Functional Characterization of Ataxin-2

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Spinocerebellar ataxias

- Clinically and genetically heterogenious
- 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
- Prevalence: 3-7 in 100.000
- Identified genetic loci: 29 named SCA1-SCA29

Spinocerebellar ataxias

Table 2 Summary of the genes and molecular defects accounting for the SCAs

SCA subtype	Genomic location	Gene/locus	Protein	Mutation	References
SCA1	6p22.3	ATXN1	Ataxin 1	CAG repeat	Orr *et al.* (1993)
SCA2	12q24.13	ATXN2	Ataxin 2	CAG repeat	Imbert *et al.* (1996)
					Pulst *et al.* (1996)
					Sanpei *et al.* (1996)
SCA3	14q32.12	ATXN3	Ataxin 3	CAG repeat	Kawaguchi *et al.* (1994)
SCA4	16q24-qter	SCA4	U	U	Flanigan *et al.* (1996)
SCA5	11q13.2	SPTBN2	Beta-III spectrin	D, MM	Ikeda *et al.* (2006)
SCA6	19p13.13	CACNAIA	CACNAIA	CAG repeat	Zhuchenko *et al.* (1997)
SCA7	3p14.1	ATXN7	Ataxin 7	CAG repeat	David *et al.* (1997)
SCA8	13q21	KLHL1AS	Kelch-like 1	CTG repeat	Koob *et al.* (1999)
SCA9	Reserved	U	U	U	–
SCA10	22q13.31	ATXN10	Ataxin 10	ATTCT repeat	Matsuura *et al.* (2000)
SCA11	15q14-q21.3	SCA11	U	U	Worth *et al.* (1999)
SCA12	5q32	PPP2R2B	PPP2R2B	CAG repeat	Holmes *et al.* (1999)
SCA13	19q13.33	KCNC3	KCNC3	MM	Waters et al. (2006)
SCA14	19q13.42	PRKCG	PRKCG	MM	Chen et al. (2003*a*)
SCA15	3p24.2-pter	U	U	U	Gardner *et al.* (2005)
SCA16	8q23-q24.1	U	U	U	Miyoshi *et al.* (2001)
SCA17	6q27	TBP	TBP	CAG repeat	Nakamura *et al.* (2001)
SCA18	7q31-q32	U	U	U	Devos *et al.* (2001)
SCA19*	1p21-q21	U	U	U	Verbeek *et al.* (2002)
SCA20	11	U	U	U	Knight *et al.* (2004)
SCA21	7p21.3-p15.1	U	U	U	Vuillaume *et al.* (2002)
SCA22*	1p21-q23	U	U	U	Chung *et al.* (2003)
SCA23	20p13-p12.2	U	U	U	Verbeek *et al.* (2004)
SCA24	1p36	U	U	U	Swartz *et al.* (2002)
SCA25	2p21-p15	U	U	U	Stevanin *et al.* (2005)
SCA26	19p13.3	U	U	U	Yu *et al.* (2005)
SCA27	13q33.1	FGF14	FGF14	MM	van Swieten *et al.*(2003)
SCA28	18p11.22-q11.2	U	U	U	Cagnoli *et al.* (2006)
DRPLA	12p13.31	ATN1	Atrophin 1	CAG repeat	Koide *et al.* (1994)
					Nagafuchi *et al.* (1994)
Undefined**	16q22.1	PLEKHG4	Puratrophin 1	5′ SNS	Ishikawa *et al.* (2005)

*SCAs 19 and 22 are likely allelic forms of the same gene.
**The gene encoding puratrophin 1 lies on the same chromosomal region where the *SCA4* gene localizes. Genes in genomic location are noted according to Ensembl. D, deletions; MM, missense mutations; SNS, single-nucleotide substitutions; U, unknown.

Duenas et al .,2006

* SCA29 3p26 Dudding et al. (2004) Source: OMIM

SCA2 overview: Disease

- SCA2 causes about 15% of the spinocerebellar ataxias
- SCA2 is caused by an expansion of a polyQ repeat in the ataxin-2 gene
- The normal polyQ repeat length varies about 14-30 CAGs whereas the disease related SCA2 protein contains more than 31 CAG repeats
- The age of onset as well as the severity of SCA2 is inversely correlated to the polyQ repeat length
- The function of the ataxin-2 protein is mostly unknown

SCA2 overview: Protein domains

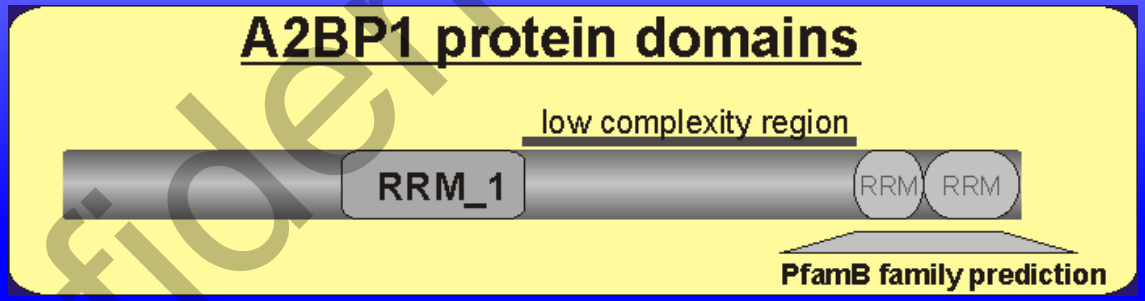
SBM	-	SH3-binding motif (protein-protein interactions)
Lsm	-	like-SM domain (RNA binding)
Sm	-	Splicing motif
Lsm-AD	-	SM associated domain with trans-Golgi-signal and ER export signal
PAM2	-	*PolyA binding protein* interacting motif

➔ RNA interaction

➔ probably Golgi associated localization or function (cell type?)

Interaction with A2BP1

- First identified interaction partner: A2BP1
- Nuclear as well as cytoplasmic localization
- mRNA binding motifs
- mRNA splicing



RRM: Eucaryotic RNA Recognition Motif

- 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

Options for functional studies

- Interaction studies with proteins of the mRNA pathway

  - mRNA transport
  - Translation
  - mRNA degradation

- Functional association studies with A2BP1

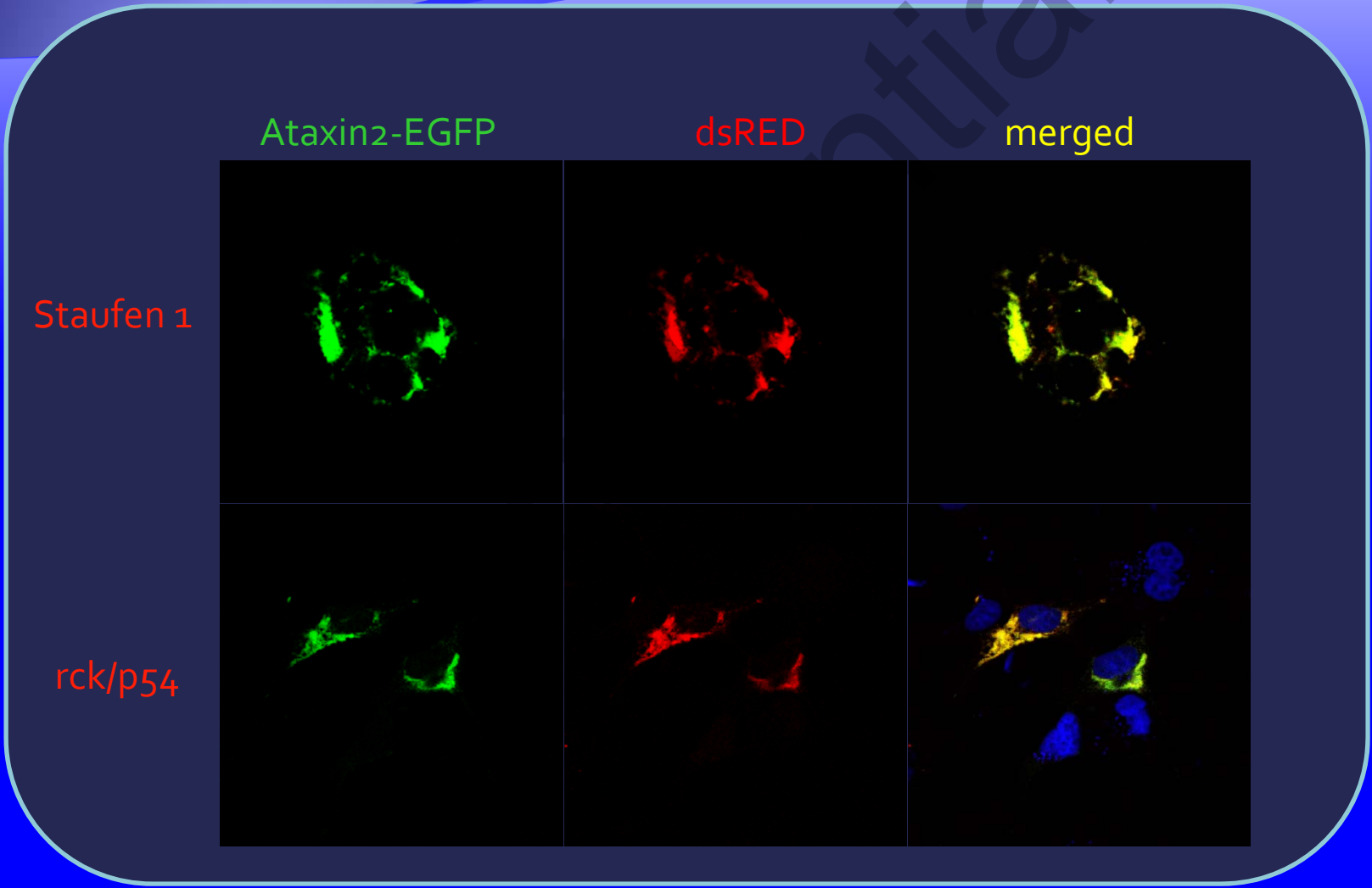
  - Interaction with wt and mutated atxn2 forms ➔ differences?
  - Splicing alterations due to atxn2 interaction

- Ataxin-2 degradation

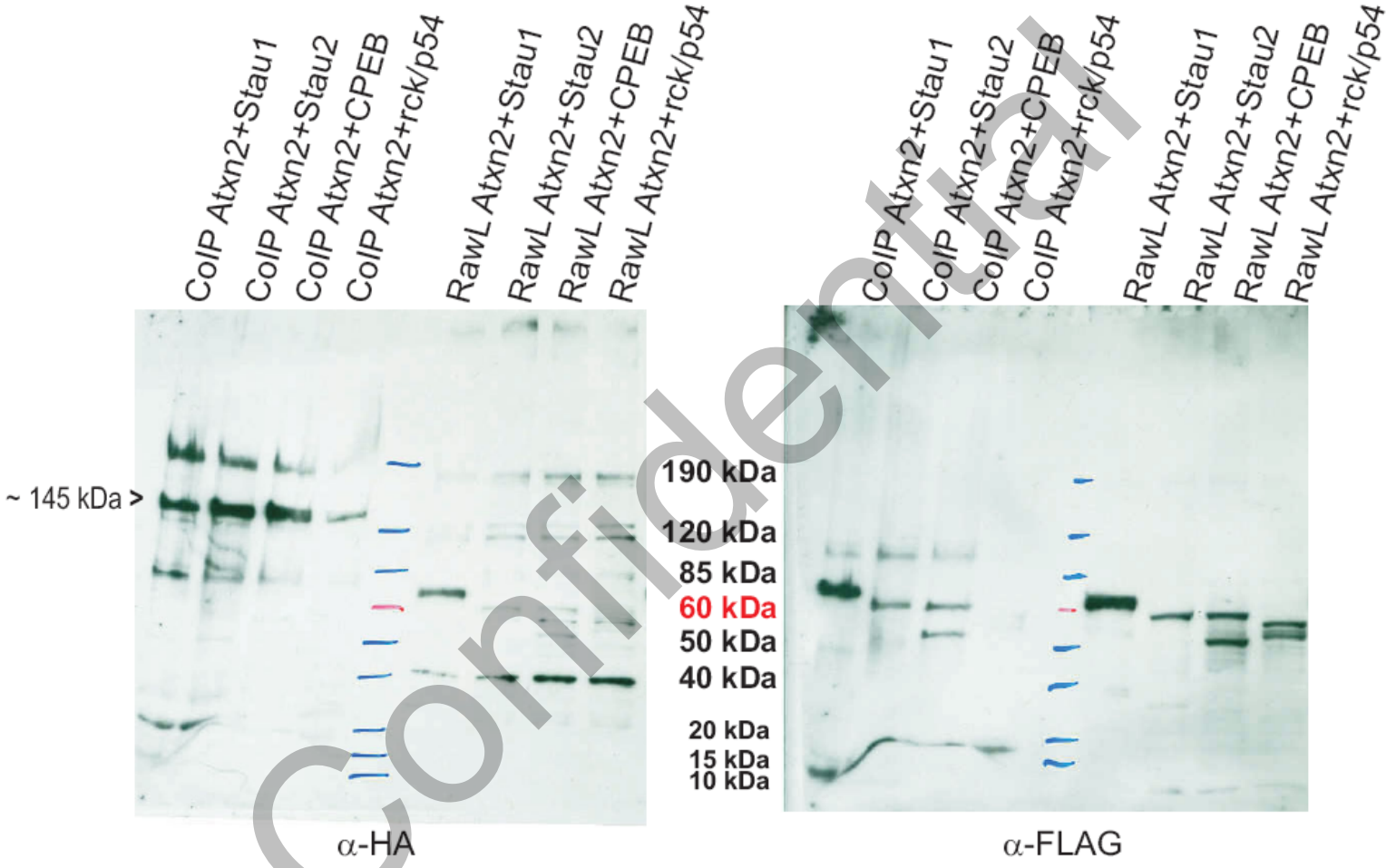
  - Are there differences between short and long polyQ forms

  ➔ Is the proteasomal degradation decreased for higher polyQ repeat

Ataxin-2 interacting proteins



SCA2 interacting proteins



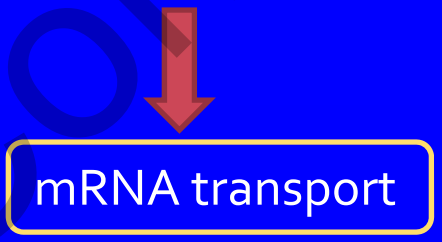
Estimated sizes:

Stau1-FLAG => ~65 kDa
Stau2-FLAG => ~61 kDa
CPEB-FLAG => ~62 kDa
rck/p54-FLAG => ~54 kDa

IP with HA-agarose

SCA2 interacting proteins

- Staufen 1	➔ mRNA transport	cytoplasm / (nucleus)
- Staufen 2	➔ mRNA transport	cytoplasm / nucleus
- rck/p54	➔ RNA helicase	cytoplasm / PBs
- CPEB	➔ translational regulator	cytoplasm / SGs



SCA2 interacting proteins

- Conclusions:

Ataxin-2 interacts with proteins of the mRNA pathway

Interaction was shown mainly with mRNA transport proteins

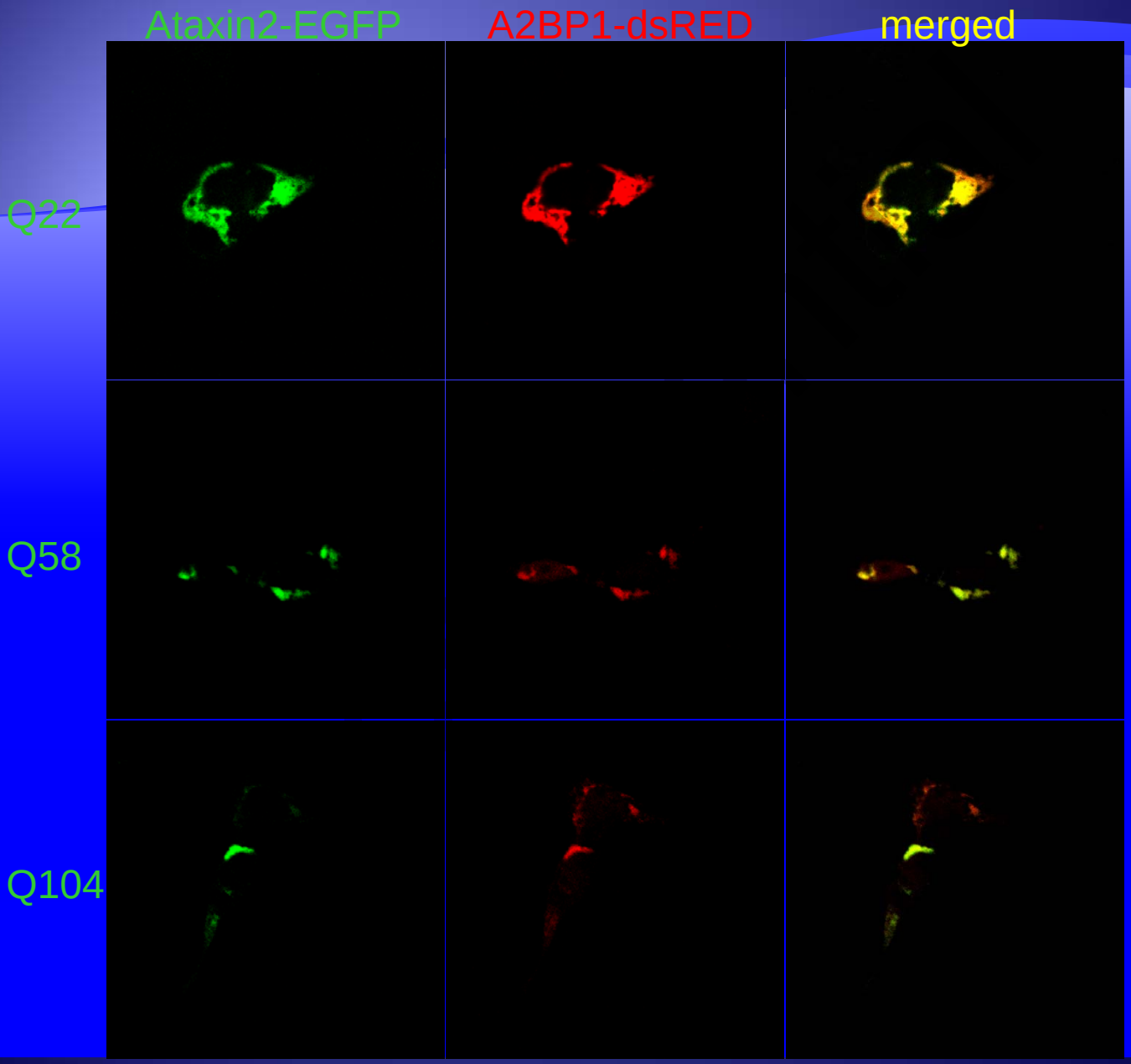
Published interactions include the translational regulator PABP

- Future :

Interaction assays with other proteins of mRNA pathways and eIF4A1, eIF4A2 and eIF4E as key proteins of translational initiation

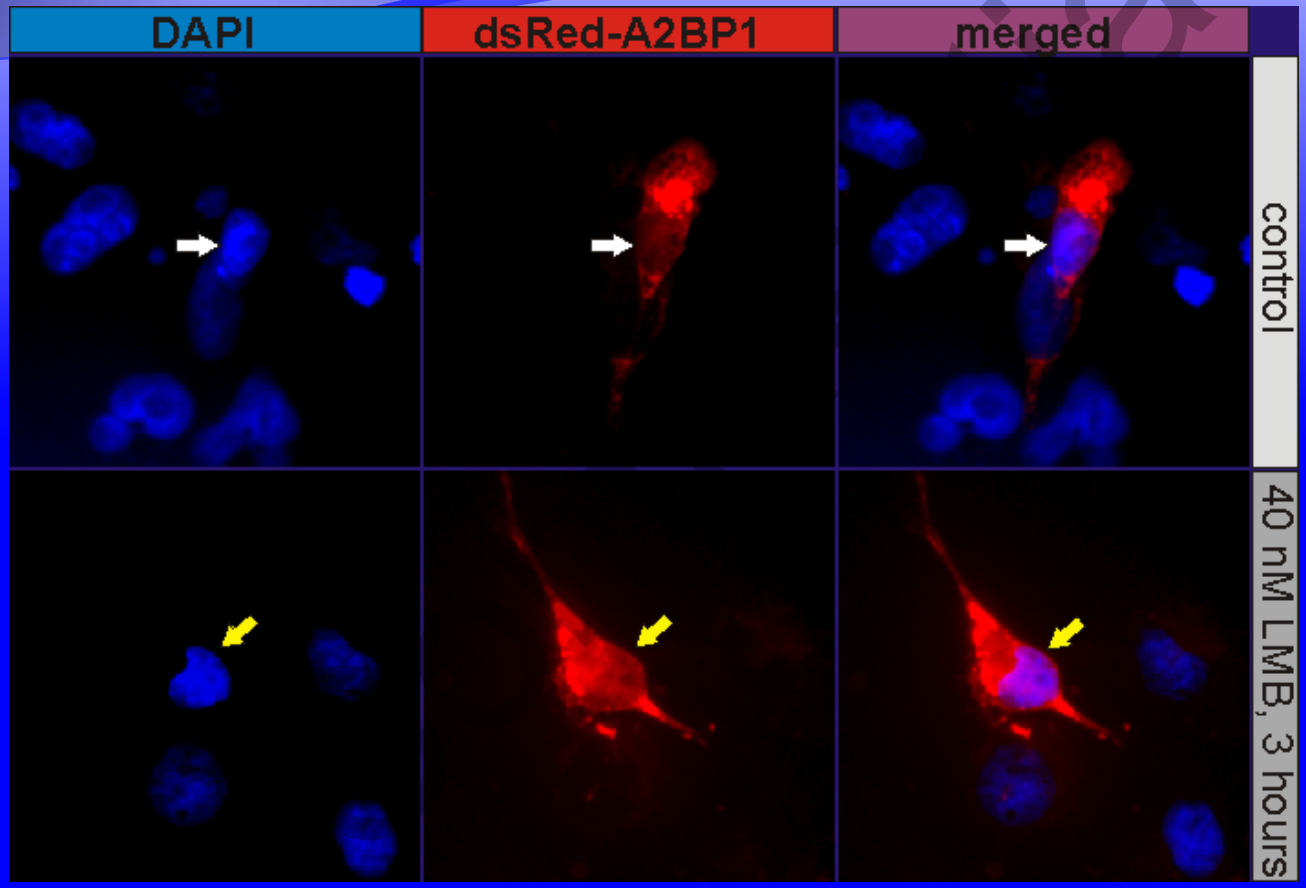
Proof of interaction with observed interaction partners
wild-type ⇔ expanded polyQs

A2BP1 interaction with ataxin-2



A2BP1 interaction

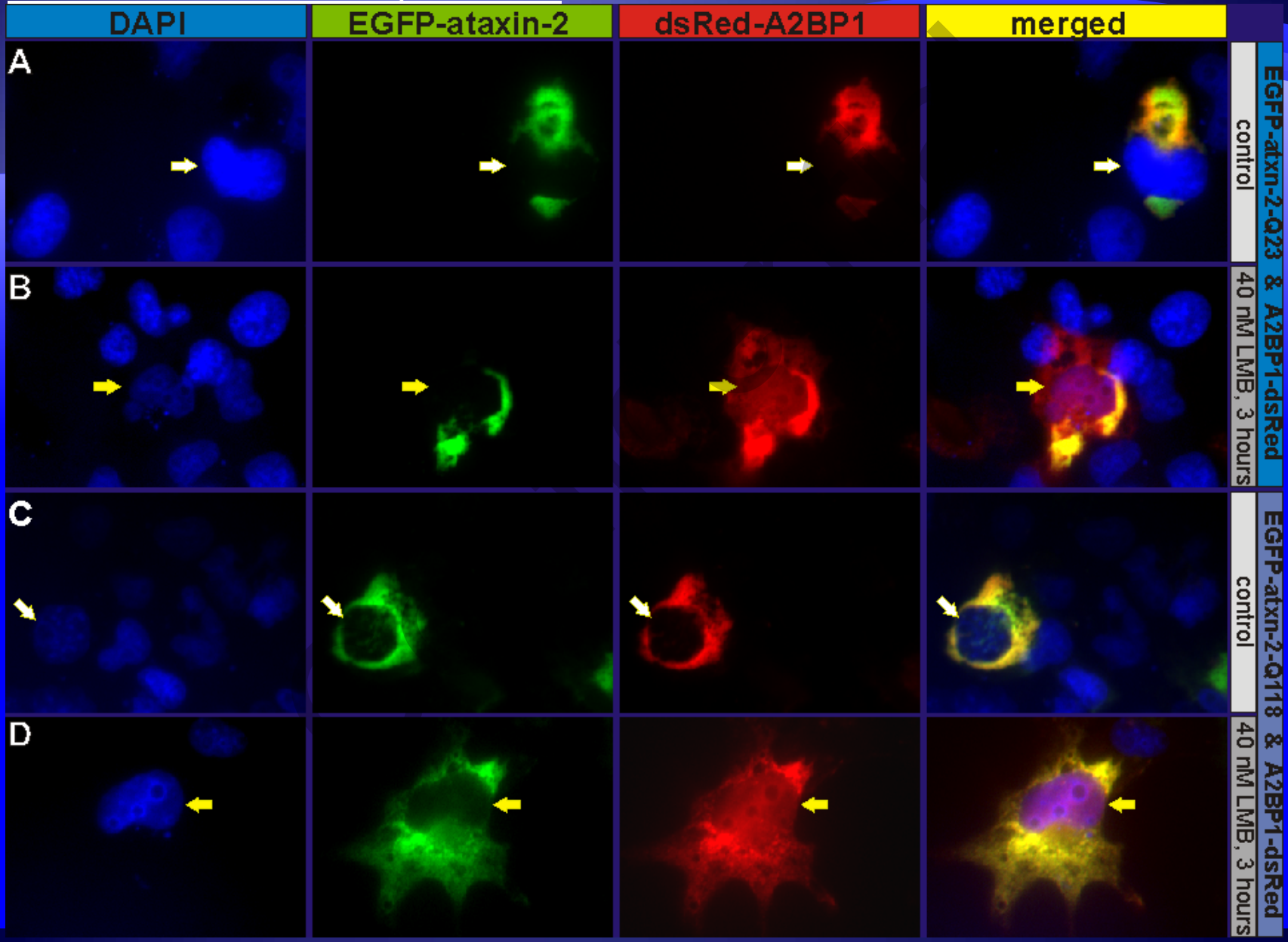
Nucleo-cytoplasmic shuttling of A2BP1



Leptomycin B (LMB): Specific nuclear export inhibitor which inhibits exportin-1

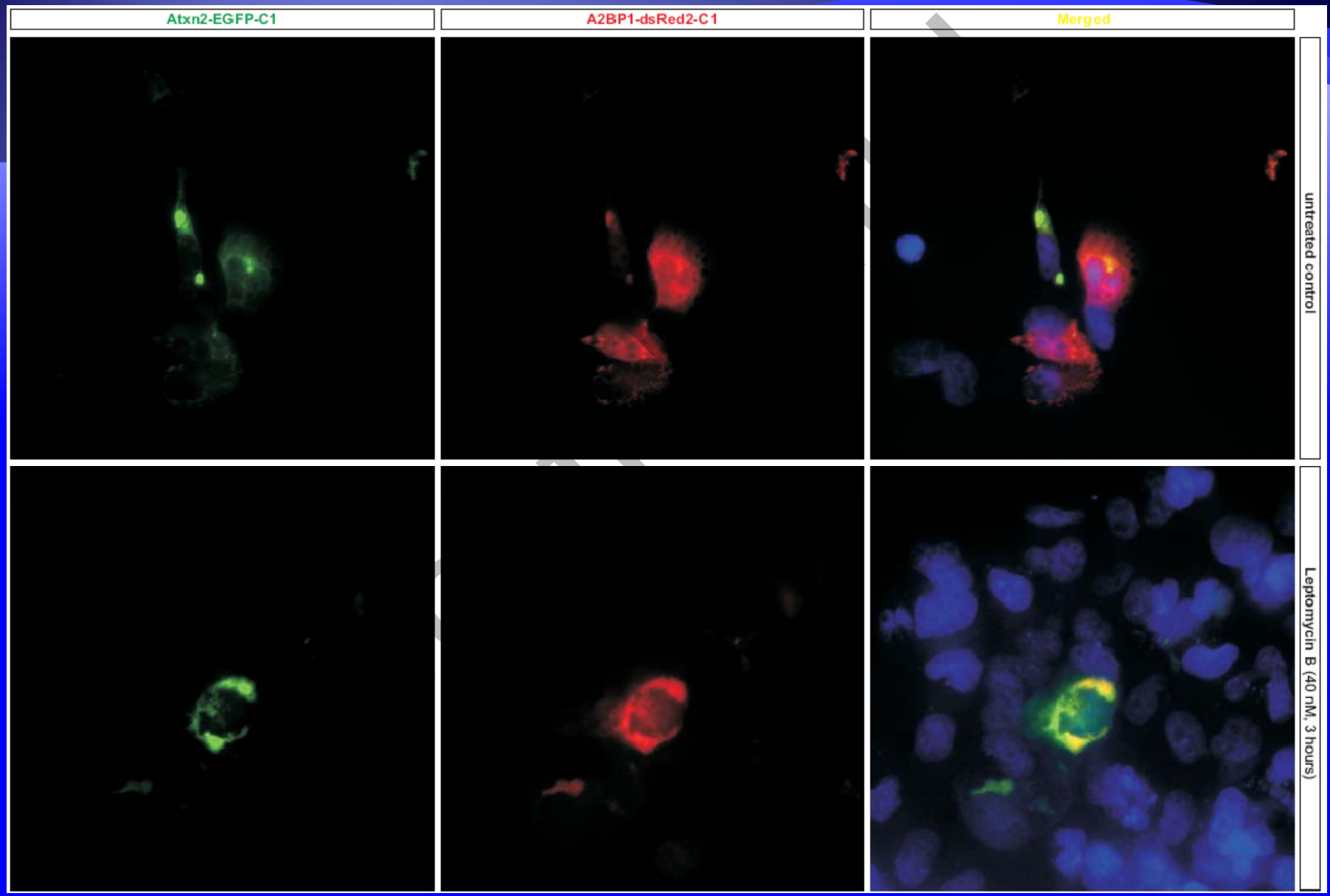
A2BP1 interaction

Recruitment of A2BP1 by ataxin-2:



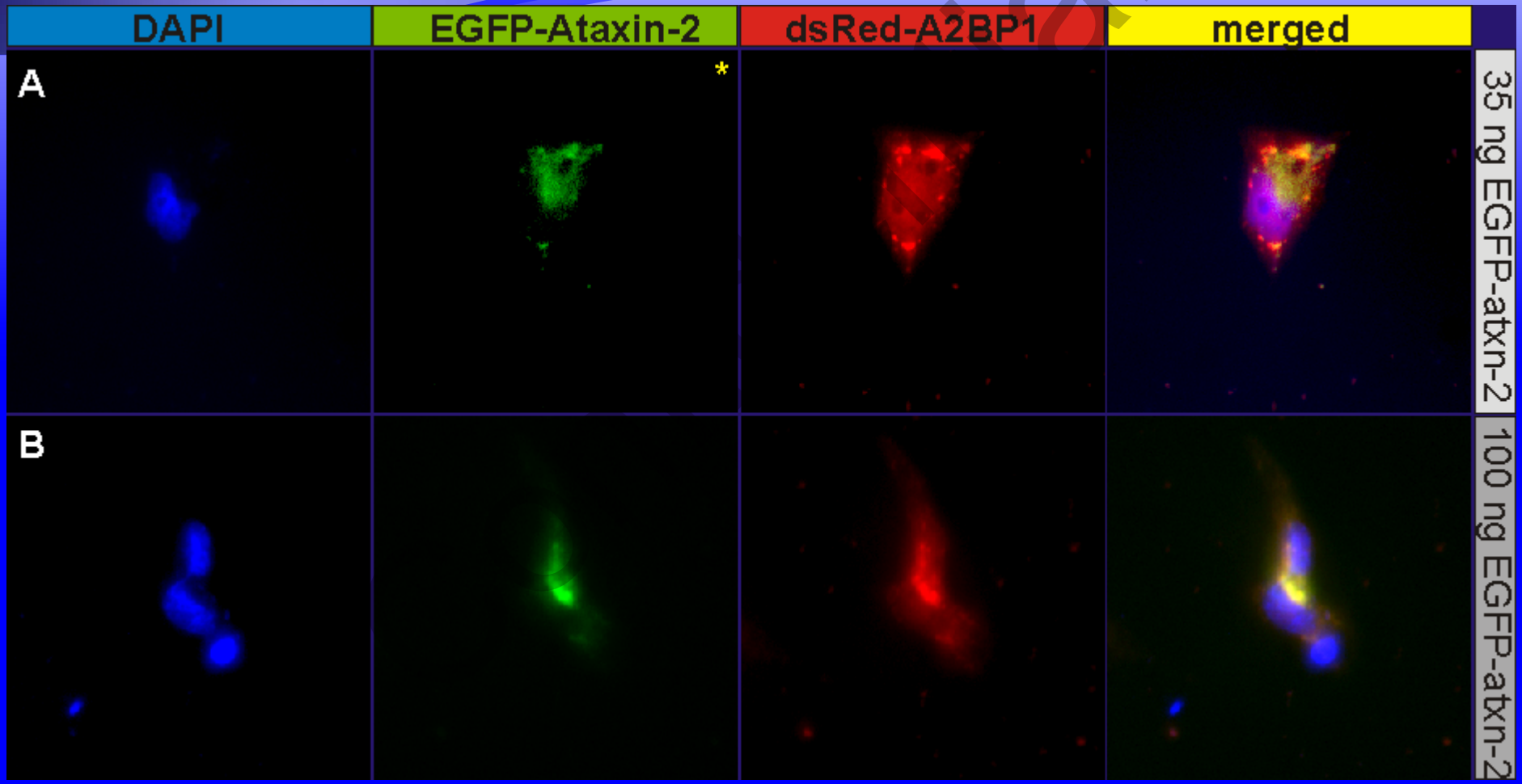
A2BP1 interaction

Recruitment of Ataxin-2-Q58:



A2BP1 interaction

Recruitment of A2BP1 by ataxin-2 is dosage depended:



* Extended exposure for EGFP staining

A2BP1 mediated splicing

1. Alter increased levels of ataxin-2 the splicing function of A2BP1?
2. Is there a functional inhibition of A2BP1 splicing for expanded polyQ repeats?

** Source: Genomics Institute of the Novartis Research Foundation/Genecards

A2BP1 mediated splicing

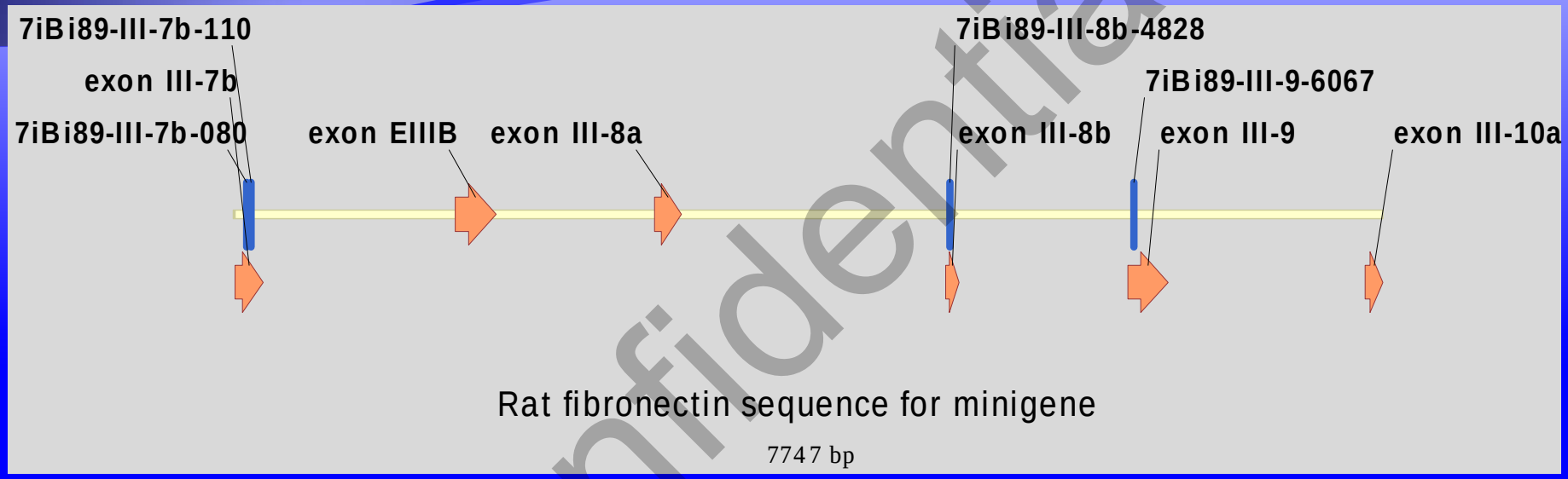
- Nucleotide recognition sequence of A2BP1/Fox-1: UGCAUGU
- A2BP1 mediates exon inclusions and exclusions
- Splicing recognition is similar for RBM9/Fox-2
- Expression pattern for RBM9/Fox-2 is ubiquitous
  A2BP1/Fox-2 has a more specific expression pattern
- A2BP1/Fox-1 is expressed in Purkinje cells and has a more nuclear pattern over fox-2 but fox-2 has the higher expression in PC's*

  A2BP1/Fox-1 is expressed in granule cells but not Fox-2**

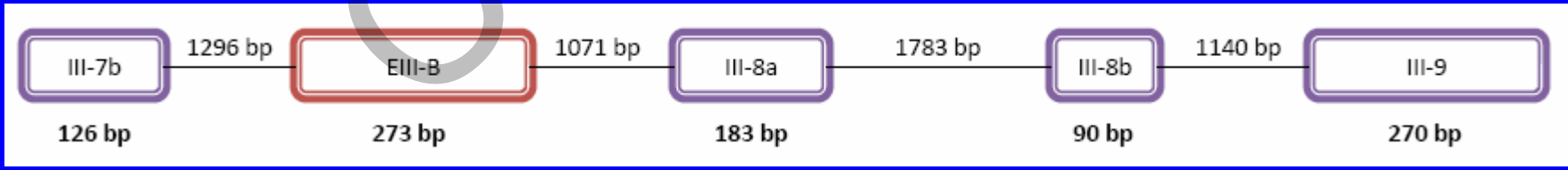
* Source: Genomics Institute of the Novartis Research Foundation/Genecards
** Source: Douglas Black, UCLA

Rat fibronectin 7iBi89 minigene

A2BP1 mediated exon inclusion

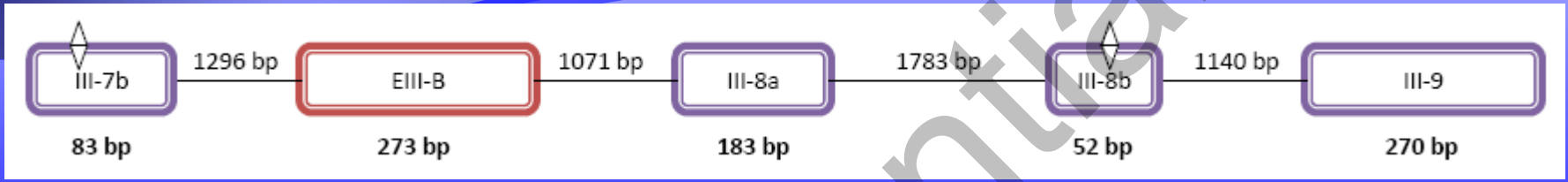


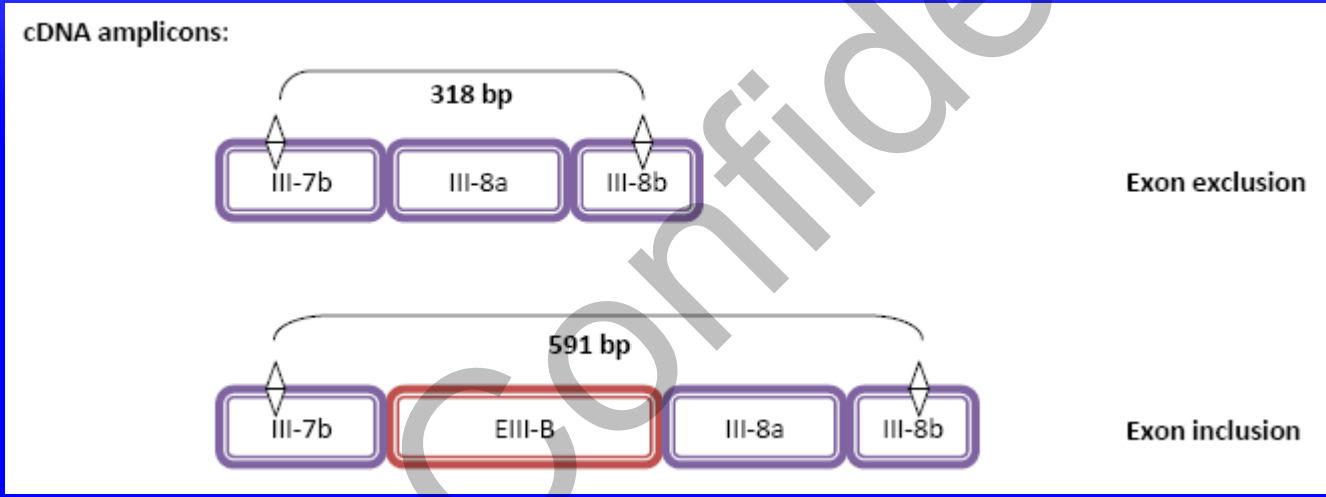
> Nested PCR instead of a hot PCR



Rat fibronectin 7iBi89 minigene

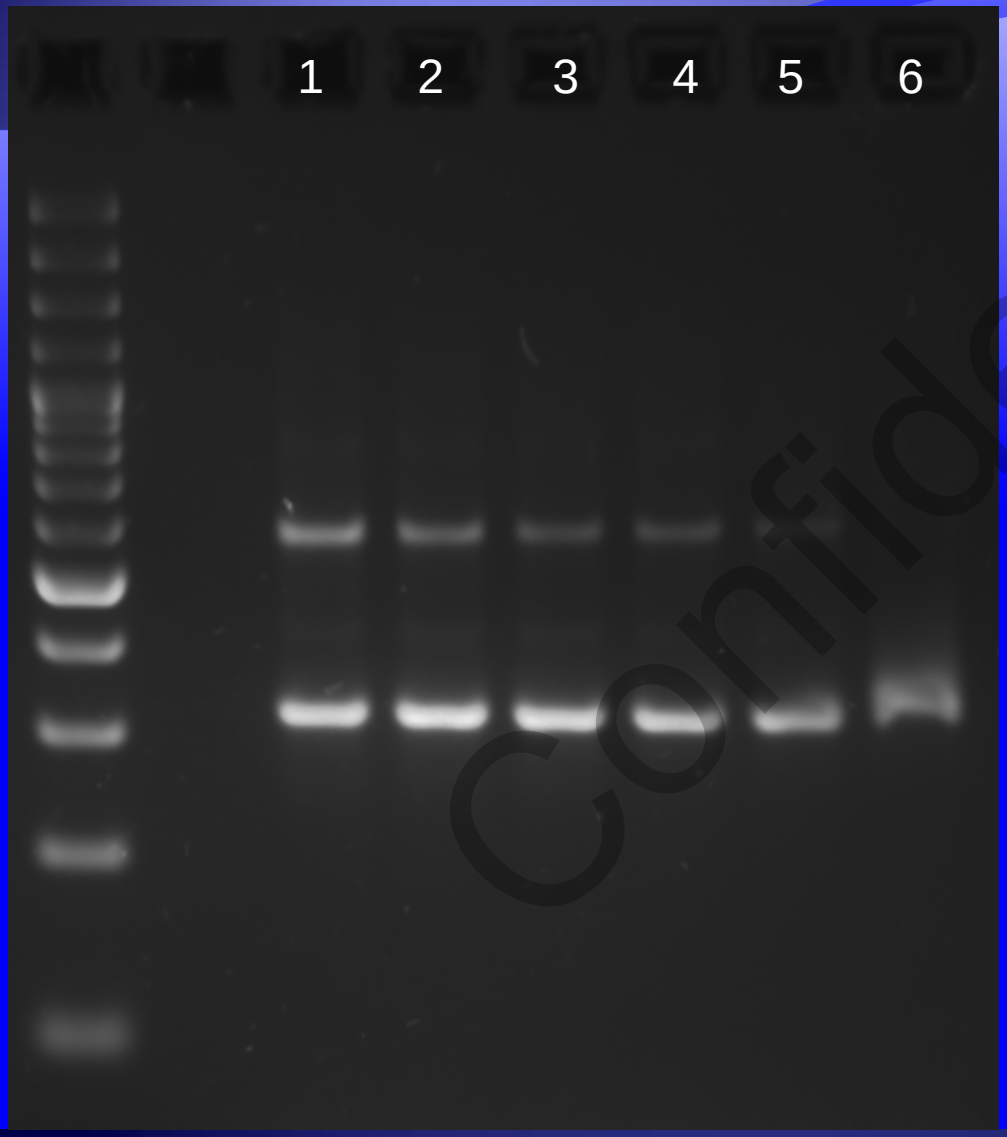
Amplicons of 2nd PCR





> A2BP1 mediated exon inclusion

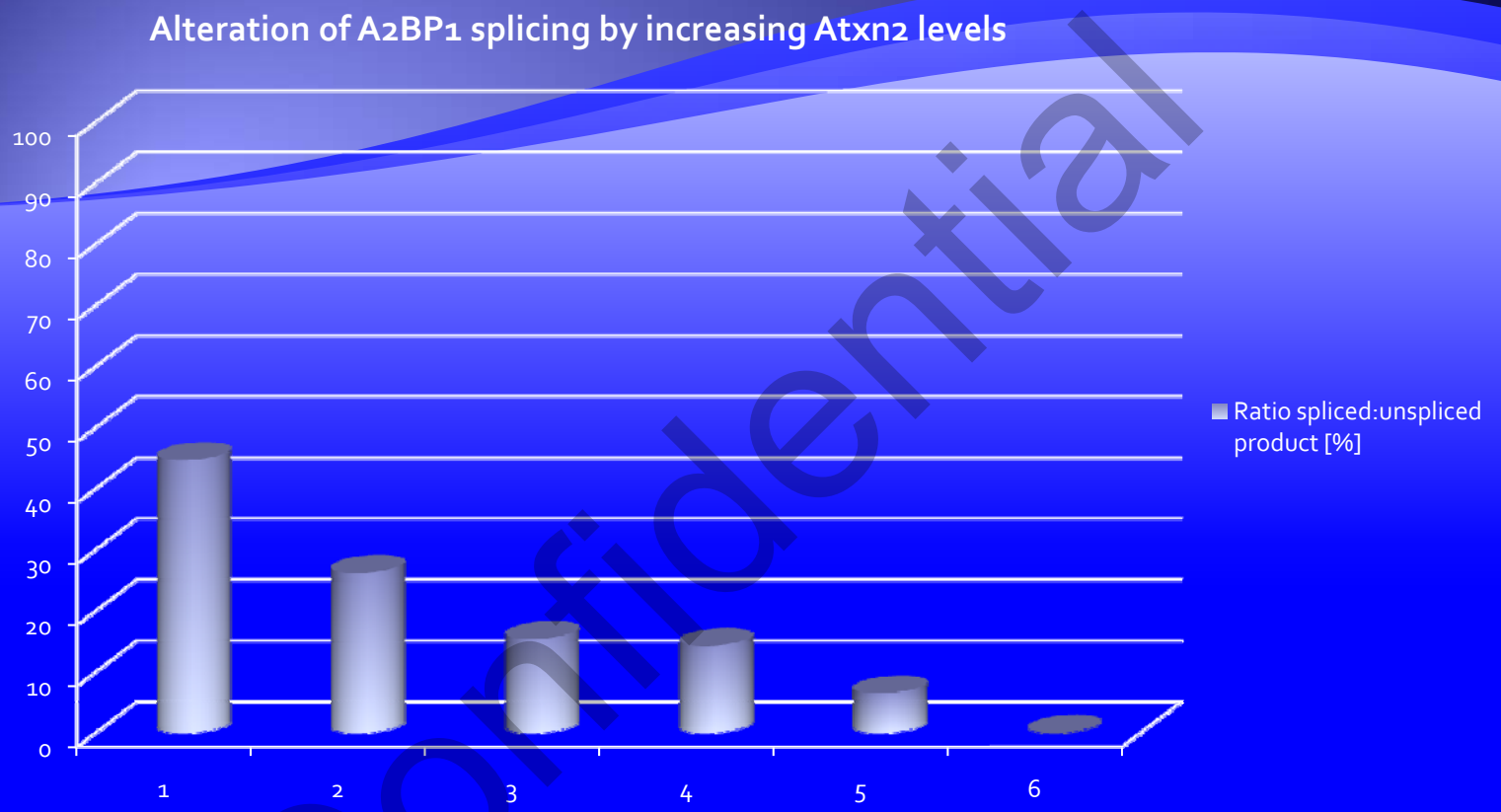
A2BP1 interaction – Ataxin-2 degradation



Cotransfection of A2BP1, ataxin-2 and the 7iBi minigene
changing the amount of ataxin-2

Lane	1	2	3	4	5	6
A2BP1	250	250	250	250	250	0
Atxn2	0	191	383	574	765	383
pCMV-HA	160	0	0	0	0	160
Ratio	0:1	0.5:1	1:1	1.5:1	2:1	1:0

A2BP1 interaction – Ataxin-2 degradation



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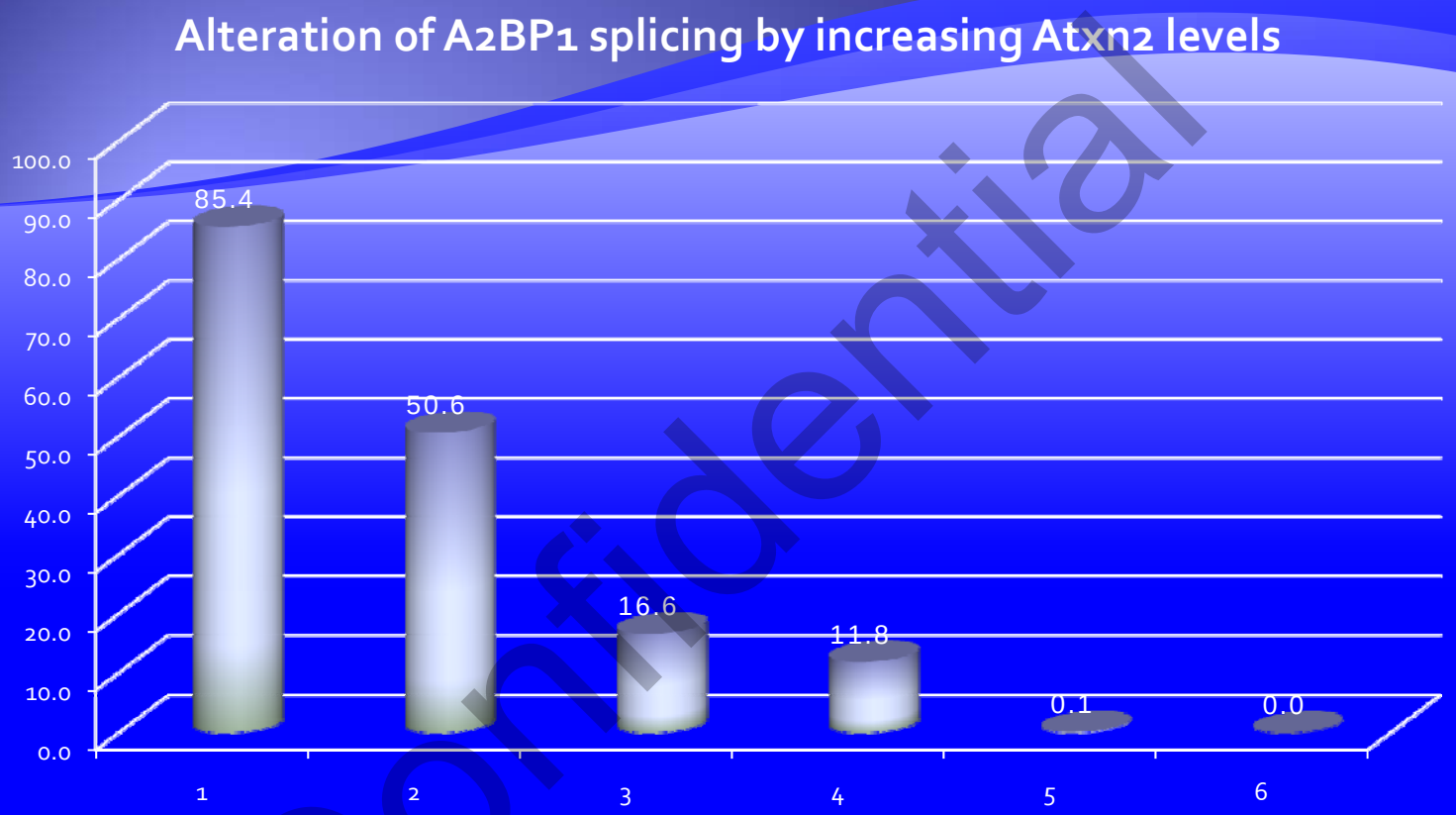

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A2BP1 interaction – Ataxin-2 degradation

If atxn2 forms with expanded polyQ repeats are more stable to the degradation pathways of the cell than wt protein, they accumulate in the cell.

➔ Elevated levels of atxn2 alter the function of A2BP1

➔ Accumulation of atxn2 and atxn2 intermediate degradation products inhibit the proteasome > Proteasomal stress

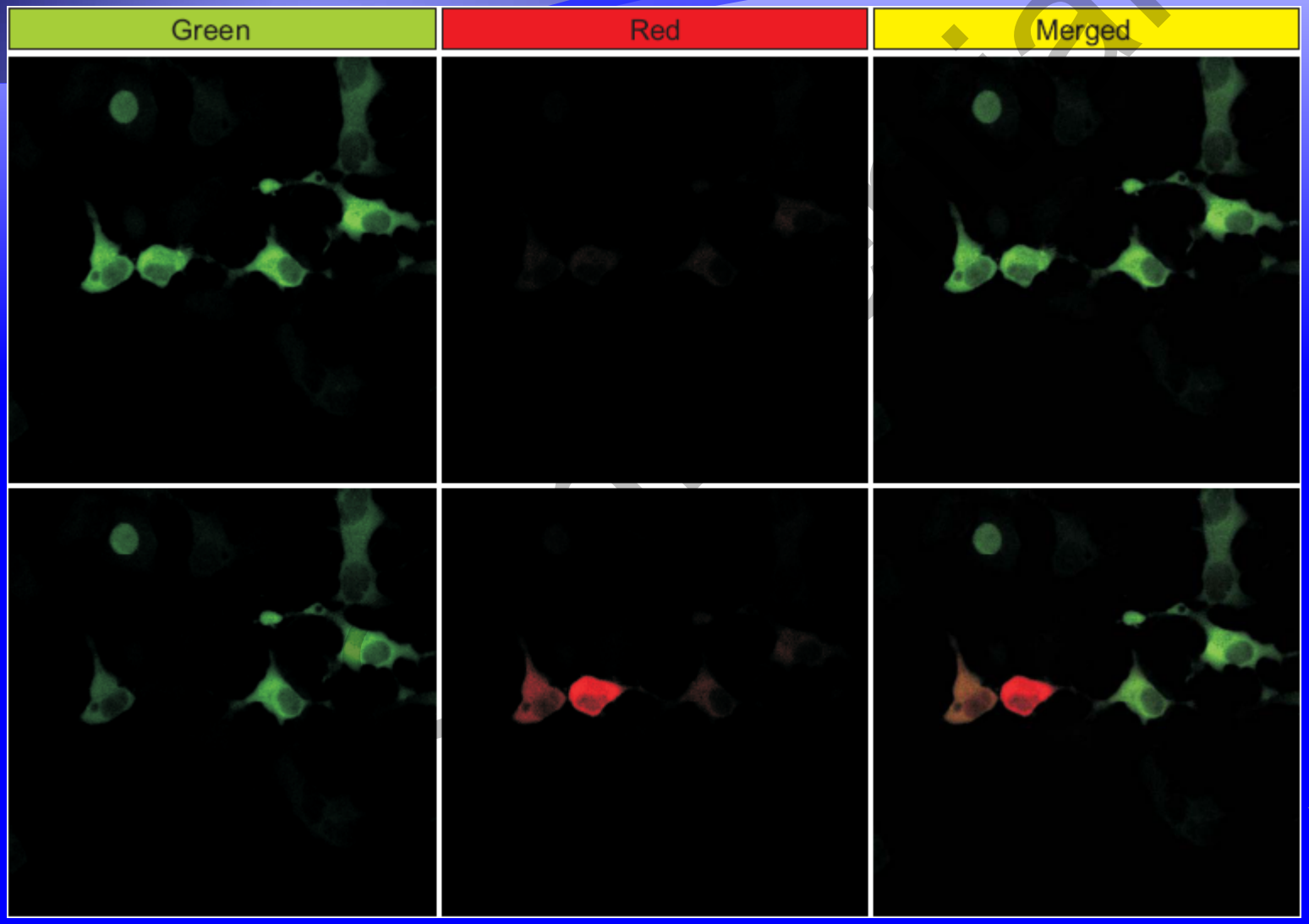
Determination of the atxn2 stability/degradation time

- Pulse-chase experiment with radioactive labelled Methionine

- Pulse-chase color changing fluorescent protein Kikume

Ataxin-2 degradation: Kikume vector

Photoconversion of Kikume-green to Kikume-red



Ataxin-2 degradation: Kikume vector

- Transfect ataxin-2-Kikume-Qxx
- Incubate for 24-48 hours — Ataxin-2
- Photoconvert ataxin-2 — Ataxin-2
- Incubate for 2, 4, 6, 8, 10, 12, 14, xx hours — Ataxin-2
  New synthesized ataxin-2 is green — Ataxin-2

  > Measure ratio between red and green ataxin-2 fluorescence

- Are there difference in the fluorescence ratio‘s for different polyQ‘s

Conclusions

- Ataxin-2 interacts with proteins of the pre-mRNA/mRNA pathway
- Ataxin-2 recruits A2BP1 and Staufen
- Overexpression of ataxin-2 alters the function of A2BP1 as a splicing regulator

Future prospects

- Interaction assays between ataxin-2 and it‘s interacting proteins to determine if different polyQ repeats alter the interaction efficiency

  ➔ Bioprocessor assays

  ➔ Protein-protein interaction core of the U

  ➔ Immobilization of recombinant atxn2
  Interacting protein is driven over the immobilized atxn2, interaction efficiency is measured by the bioprocessor

Future prospects

- Locating A2BP1 splicing targets which are expressed in Purkinje cells

➔ Splicing/gene expression chips for overexpressed atxn2...

Target for chip and PCR splicing assay

Mouse brain wt <> Q58/Q127
or
cell line which expresses a variety of the target genes

Future prospects

- Interaction studies between ataxin-2 and RBM9/Fox-2
  as A2BP1 and RBM9 have the same recognition sequence and could rescue a misfunction of the other protein, expression levels of RBM9 in the cerebellum (mouse) and interaction possibities between Atxn2 and RBM9 must be determined

  ➔ Bioprocessor and/or standard IP and colocalization via microscopy

  → If interaction is positive, determine if atxn2 isable to recruit RBM9
  → If not, IHC to show if RBM9 is expressed in Purkinje cells

  → Determine if A2BP1 expression is elevated under stress conditions