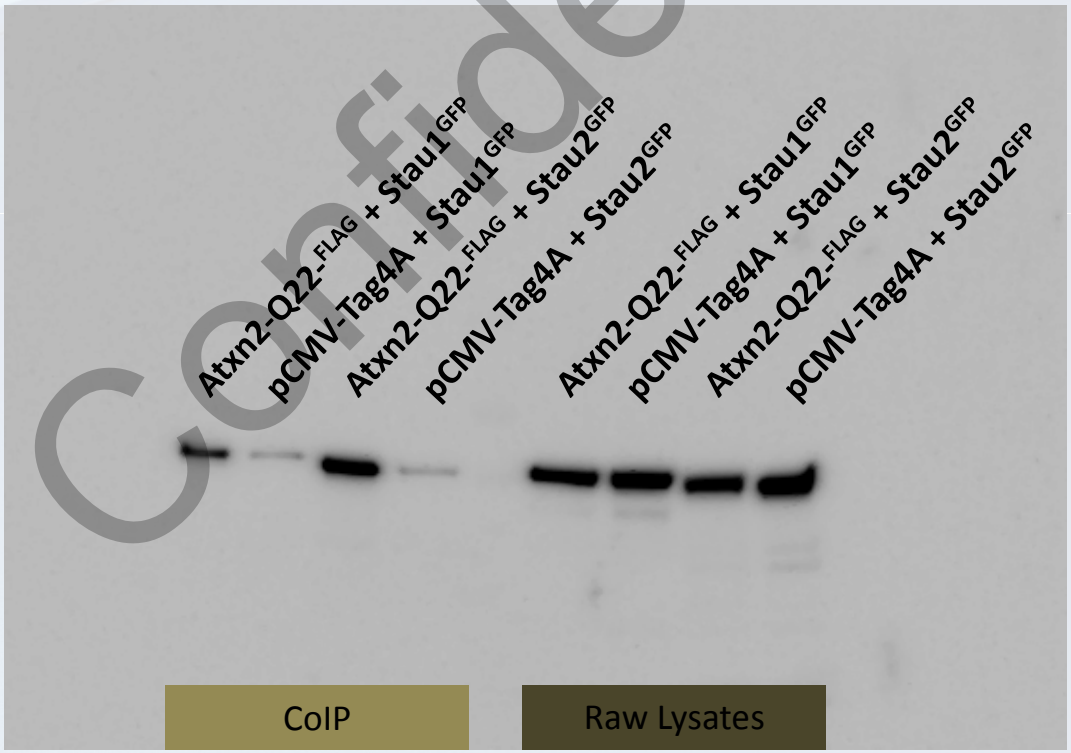


More Atxn2 Interactions

Interaction with Stau1 and Stau2

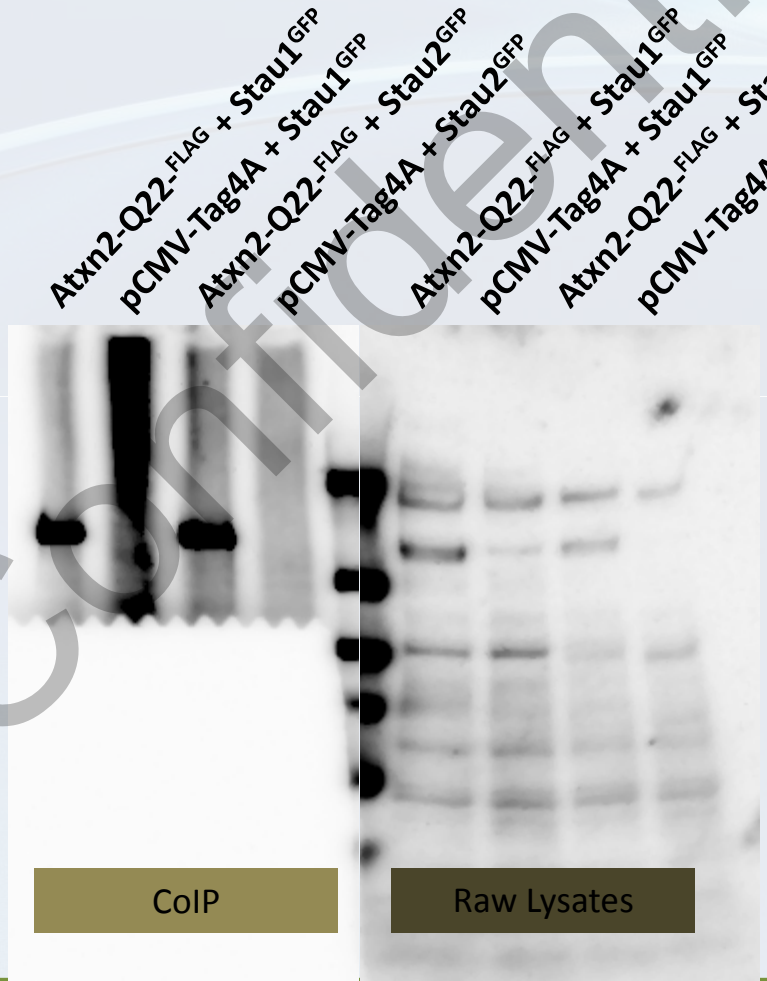
Pulldown of atxn2 with FLAG agarose

Detection with α-GFP antibody



More Atxn2 Interactions

Detection with α-FLAG antibody



Endogenous Expression Levels of A2BP1, Atxn2 and RBM9

CT values in different cell lines

	RBM9	A2BP1	Atxn2	
HEK293	23.27	0	27.37	
H1299	23.17	0	26.62	
A2058	22.78	0	26.7	
HTB10	21.16	35.3	25.67	
MG-63	23.51	0	28.11	
SH-SY5Y	23.30	31.42	27.63	
IMR-90	22.2	0	27.53	
MB-231	21.83	0	25.25	
HEK-**A2BP1**	23.85	**18.79**	27.84	
HEK-**Atxn2**	23.64	0	**20.73**	

- ➔ RBM 9 and Ataxin-2 are expressed in each cell line > housekeeping genes?
- ➔ A2BP1 is only expressed in neuronal cell lines at a very low level
- ➔ A2BP1 has a much higher exogenous expression level than Atxn2

Atxn2 is less potent for transfection than A2BP1

Endogenous Expression of A2BP1 and Atxn2 in the Cerebellum

Allen Brain Atlas

Open Questions – To Do List

- Is RBM9 interfering with A2BP1 (Co-localization with increasing levels of RBM9)
- Does atxn2 interact with RBM9, does it recruit RBM9
- Are there any cerebellar splicing differences in Q127 transgenic mice compared to wt
-