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Emanuele Buratti

Taupatie vs TDP-patie: meccanismi di base

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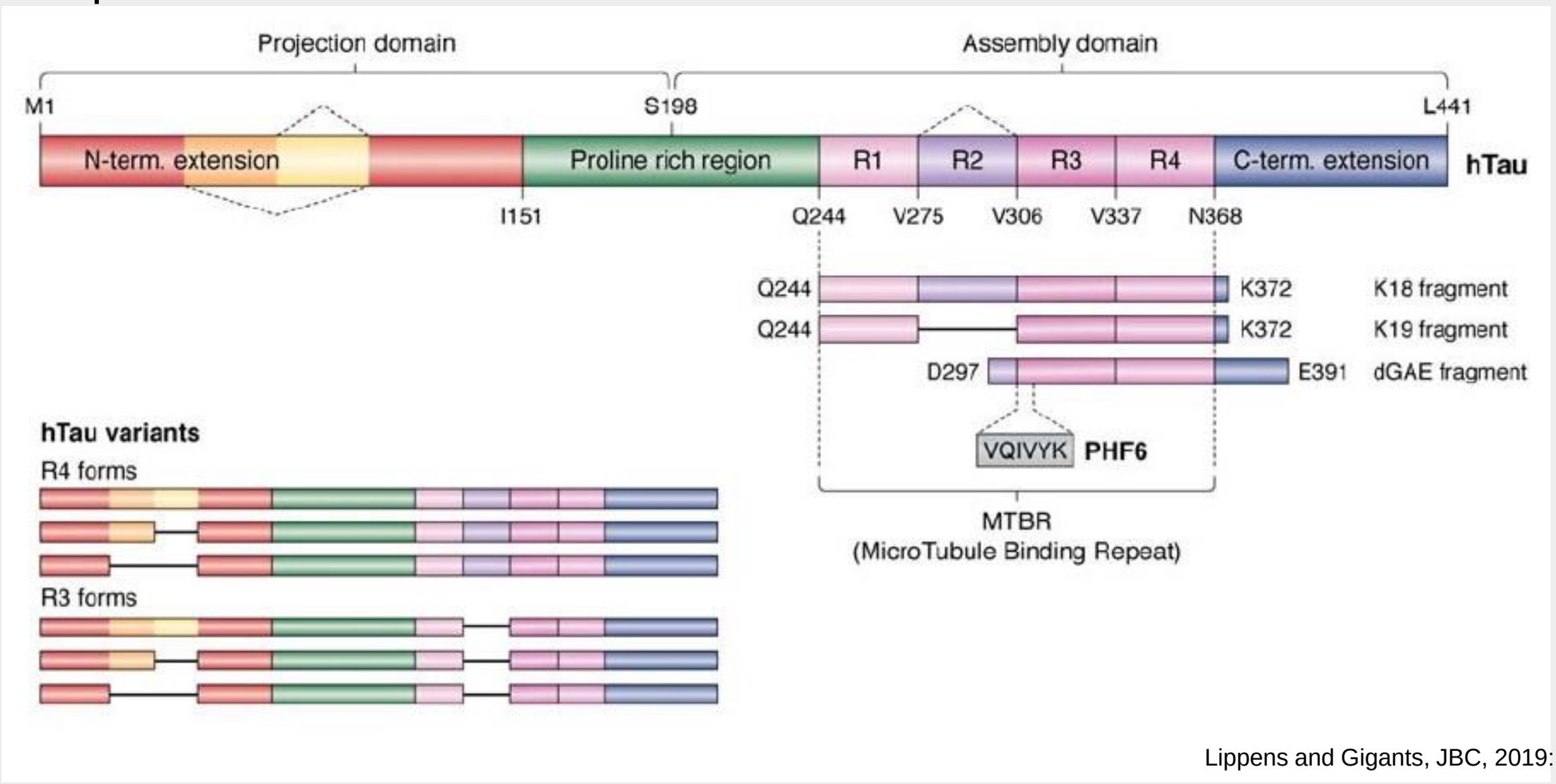
Uruguay

Member States (57)
Signatory States (77)

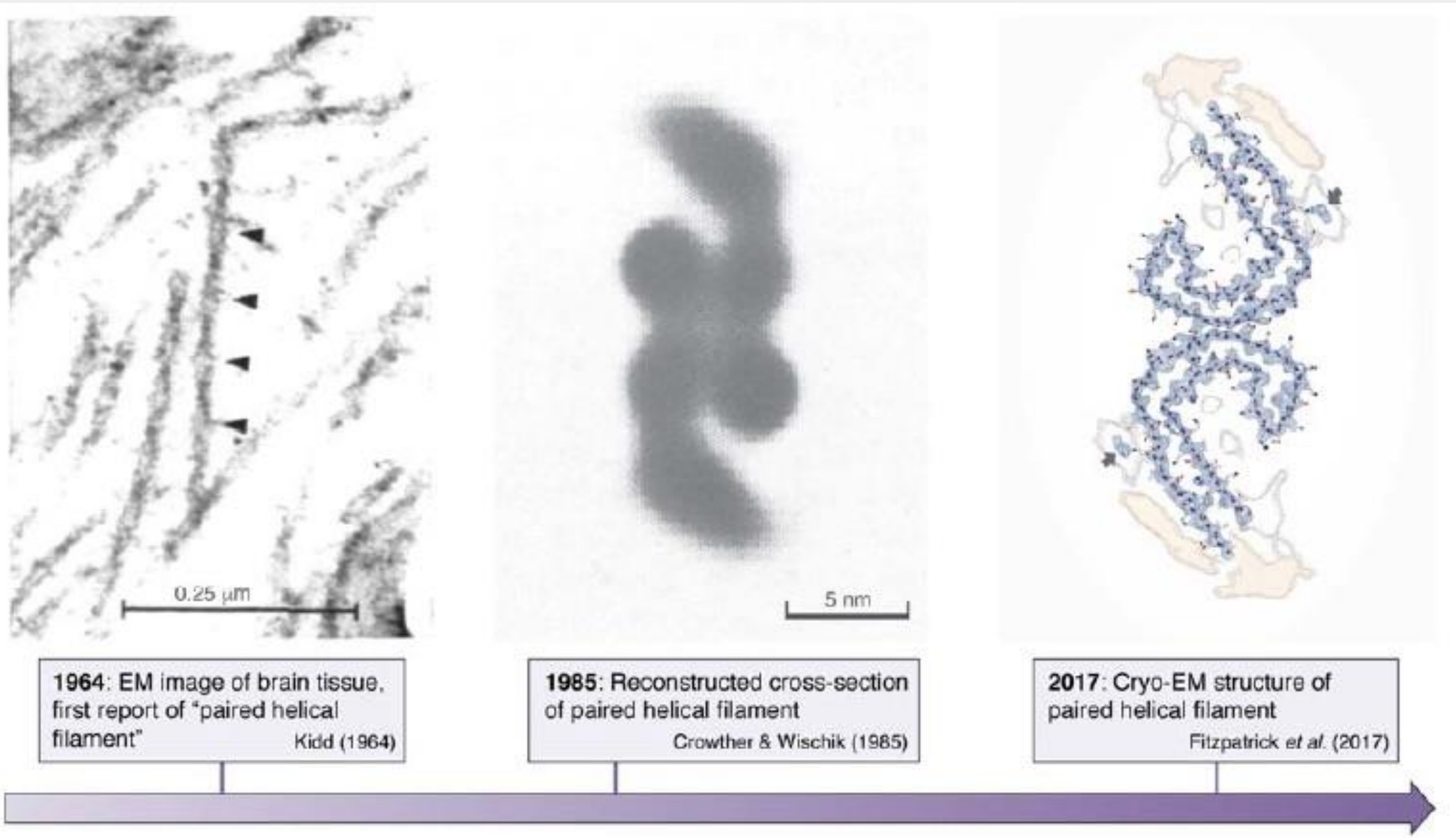
Structure and functions of TDP-43 and Tau

Tau protein:



Lippens and Gigants, JBC, 2019:

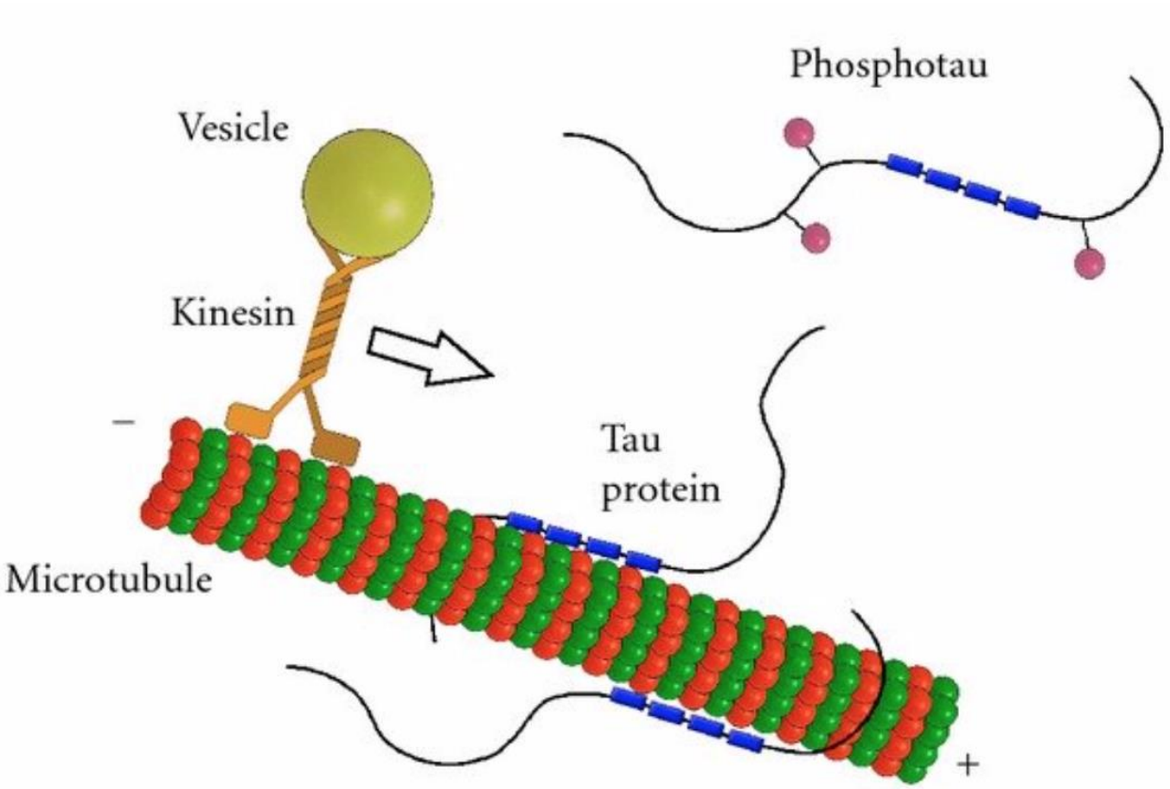
Figure 1 Primary structure of the longest isoform of human Tau, with its different domains. Splice variants occur through the omission of one or two N-terminal inserts or of the second repeat in the MTBR.



Negative staining electron microscopy image of brain tissue showed the first "Paired Helical Filaments" (PHFs). (Middle) Reconstructed cross-section of the paired helical filament. (Right) Atomic model of the same cross section obtained by cryo-electron microscopy.

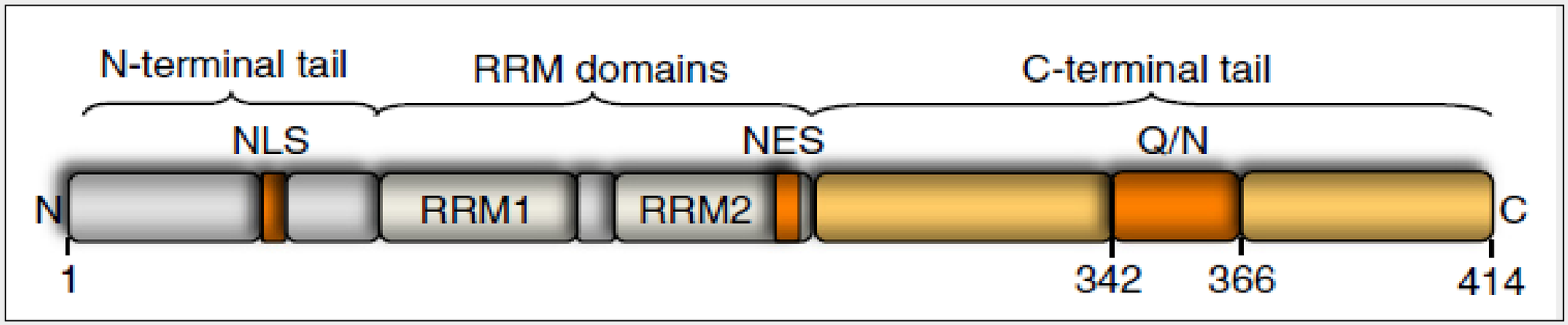
Lippens and Gigants, JBC, 2019:

Tau was originally defined by its ability to bind and stabilize microtubules. However, it is now becoming evident that the functions of tau extend beyond its ability to modulate microtubule dynamics. Tau plays a role in mediating axonal transport, synaptic structure and function, and neuronal signaling pathways.



Binding of tau protein to the microtubules is maintained in equilibrium by coordinated actions of kinases and phosphatases. The phosphorylation of tau (pink balls) regulates its activity to bind to microtubules and can affect axonal transport. Tau protein may inhibit the plus-end-directed transport of vesicles along microtubules by kinesin

TDP-43 protein:



414 amino acid nuclear protein

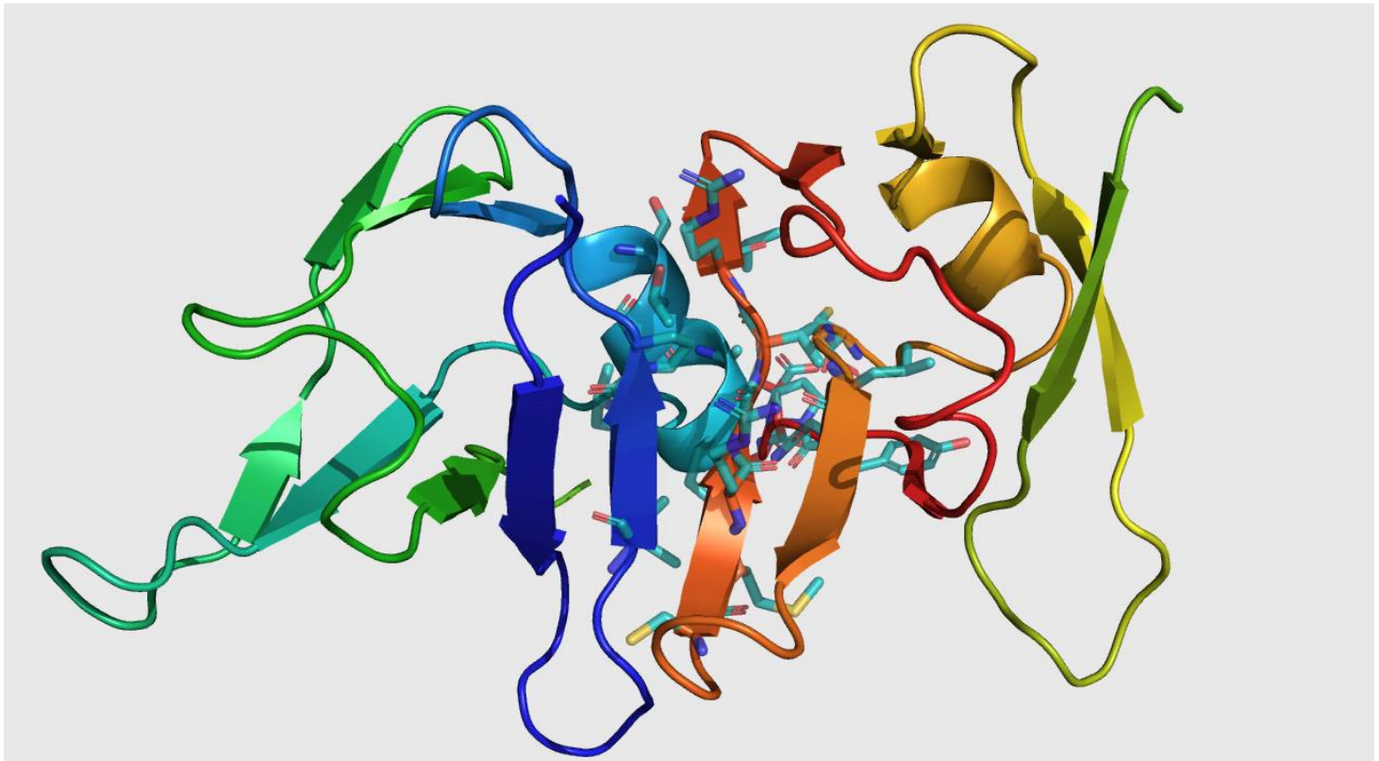
Ubiquitously expressed DNA-/RNA-binding protein

Encoded by the TARDBP gene on chromosome 1

Family of *hnRNPs*

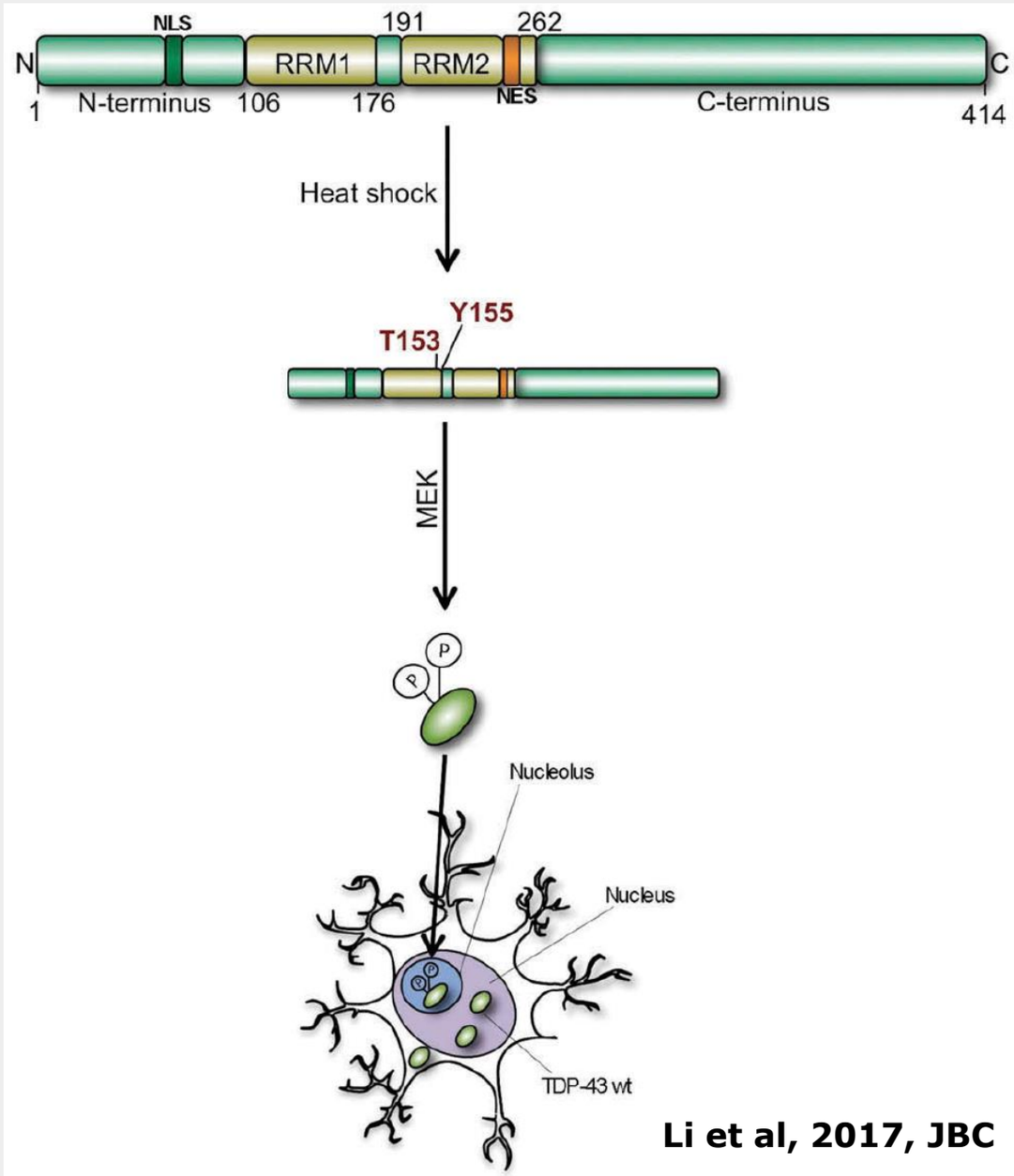
Mostly nuclear (although up to ~ 30% of TDP-43 protein can be found in the cytoplasm

Physiological phosphorylation can affect TDP-43 oligomerization and cellular loca lization



Wang et al, 2018, EMBO J

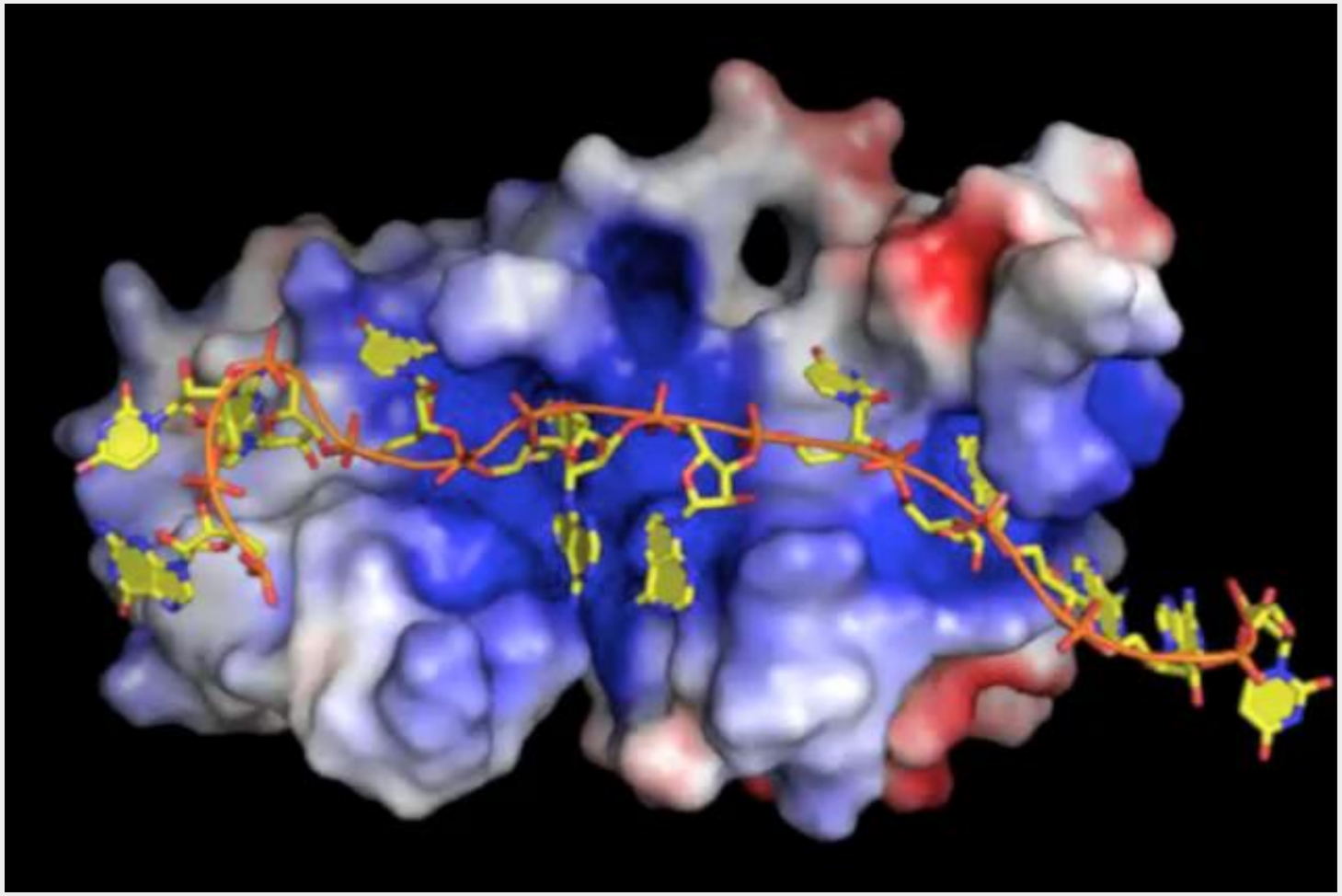
NTD oligomerization promotes TDP-43 Liquid/Liquid Phase Separation, but that phosphorylation on the co nserved Ser 48* prevents this.



Li et al, 2017, JBC

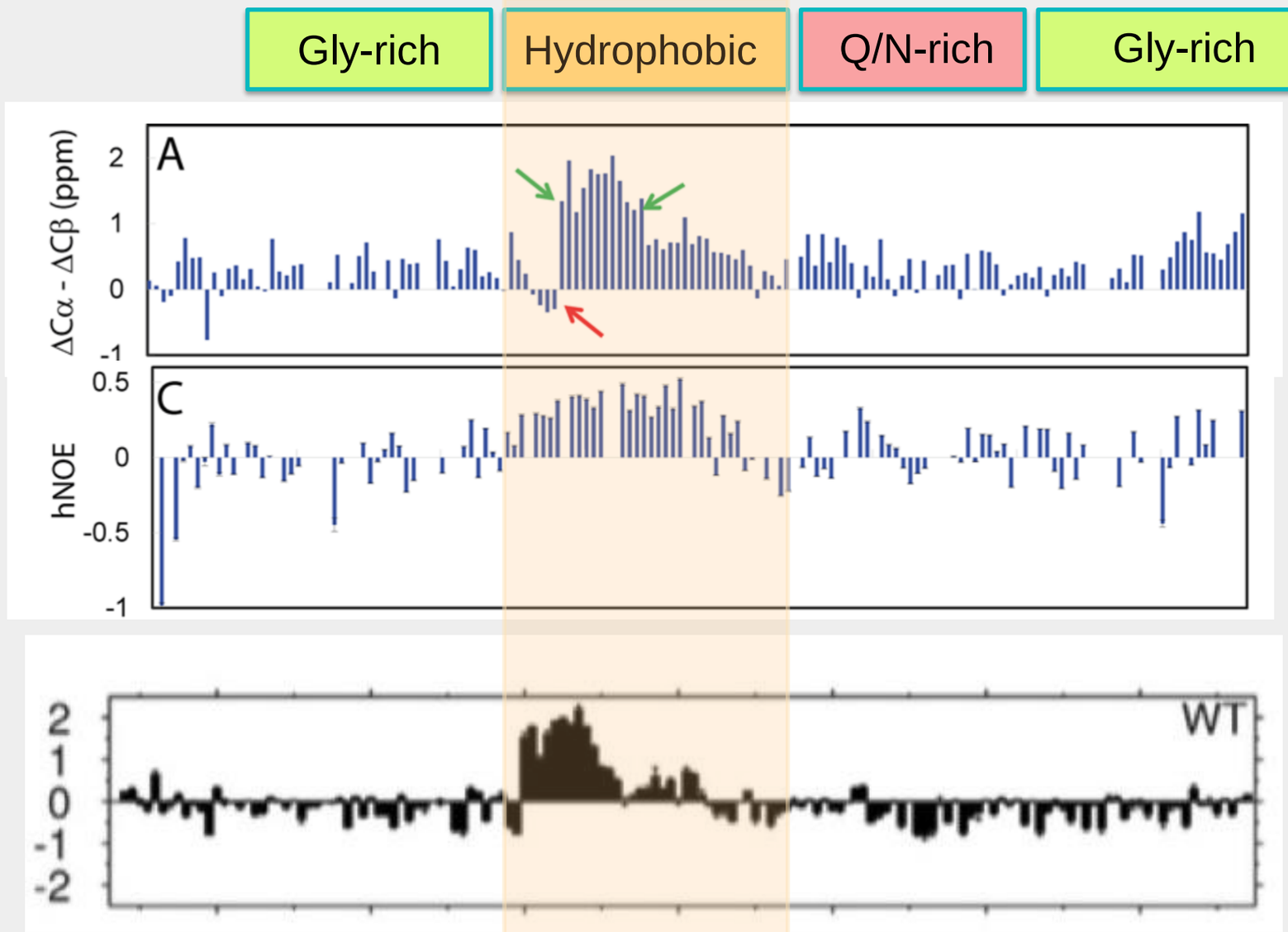
TDP-43 likes to bind UG-repeated motifs in a sequence specific manner using both RRMs:

- RRM1 specifically recognizes one GU repeat and RRM2 recognizes also one UG repeat plus additional nucleotides that are not specifically recognized. Thus the emerging binding consensus sequence is 5'-N(N)GUGN(N)UGN-3'.
- A unique feature of the TDP-43-RNA complex is the reversed binding of the UG-rich RNA with the 5'end being bound on RRM1 rather than RRM2 as normally seen in tandem RRMs.
- TDP-43 binding to UG-rich RNA also showed a very clear correlation between binding affinity and inhibitory splicing function for interaction on RRM1, but not for RRM2 in which alanine mutations in the recognition sites on RRM2 has only little impact on the overall RNA binding affinity of TDP-43. Nevertheless, these interactions are of functional importance and required for the splicing function of TDP-43. Thus the role of RRM2 could be to direct the UG-rich RNA path for productive TDP-43 dimerization on pre-mRNA target sites.



Lukavsky et al. 2013

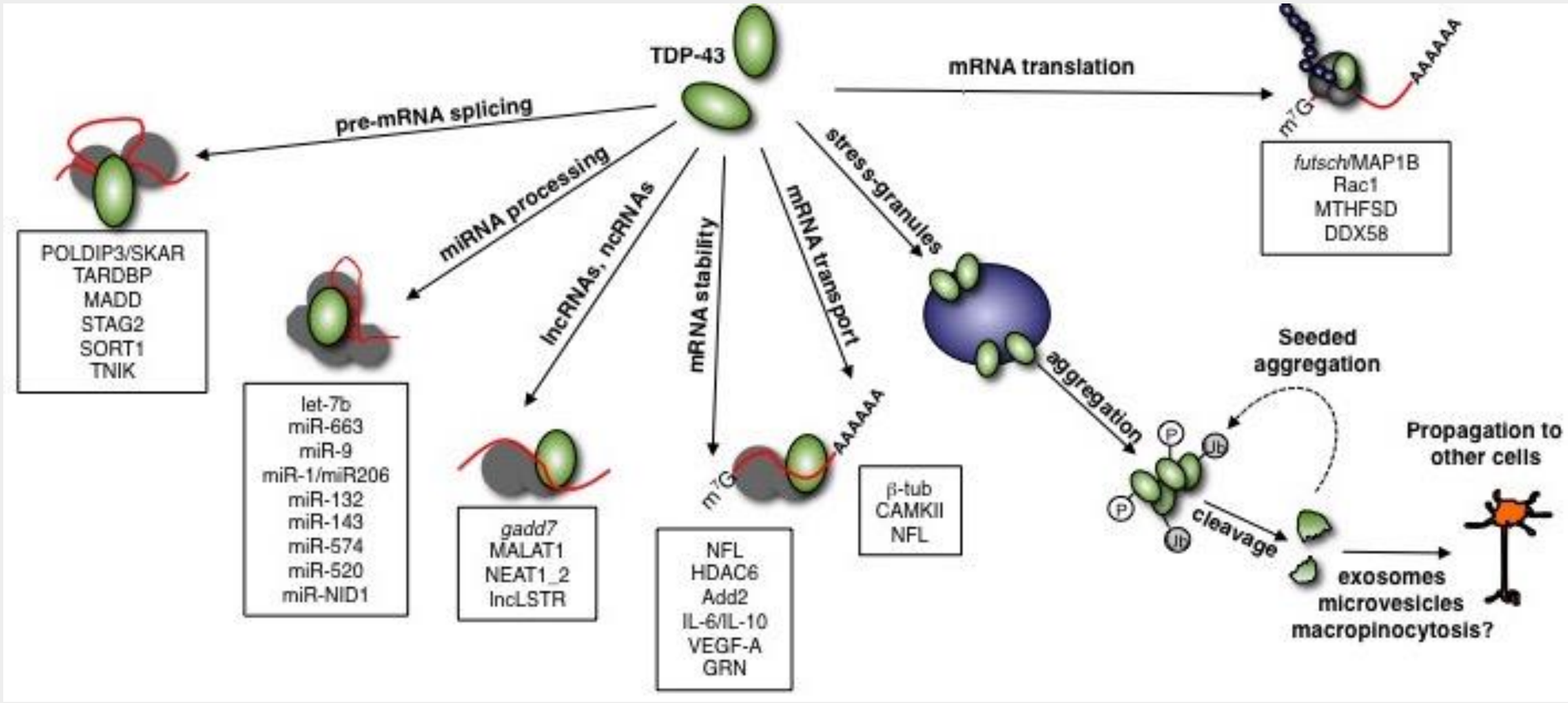
TDP-43's Hydrophobic stretch is important for regulating LLPS properties.



In 2016, Song and co-workers found that the hydrophobic stretch, which is conserved in evolution, can form a partially populated helices.

Confirmed and extended in Fawzi's lab in 2017 who advanced that the CTD of TDP-43 undergoes liquid/liquid phase separation, that the hydrophobic helix is key for

What does TDP-43 do?:



Ratti and Buratti, J. Neurochem., 2016

Involvement in disease

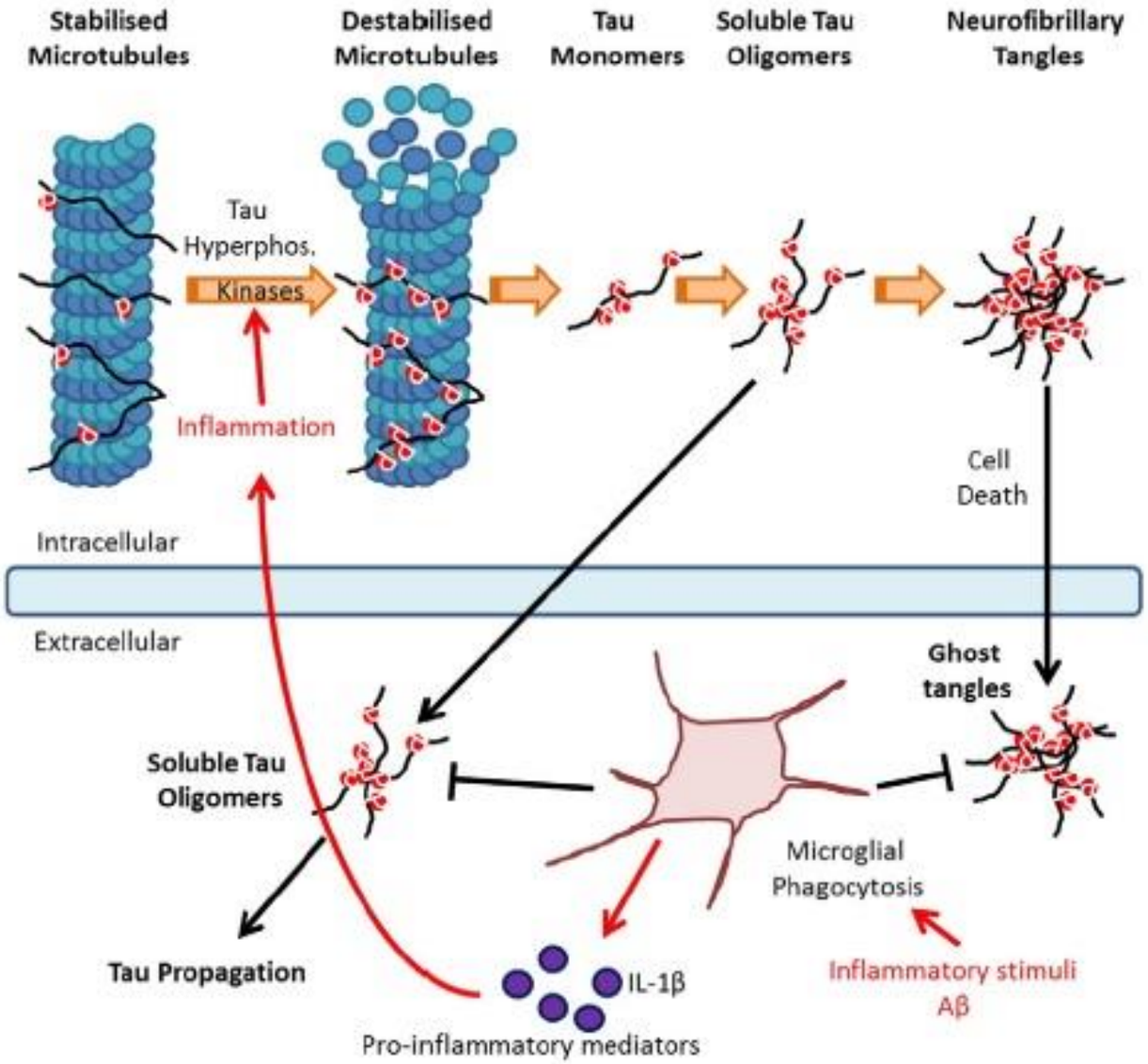
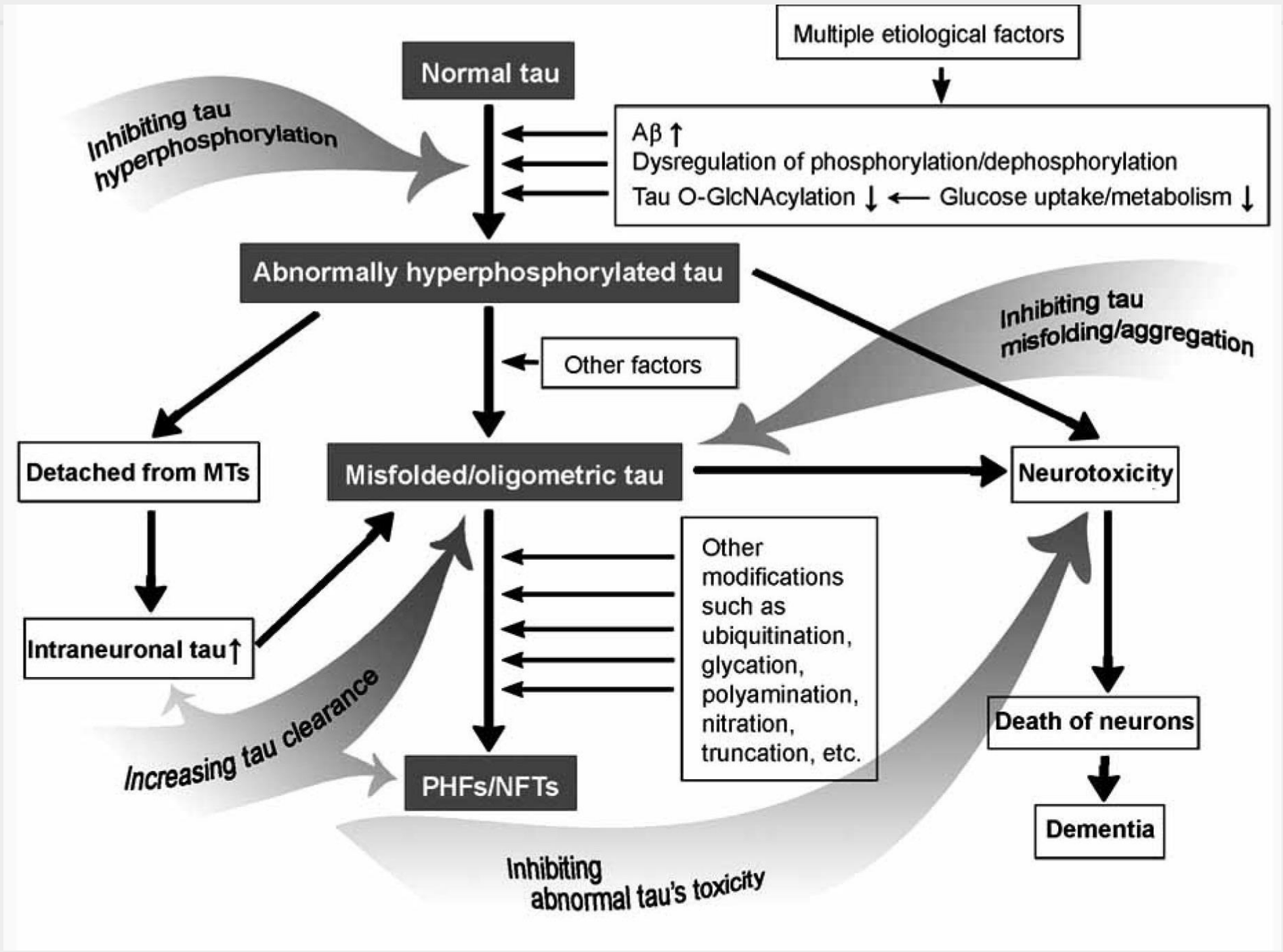
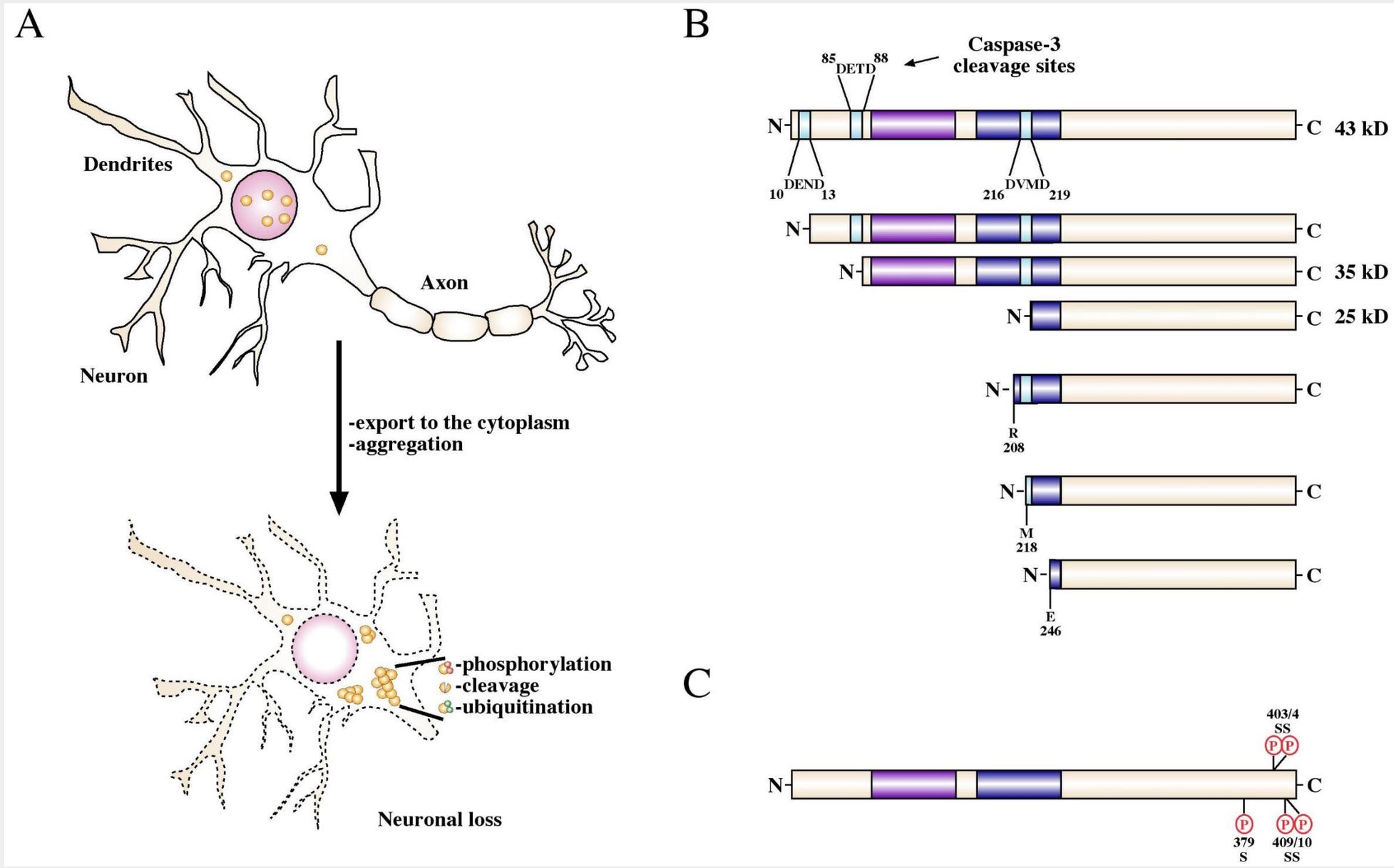


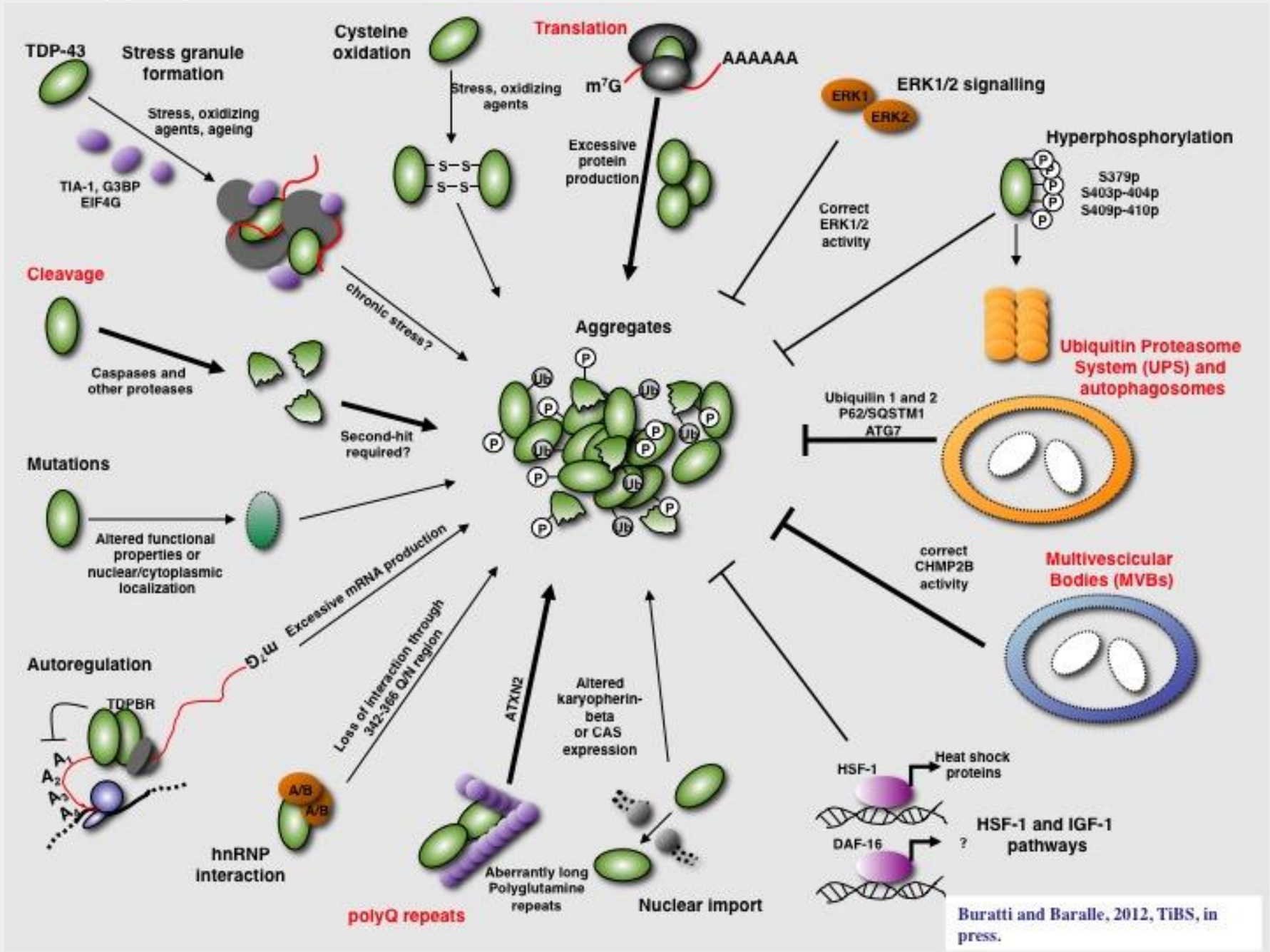
Fig. 1. Progression of tau pathology: Under physiological conditions tau regulates microtubule stabilisation. In tauopathies, tau hyperphosphorylation triggers a loss in microtubule affinity. Soluble tau aggregates into pathological soluble tau oligomers, ultimately forming pathological insoluble neurofibrillary tangles (NFT). Tau oligomers are secreted into the extracellular compartment contributing to the propagation of tau pathology into neighbouring neurons. Inflammatory stimuli, such as Aβ, stimulate microglial production of pro-inflammatory mediators such as IL-1β leading to the up-regulation of kinases involved in tau phosphorylation and exacerbation of the pathology. However, inflammation can have beneficial effects on tau pathology by inducing microglial phagocytosis of extracellular tau species. Image adapted from National Institute of Ageing.


Multiple etiological factors
Normal tau
Inhibiting tau hyperphosphorylation
Aβ ↑
Dysregulation of phosphorylation/dephosphorylation
Tau O-GlcNAcylation ↓ ← Glucose uptake/metabolism ↓
Abnormally hyperphosphorylated tau
Inhibiting tau misfolding/aggregation
Other factors
Detached from MTs
Misfolded/oligometric tau
Neurotoxicity
Other modifications such as ubiquitination, glycation, polyamination, nitration, truncation, etc.
Intraneuronal tau↑
Increasing tau clearance
Death of neurons
PHFs/NFTs
Dementia
Inhibiting abnormal tau's toxicity



Buratti and Baralle., 2009
Adv.Gen, 66:1

Why does TDP-43 aggregate?



Buratti and Baralle, 2012, TiBS, in press.

Loss of function against gain of function?

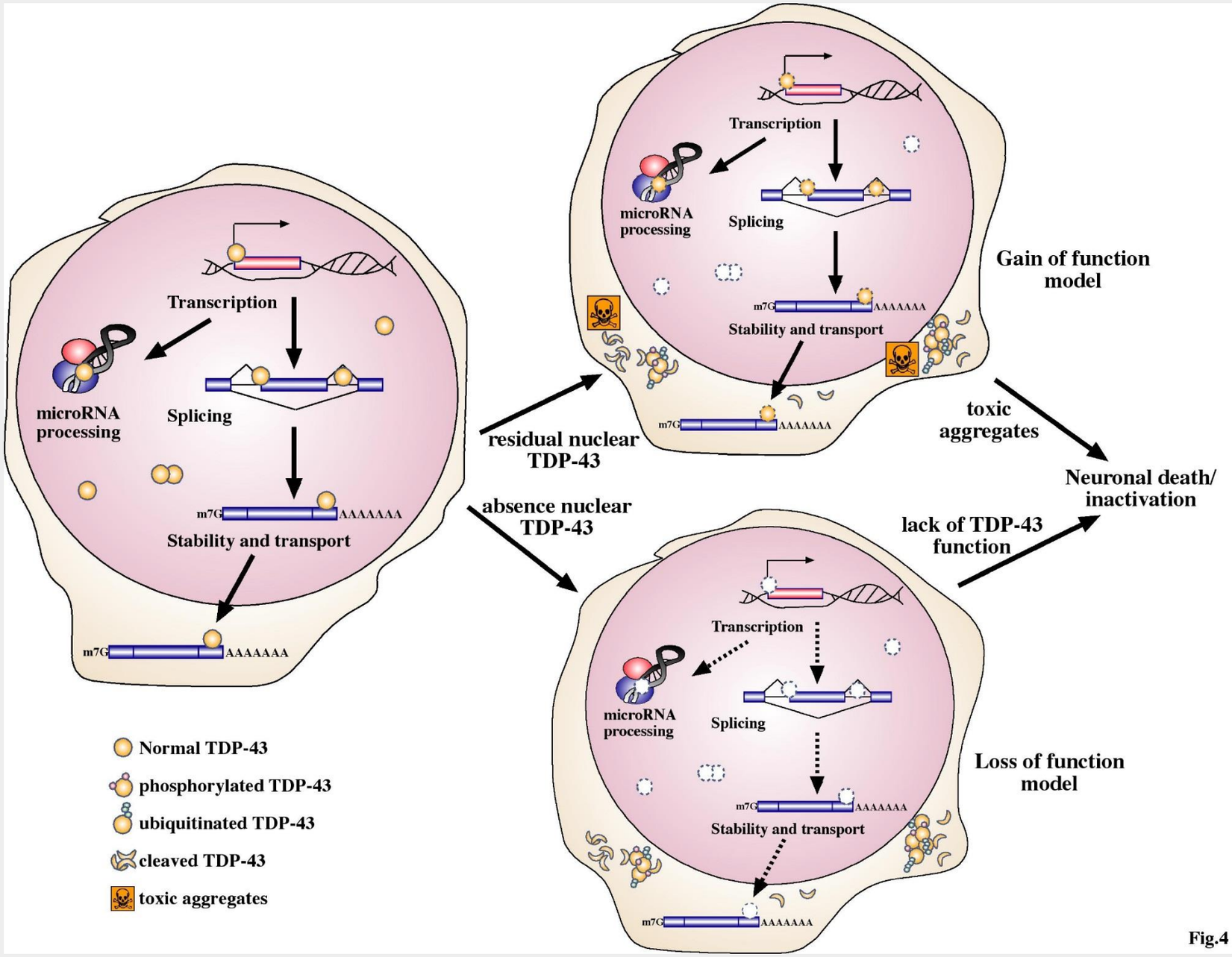
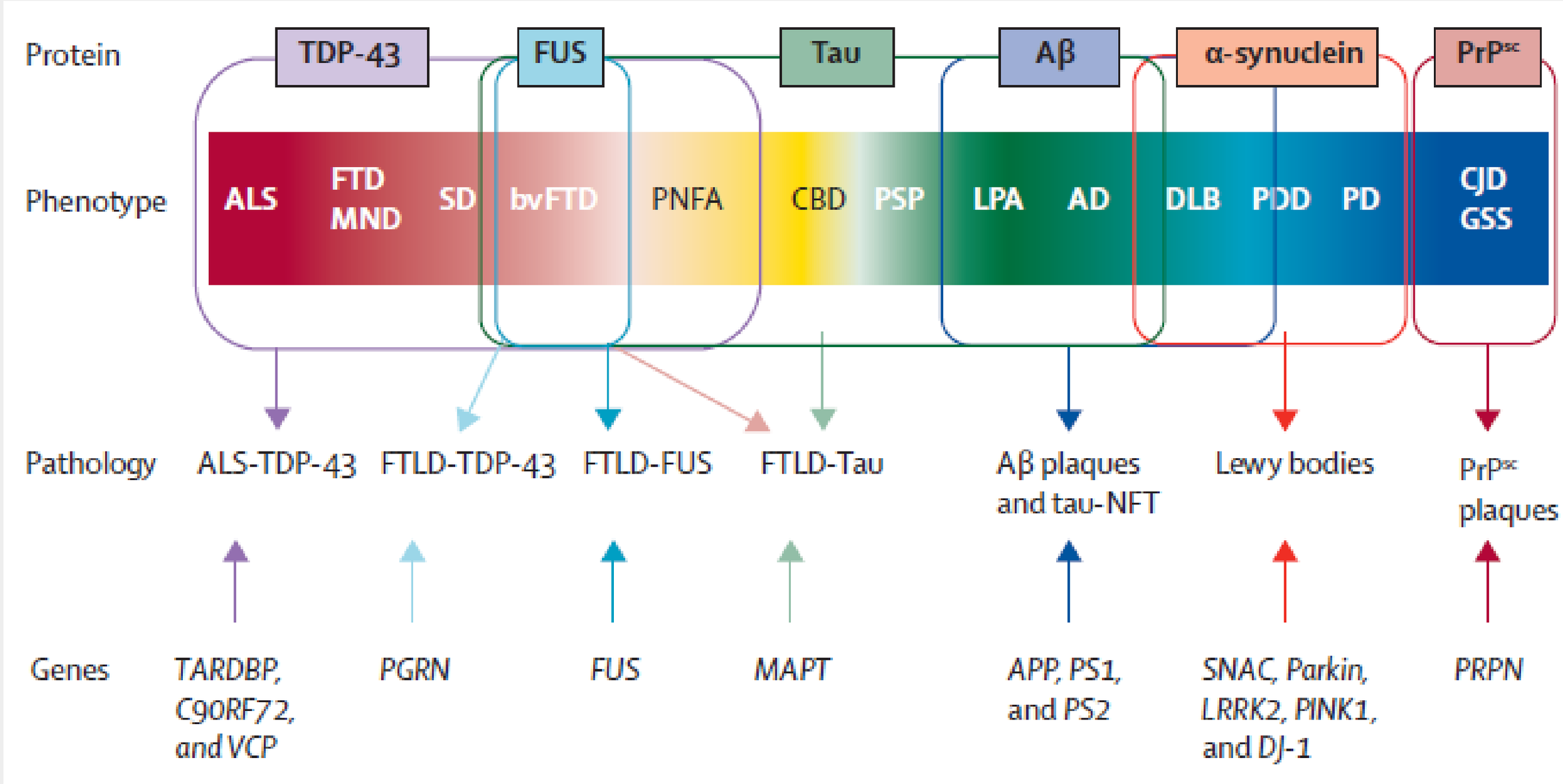


Fig.4

Buratti and Baralle., 2009 Adv.Gen, 66:1

TDP-43 and Tau pathology

Disorders of misfolded proteins



Molecular mechanisms that contribute to FTD pathology

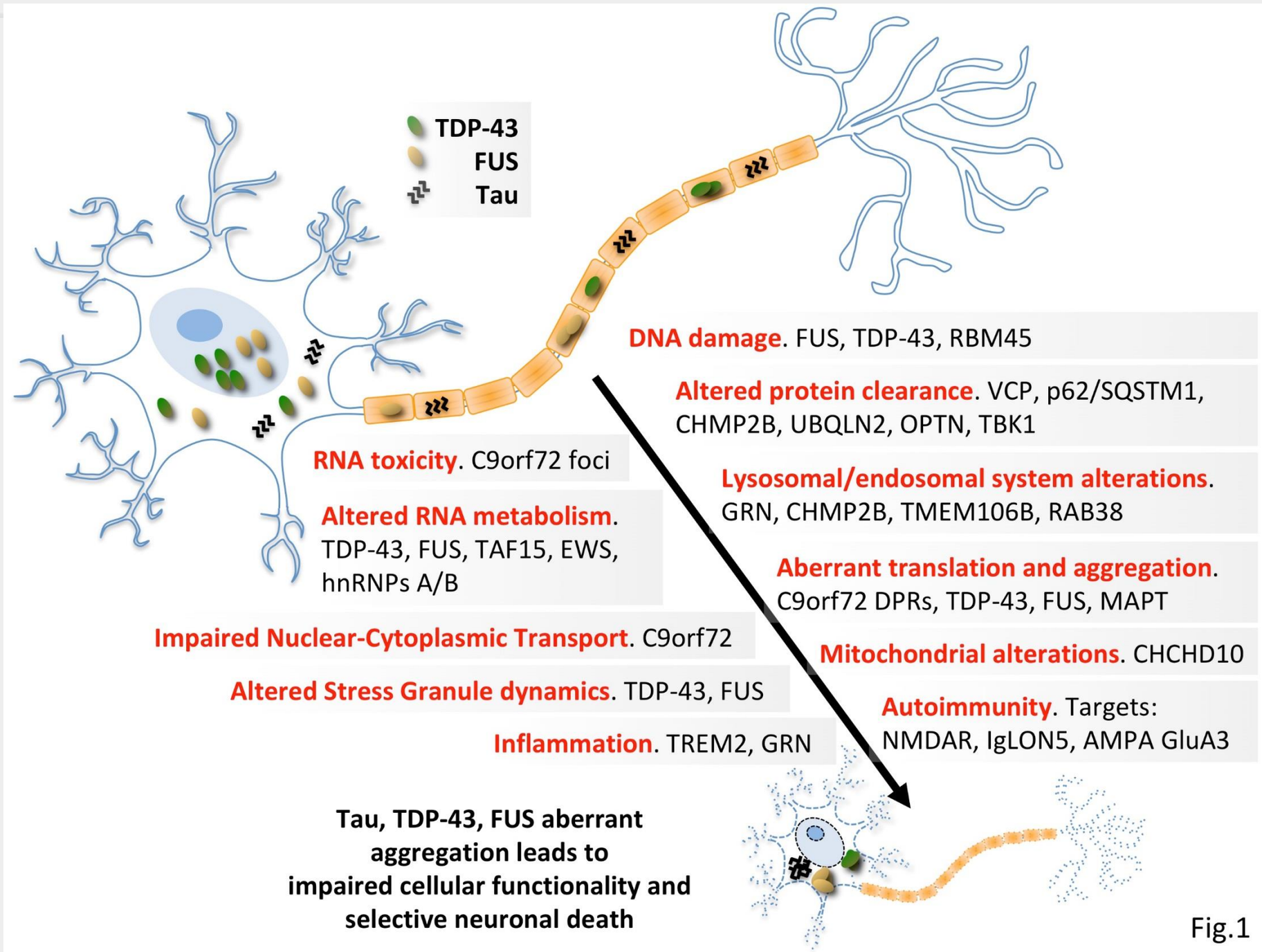


Fig.1

Connections

Mixed TDP-43 proteinopathy and tauopathy in frontotemporal lobar degeneration: nine case series

Eun-Joo Kim[1,2] · Jesse A. Brown[1] · Jersey Deng[1] · Ji-Hye L. Hwang[1] · Salvatore Spina[1] · Zachary A. Miller[1] · Mary G. DeMay[1] · Victor Valcour[1] · Anna Karydas[1] · Eliana Marisa Ramos[3,4] · Giovanni Coppola[3,4] · Bruce L. Miller[1] · Howard J. Rosen[1] · William W. Seeley[1,5] · Lea T. Grinberg[1,5]

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Abstract

Objectives To determine the clinical, anatomical, genetic and pathological features of dual frontotemporal lobar degeneration (FTLD) pathology: FTLD-tau and FTLD-TDP-43 in a large clinicopathological cohort.

Methods We selected subjects with mixed FTLD-TDP and FTLD-tau from 247 FTLD cases from the University of California, San Francisco, Neurodegenerative Disease Brain Bank collected between 2000 and 2016 and compared their clinical, anatomical, genetic, imaging and pathological signatures with those of subjects with pure FTLD.

Results We found nine cases (3.6%) with prominent FTLD-TDP and FTLD-tau. Six cases were sporadic, whereas one case had a *C9ORF72* expansion, another had a *TARDBP* A90V variant, and the other had an *MAPT* p.A152T variant. The subtypes of FTLD-TDP and FTLD-tau varied. Mixed FTLD cases were older and tended to show a higher burden of Alzheimer disease pathology (3/9, 33%). The neuroimaging signature of mixed cases, in general, included more widespread atrophy than that of pure groups. Specifically, cases of mixed corticobasal degeneration (CBD) with FTLD-TDP showed more prominent asymmetric left-sided atrophy than did those of pure CBD. However, the clinical phenotype of mixed cases was similar to that seen in pure FTLD.

Conclusions Although patients with mixed FTLD-TDP and FTLD-tau are rare, in-depth clinical, pathological and genetic investigations may shed light on the genetic and biochemical pathways that cause the accumulation of multiple proteinaceous inclusions and inform therapeutic targets that may be beneficial to each one of these abnormal protein misfoldings.

Keywords Frontotemporal lobar degeneration · TAR-DNA binding protein-43 · Tau

Acta Neuropathol (2013) 126:39–50
DOI 10.1007/s00401-013-1123-8

ORIGINAL PAPER

Robust cytoplasmic accumulation of phosphorylated TDP-43 in transgenic models of tauopathy

Amy K. Clippinger · Simon D'Alton · Wen-Lang Lin · Tania F. Gendron · John Howard · David R. Borchelt · Ashley Cannon · Yari Carlomagno · Paramita Chakrabarty · Casey Cook · Todd E. Golde · Yona Levites · Laura Ranum · Patrick J. Schultheis · Guilian Xu · Leonard Petrucelli · Naruhiko Sahara · Dennis W. Dickson · Benoit Giasson · Jada Lewis

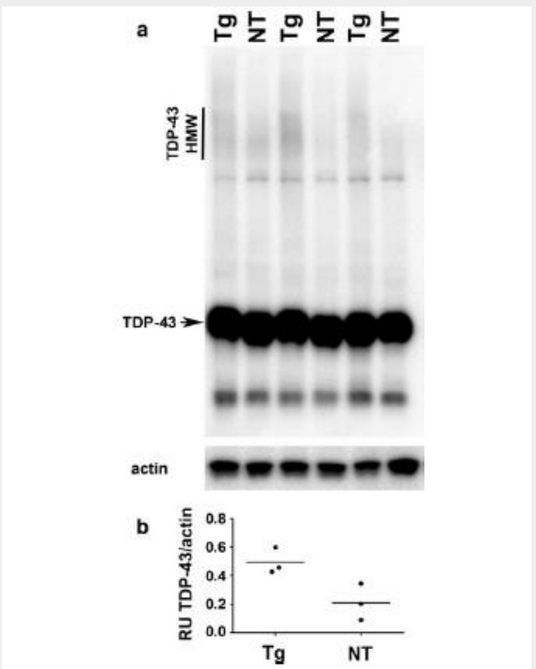


Fig. 5 Higher molecular weight species of TDP-43 is elevated in the soluble fraction of rTg4510 compared to non-transgenic mice. (**a**) 10-month-old rTg4510 (Tg) and non-transgenic (NT) mice have equivalent expression of full-length TDP-43 protein (*arrow*). (**a**, **b**) rTg4510 mice have increased levels of high molecular weight TDP-43 protein (*line*, TDP-43 HMW) in the soluble fraction compared to NT mice ($p = 0.03$, unpaired t-test). β-Actin was used as a loading control. RU stands for relative units

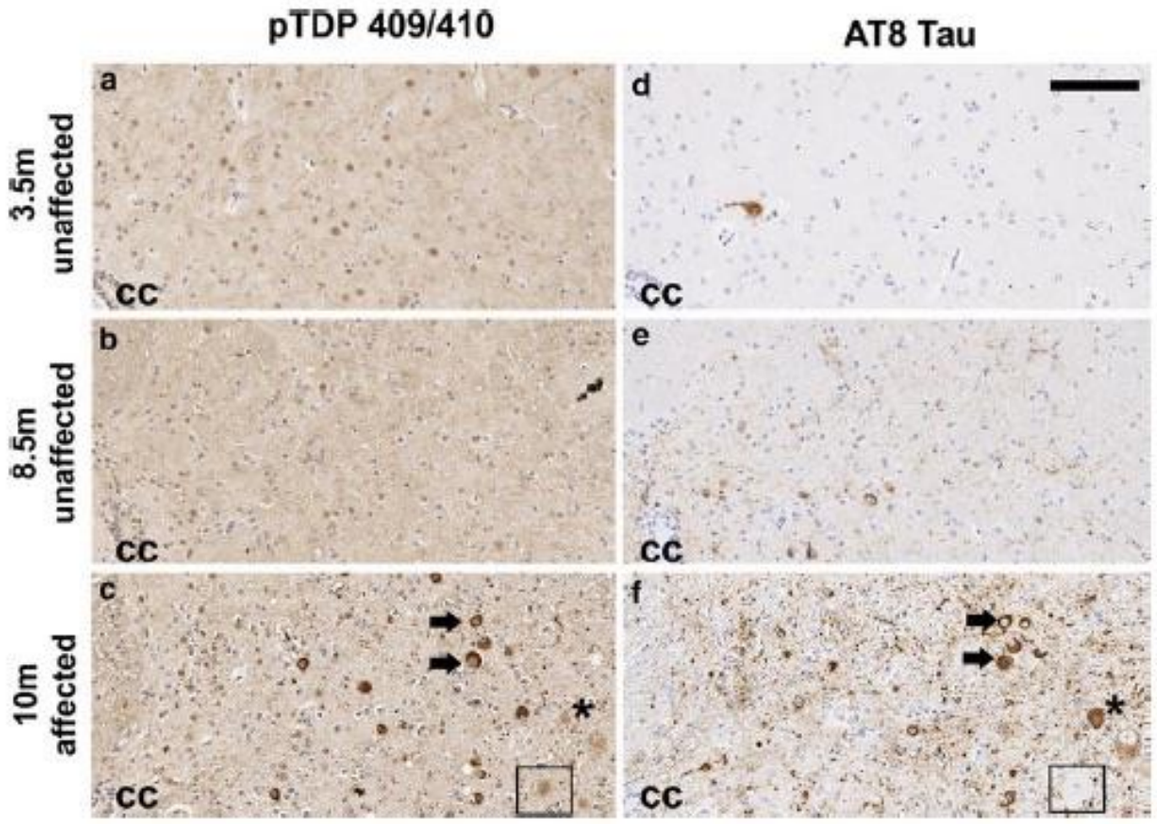


Fig. 2 Tau pathology generally precedes the cytoplasmic accumulation of phosphorylated TDP-43 in the JNPL3 mouse model of tauopathy. Serial sections of spinal cord tissue from (**a**, **d**) 3.5, (**b**, **e**) 8.5 and (**c**, **f**) 10-month-old JNPL3 mice was immunostained for (**a**–**c**) TDP-43 phosphorylated at S409/410 and (**d**–**f**) tau phosphorylated at S202/T205 (AT8 antibody). JNPL3 mice at 3.5 months of age show (**a**) normal nuclear localization of pTDP-43 and (**d**) minimal tau pathology. (**b**) pTDP-43 remains localized in the neuronal nuclei as (**e**) tau pathology slowly accumulates in the spinal cord of 8.5-month-old JNPL3 lacking a motor phenotype. (**c**) Serial sectioning of a JNPL3 mouse with motor phenotype shows neurons with cytoplasmic relocalization of pTDP-43 (**c**, *arrows*) that also show prominent tau pathology (**f**, *arrows*). In addition, normal nuclear localization of pTDP-43 (**c**, *asterisk*) can be seen in cells with prominent tau pathology (**f**, *asterisk*). A healthy neuron without (**c**, *square*) cytoplasmic pTDP-43 or (**f**, *square*) tau pathology can also be seen. The central canal (*cc*) has been noted. The *bar* indicates 100 μm

JBC ARTICLE

Transactive response DNA-binding protein 43 (TDP-43) regulates alternative splicing of tau exon 10: Implications for the pathogenesis of tauopathies

Received for publication, February 27, 2017, and in revised form, May 8, 2017 Published, Papers in Press, May 9, 2017, DOI 10.1074/jbc.M117.783498

Jianlan Gu[‡§¶], Feng Chen[‡§], Khalid Iqbal[§], Cheng-Xin Gong[‡§], Xinglong Wang[‖], and Fei Liu[‡§1]

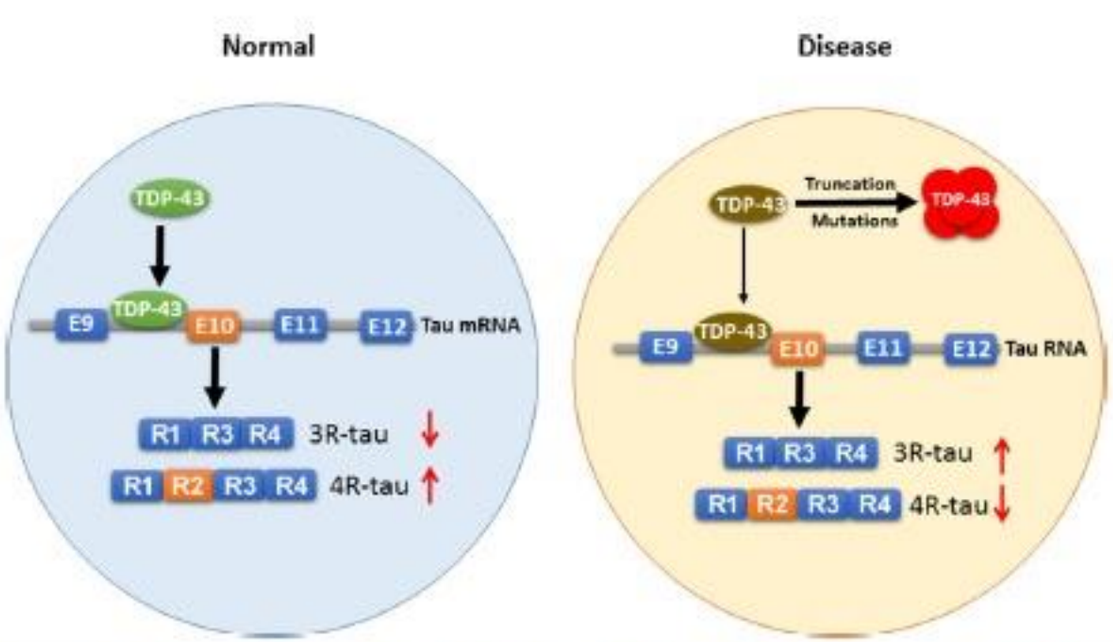


Figure 6. The proposed mechanism by which TDP-43 regulates tau expression. TDP-43 acts on intron 9 and promotes tau exon 10 inclusion in physiological condition. In the disease condition, truncations or mutations lead to its cytoplasmic aggregation and nuclear depletion. Loss of function of TDP-43 (as a result of the nuclear depletion) leads to suppression of tau exon 10 inclusion and 4R-tau expression, which consequently may contribute to the neurofibrillary pathology.

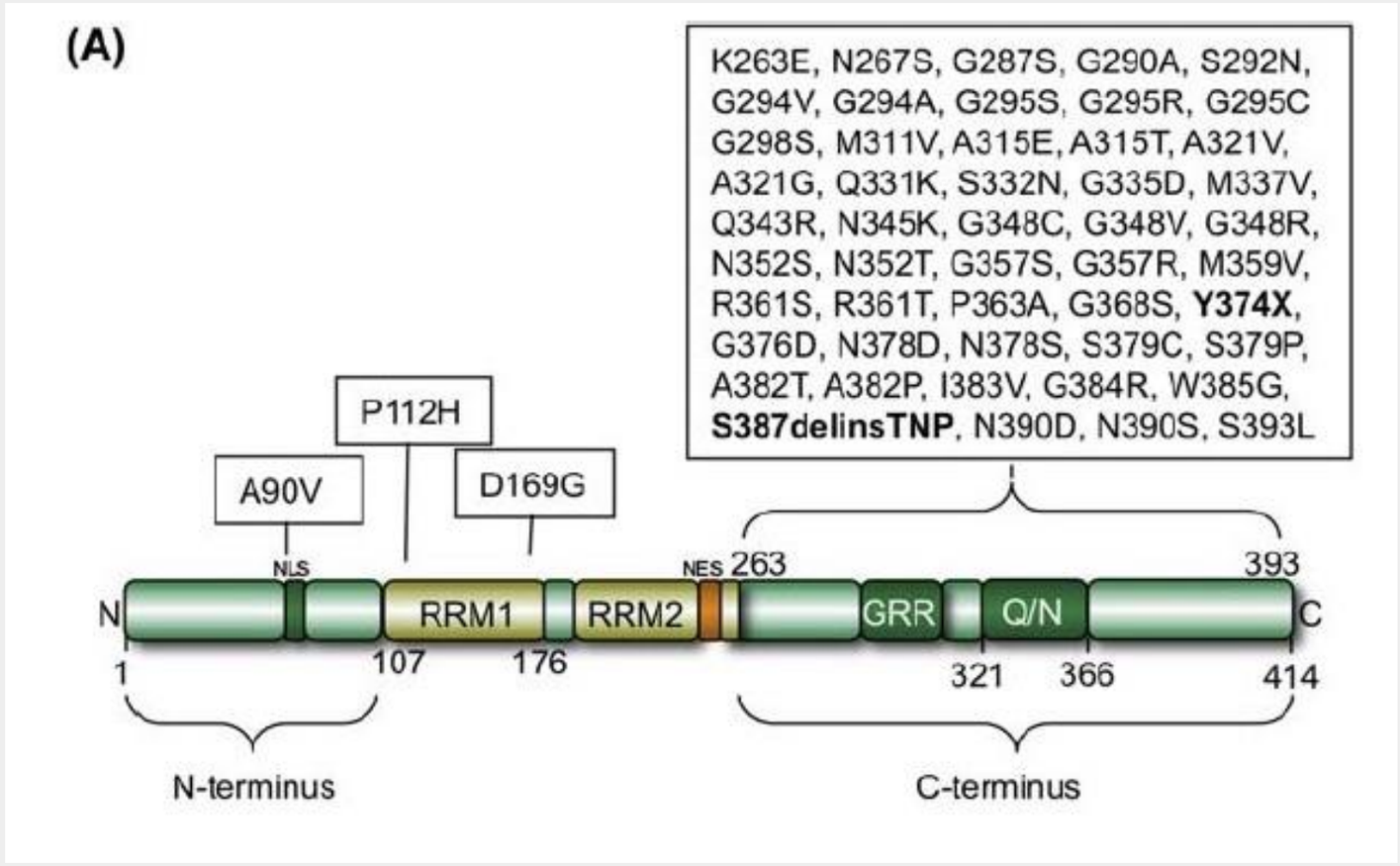
Importance of studying mutations

008- first reports of disease-associated mutations in TDP-43

ScienceExpress Report

TDP-43 Mutations in Familial and Sporadic Amyotrophic Lateral Sclerosis

Jemeen Sreedharan,[1]* Ian P. Blair,[3,4]* Vineeta B. Tripathi,[1]* Xun Hu,[1] Caroline Vance,[1] Boris Rogelj,[1] Steven Ackerley,[1,2] Jennifer C. Durnall,[3] Kelly L. Williams,[3] Emanuele Buratti,[5] Francisco Baralle,[5] Jacqueline de Belleroche,[6] J. Douglas Mitchell,[7] P. Nigel Leigh,[1] Ammar Al-Chalabi,[1] Christopher C. Miller,[1,2] Garth Nicholson,[3,4,8]* Christopher E. Shaw[1]*†



Buratti E., Adv. Genetics 2016

S375G is a rare variant recently reported in databases
And has been classified as, neutral, possibly benign.

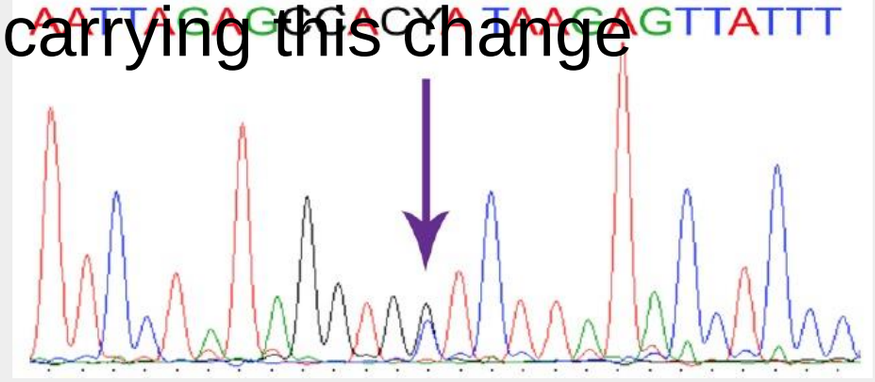
ALS onset is influenced by the burden of rare variants in known ALS genes

Janet Cady, BS[1], Peggy Allred, DPT[2], Taha Bali, MD[1], Alan Pestronk, MD[1], Alison Goate, PhD[1,3,4], Timothy M. Miller, MD, PhD[1,4], Rob Mitra, PhD[5], John Ravits, MD[6], Matthew B. Harms, MD[1,4], and Robert H. Baloh, MD, PhD[2]

	Gene Name	Genomic location[a]	dbSNP ID[b]	Predicted cDNA change[c]	Predicted protein change[c]	FALS	SALS
	DAO	12:109278977	-	c.194+1G>A	Splice donor	0/84	1/698
	DCTN1	2:74388653	-	c.3810C>A	H1270Q	0/84	1/698*
	DCTN1	2:74390527	-	c.3219C>T	S1080F	0/84	1/698
	EWSR1	22:29682932	-	c.620C>G	T207S	0/84	1/698
	FIG4	6:110087935	-	c.1588_1589delTT	F530Ter	0/84	1/698
	FUS	16:31202282	-	c.1394-2delA	Splice site	1/84	0/698
	OPTN	10:13160964	-	c.70C>T	Q23.5Ter	0/84	1/698
Category 3:	*SETX*	9:135202223	-	c.4762G>A	A1588T	0/84	1/698
• Not reported in ALS	*SETX*	9:135203632	-	c.3353C>A	T1118K	0/84	1/698
• Not in databases	*SETX*	9:135206694	-	c.980A>T	E327V	0/84	1/698
	SETX	9:135210013	-	c.820A>G	M274V	0/84	1/698*
	SETX	9:135211748	-	c.658A>C	K220Q	0/84	1/698
	SETX	9:135211898	-	c.503G>A	R168Q	0/84	1/698*
	SETX	9:135224775	-	c.41C>T	T14I	0/84	1/698
	SOD1	21:33038791	-	c.199C>G	P67A	1/84*	0/698
	SQSTM1	5:179248079	-	c.143T>T	L48P	0/84	1/698
	TARDBP	1:11082589	-	c.1123A>G	S375G	0/84	1/698

This mutation was recently identified in a very early-onset ALS case (female, 22 year old)

carrying this change



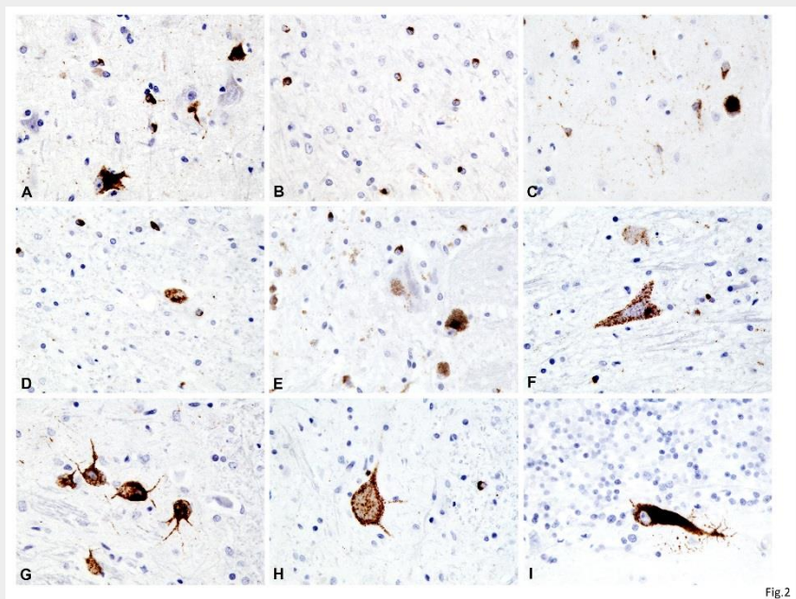


Fig.2

S375G can affect the nuclear localization of TDP-43

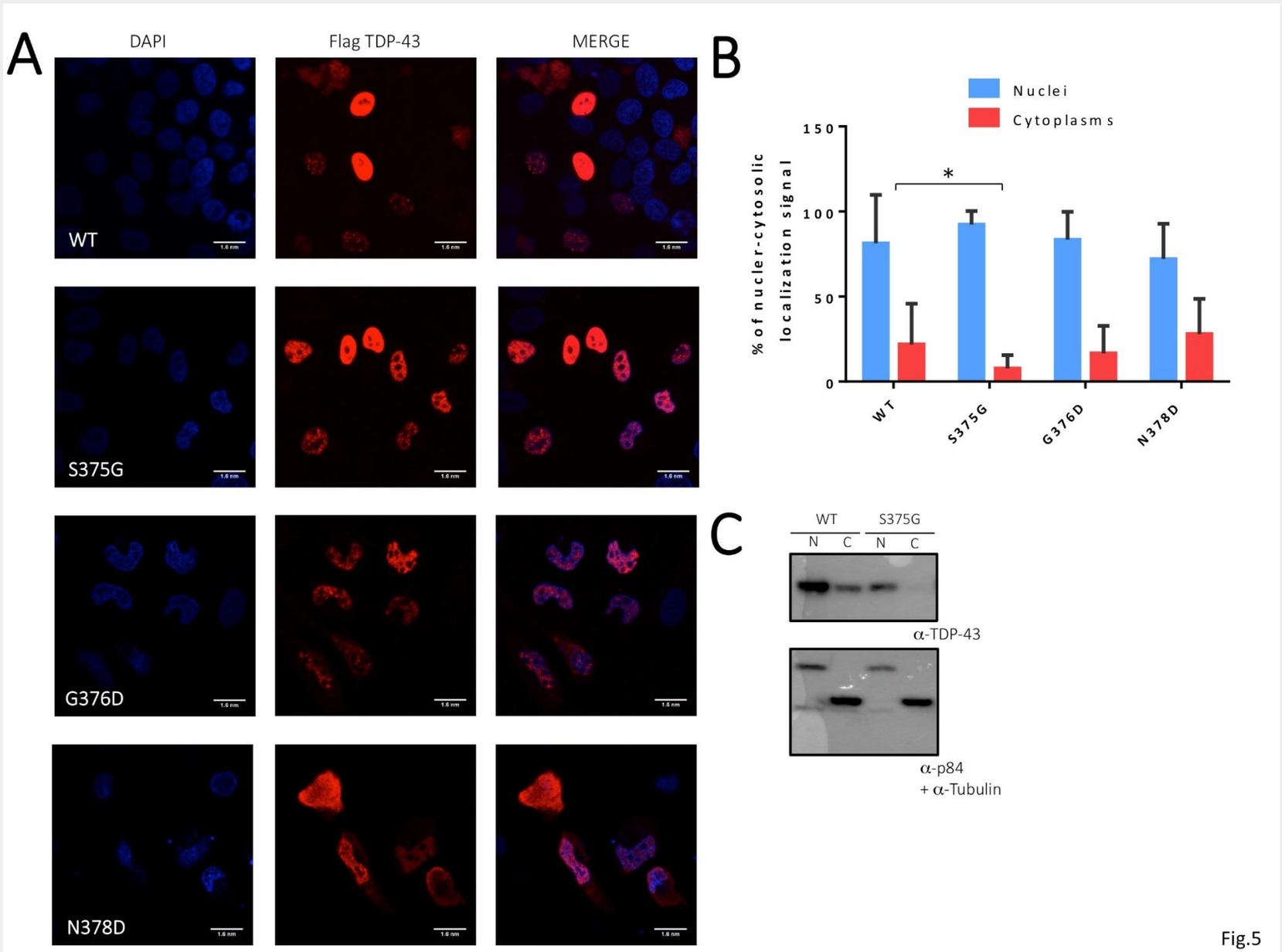


Fig.5

When expressed in HeLa cells S375G is more toxic than nearby disease-assotiate mutations

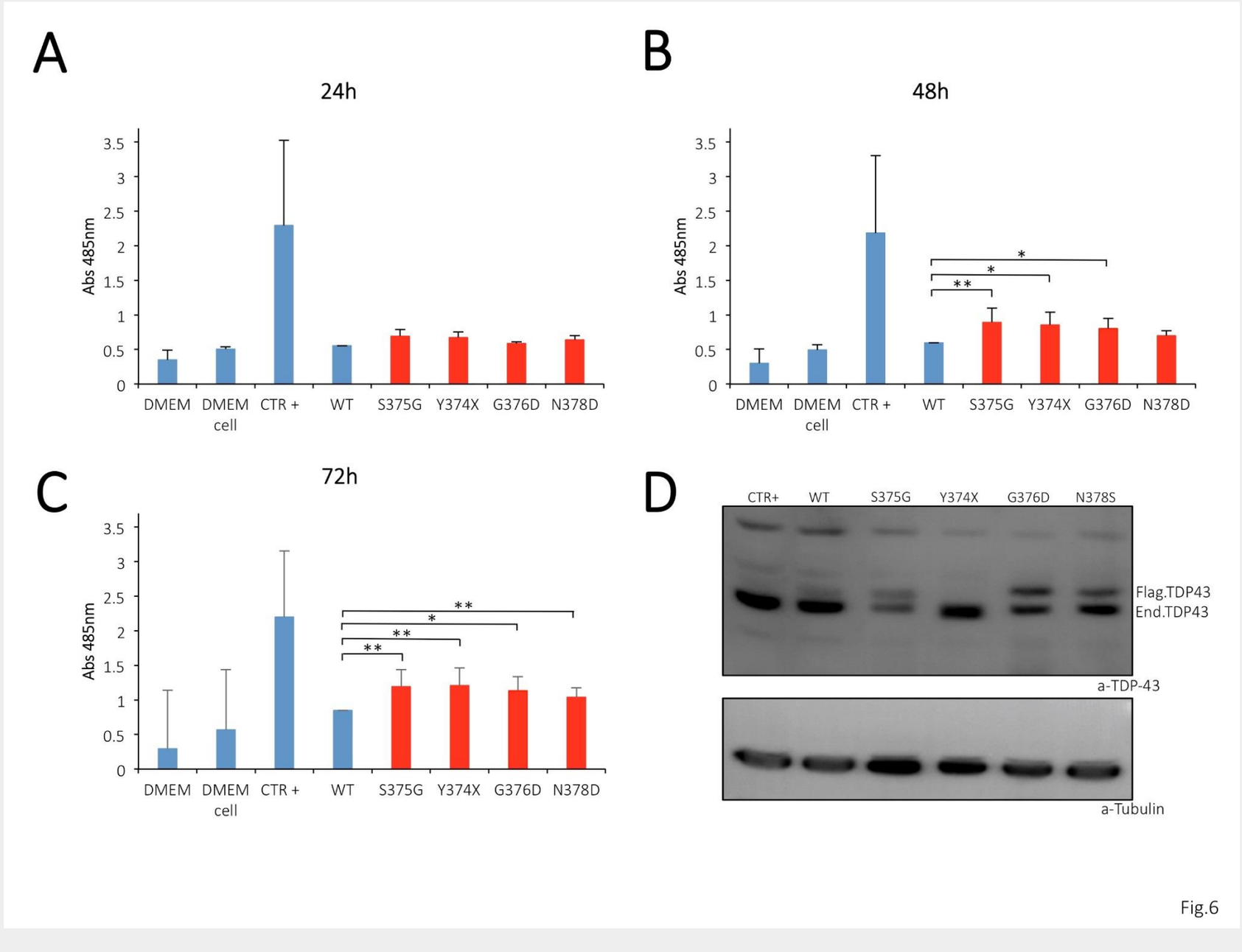


Fig.6

The Serine in position 375 could represent an important phosphorylation site both and in vivo

Identification of casein kinase-1 phosphorylation sites on TDP-43

Fuyuki Kametani [a,*], Takashi Nonaka [a], Takehiro Suzuki [c], Tetsuaki Arai [b], Naoshi Dohmae [c], Haruhiko Akiyama [b], Masato Hasegawa [a]

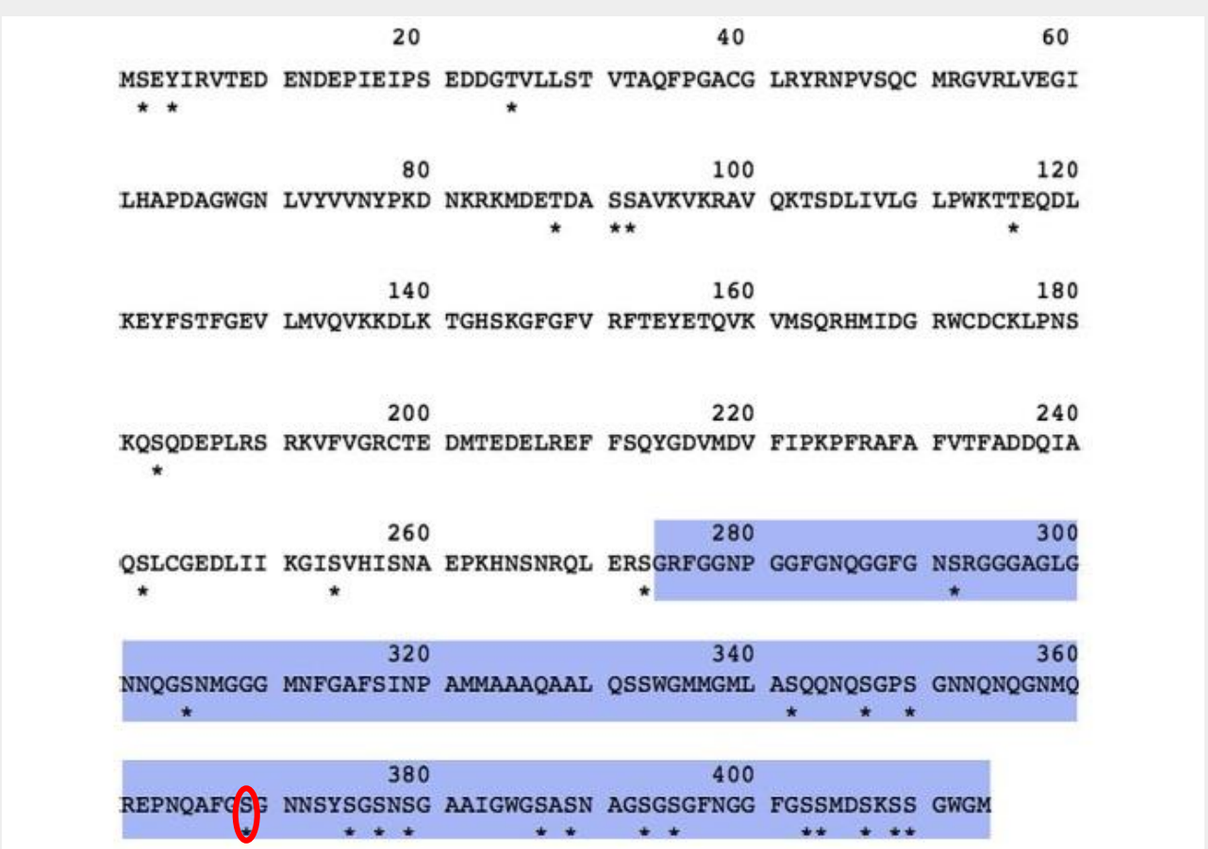


Fig. 2. CK1 phosphorylation sites on recombinant TDP-43. Asterisks show phosphorylation sites. The boxed region is the C-terminal Gly-rich region (273–414).

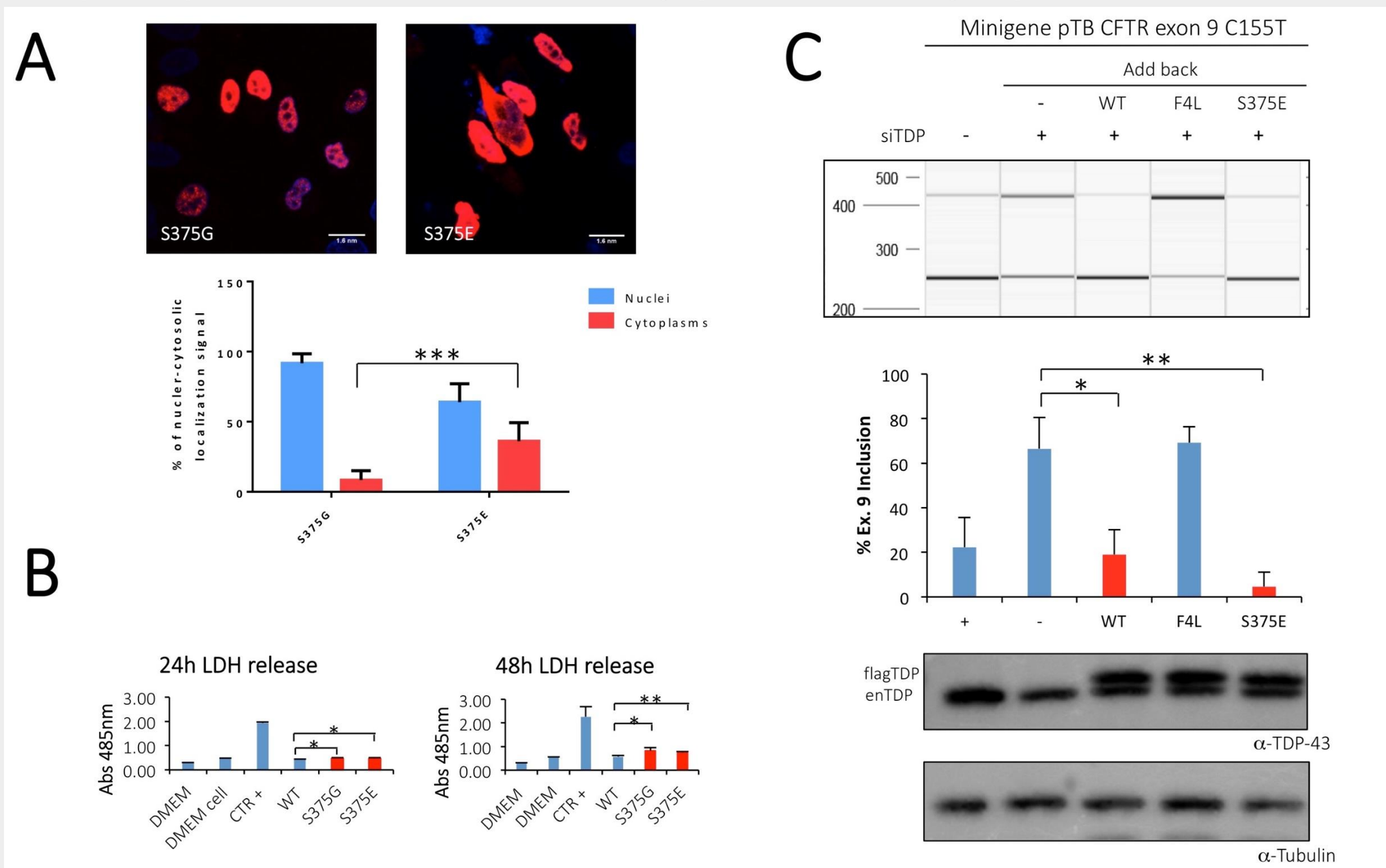
Mass spectrometric analysis of accumulated TDP-43 in amyotrophic lateral sclerosis brains

Fuyuki Kametani[1], Tomokazu Obi[2], Takeo Shishido[2], Hiroyasu Akatsu[3], Shigeo Murayama[4], Yuko Saito[5], Mari Yoshida[6] & Masato Hasegawa[1]

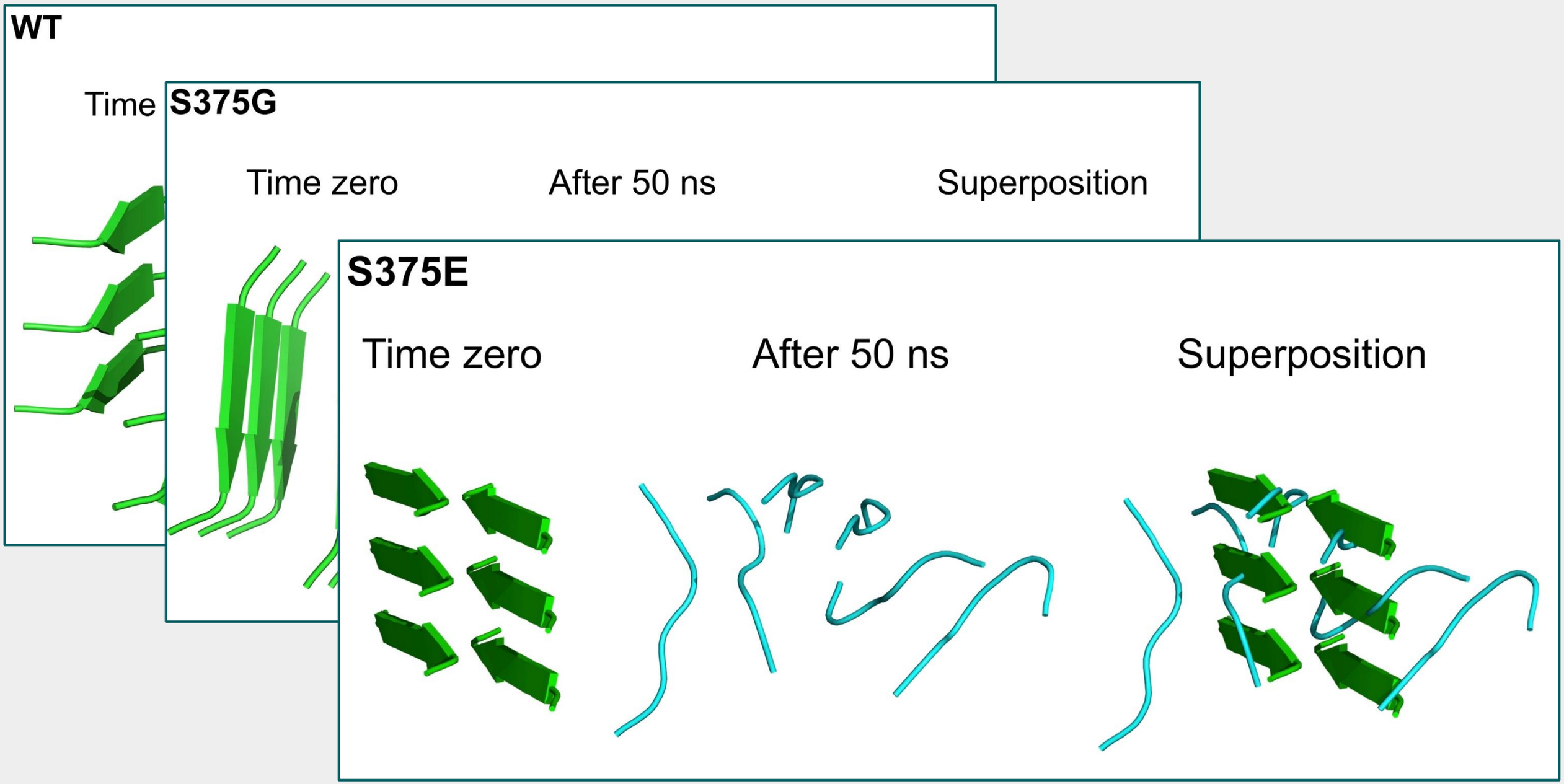
ALS case 1

ALS case 2

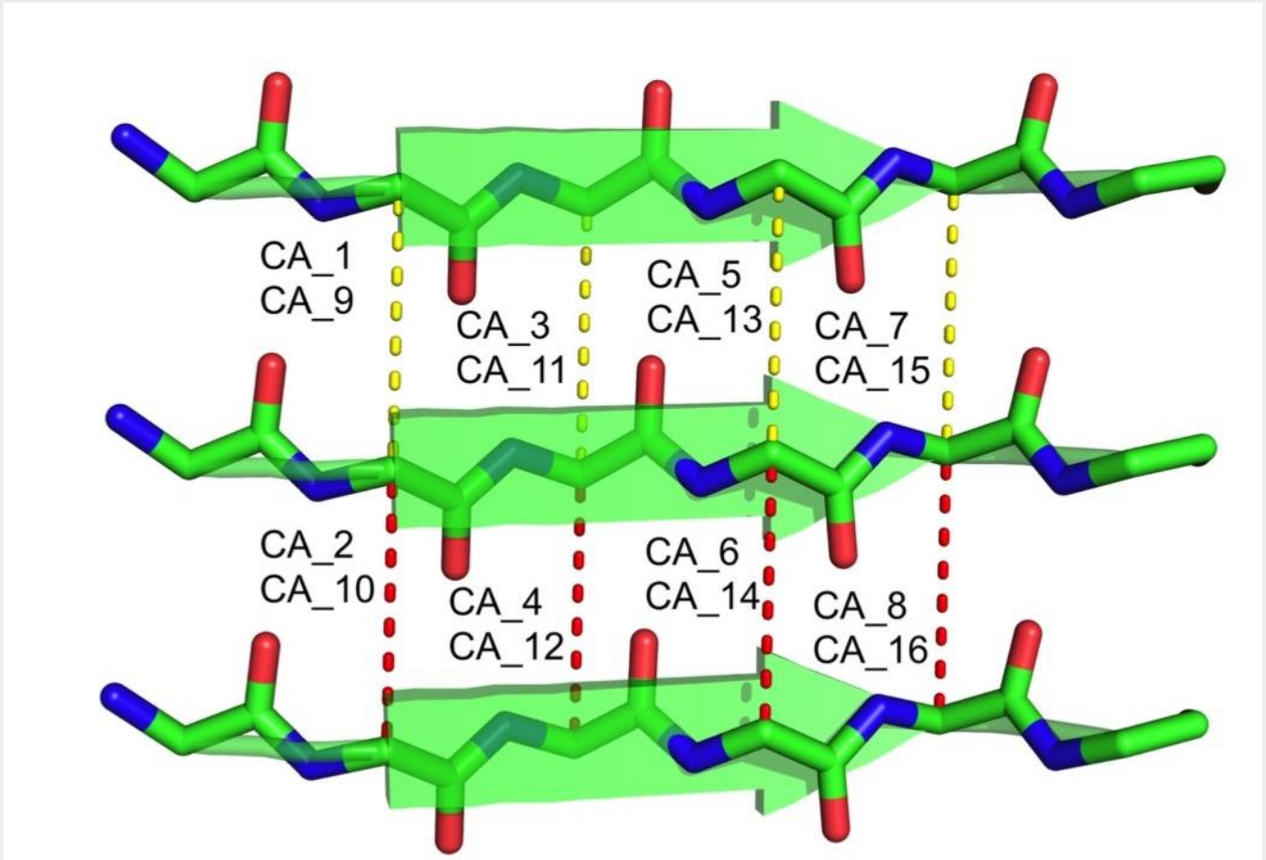
A S375E phosphomimic shows a very prominent cytoplasmic localization in 50% c

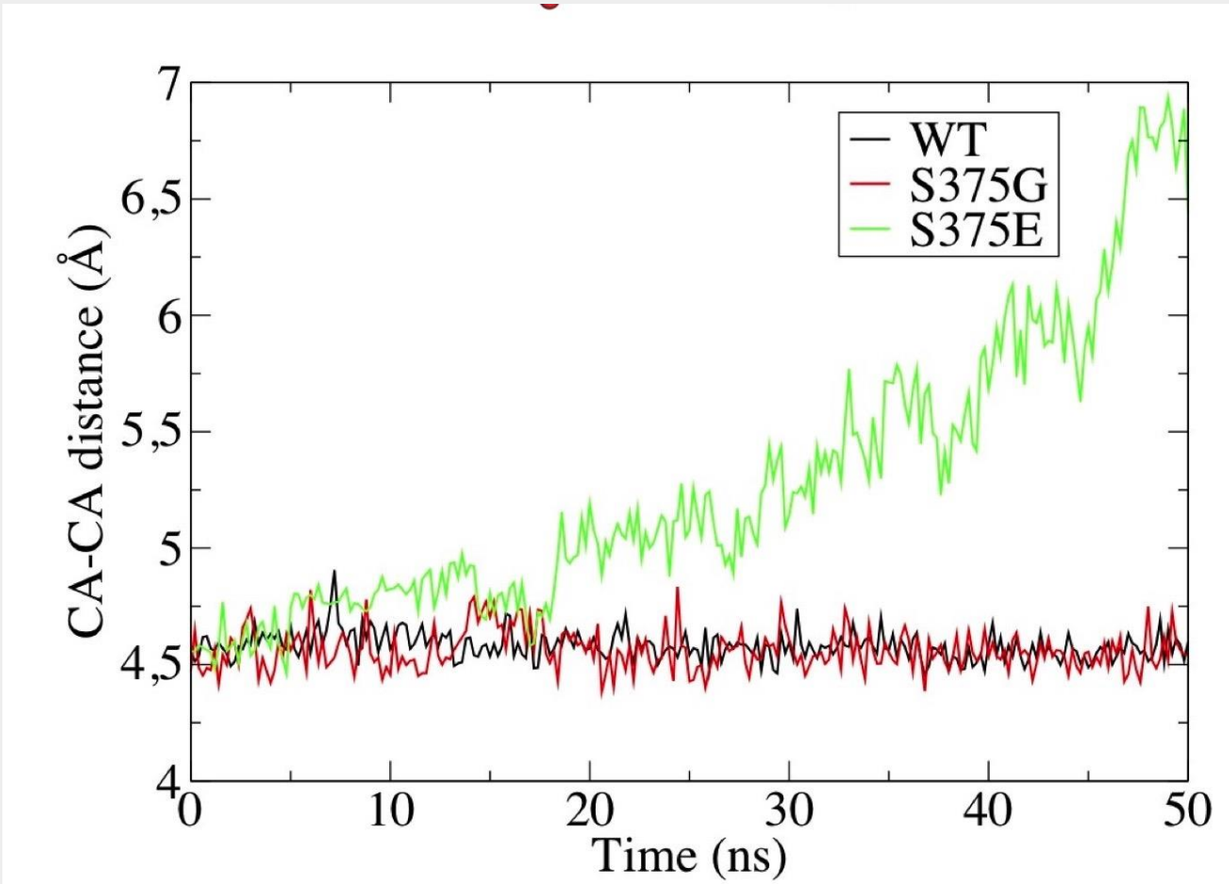


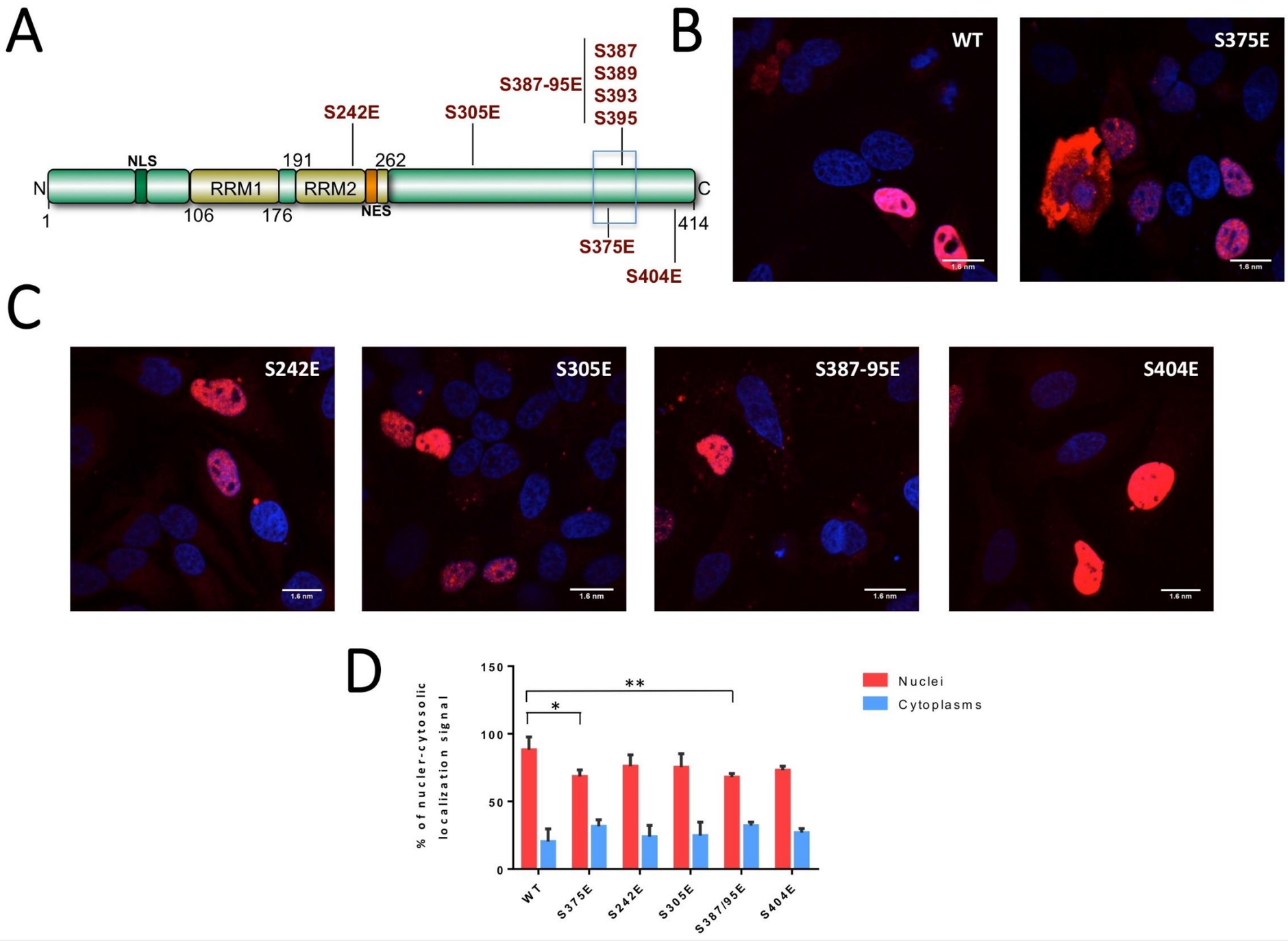
Molecular modelling confirms this possibility:



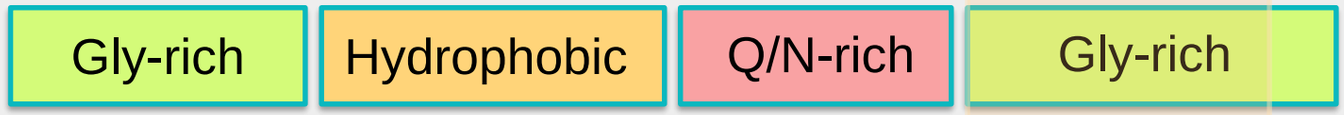
This is confirmed at the distance level between the beta strands:



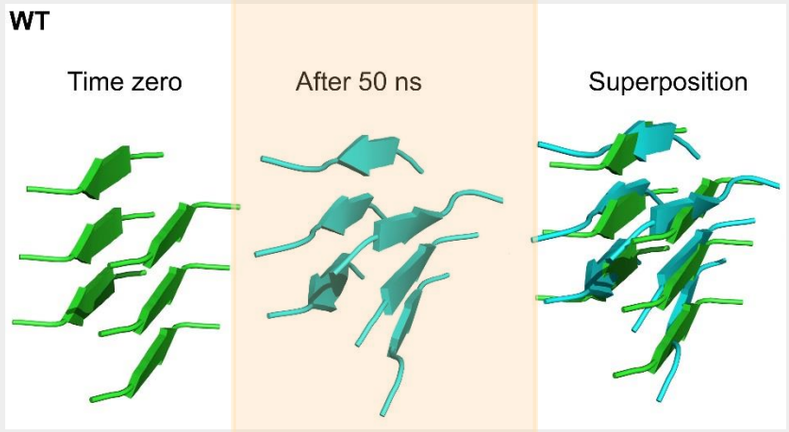



A
S387
S389
S393
S395
S387-95E
S242E
S305E
NLS
191
262
N
RRM1
RRM2
C
1
106
176
NES
414
S375E
S404E
B
WT
S375E
1.6 nm
C
S242E
S305E
S387-95E
S404E
D
% of nucler-cytosolic localization signal
150
100
50
0
**
*
Nuclei
Cytoplasms
WT
S375E
S242E
S305E
S387/95E
S404E

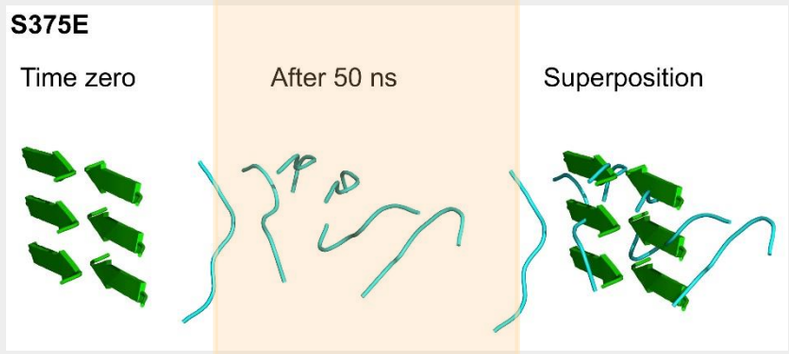
TDP-43's region from residues S375 to S395 is important to regulate nuclear-cytoplasmic shuttling through reversible phosphorylation.



Small sequences in the last Gly-rich domain can also fold to form beta-sheets.



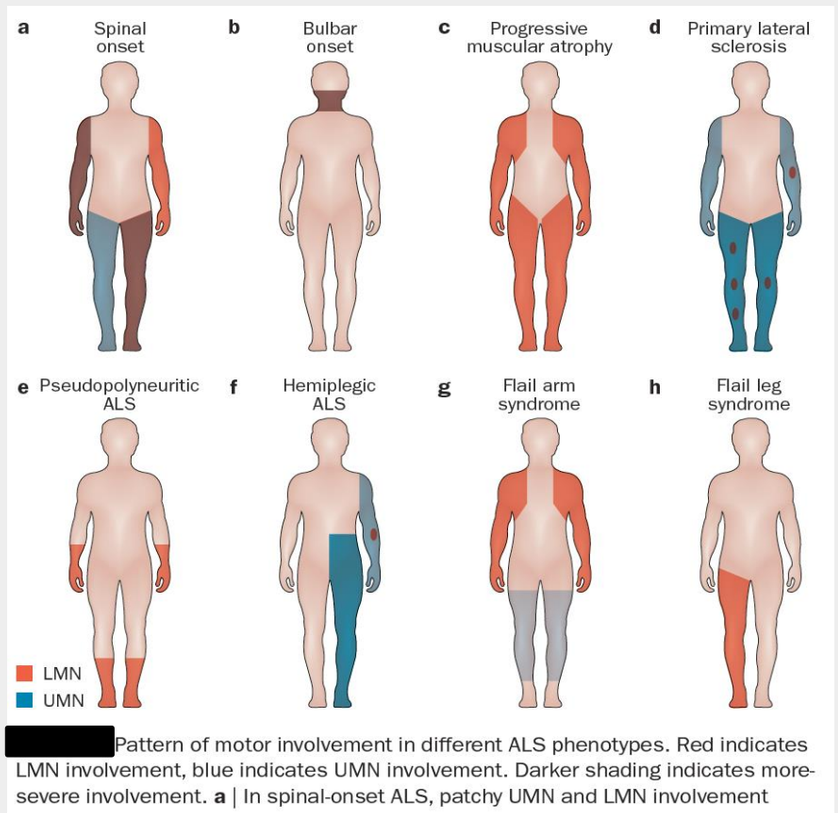
Phosphorylation can transiently affect this process, controlling TDP-43 localization in cellular nucleus/cytoplasm



Importance of studying the surrounding environment

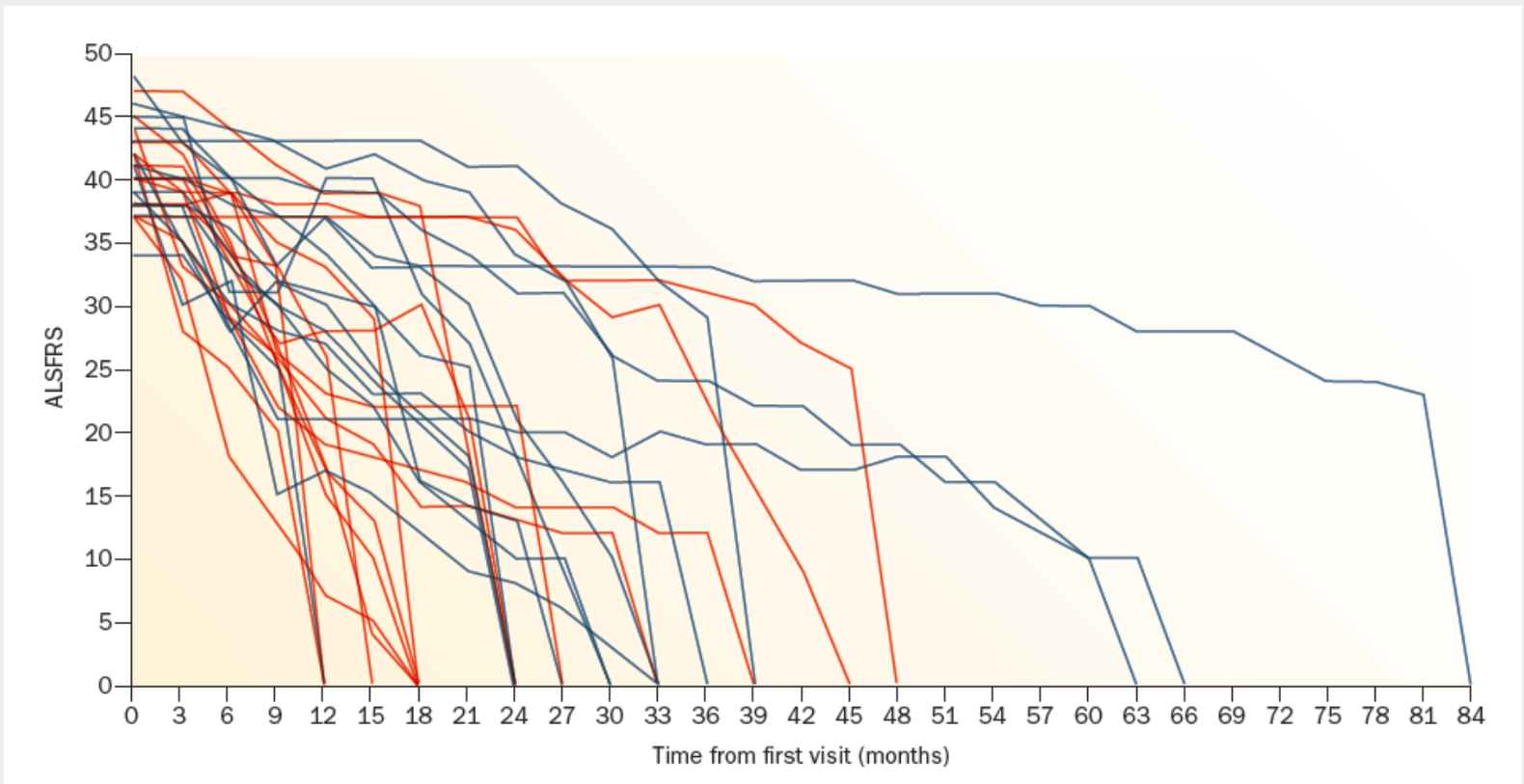
ALS is a very heterogenous disease, both regarding the clinical onset and the progression.

Clinical onset



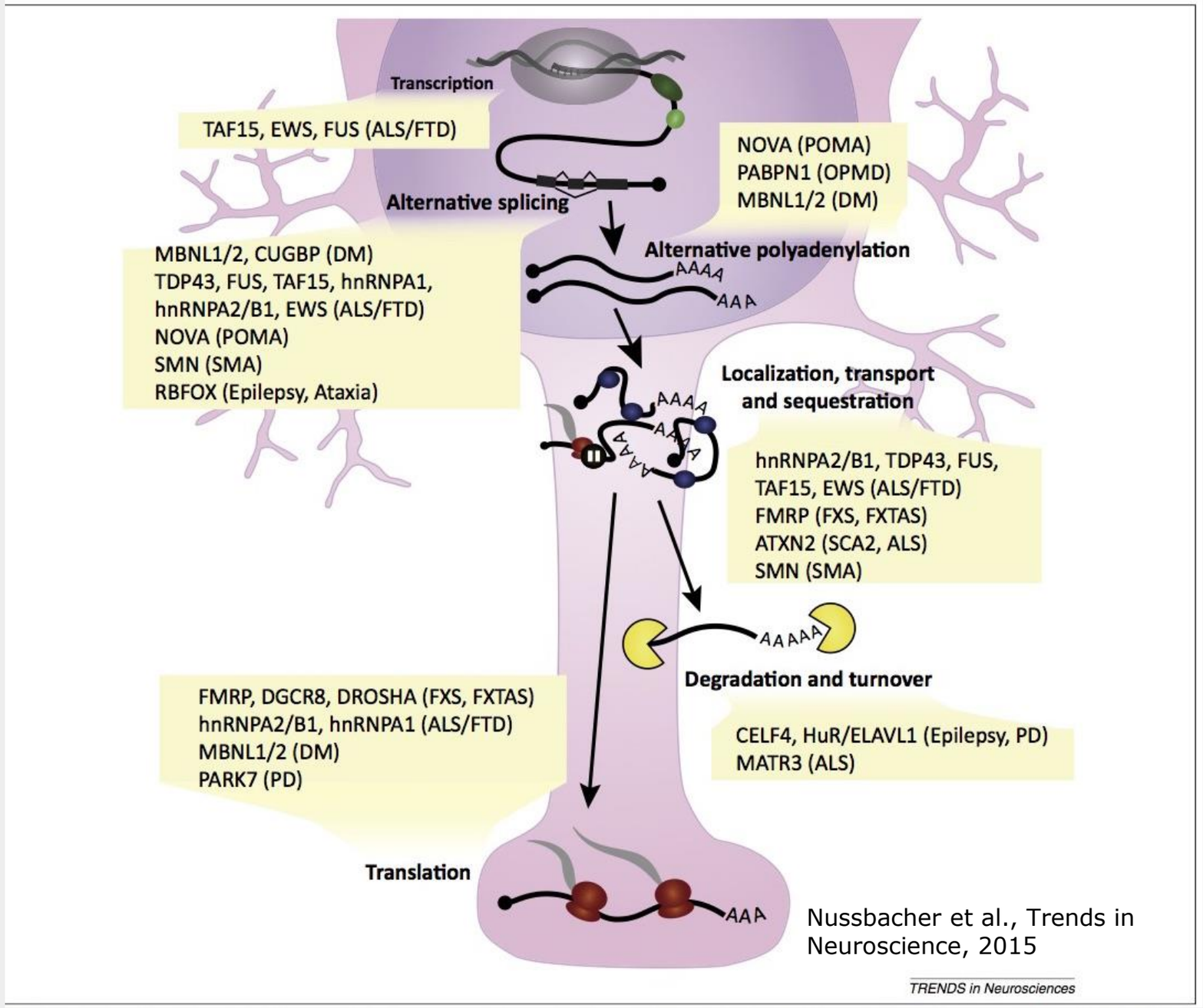
Pattern of motor involvement in different ALS phenotypes. Red indicates LMN involvement, blue indicates UMN involvement. Darker shading indicates more-severe involvement. **a** | In spinal-onset ALS, patchy UMN and LMN involvement

Progression



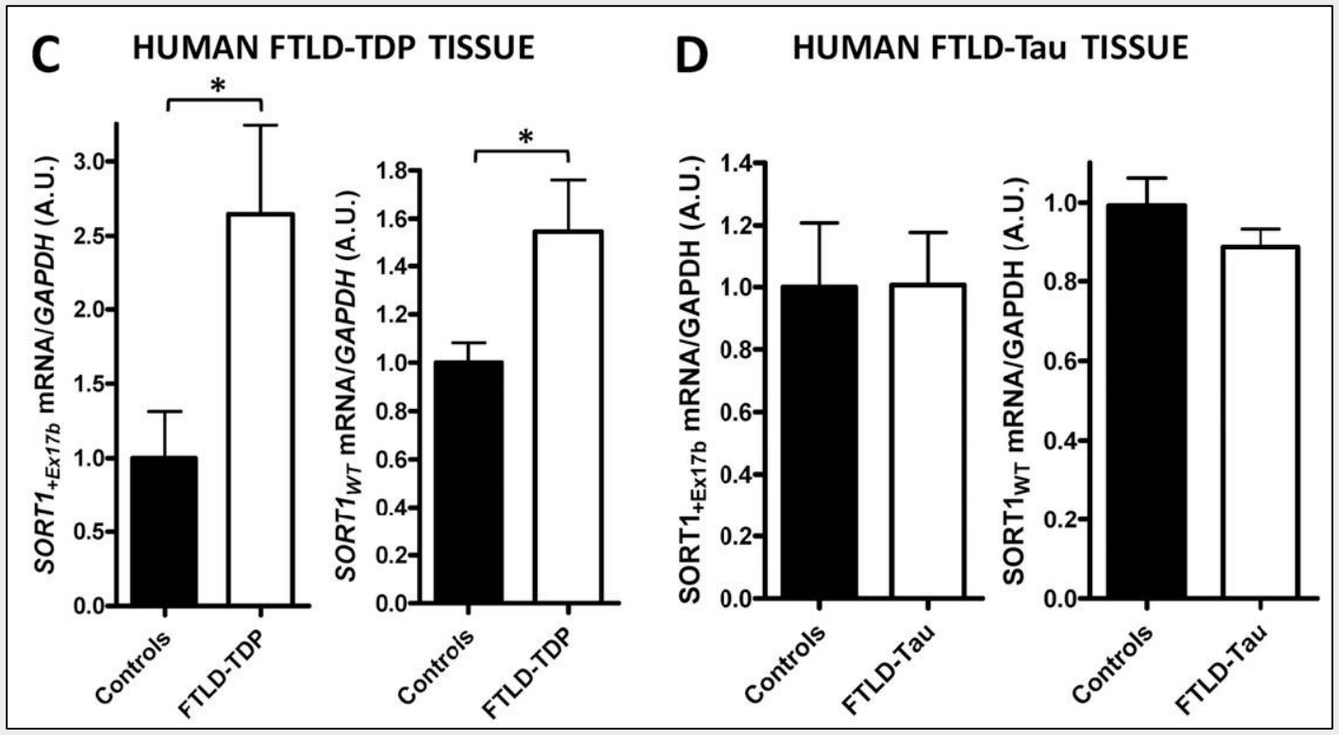
Swinnen and Robberecht, 2014

RNA binding proteins are particularly abundant within the neuronal body, axons, and synapses:

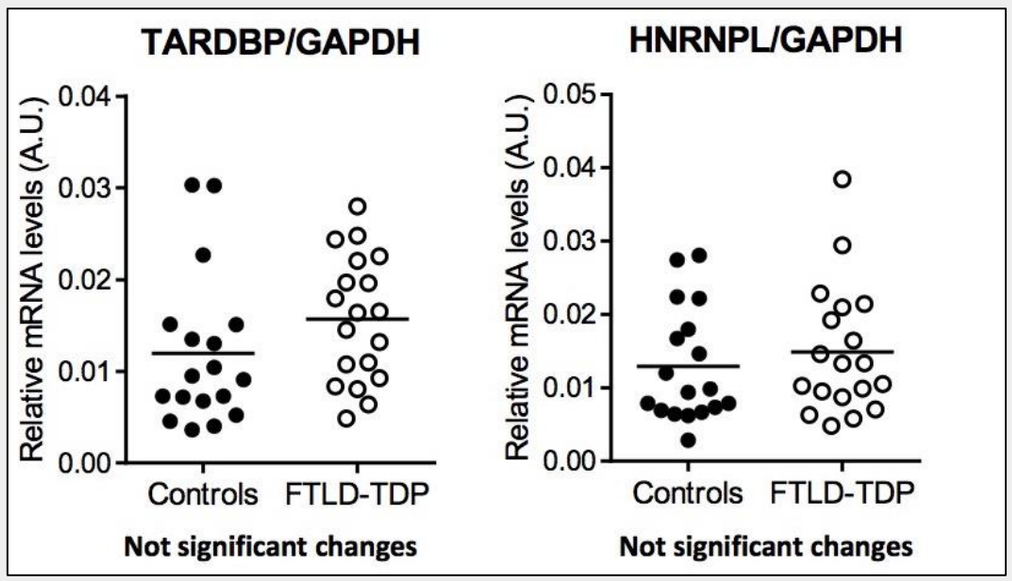


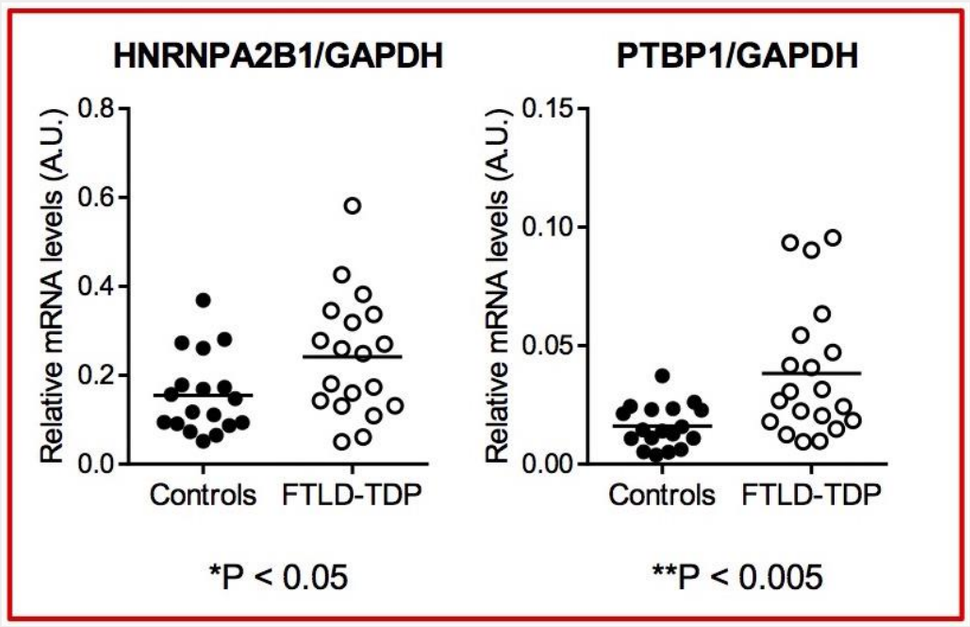
Nussbacher et al., Trends in Neuroscience, 2015

What happens in patients?, in FTLD-TDP the expression of these proteins can vary a lot both between and among healthy controls and diseased patients (work in collaboration with Leo Petrucelli and Mercedes Prudencio at the Mayo Clinic, Jacksonville):

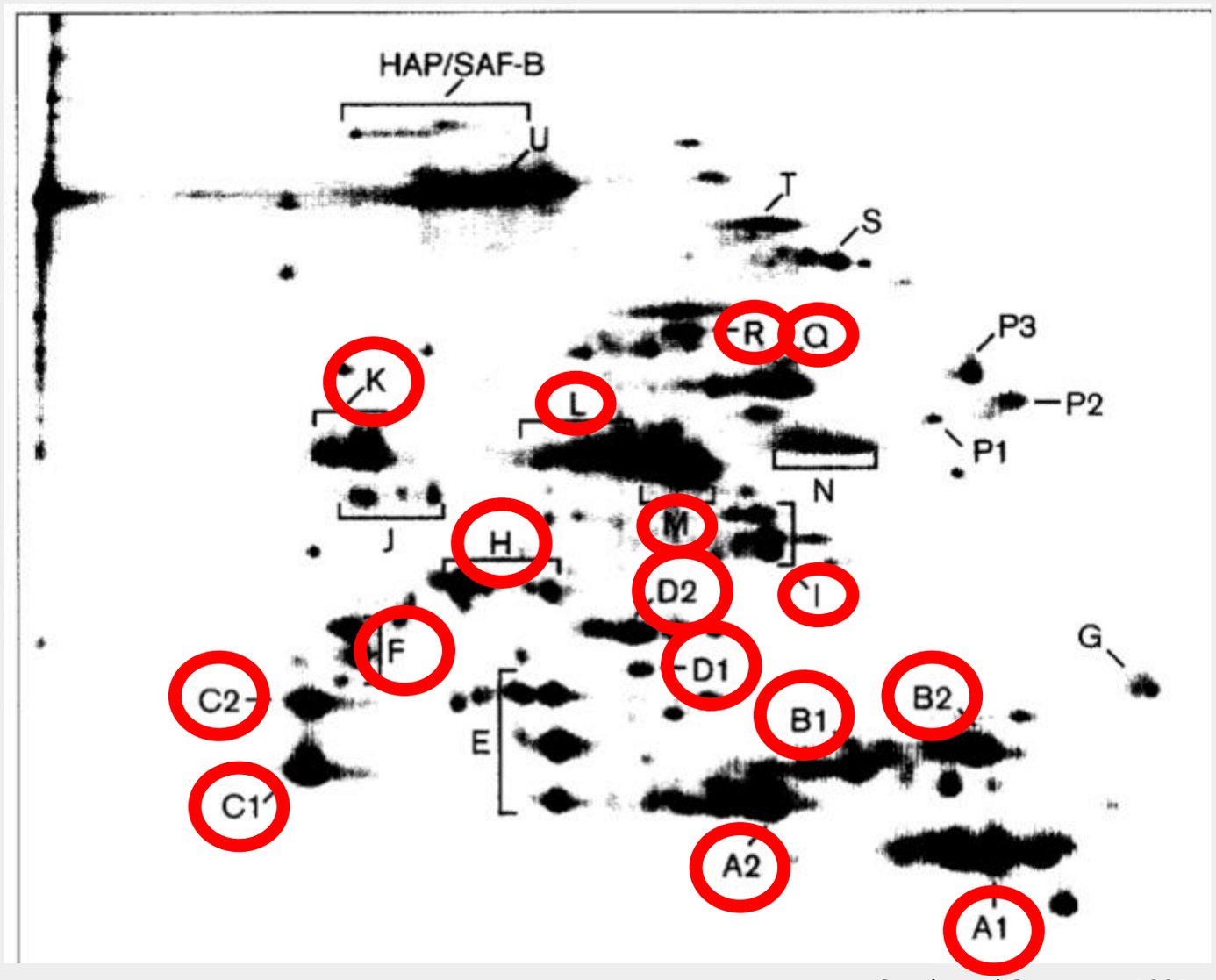


Mohagheghi et al., 2015





Most of the major hnRNP proteins in human cells are conserved in Drosophila



Crecic and Swanson, 1991

Human gene	Fly gene symbol	ID RNAi
hnRNP U	-/CG30122	106984 KK
hnRNP K	Bl/CG13425	2912 GD 105271 KK
hnRNP D	Sqd/CG16901	32395 GD
DAZAP1	Hrb27c/CG10377	16040 GD 16041 GD 101555KK
hnRNP C	-/CG42458	47828 GD 108072 KK
hnRNP F/H	Glo/CG6946	27752 GD 110653 KK
hnRNP R/Q	Syp/CG17838	33011 GD 33012 GD 110542 KK
hnRNP M	Rump/CG9373	44658 GD 44659 GD 1000001KK
hnRNP I	Heph/CG31000	33735 GD 110749 KK
hnRNP A3	Hrb87F/CG12749	100732 KK
hnRNP L	Sm/CG9218	28117 GD 108351 KK

In collaboration with the Neurology group we have started a search for hnRNP modifiers of TDP-43 pathology:

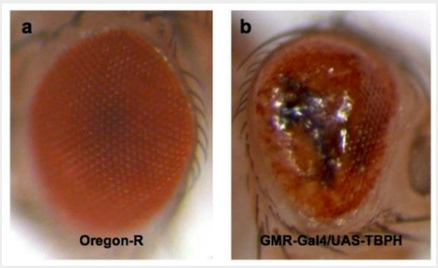
Loss-of-function fly disease model

Flies expressing 50% normal TDP-43 levels (haploinsufficient)

Flies expressing 1-2% normal TDP-43 levels (hypomorphic)

Gain-of-function fly disease model

Flies overexpressing wild-type TDP-43 in eye (hypomorphic)



Crossing with fly strains that can be silenced for all major hnRNPs conserved between flies and humans:

Human gene	Fly gene symbol	ID RNAi
hnRNP U	-/CG30122	106984 KK
hnRNP K	Bl/CG13425	2912 GD 105271 KK
hnRNP D	Sqd/CG16901	32395 GD
DAZAP1	Hrb27c/CG10377	16040 GD 16041 GD 101555KK
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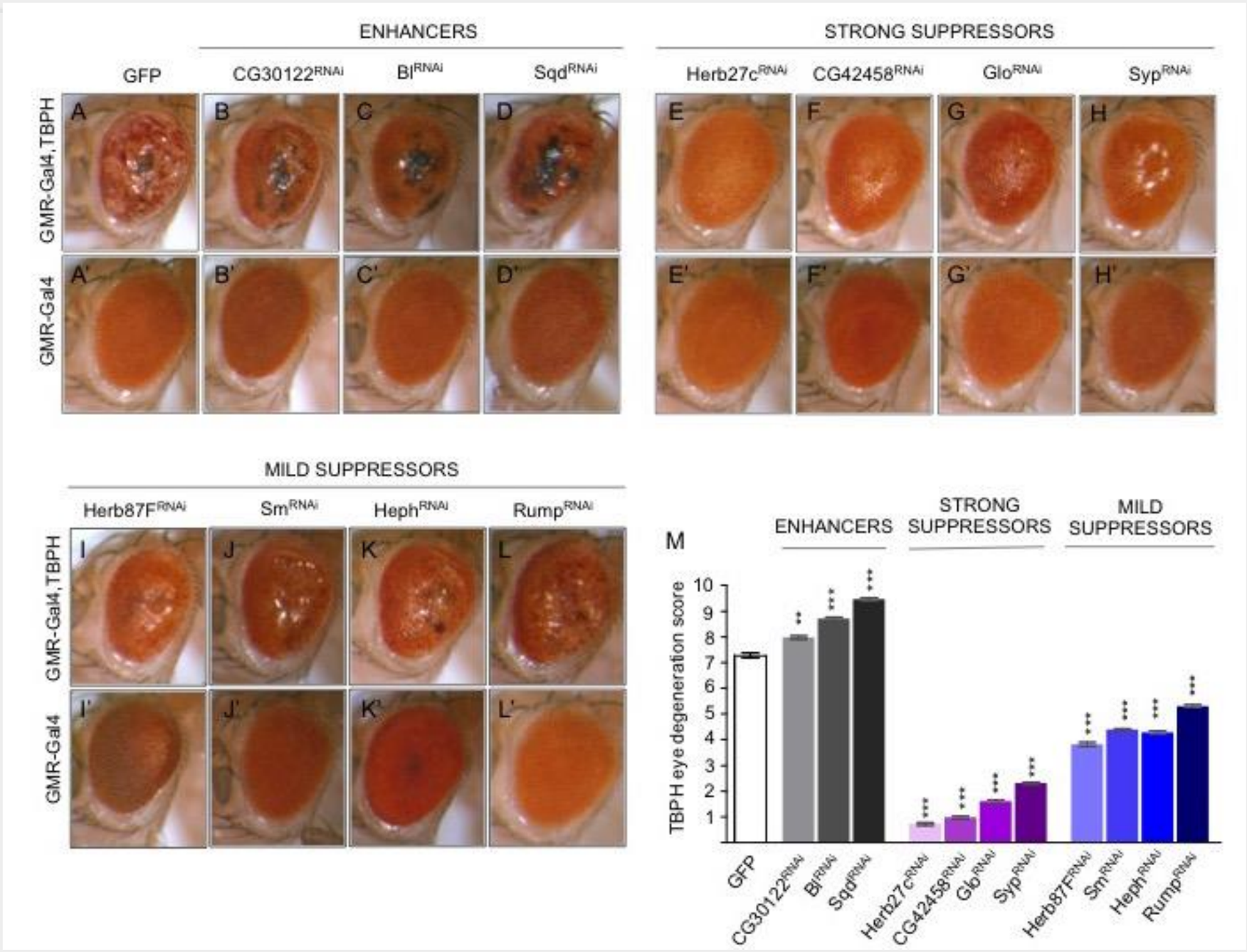
hnRNP I	Heph/CG31000	33735 GD 110749 KK
hnRNP A3	Hrb87F/CG12749	100732 KK
hnRNP L	Sm/CG9218	28117 GD 108351 KK

Validation of functional relationship in Human neuronal cells

RNA sequencing analysis of promising transcripts

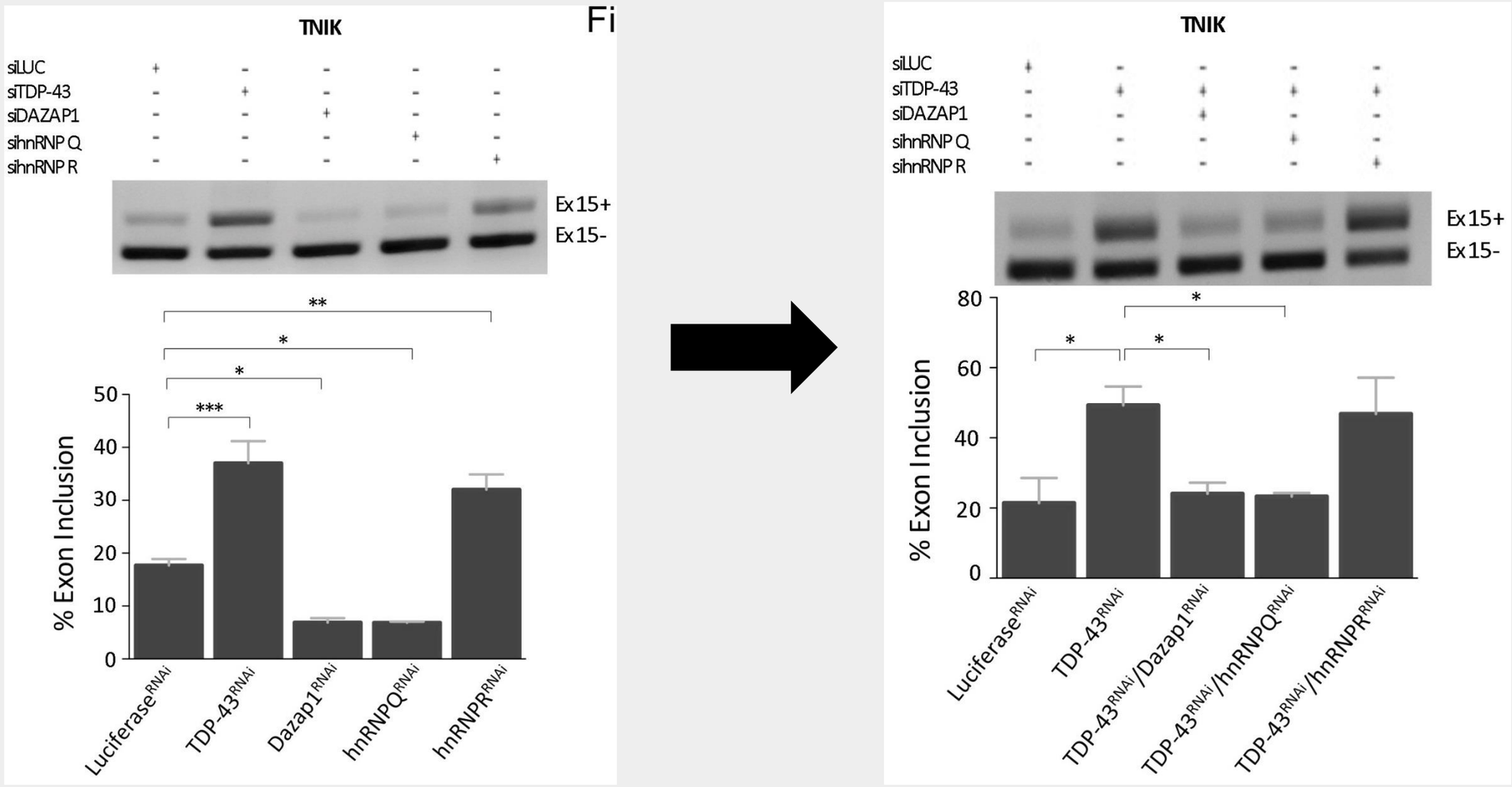
Validate the effect of individual transcript to modulate TDP43-associated pathology

In addition to a small group of phenotype enhancers we were able to find a group of strong suppressors (Hrb27c, CG42458, Glo and Syp) which silencing, rescued almost completely TBPH phenotype (Fig. 1 E-H, M) and a group of mild suppressors (Herb87F, Sm, Heph and Rump) that recovered only partially TBPH defects (Fig. I-L, M) .

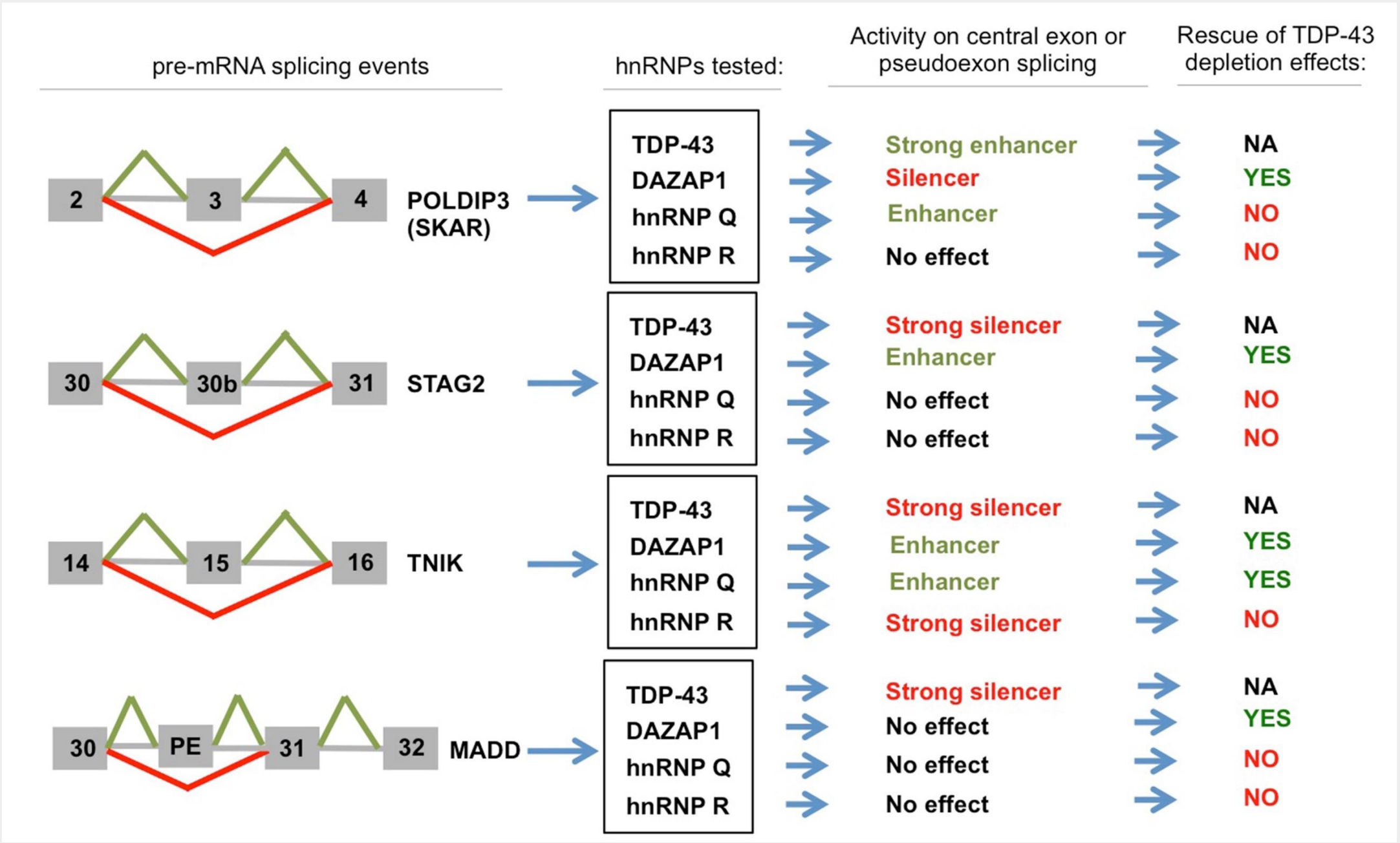


Appocher et al. 2017

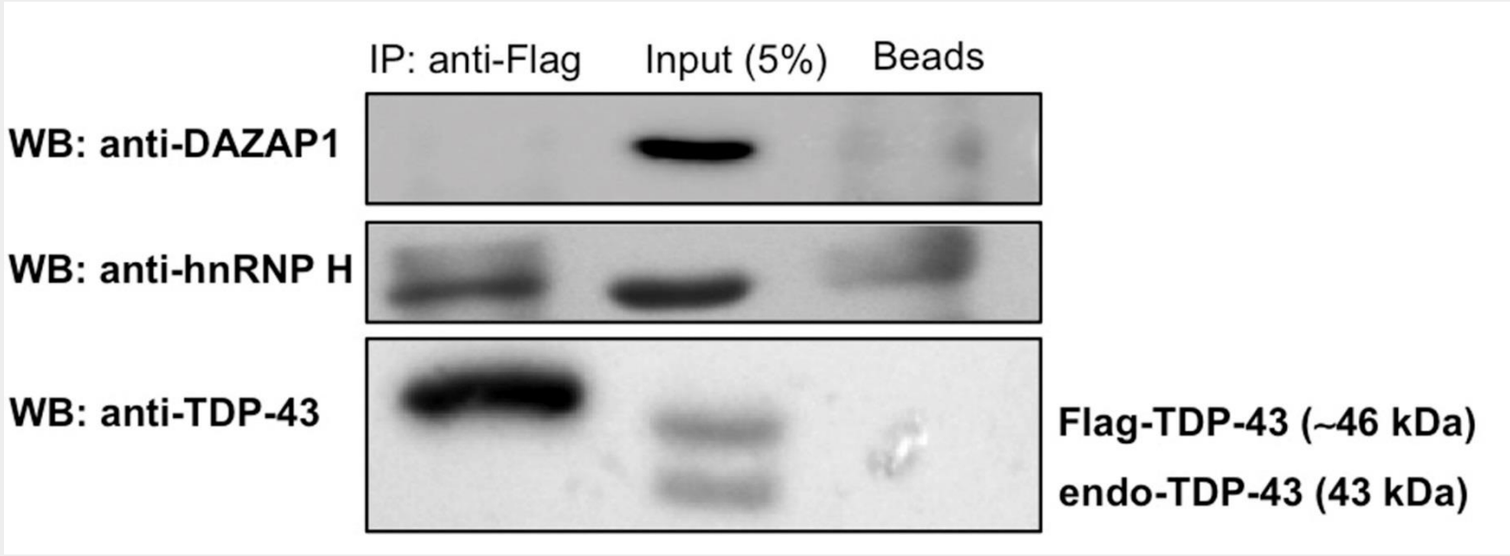
Can the human homologues of these proteins affect human TDP-43 functions as well?.



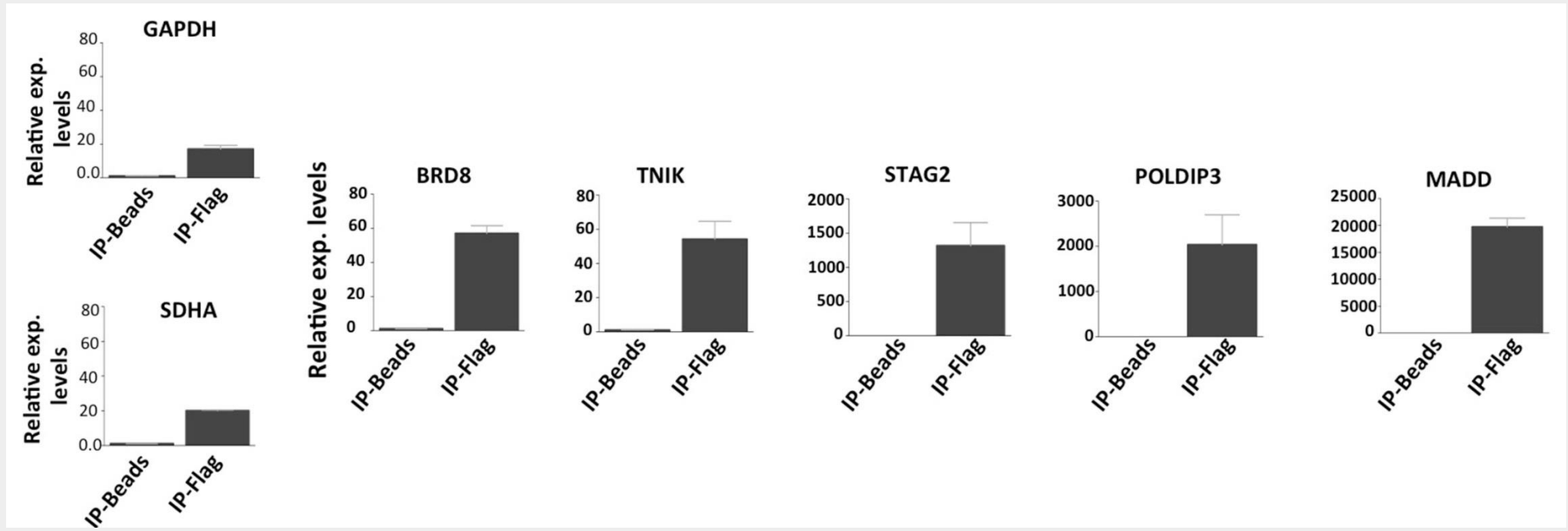
This has also been seen for several other examples of TDP43-controlled genes.



DAZAP1 does not bind to TDP-43.



However, it binds to all these TDP-43 controlled transcripts.



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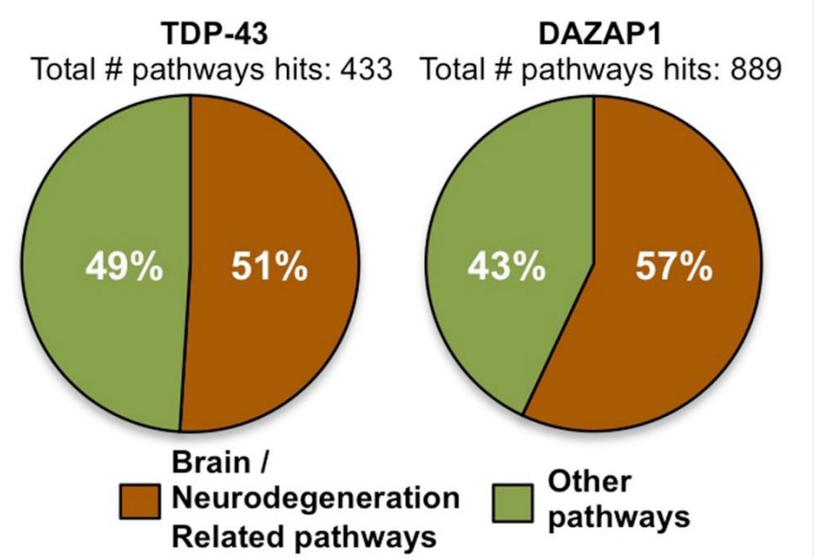
Performing RNAseq analysis to find the co-targets and affected pathways of TDP-43 and DAZAP1 in SH-SY-5Y cells:



Total genes analyzed	17147
Downregulated (<0.7 vs siLuc):	
siTDP-43	1173
siDAZAP1	3244
siTDP-43/siDAZAP1 common	484

Total genes analyzed	17147
Upregulated (>1.3 vs siLuc):	
siTDP-43	2360
siDAZAP1	4327
siTDP-43/siDAZAP1 common	215

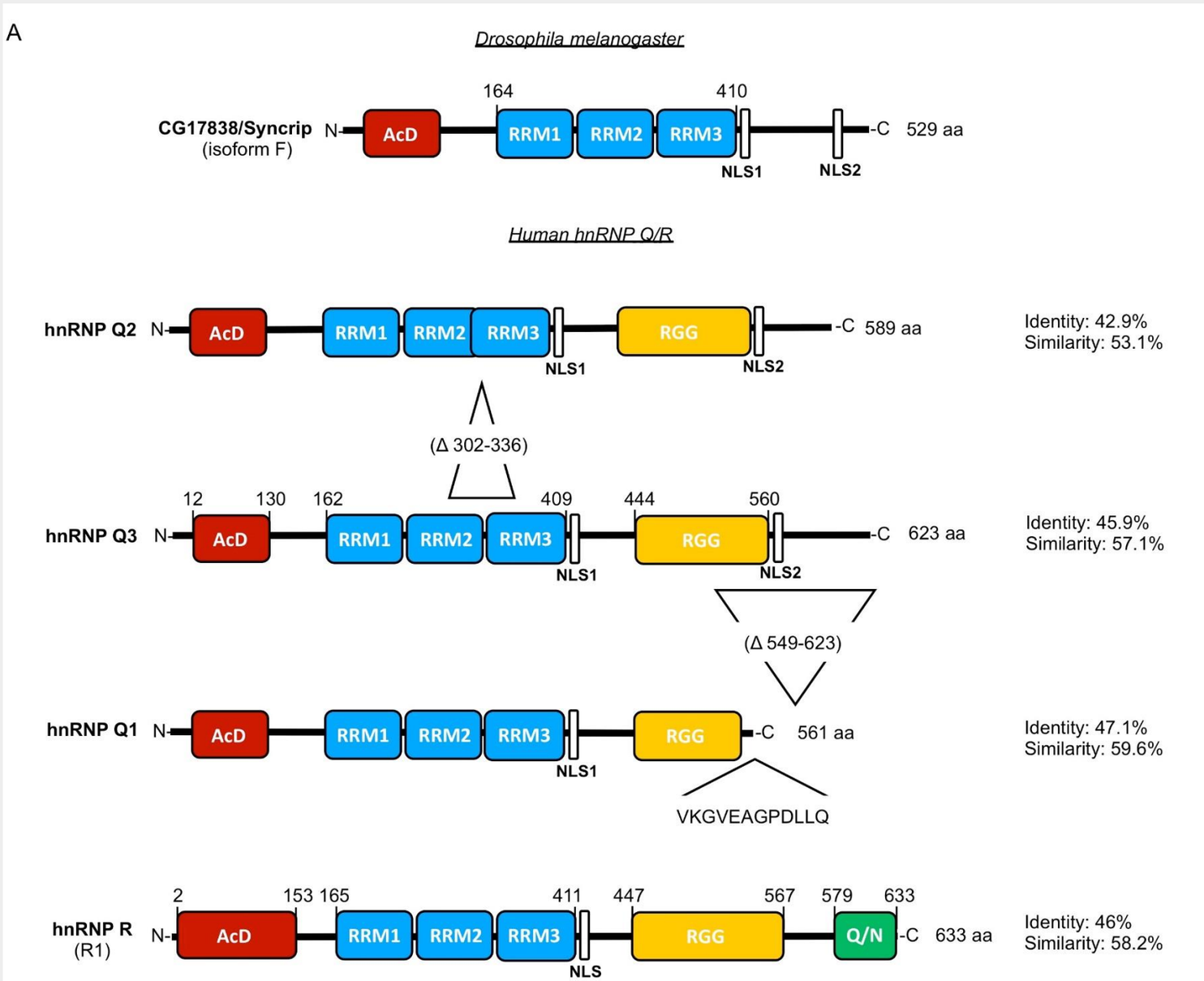
Pathways enrichment of common Downregulated genes (<0.7 vs siLuc)

	Total # mapped genes: 309	Total # pathways hits: 165
Genes related with:		%
Inflammation	48	29.1
Neurodegenerative diseases	12	7.3
Nervous system	29	17.6

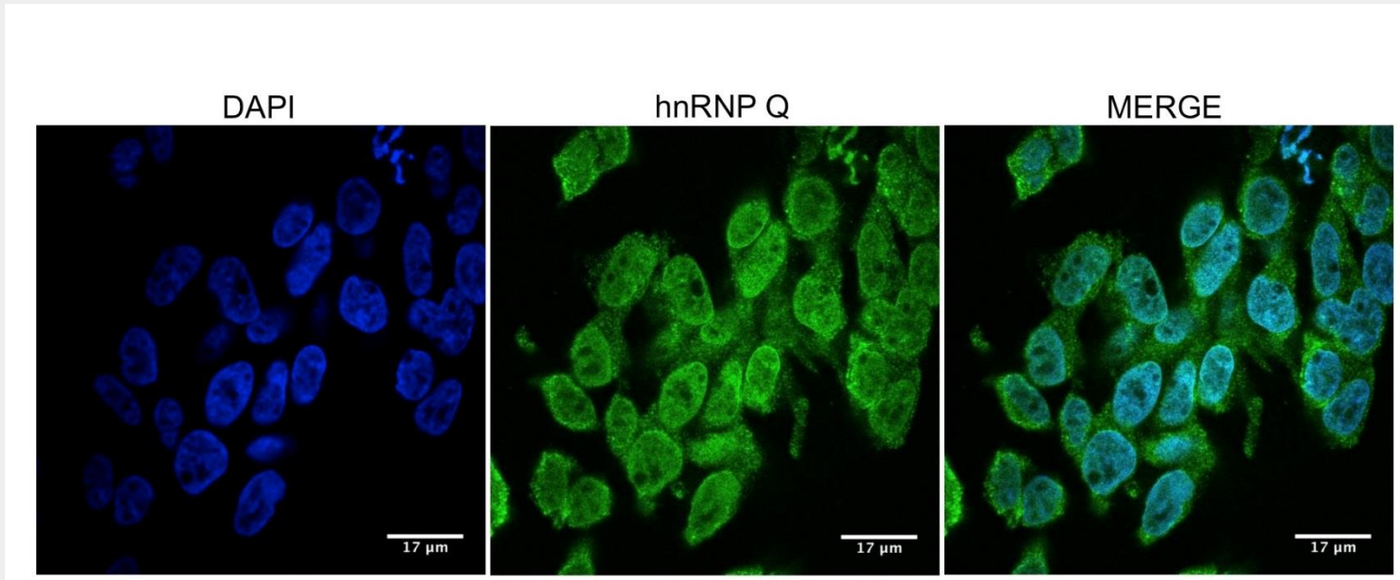
Pathways enrichment of common Upregulated genes (>1.3 vs siLuc)

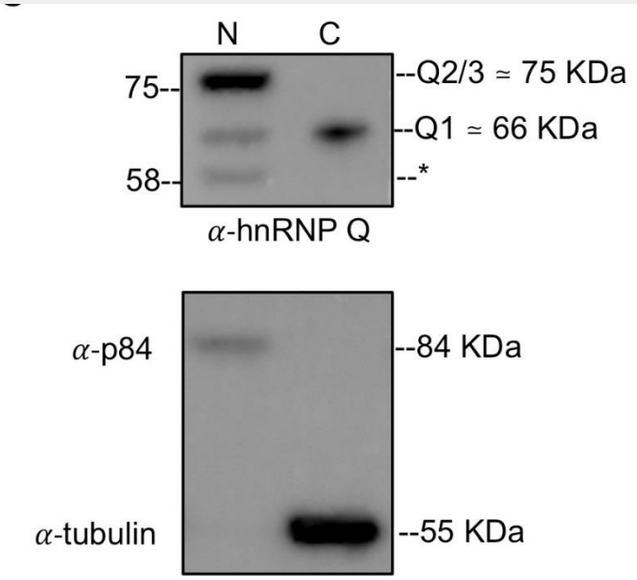
	Total # mapped genes: 918	Total # pathways hits: 337
Genes related with:		%
Inflammation	95	28.2
Neurodegenerative diseases	40	11.9
Nervous system	35	10.4

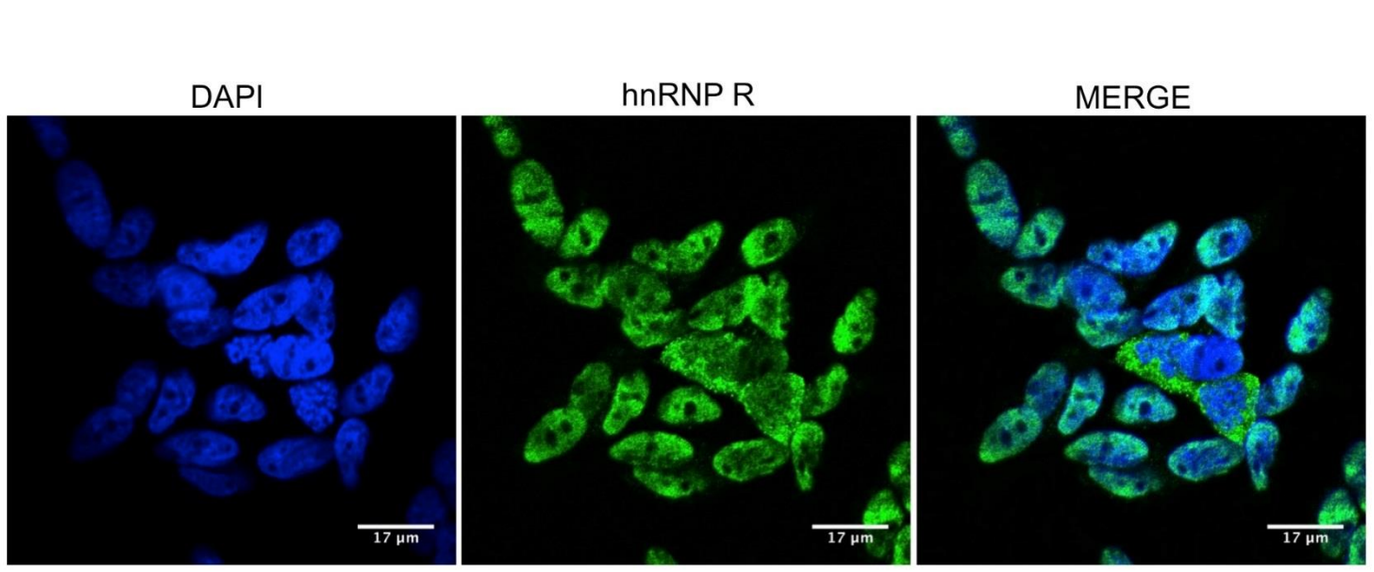
hnRNP Q and R proteins share considerable similarity with the fly Syp protein

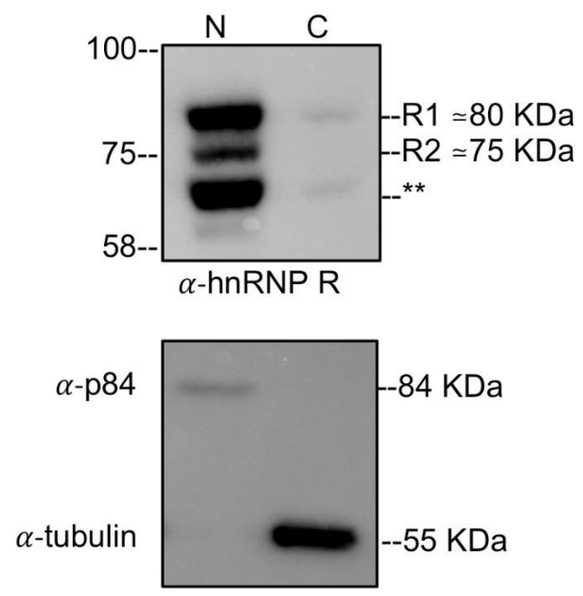


However, these two proteins show marked diferences in cellular subdistribution

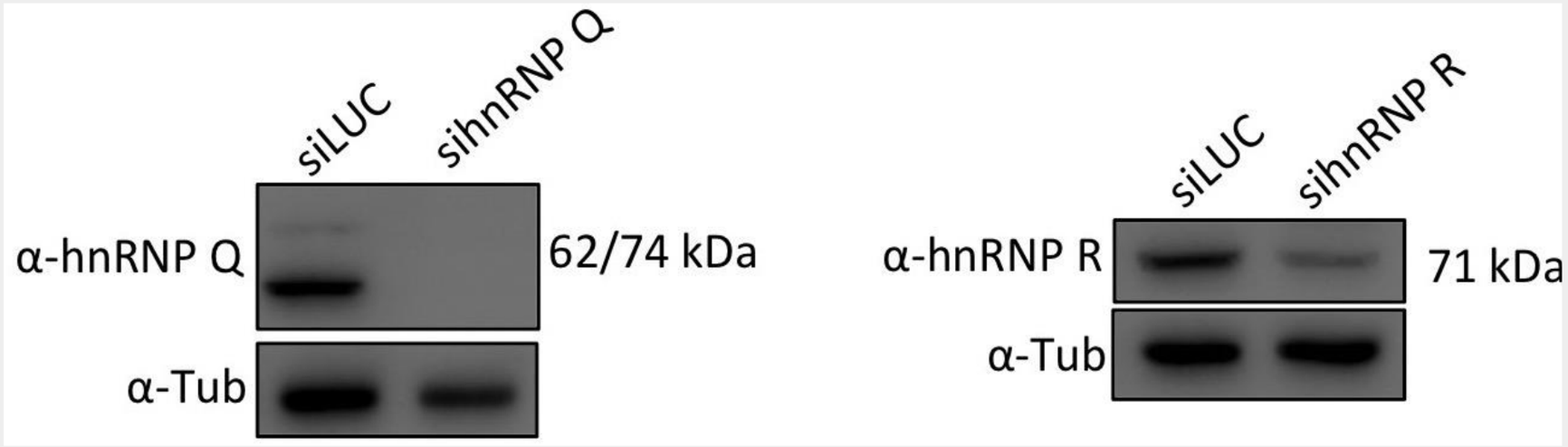








RNAseq analysis of hnRNP Q and hnRNP R in SH-SY5Y cells



hnRNP Q

Total gene analyzed	17147
DEGs (30% cut-off vs siLUC; pValue <0.05):	
Upregulated	1380 (49%)
Downregulated	1439 (51%)

hnRNP R

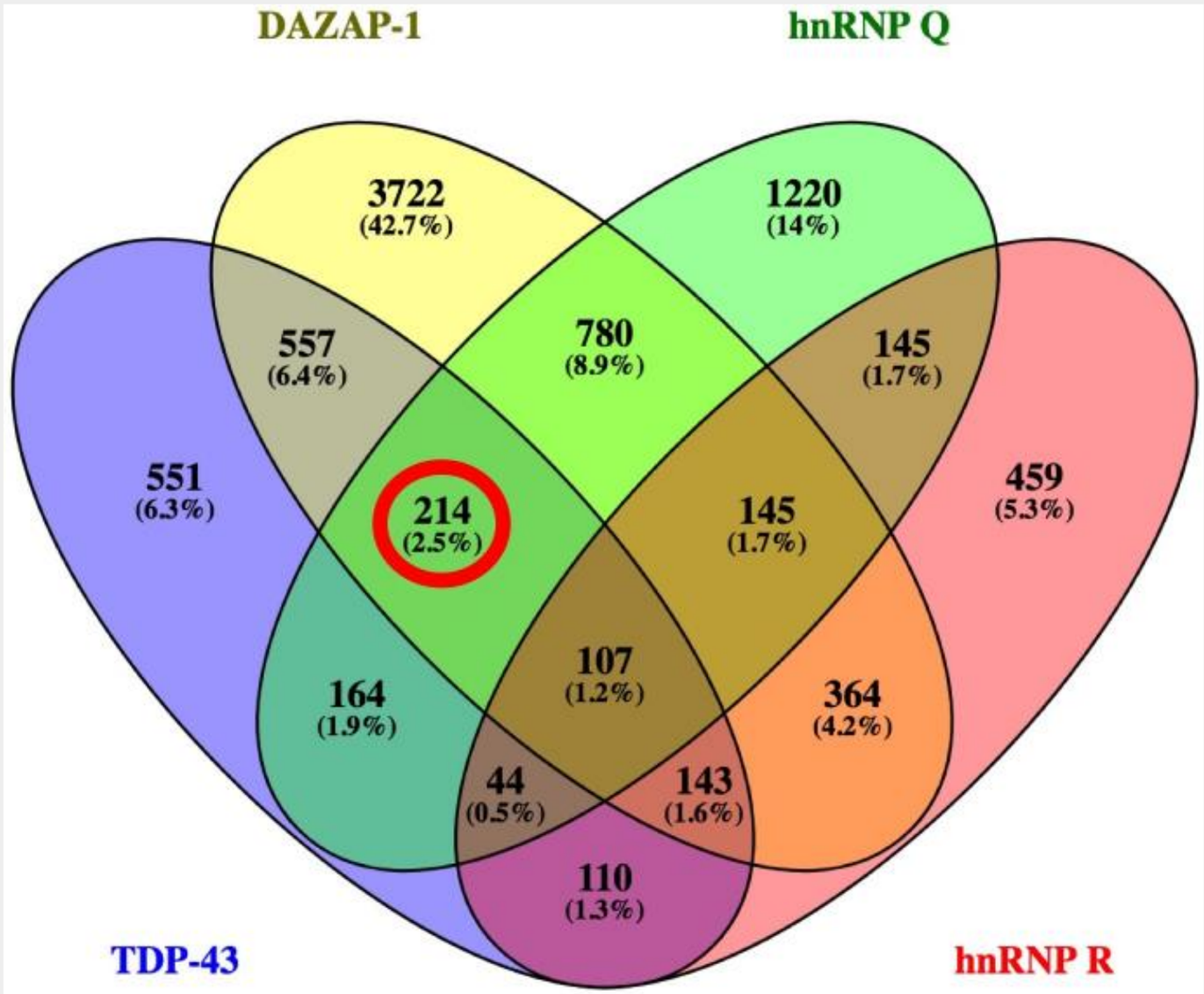
Total gene analyzed	17147
DEGs (30% cut-off vs siLUC; pValue <0.05):	
Upregulated	957 (63%)
Downregulated	560 (37%)

Top regulated DEGs that relate to neurodegeneration do not correspond:

ENSEMBL	Gene Symbol	Description	RNAseq
ENSG00000232810	TNF	TNF, tumor necrosis factor	24.52
ENSG00000090339	ICAM1	ICAM1, intercellular adhesion molecule 1	17.45
ENSG00000181195	PENK	PENK, proenkephalin	15.96
ENSG00000049249	TNFRSF9	TNFRSF9, tumor necrosis factor receptor superfamily, member 9	9.87
ENSG00000136826	KLF4	KLF4, Kruppel-like factor 4 (gut)	8.28
ENSG00000102271	KLHL4	KLHL4, kelch-like family member 4	7.95
ENSG00000185737	NRG3	NRG3, neuregulin 3	5.57
ENSG00000167964	RAB26	RAB26, RAB26, member RAS oncogene family	0.18
ENSG00000147256	ARHGAP36	ARHGAP36, Rho GTPase activating protein 36	0.14
ENSG00000169551	CT55	CT55, cancer/testis antigen 55	0.12

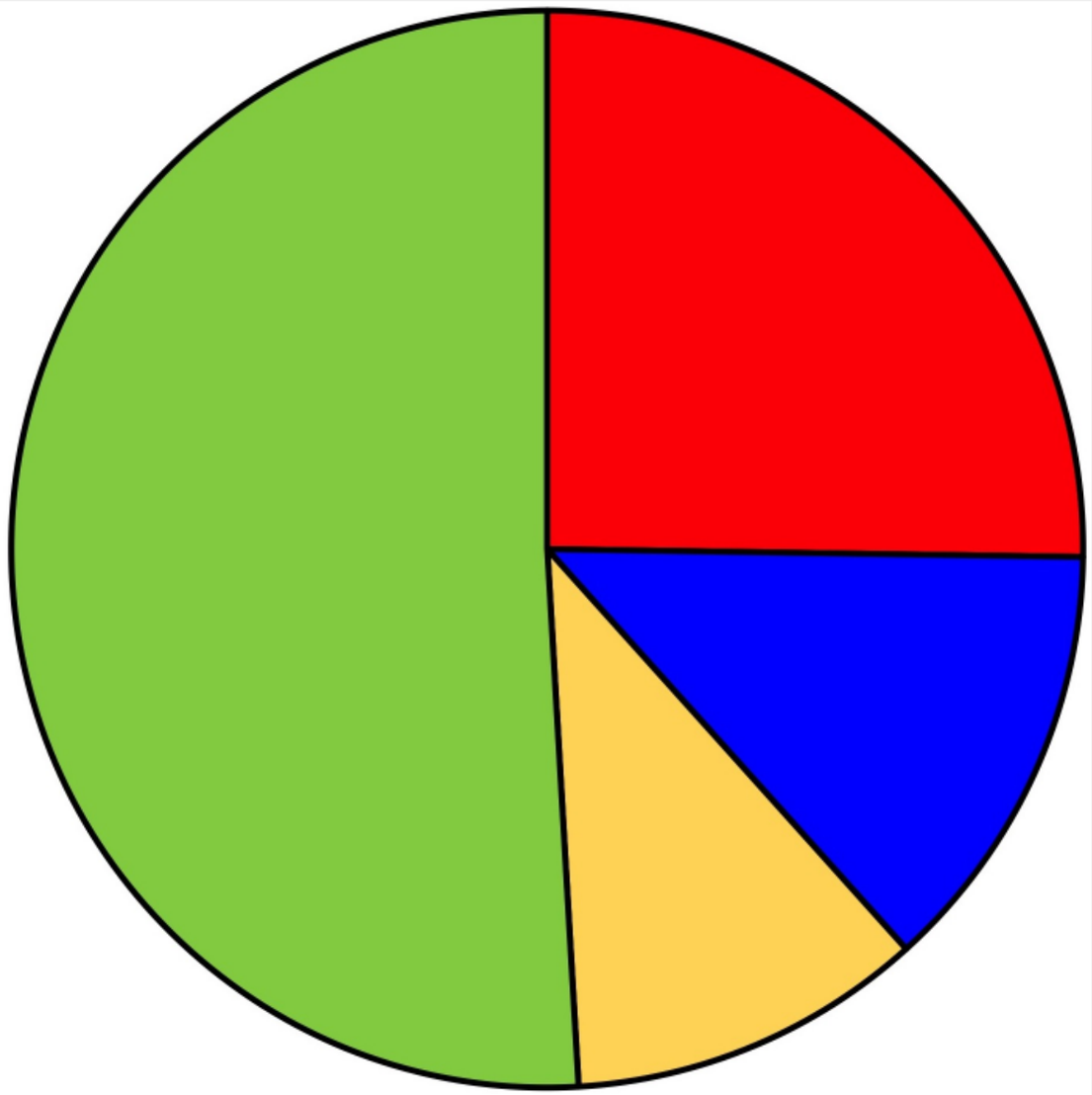
ENSEMBL	Gene Symbol	Description	RNAseq
ENSG00000164326	CARTPT	CARTPT, CART prepropeptide	24.59
ENSG00000125740	FOSB	FOSB, FBJ murine osteosarcoma viral oncogene homolog B	10.96
ENSG00000101384	JAG1	JAG1, jagged 1	5.70
ENSG00000105376	ICAM5	ICAM5, intercellular adhesion molecule 5, telencephalin	4.34
ENSG00000140254	DUOXA1	DUOXA1, dual oxidase maturation factor 1	3.91
ENSG00000100292	HMOX1	HMOX1, heme oxygenase (decycling) 1	3.36
ENSG00000169282	KCNAB1	KCNAB1, potassium voltage-gated channel, shaker-related subfamily, beta member 1	0.31
ENSG00000102575	ACP5	ACP5, acid phosphatase 5, tartrate resistant	0.25
ENSG00000125775	SDCBP2	SDCBP2, syndecan binding protein (syntenin) 2	0.18
ENSG00000115380	EFEMP1	EFEMP1, EGF containing fibulin-like extracellular matrix protein 1	0.18

Venn diagram comparing the number of transcripts altered in TDP-43, DAZAP1, hnRNP Q, and hnRNP R depleted SH-SY5Y cells.

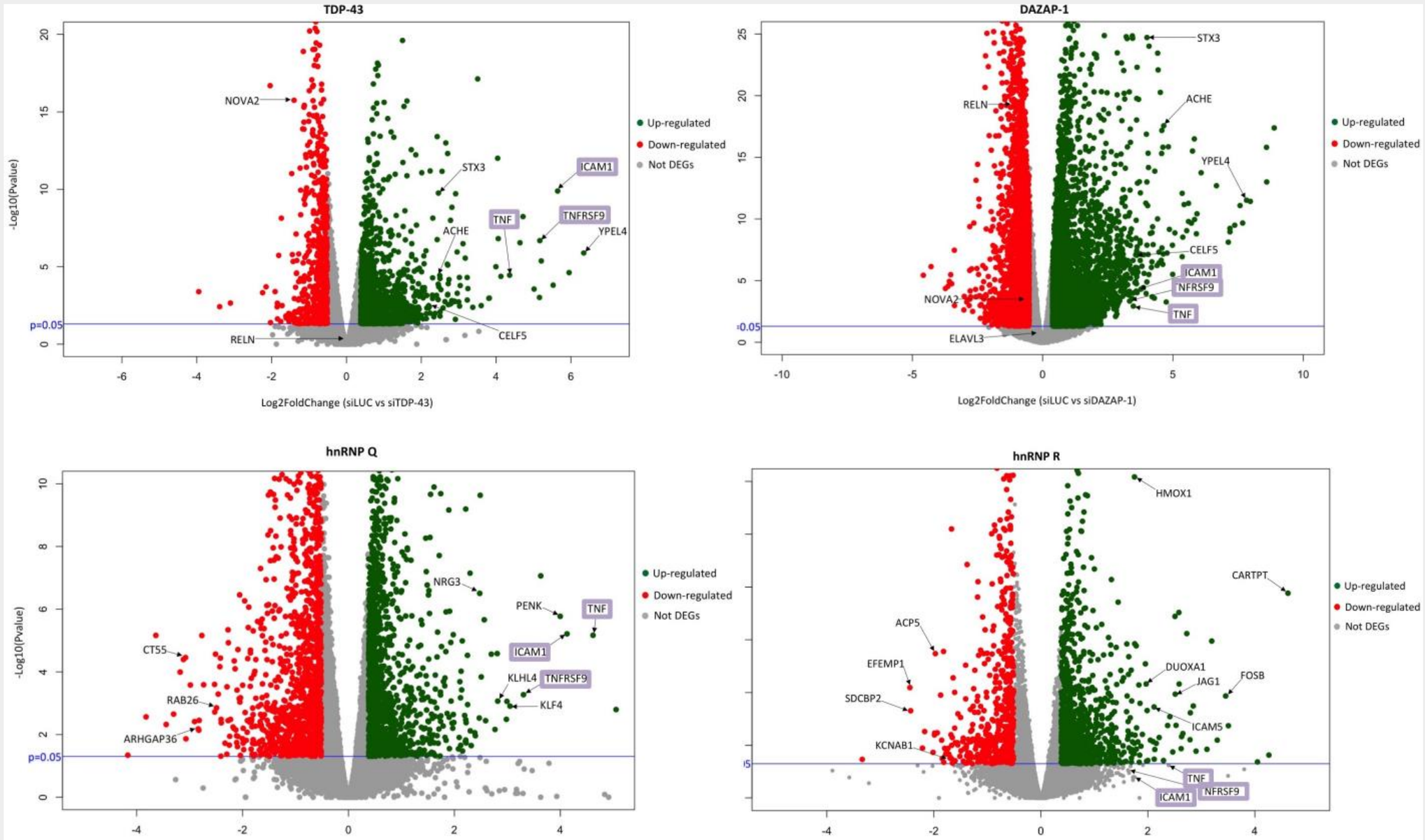


Pathways of genes co-regulated only by TDP-43, DAZAP1 and hnRNPQ. The classification of the 214 genes whose expression is co-regulated in TDP-43, DAZAP1, hnRNP Q, but not hnRNP R depleted SH-SY5Y cells according to the Gene Ontology categorization. systems .

Pathways	TDP-43/DAZAP1/hnRNPQ co-regulated genes
Neurological functions	54
Immune-related	28
Apoptosis-related	23
Other pathways	109

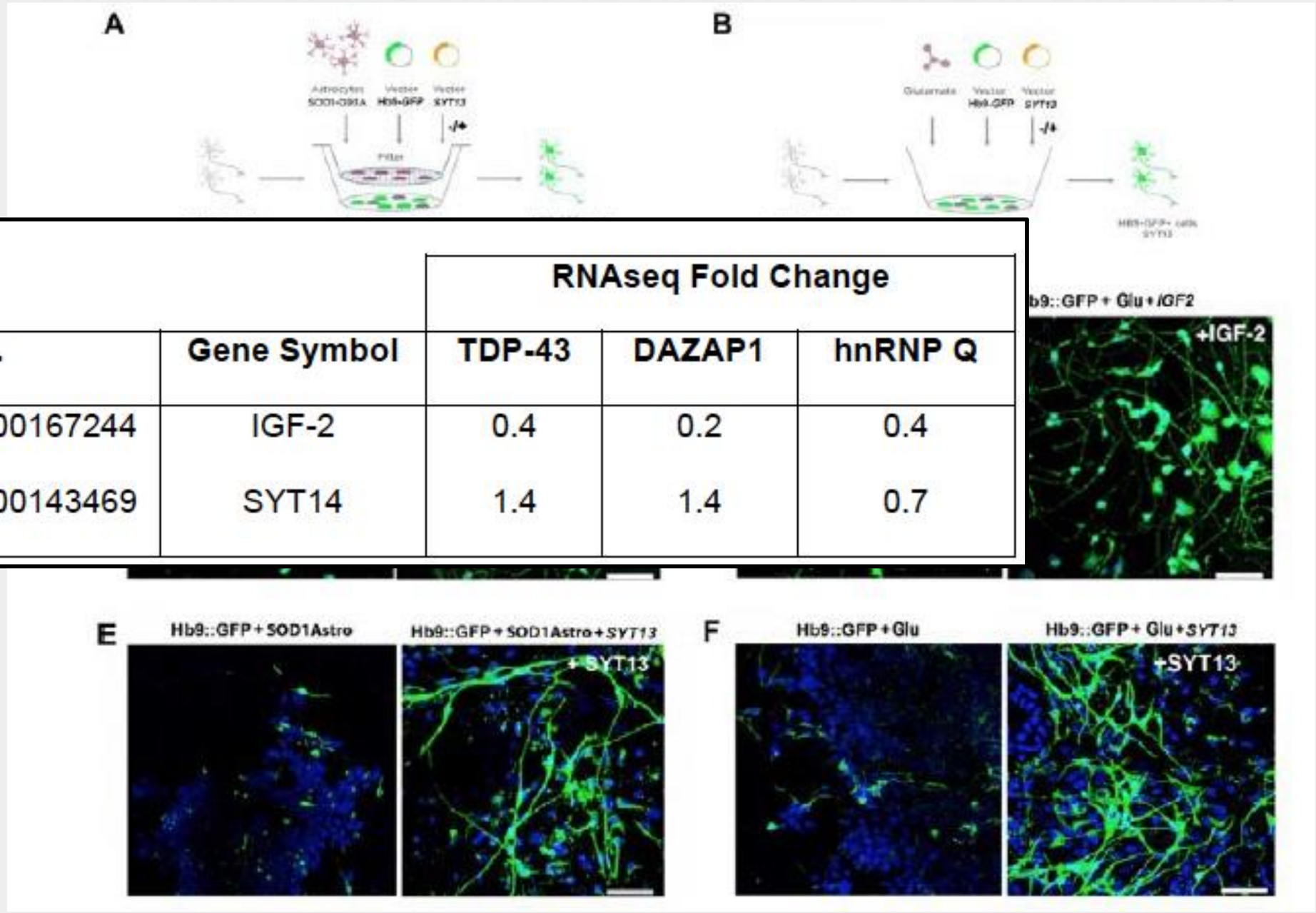


Volcano plots of differentially expressed genes (DEGs) following depletion of TDP-43, DAZAP1, hnRNP Q and hnRNP R in SH-SY5Y cells. The immune connection



Another important hit in these 214 common genes is represented IGF-2 that h been previo shown to im survivability MNs from A degeneratio vitro. In our list, SYT13 was not present, however, there is another member of this family SYT14.

ENSEMBL	Gene Symbol	RNAseq Fold Change		
		TDP-43	DAZAP1	hnRNP Q
ENSG00000167244	IGF-2	0.4	0.2	0.4
ENSG00000143469	SYT14	1.4	1.4	0.7



Conclusions

1) Tau and TDP-43 share a common type of disease (FTD) although they are very different proteins. The challenge in future times will be to identify the common pathways af fected by these two proteins

2) Apparently benign variants can alter basic TDP-43 basic properties such as the balance between nuclear and cytoplasmic localisation. In particular, through their study we have i dentified a specific region of TDP-43 (S375-395) that may be important to physiologically c ontrol this process.

3) Post-translational modifications can reversibly affect the behaviour of TDP-43 intracellular ly

4) The functionality of TDP-43 can be robustly modified by the surrouding RBP proteins pre sent in the cell.

5) Comparing the transcriptomic profiles of hnRNP proteins that can rescue TDP-43 toxicity may allow to identify the common targets.

6) These targets could represent a more “druggable” target than TDP-43 itself

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Molecular Pathology (ICGEB)

Kathy Newell
University of Kansas School of Medicine, Kansas City, KS
Dino Ghetti
Indiana University School of Medicine, Indianapolis, IN
Doug Laurents
Instituto de Química Física "Rocasolano" CSIC, Madrid
Miguel Mompen
IQFR, Madrid
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