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# Prospective analyses of neurodegeneration and disease mechanisms

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Sami Barmada  
Department of Neurology  
University of Michigan

# Diseases of protein misfolding

<u>Disease</u>	<u>Protein</u>	<u>Disease</u>	<u>Protein</u>
Alzheimers disease	A $\beta$ Tau	Huntington's disease	Huntingtin
Frontotemporal dementia	Tau TDP-43 FUS	Corticobasilar degeneration	Tau
Lewy body dementia	$\alpha$ -synuclein	Progressive supranuclear palsy	Tau
ALS	TDP-43 SOD1 FUS	Multiple system atrophy	$\alpha$ -synuclein

**Disease**

**Protein**

**Disease**

**Protein**

**a**

**b**

**c**

**d**

**Huntingtin**

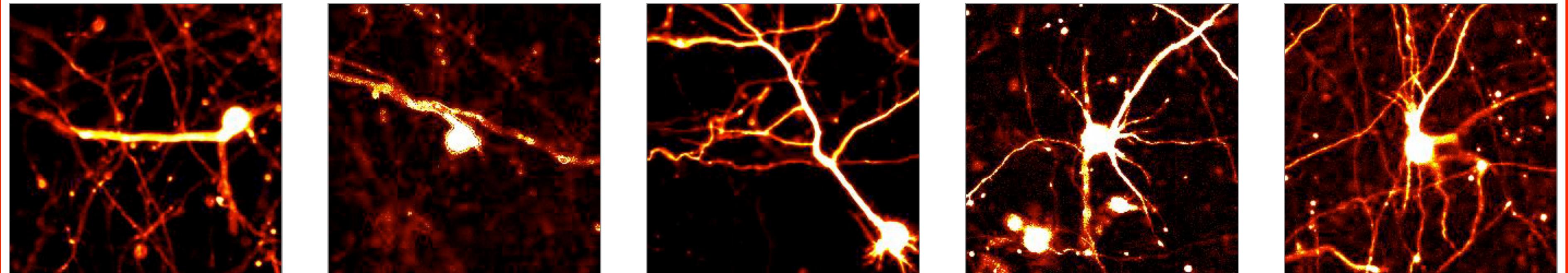
**Tau**

**Tau**

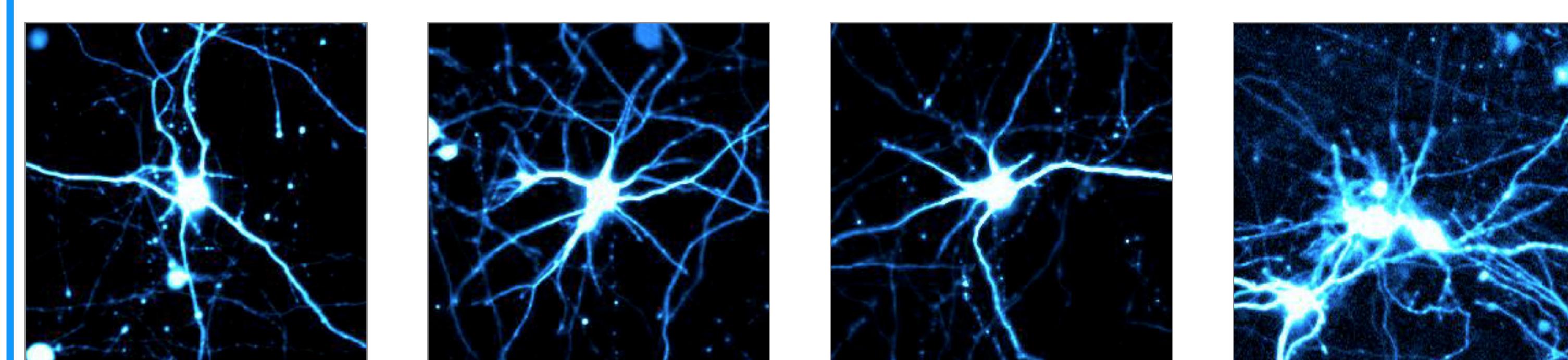
**Tau**

**$\alpha$ -synuclein**

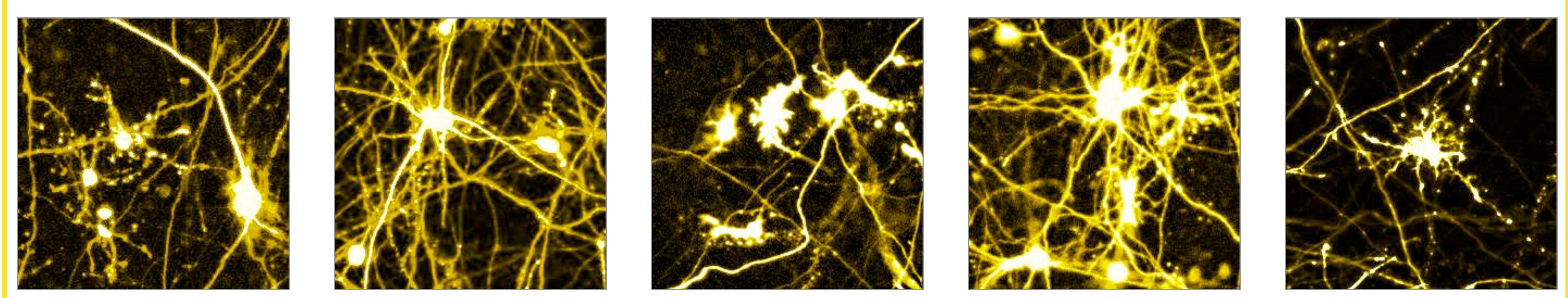
Degeneration



Survival

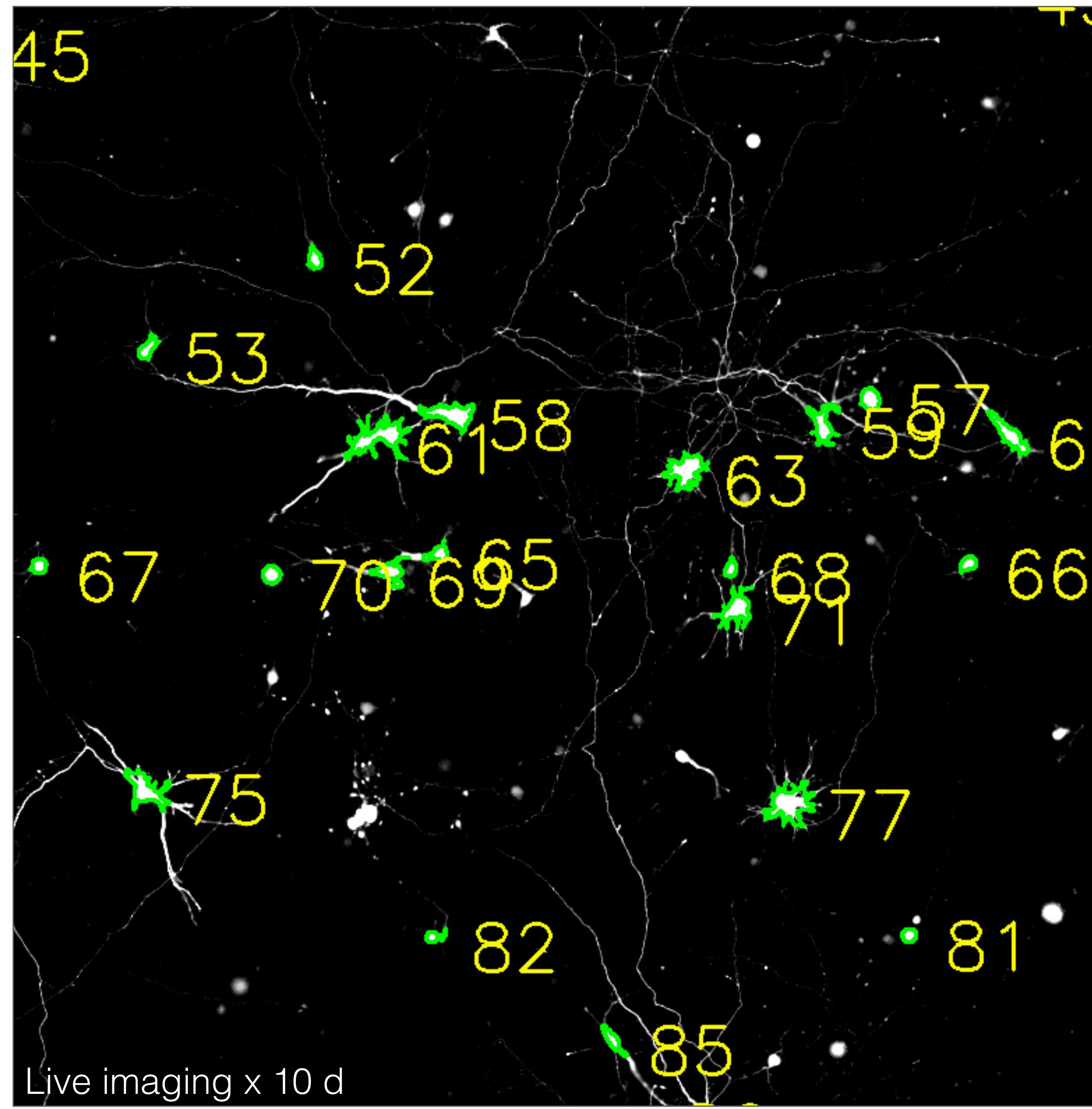


Glia



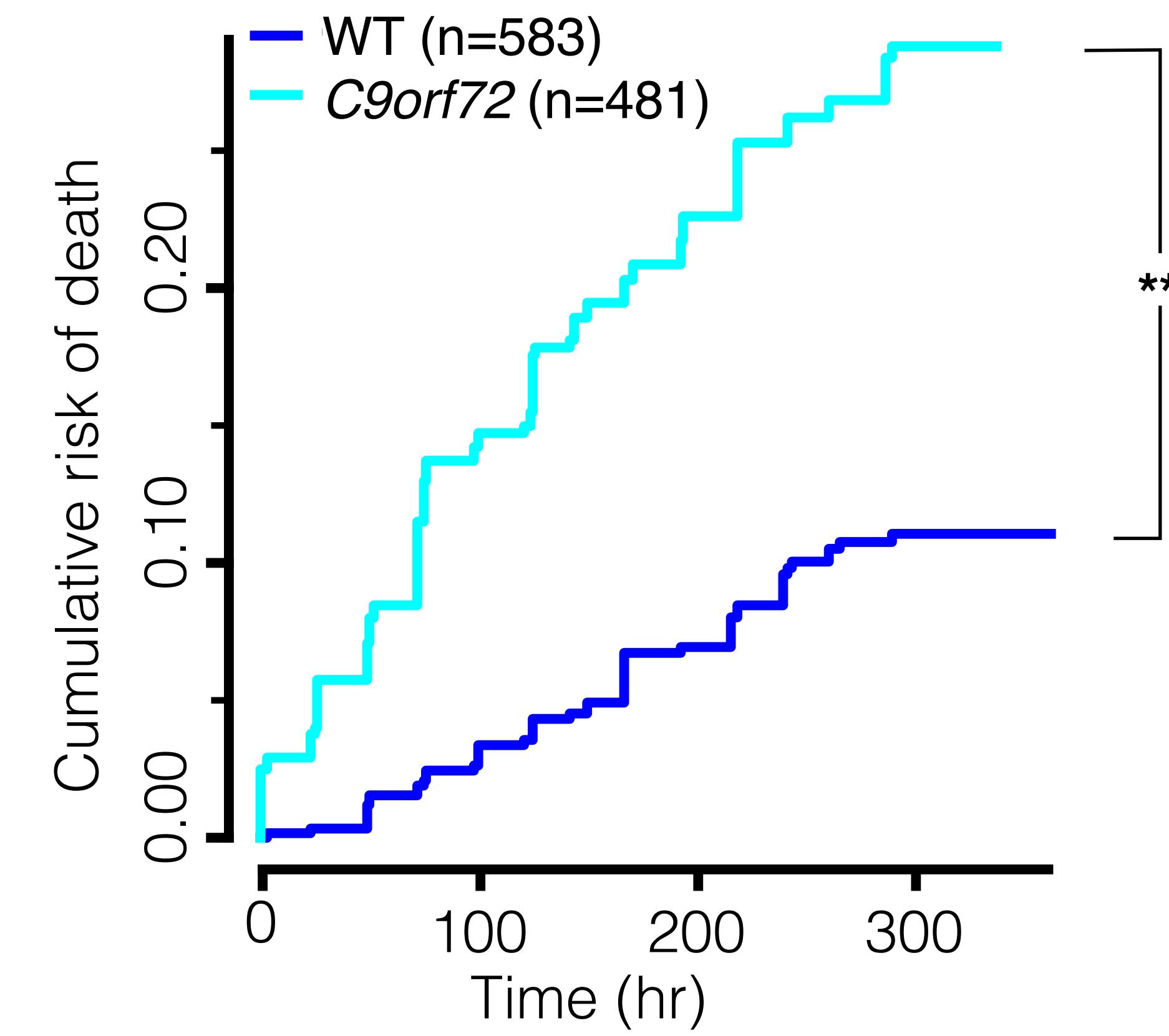
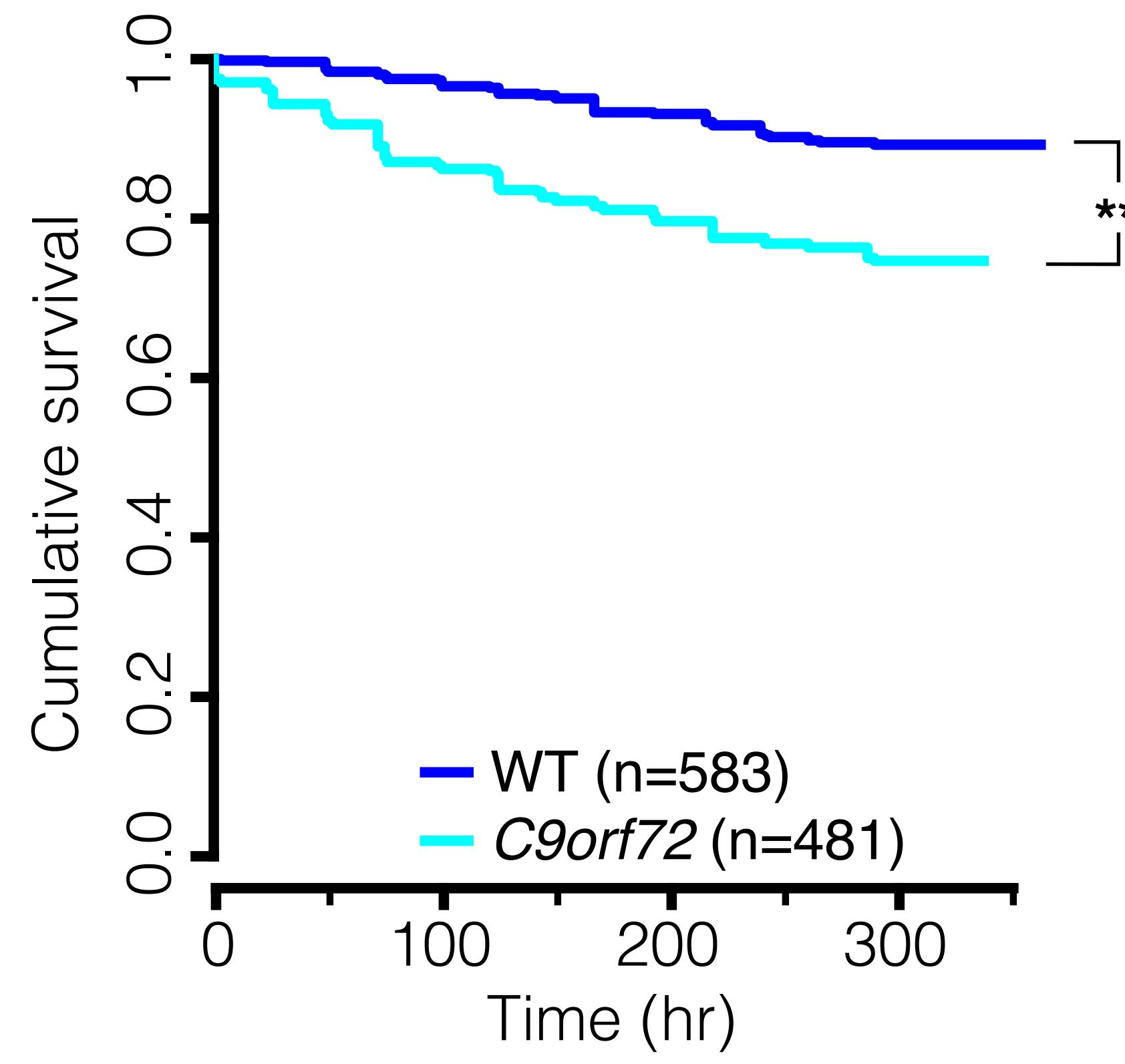
# Automated microscopy

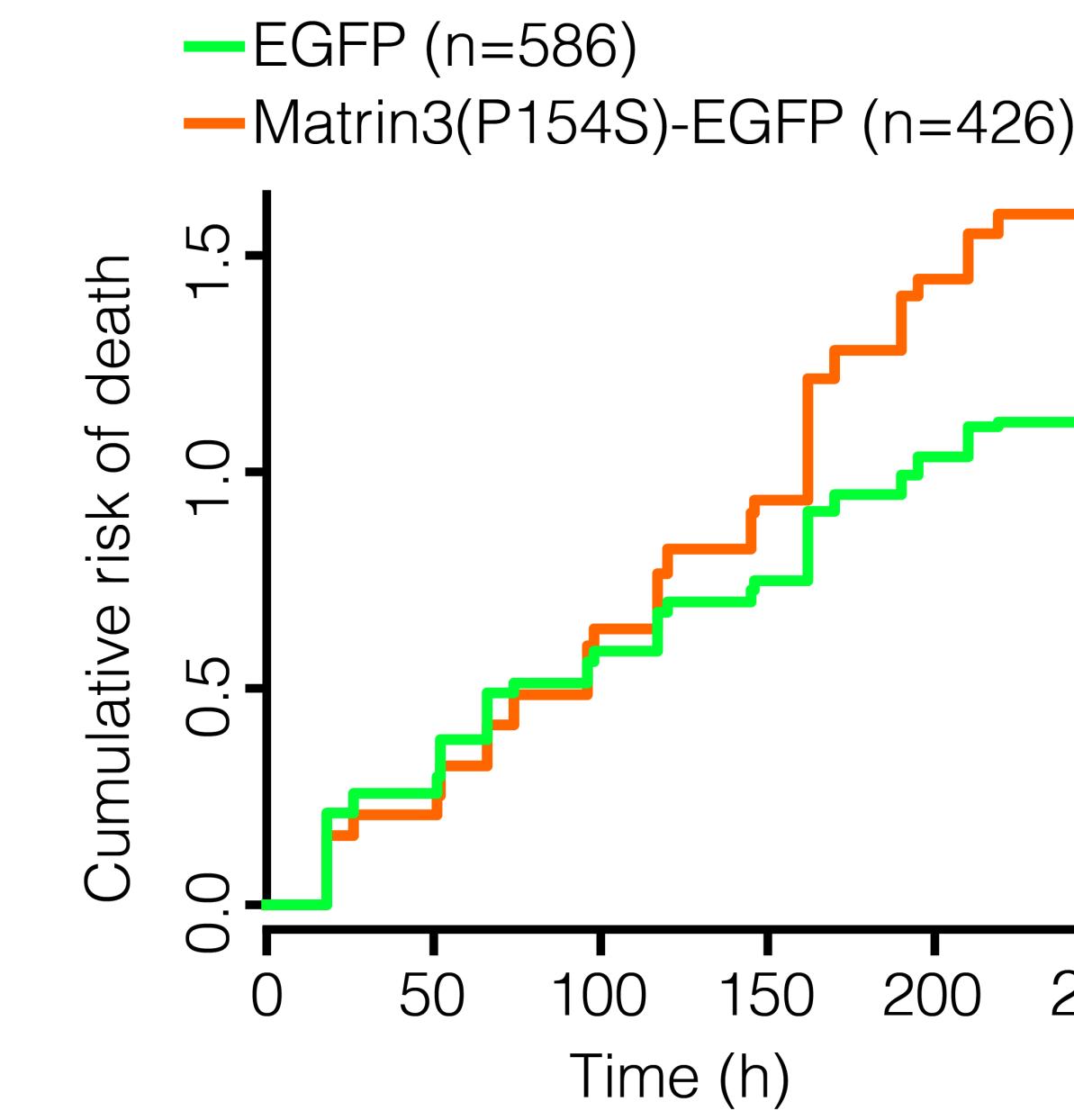
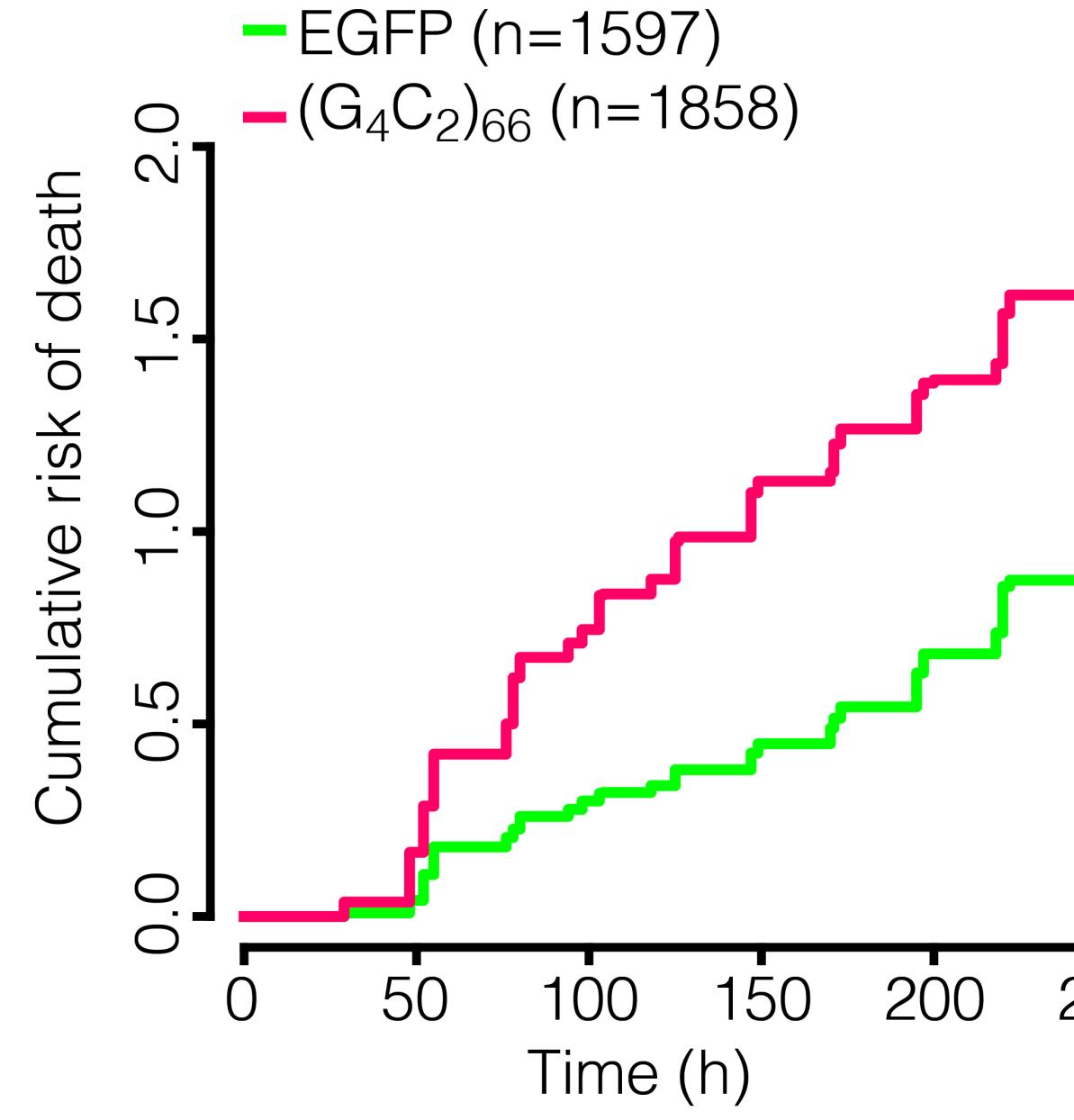
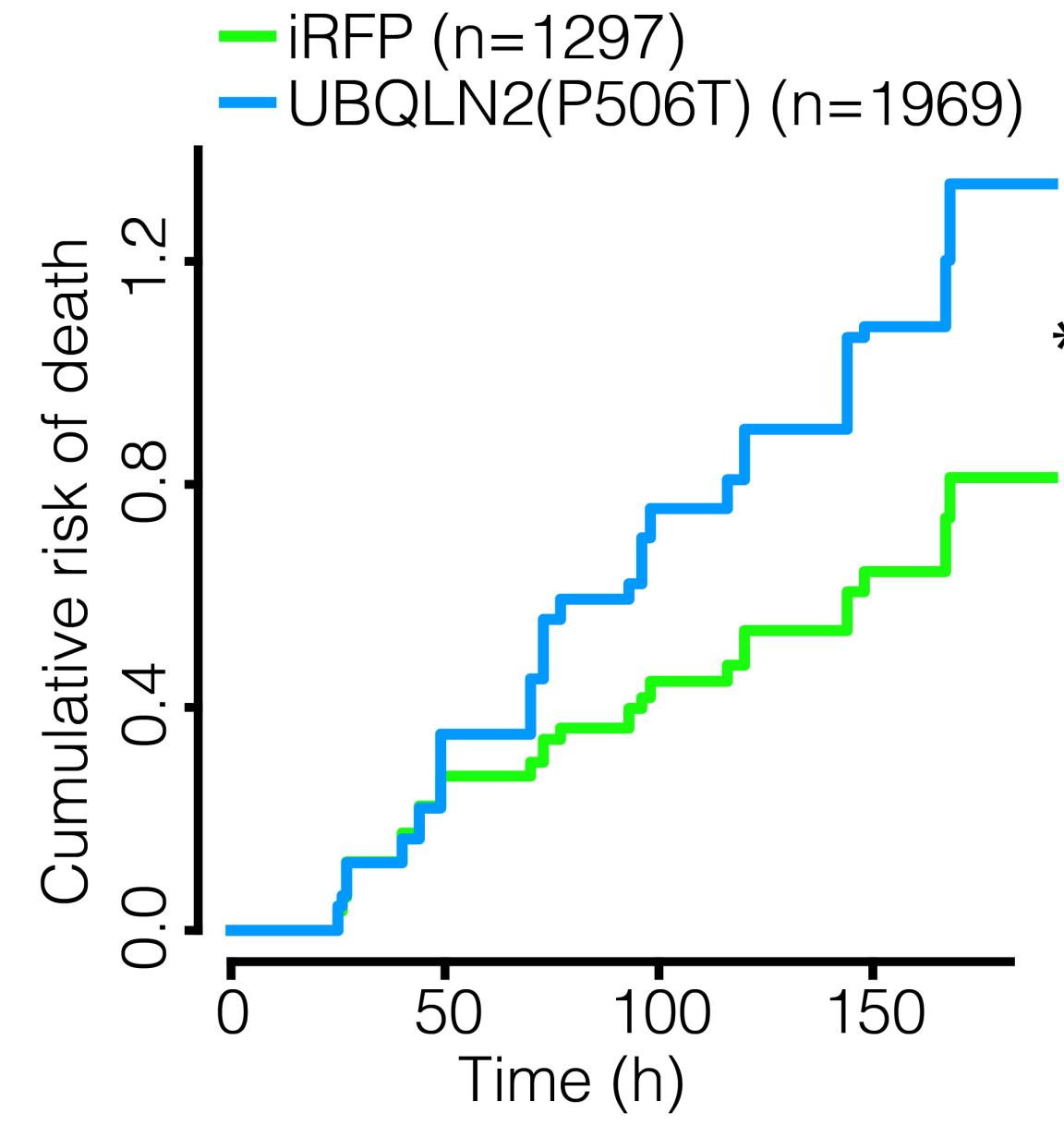
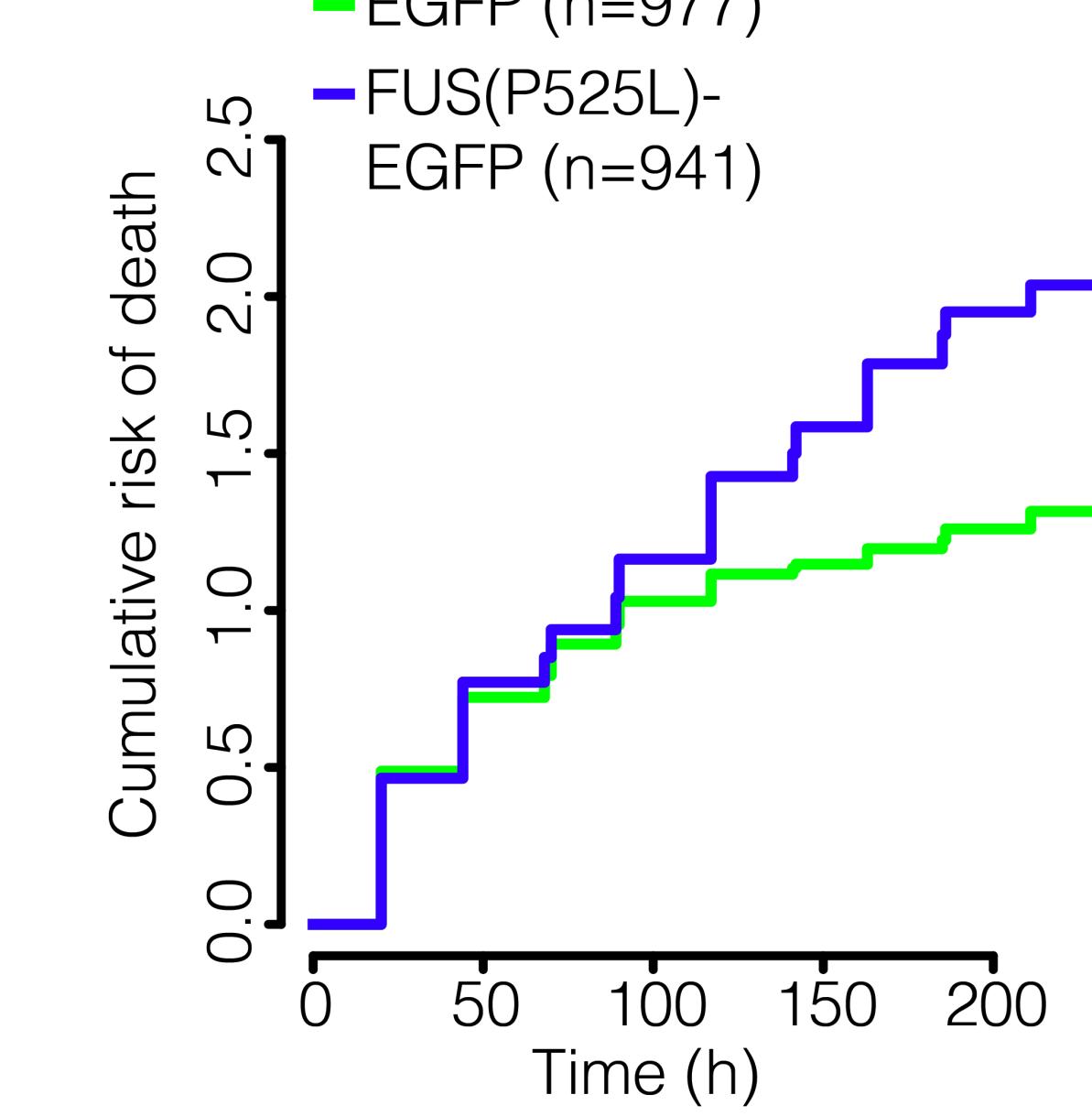
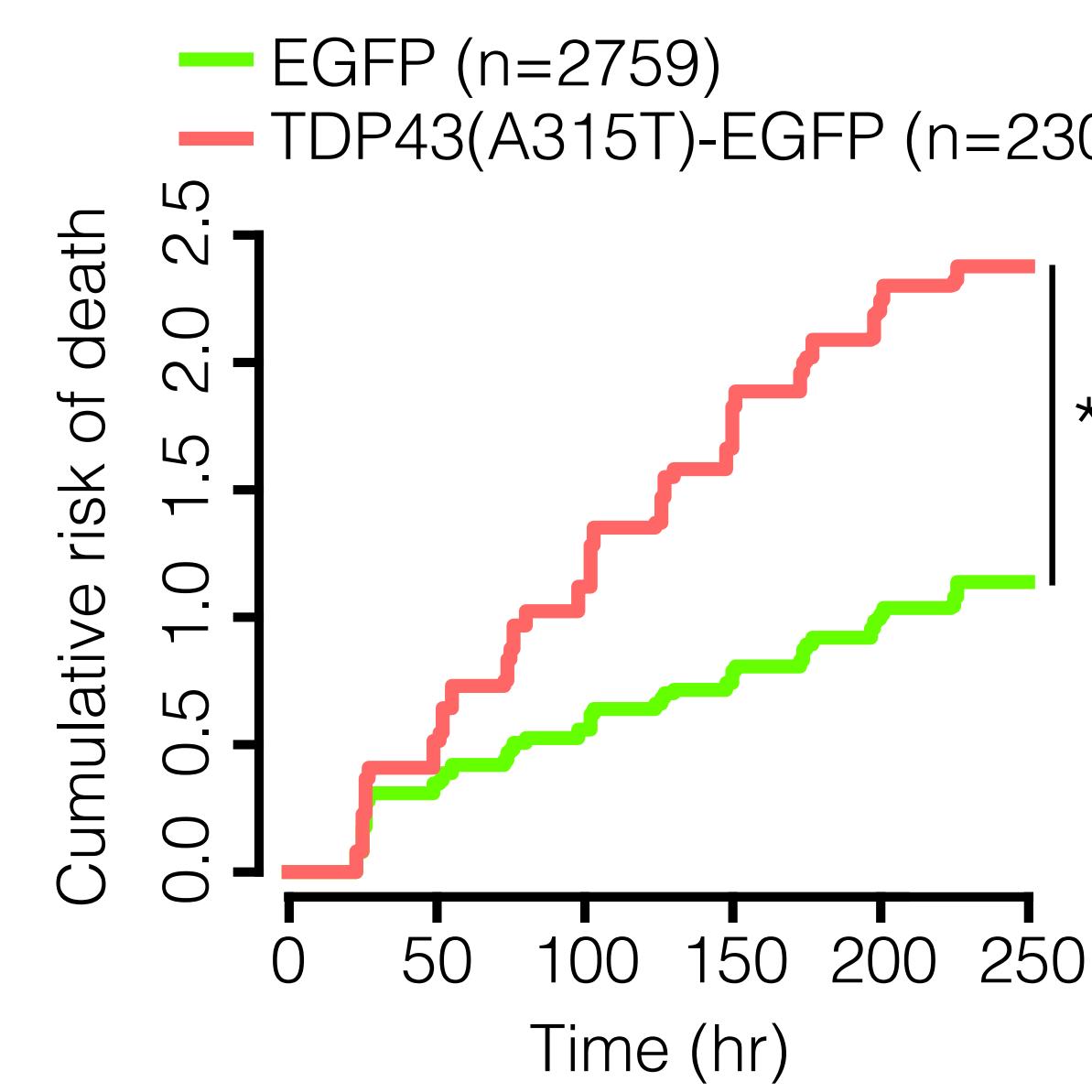
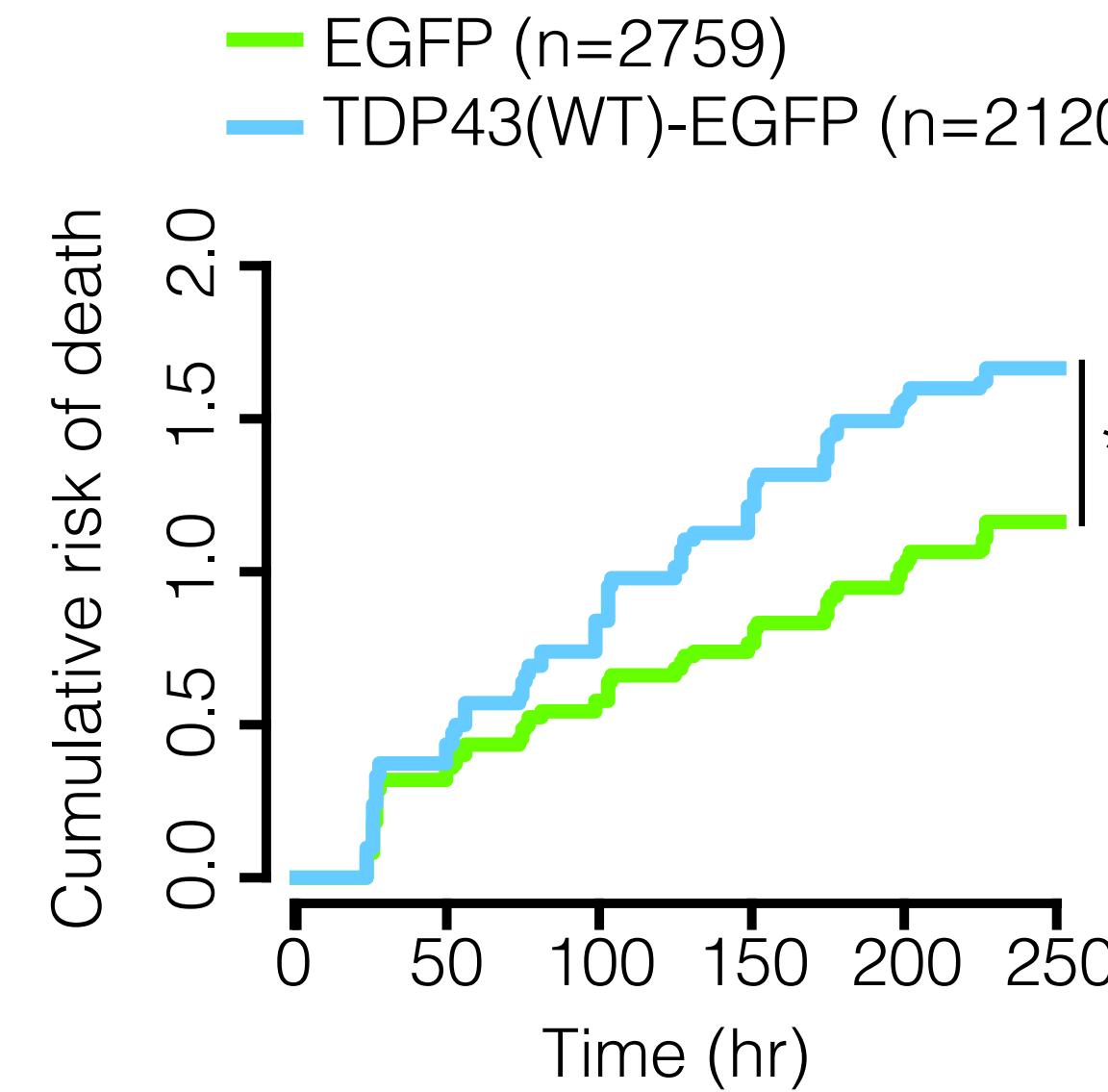
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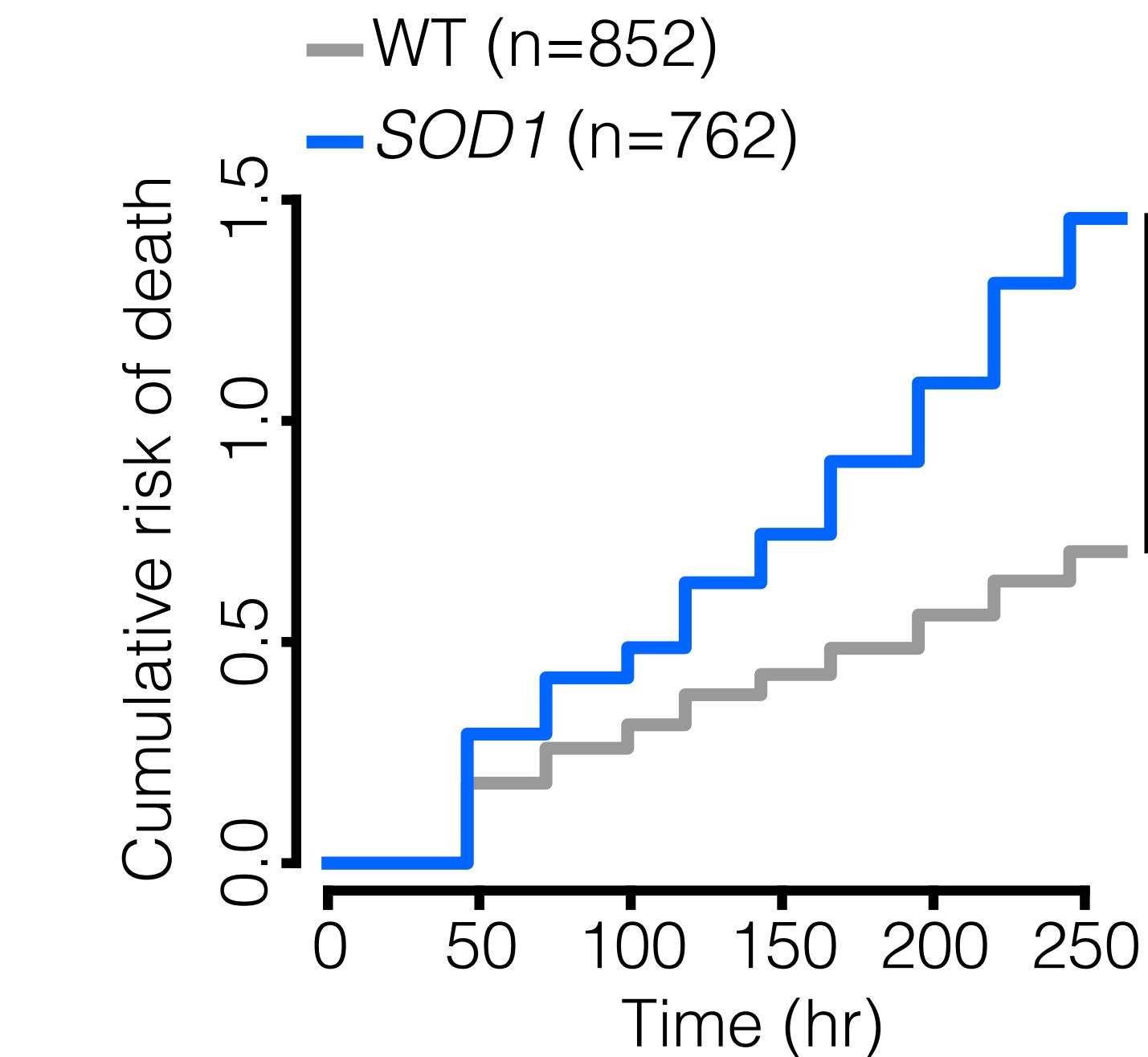
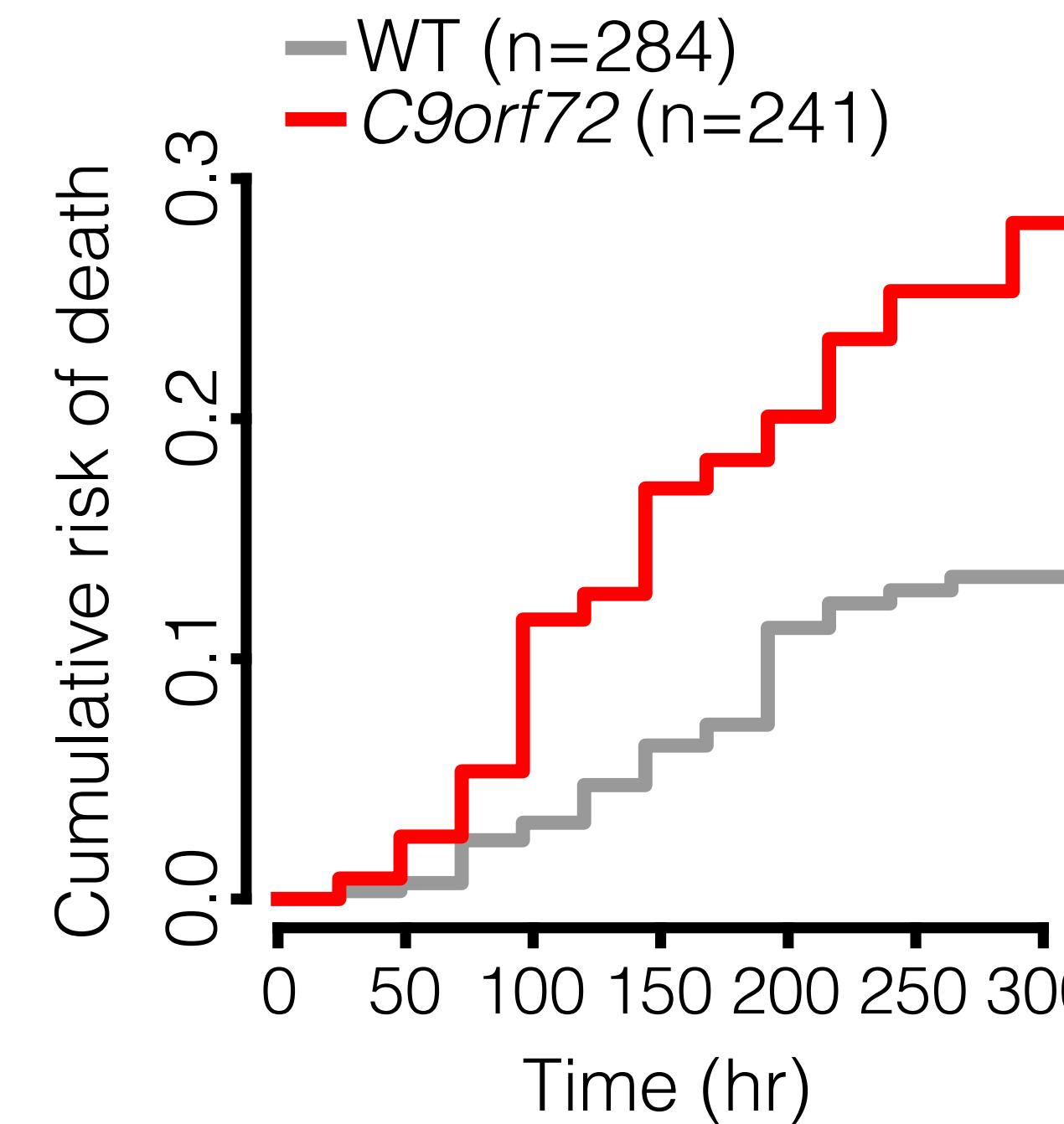
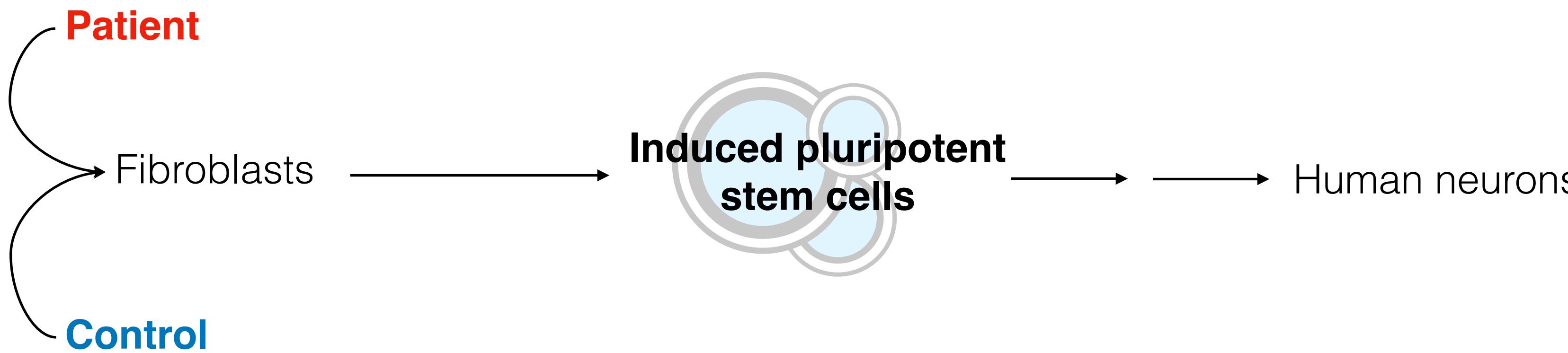
# Automated microscopy and survival analysis

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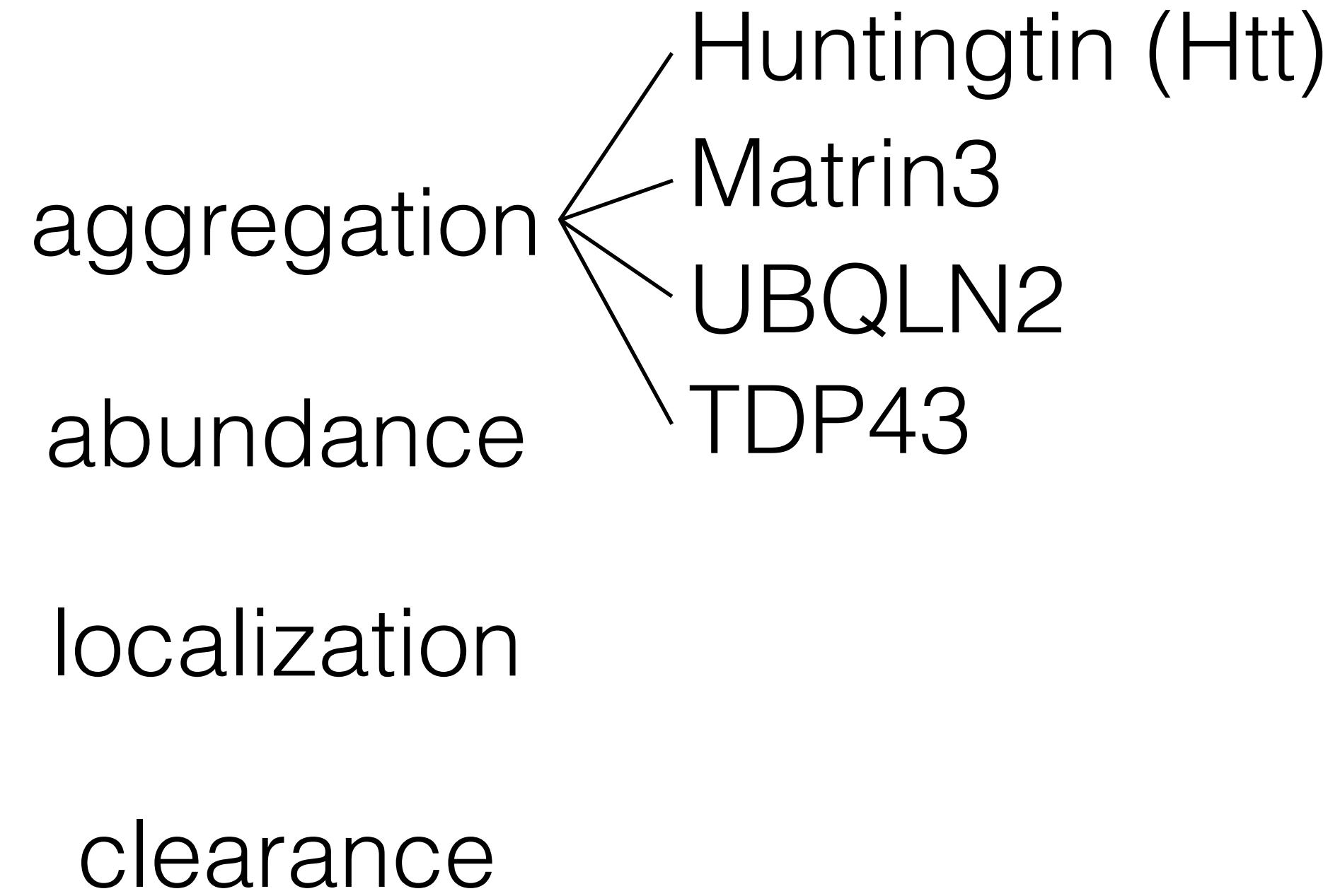


# Human neurons

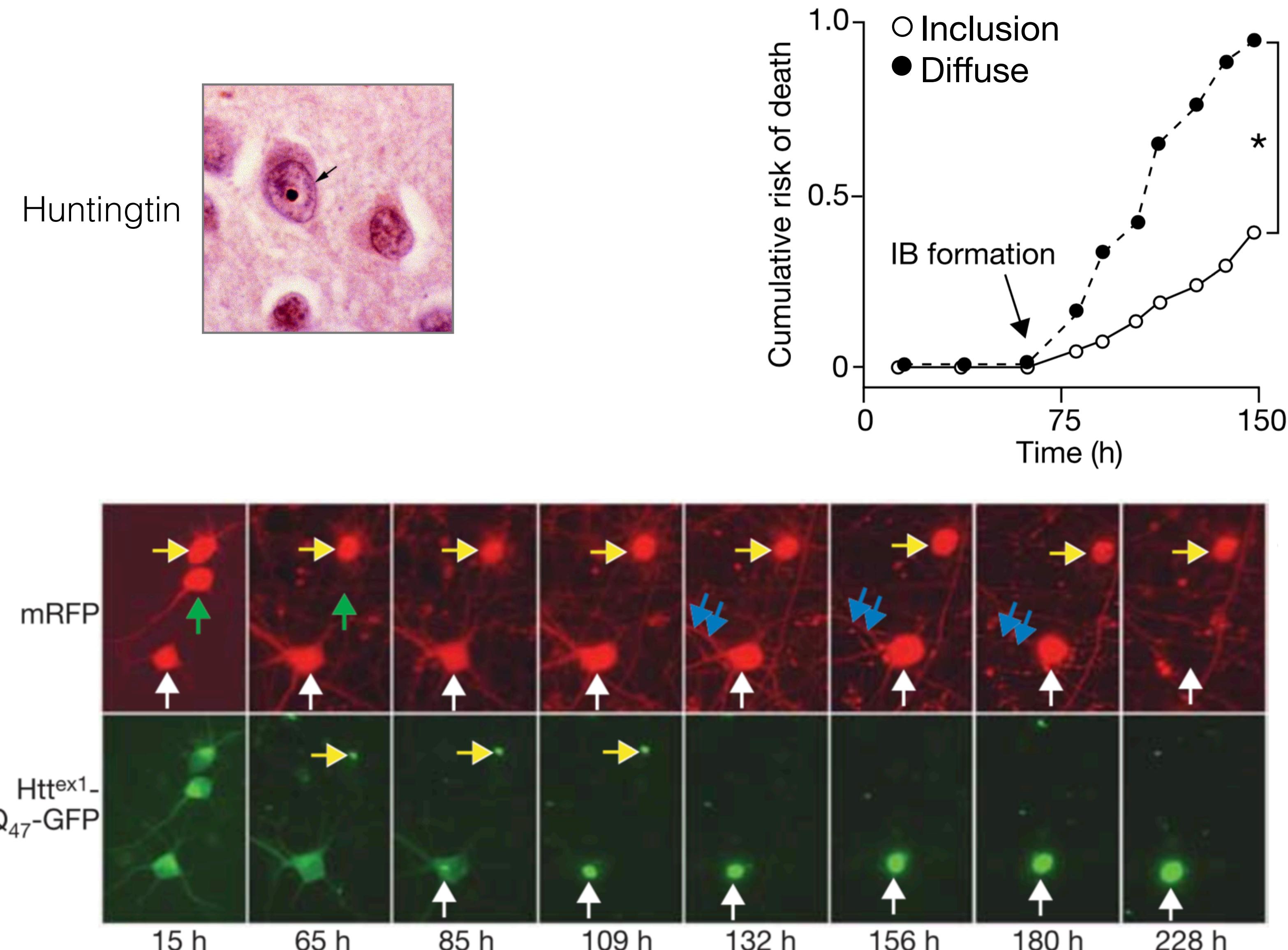


# Determinants of neurodegeneration

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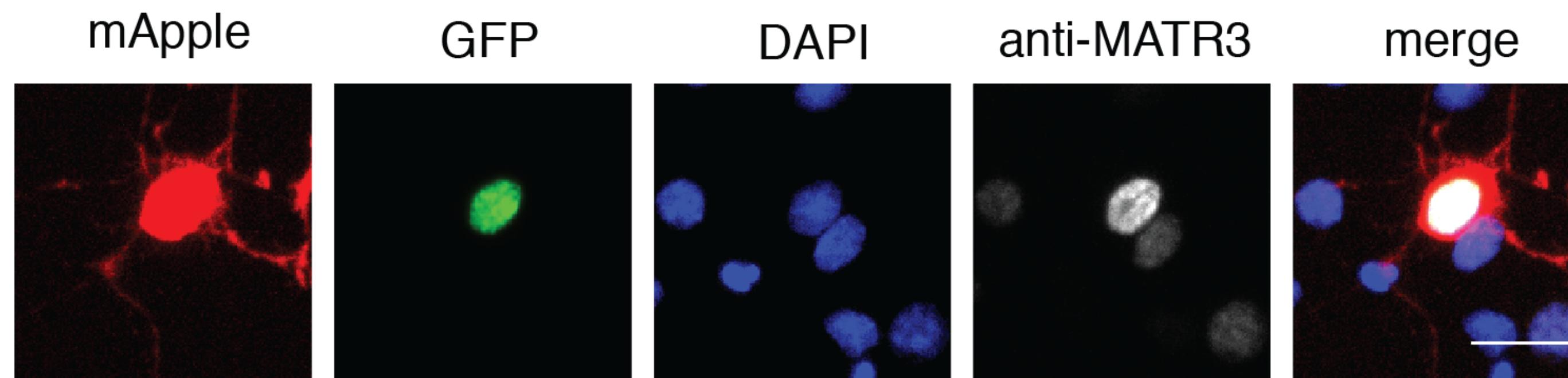
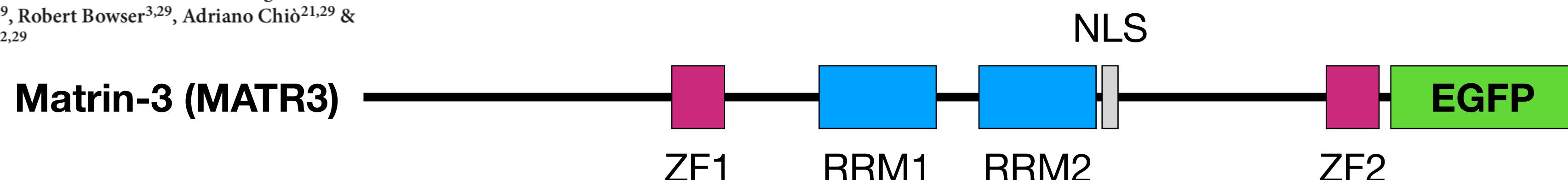
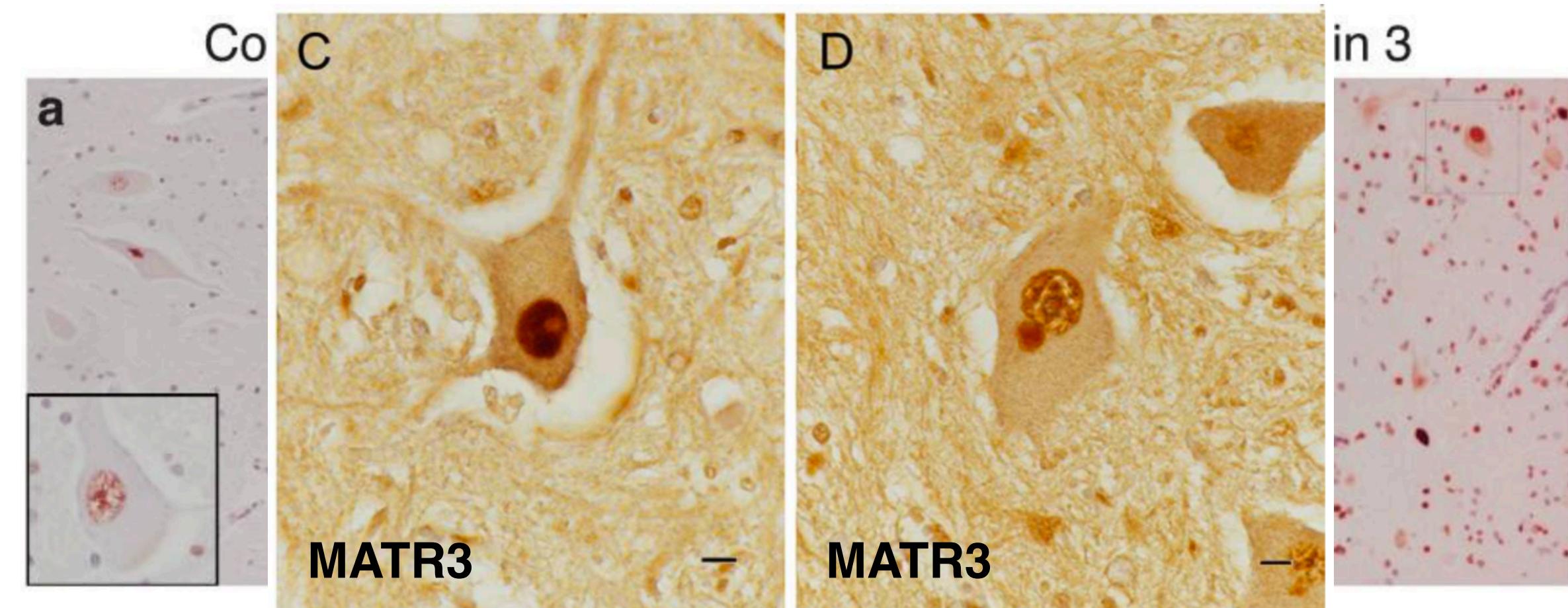
# Htt aggregation may be beneficial



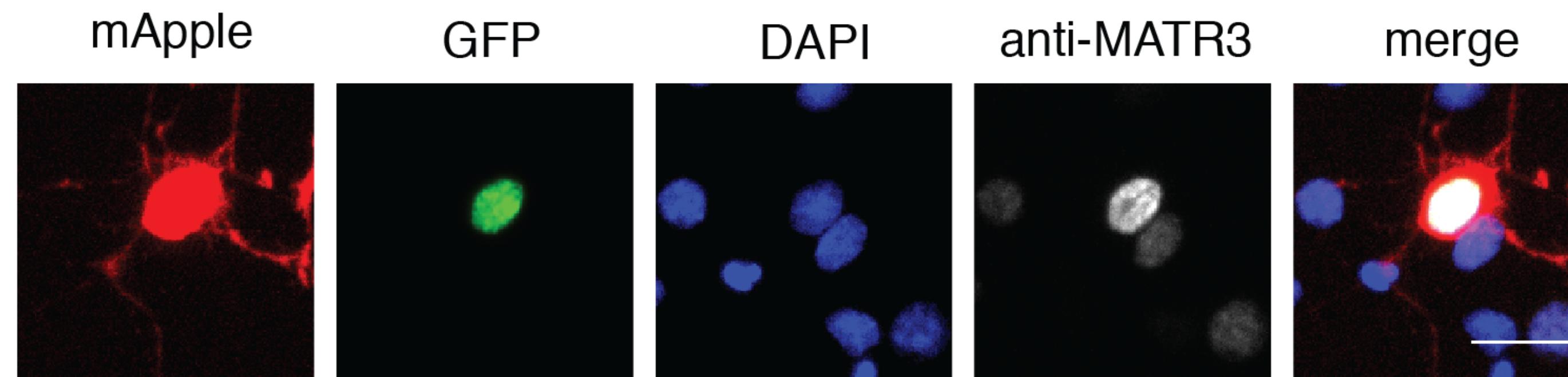
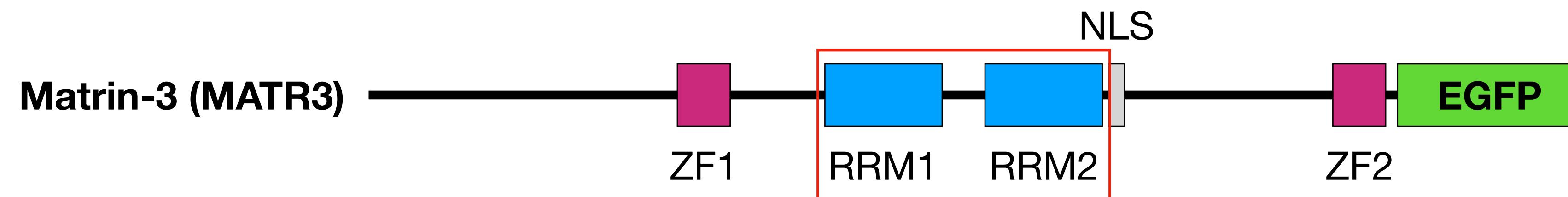
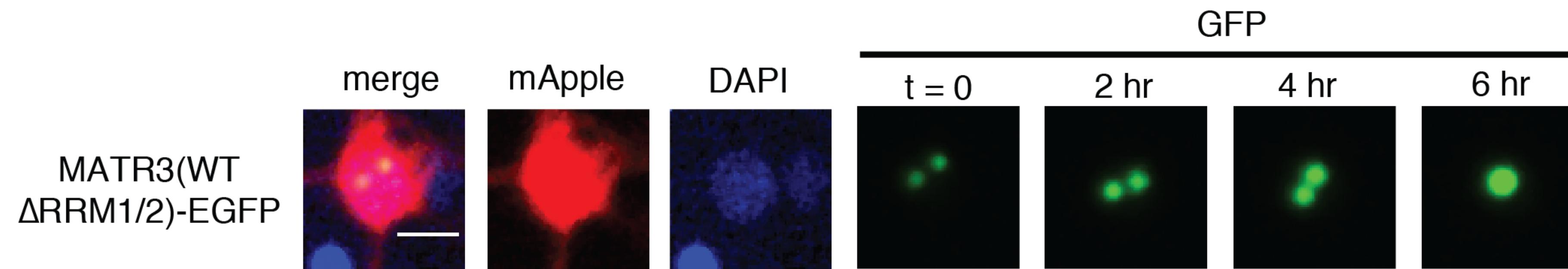
# Matrin3 deposition

Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis

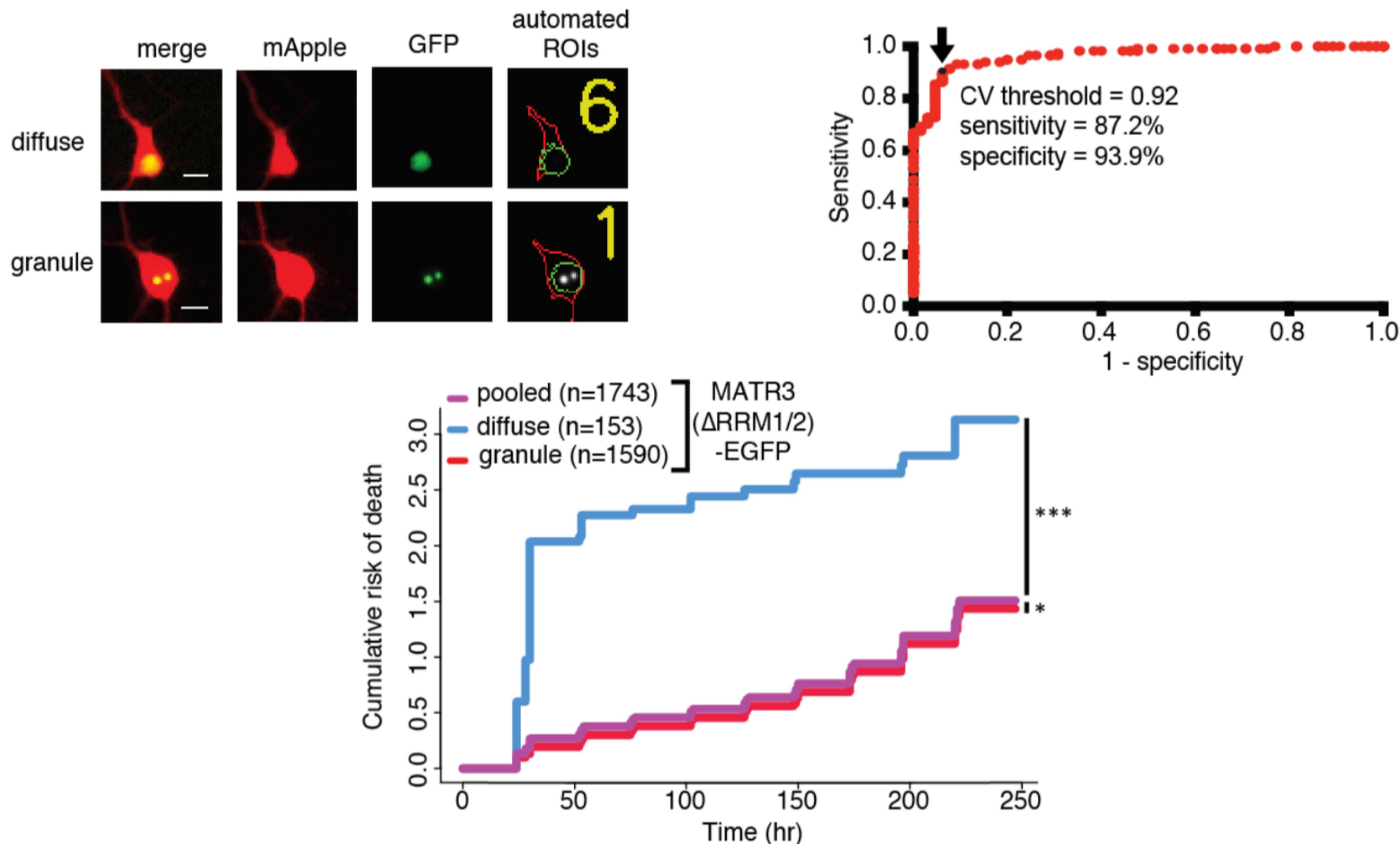
Janel O Johnson<sup>1,28</sup>, Erik P Pioro<sup>2,28</sup>, Ashley Boehringer<sup>3,28</sup>, Ruth Chia<sup>4,28</sup>, Howard Feit<sup>5</sup>, Alan E Renton<sup>1</sup>, Hannah A Pliner<sup>1</sup>, Yevgeniya Abramzon<sup>1</sup>, Giuseppe Marangi<sup>1,6</sup>, Brett J Winborn<sup>7</sup>, J Raphael Gibbs<sup>8,9</sup>, Michael A Nalls<sup>10</sup>, Sarah Morgan<sup>9</sup>, Maryam Shoai<sup>9</sup>, John Hardy<sup>9</sup>, Alan Pittman<sup>9</sup>, Richard W Orrell<sup>11</sup>, Andrea Malaspina<sup>12</sup>, Katie C Sidle<sup>9</sup>, Pietro Fratta<sup>13</sup>, Matthew B Harms<sup>14</sup>, Robert H Baloh<sup>15</sup>, Alan Pestronk<sup>14</sup>, Conrad C Weihl<sup>14</sup>, Ekaterina Rogaeva<sup>16</sup>, Lorne Zinman<sup>17</sup>, Vivian E Drory<sup>18</sup>, Giuseppe Borghero<sup>19</sup>, Gabriele Mora<sup>20</sup>, Andrea Calvo<sup>21</sup>, Jeffrey D Rothstein<sup>22</sup>, ITALSGEN<sup>23</sup>, Carsten Drepper<sup>24,25</sup>, Michael Sendtner<sup>24</sup>, Andrew B Singleton<sup>10</sup>, J Paul Taylor<sup>7</sup>, Mark R Cookson<sup>4</sup>, Gabriella Restagno<sup>26,29</sup>, Mario Sabatelli<sup>27,29</sup>, Robert Bowser<sup>3,29</sup>, Adriano Chiò<sup>21,29</sup> & Bryan J Traynor<sup>1,22,29</sup>



# Matrin3 droplet formation



# Matrin3 droplet formation may be beneficial

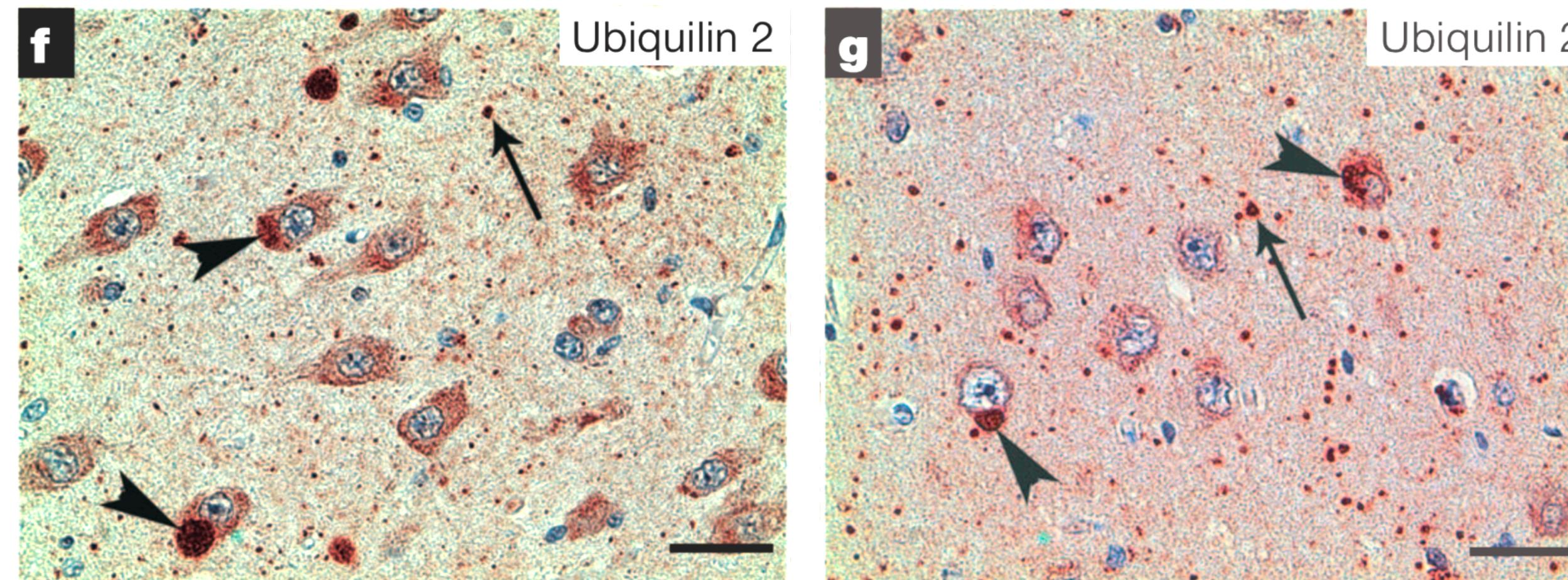


## LETTER

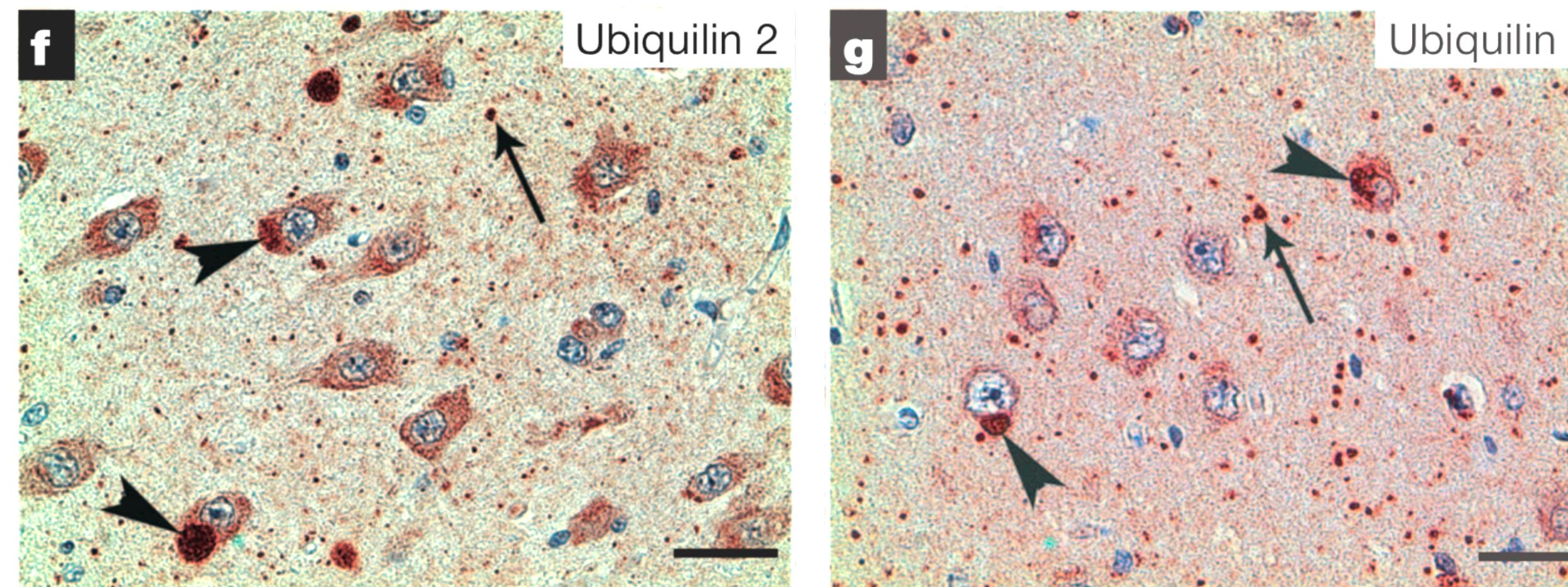
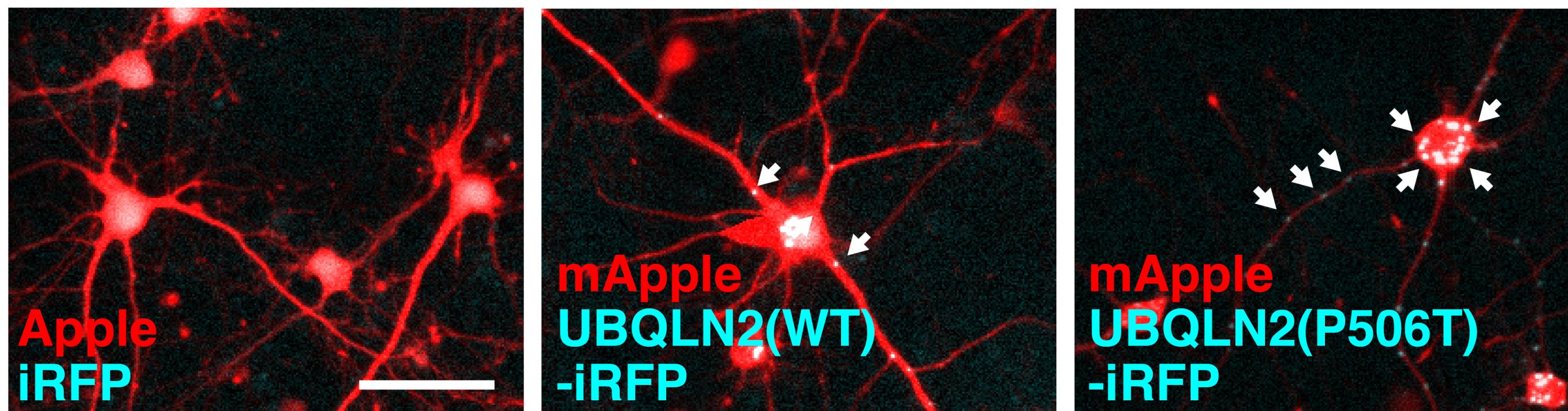
doi:10.1038/nature10353

### Mutations in *UBQLN2* cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia

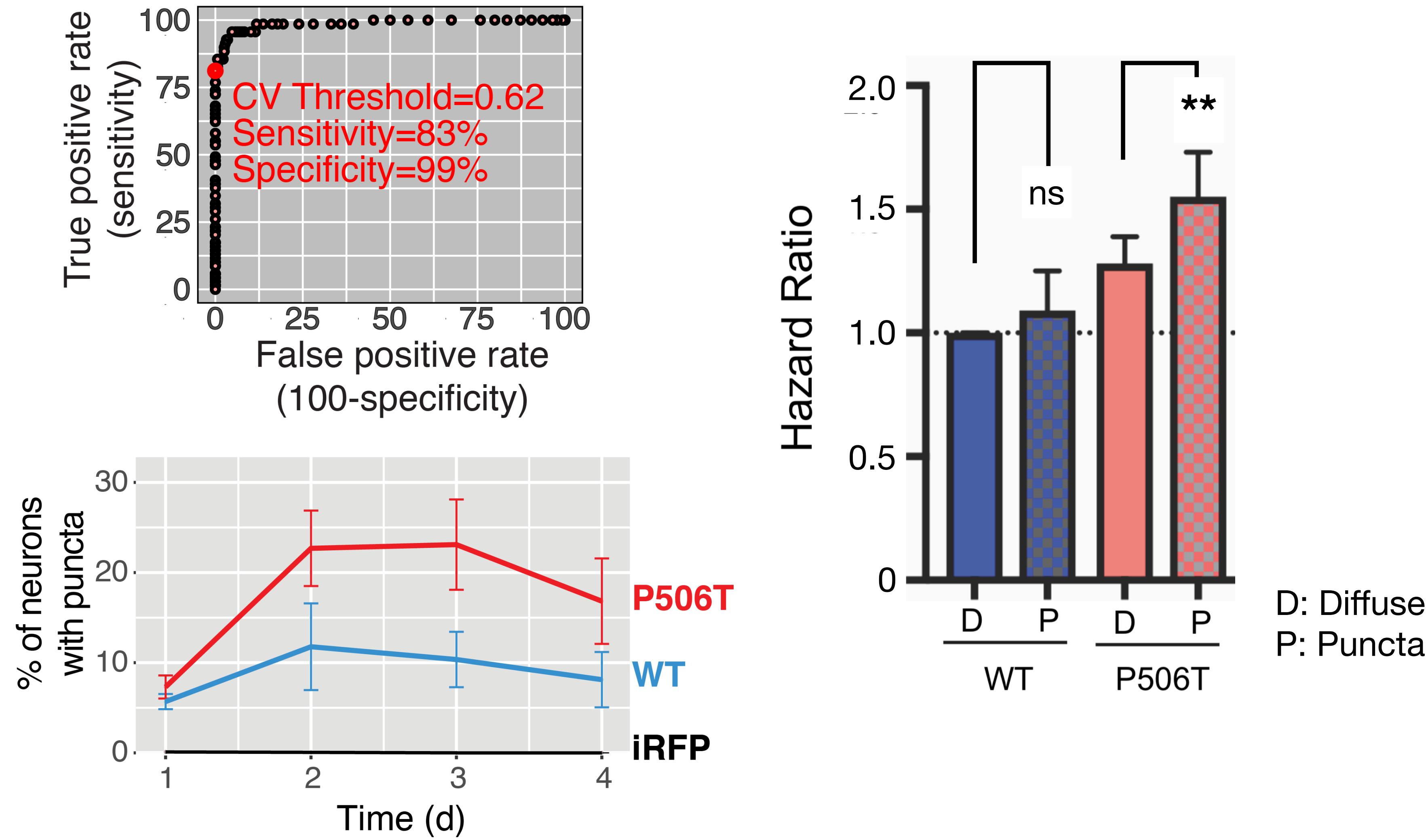
Han-Xiang Deng<sup>1\*</sup>, Wenjie Chen<sup>1\*</sup>, Seong-Tshool Hong<sup>1†</sup>, Kym M. Boycott<sup>2</sup>, George H. Gorrie<sup>1†</sup>, Nailah Siddique<sup>1</sup>, Yi Yang<sup>1</sup>, Faisal Fecto<sup>1,3</sup>, Yong Shi<sup>1</sup>, Hong Zhai<sup>1</sup>, Hujun Jiang<sup>1†</sup>, Makito Hirano<sup>1†</sup>, Evadnie Rampersaud<sup>4</sup>, Gerard H. Jansen<sup>5</sup>, Sandra Donkervoort<sup>1</sup>, Eileen H. Bigio<sup>6</sup>, Benjamin R. Brooks<sup>7</sup>, Kaouther Ajroud<sup>1</sup>, Robert L. Sufit<sup>1</sup>, Jonathan L. Haines<sup>8</sup>, Enrico Mugnaini<sup>3,9</sup>, Margaret A. Pericak-Vance<sup>4</sup> & Teepu Siddique<sup>1,3,9</sup>



# UBQLN2 aggregation

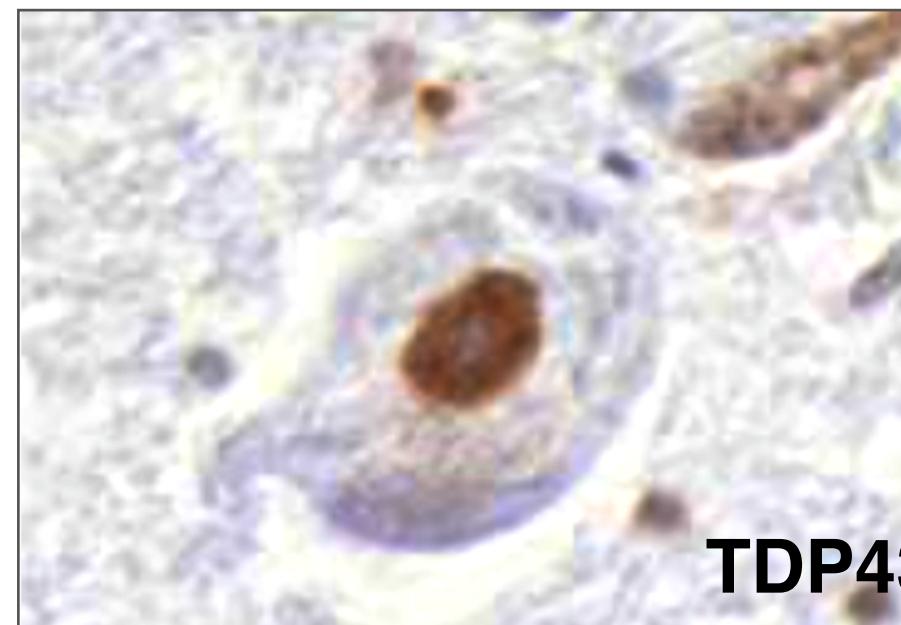


# UBQLN2 aggregation is not protective, and may be toxic



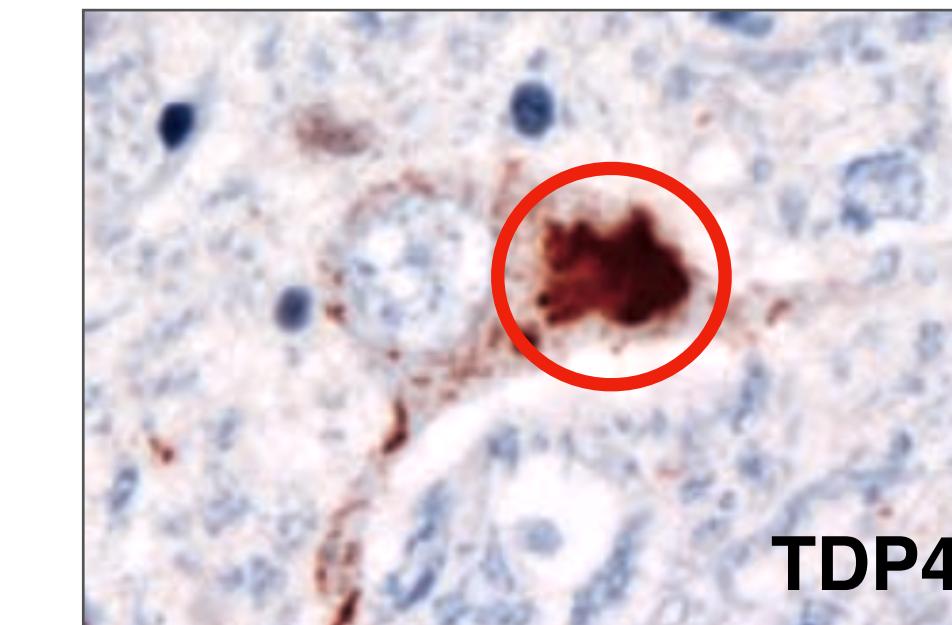
# TDP43 aggregation is neither protective nor toxic

Healthy control

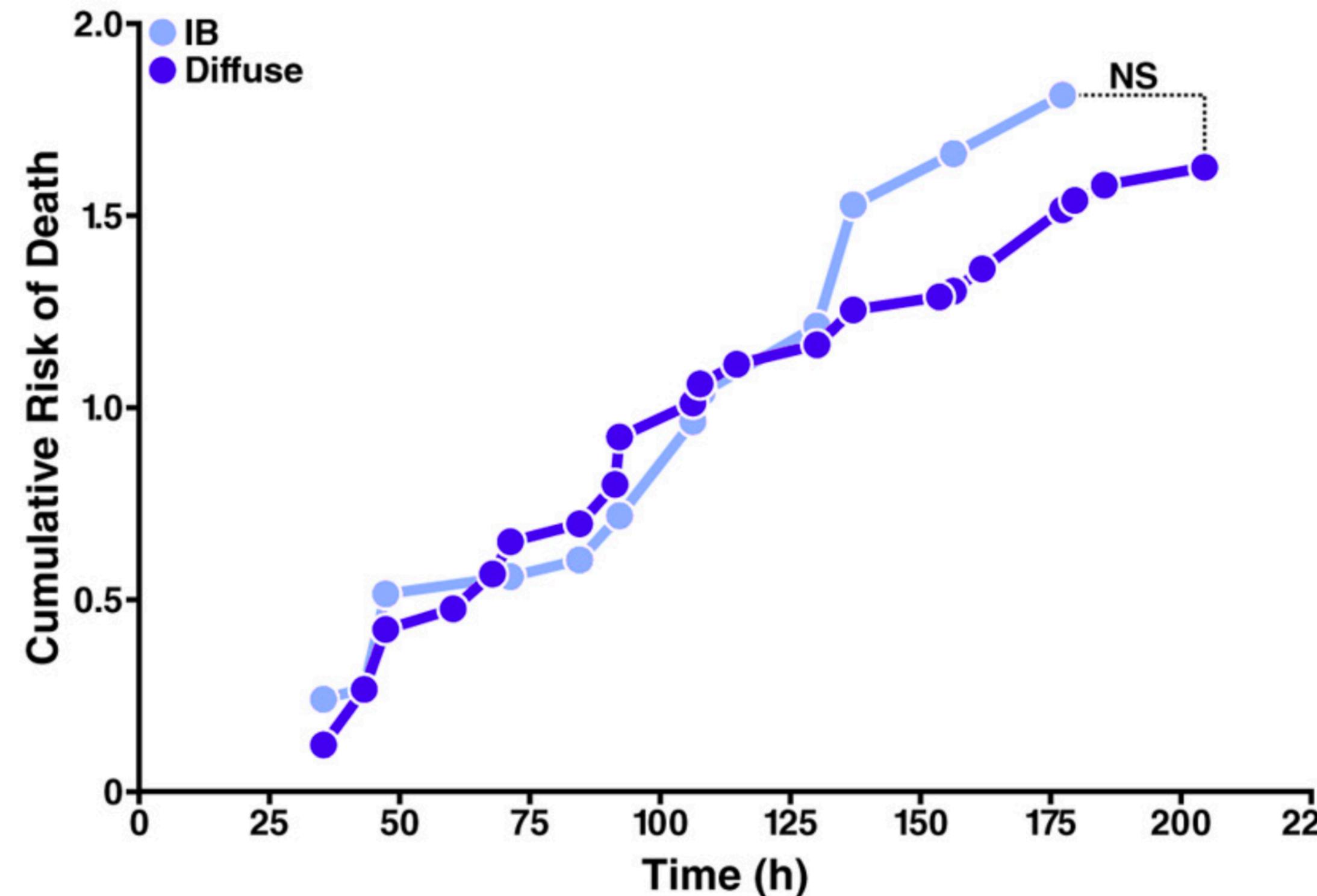


Curr Op Neurol, 2008, 21(6), 693

ALS/FTLD-TDP

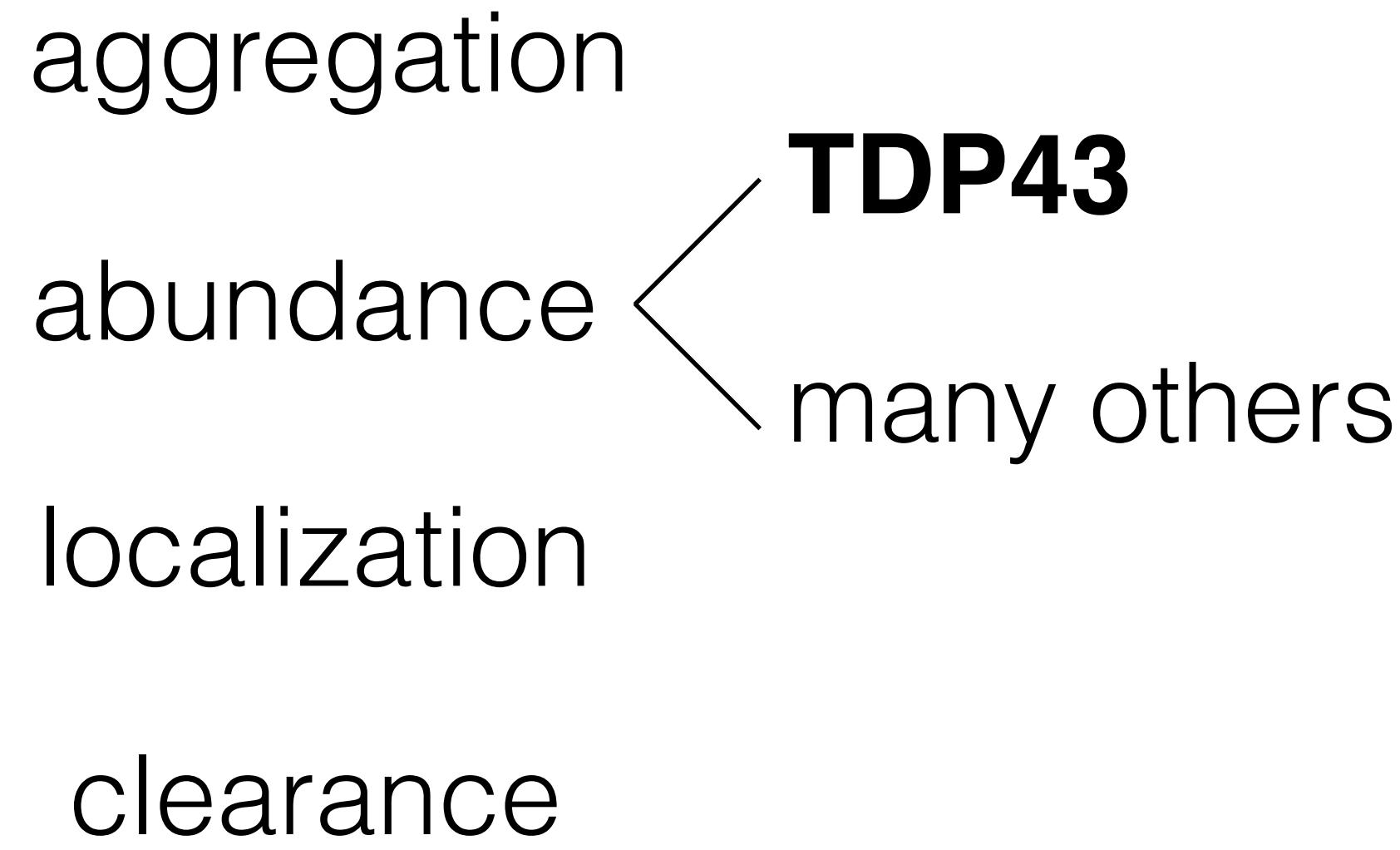


Hum Mol Genet, 2009, Vol. 18, 2

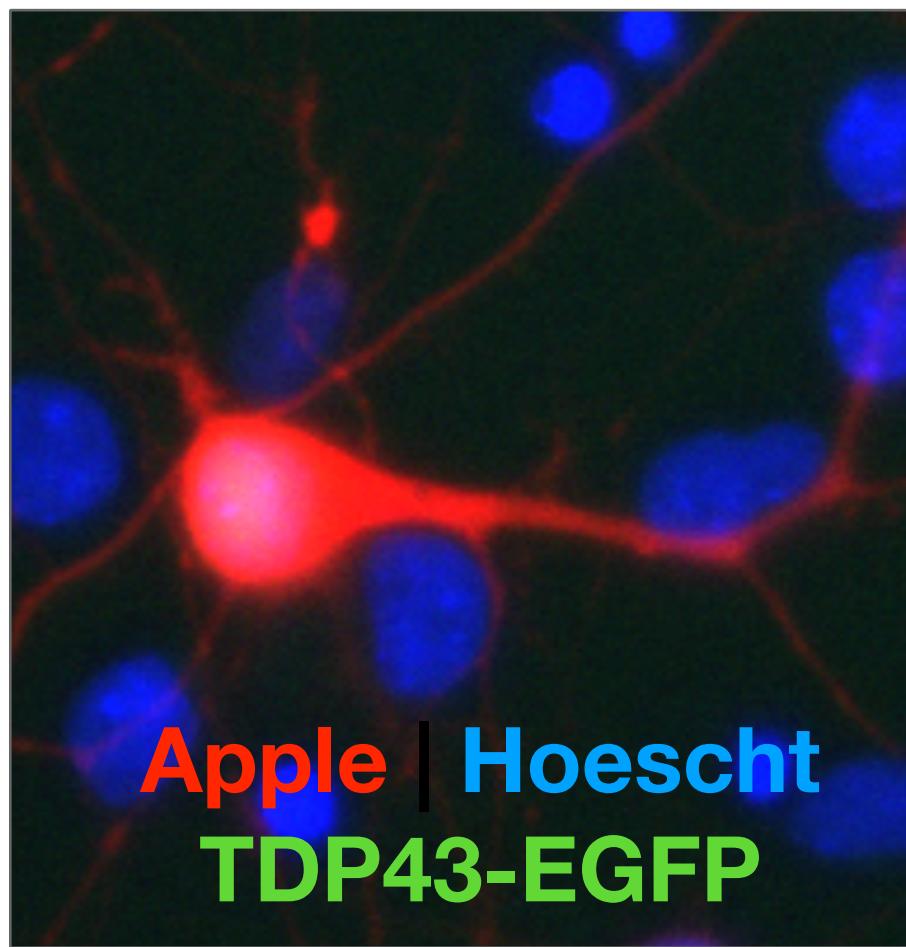


# Determinants of neurodegeneration

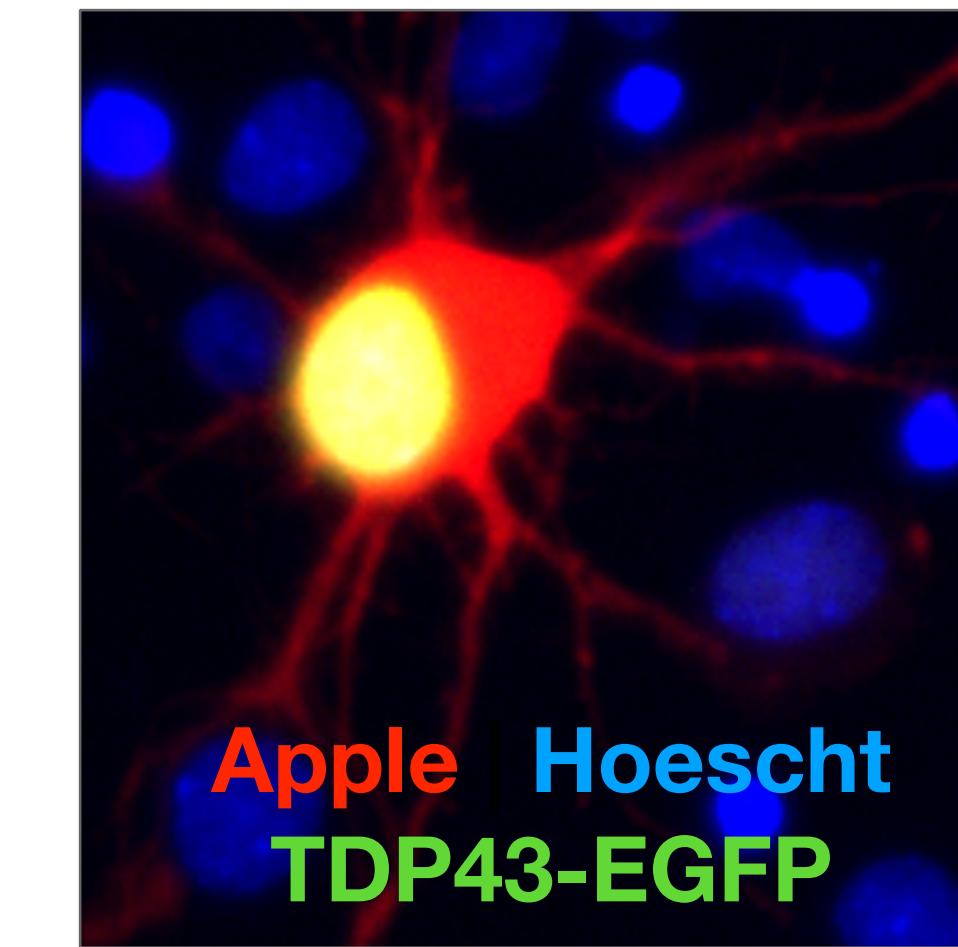
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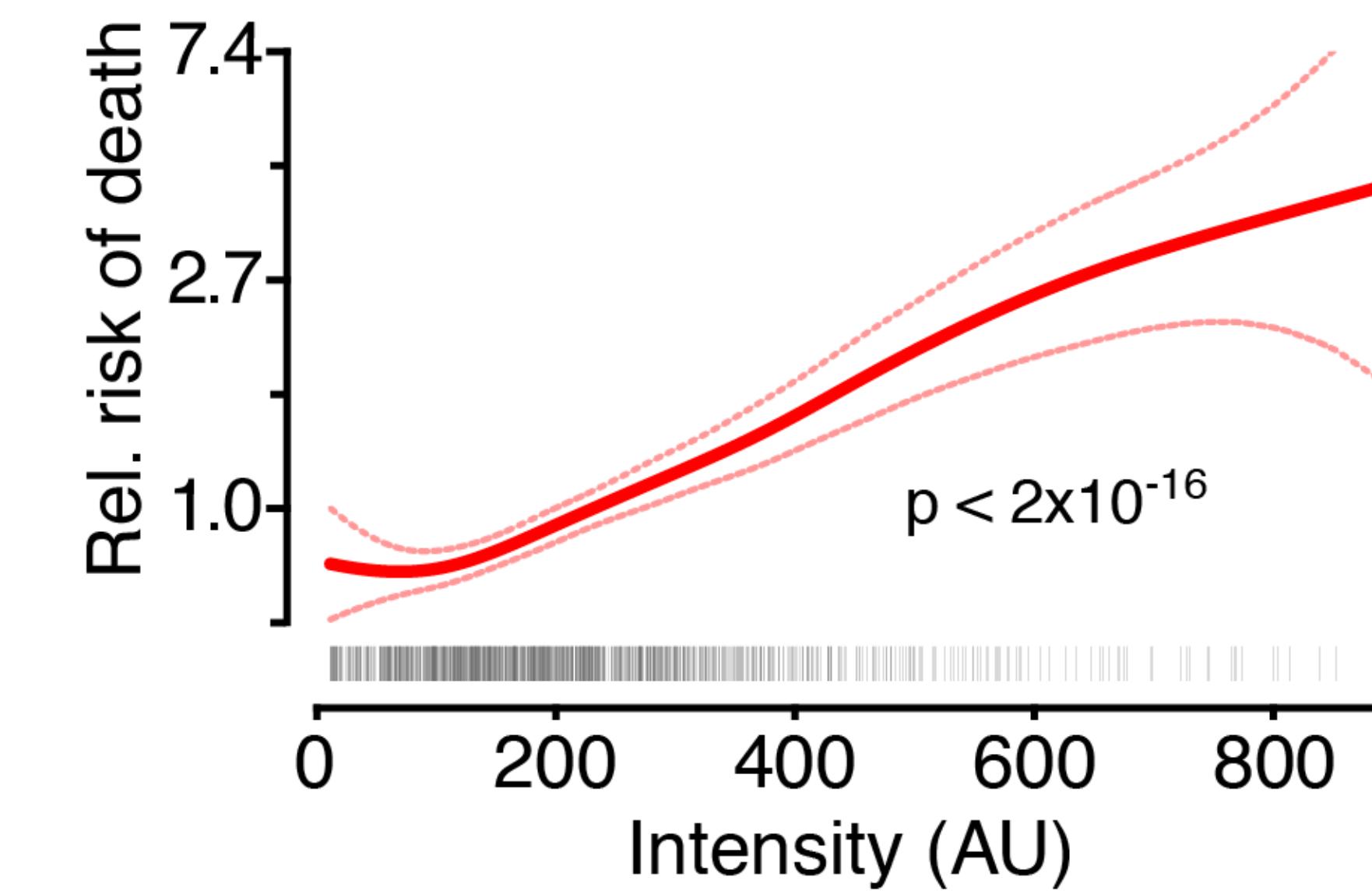
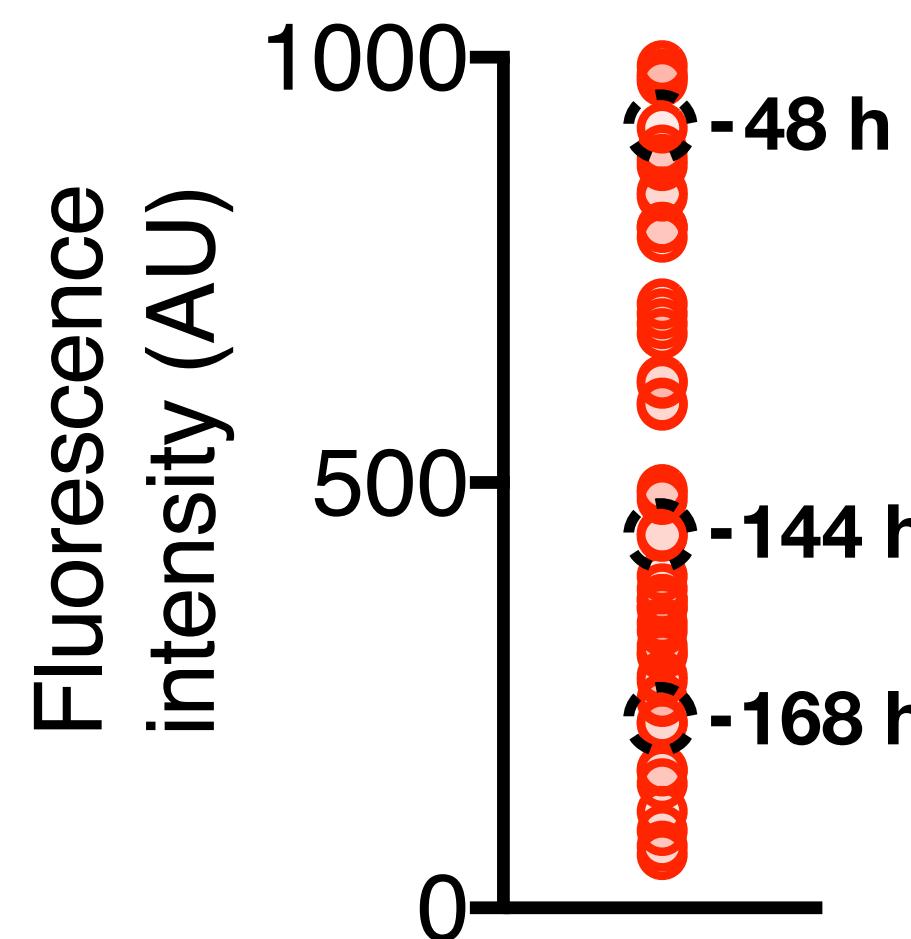
# Dose-dependent TDP43 toxicity



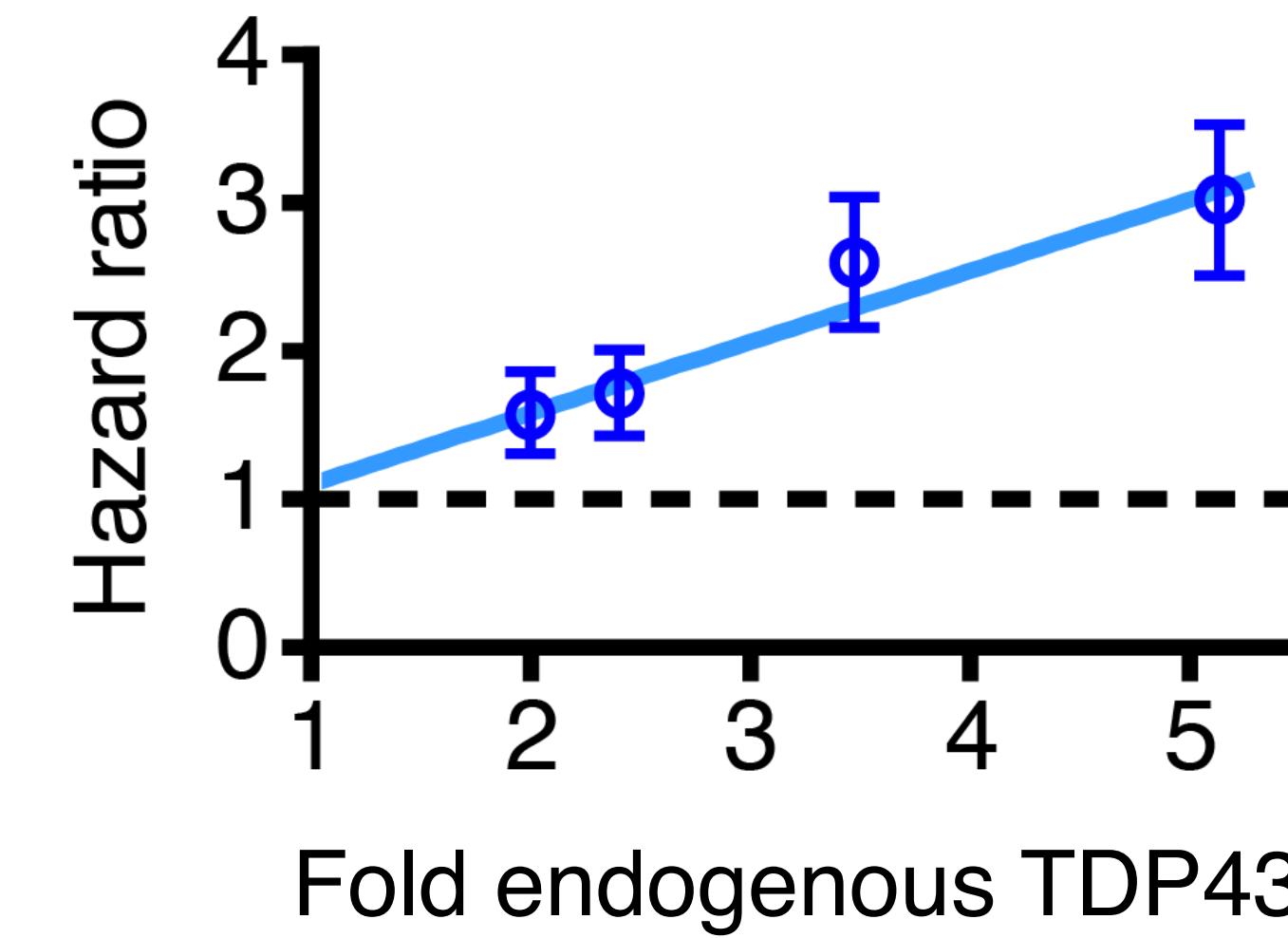
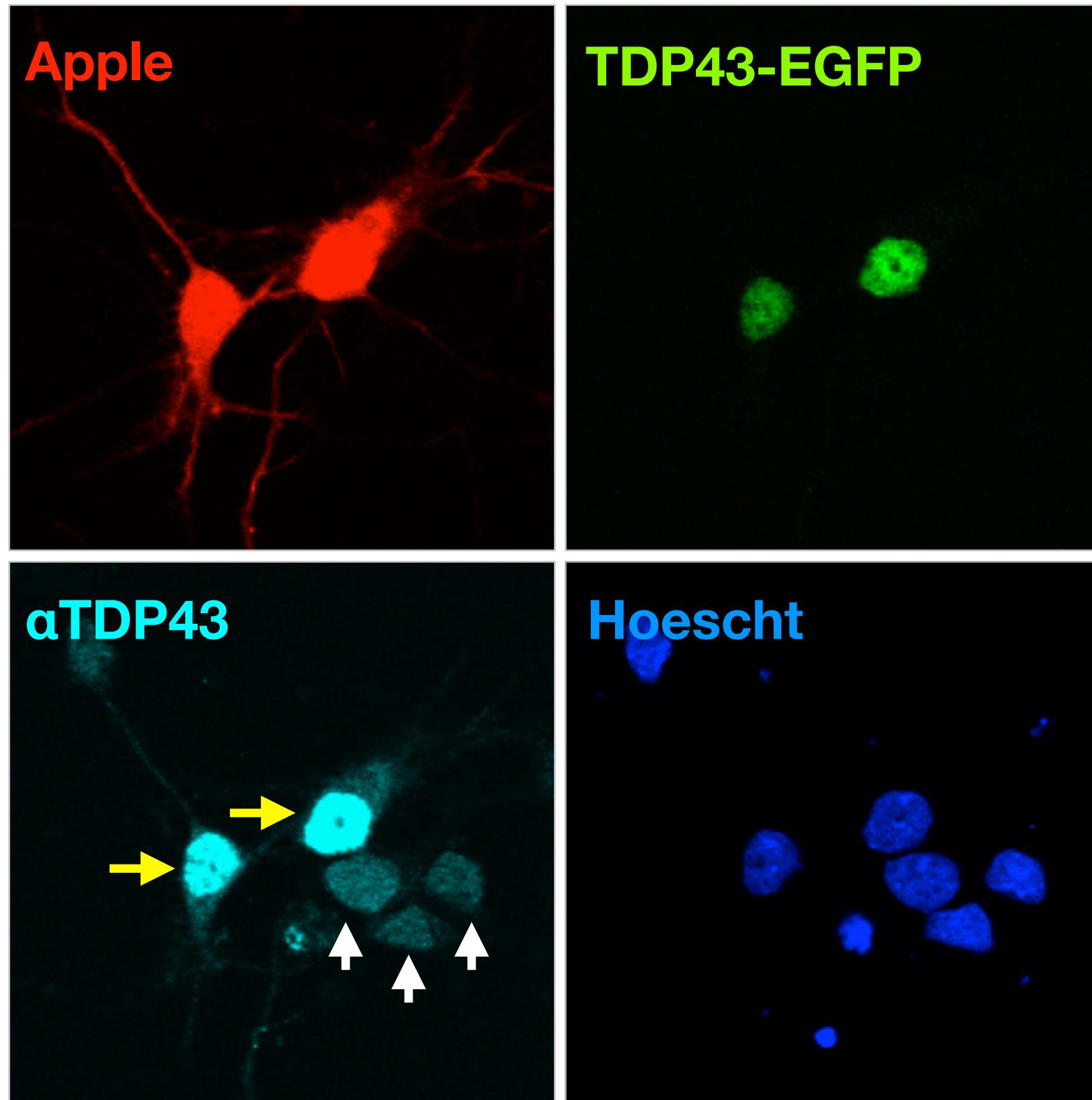
Low TDP43 expression



High TDP43 expression



# Dose-dependent TDP43 toxicity



Dose-dependent effects also noted for **Htt**, **MATR3**, **UBQLN2**, **C9orf72**, **FUS** and others

# Determinants of neurodegeneration

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aggregation

abundance

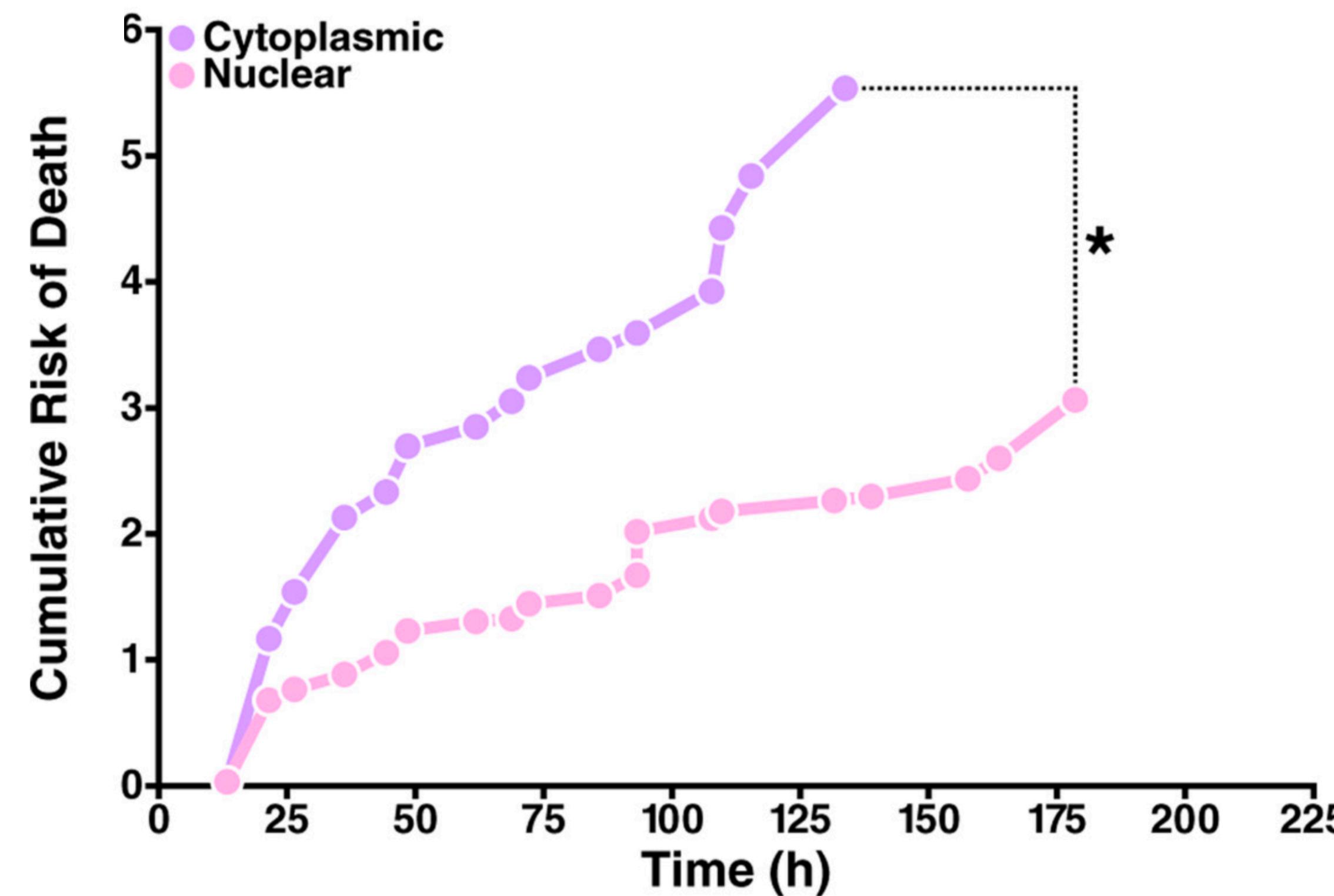
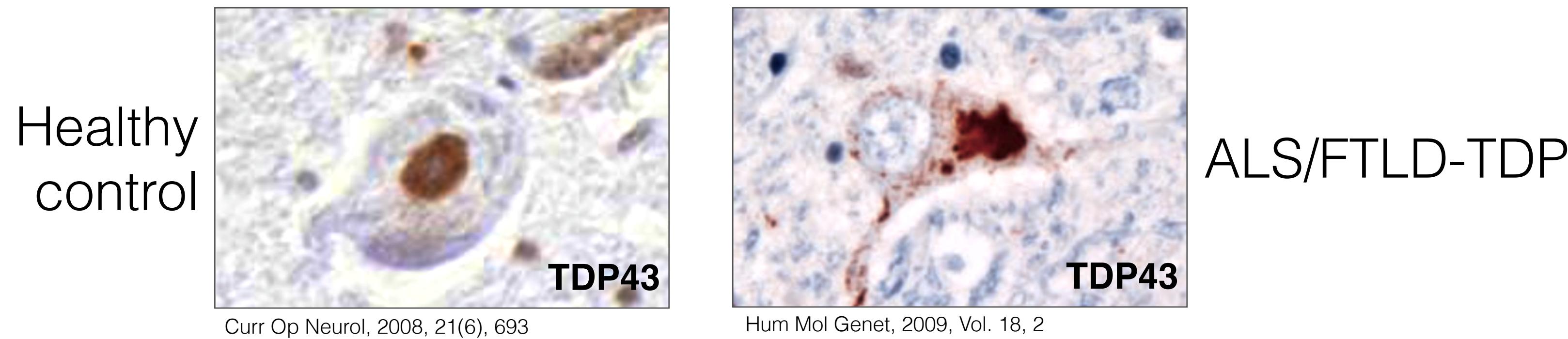
localization

clearance

**TDP43**

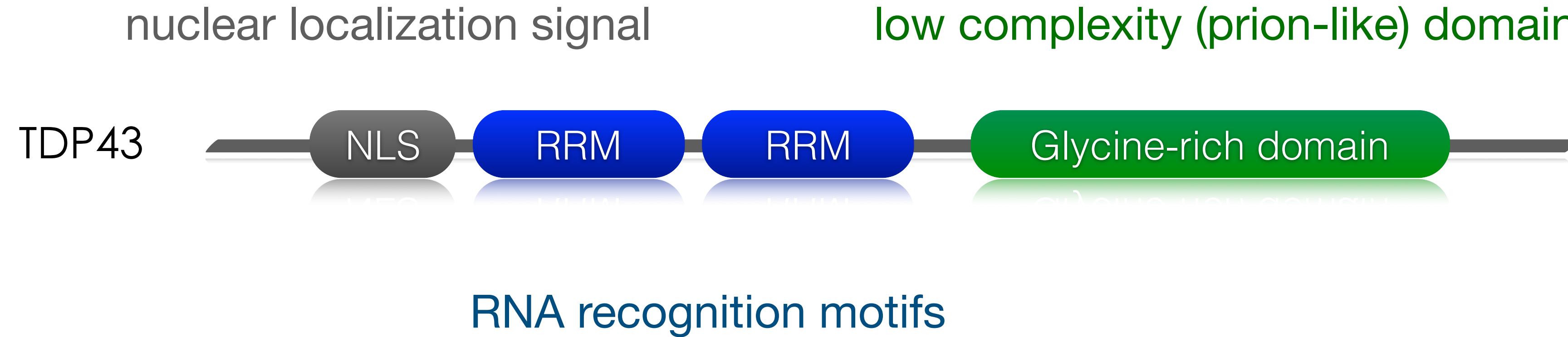
Matrin3

# Cytoplasmic TDP43 mislocalization enhances toxicity

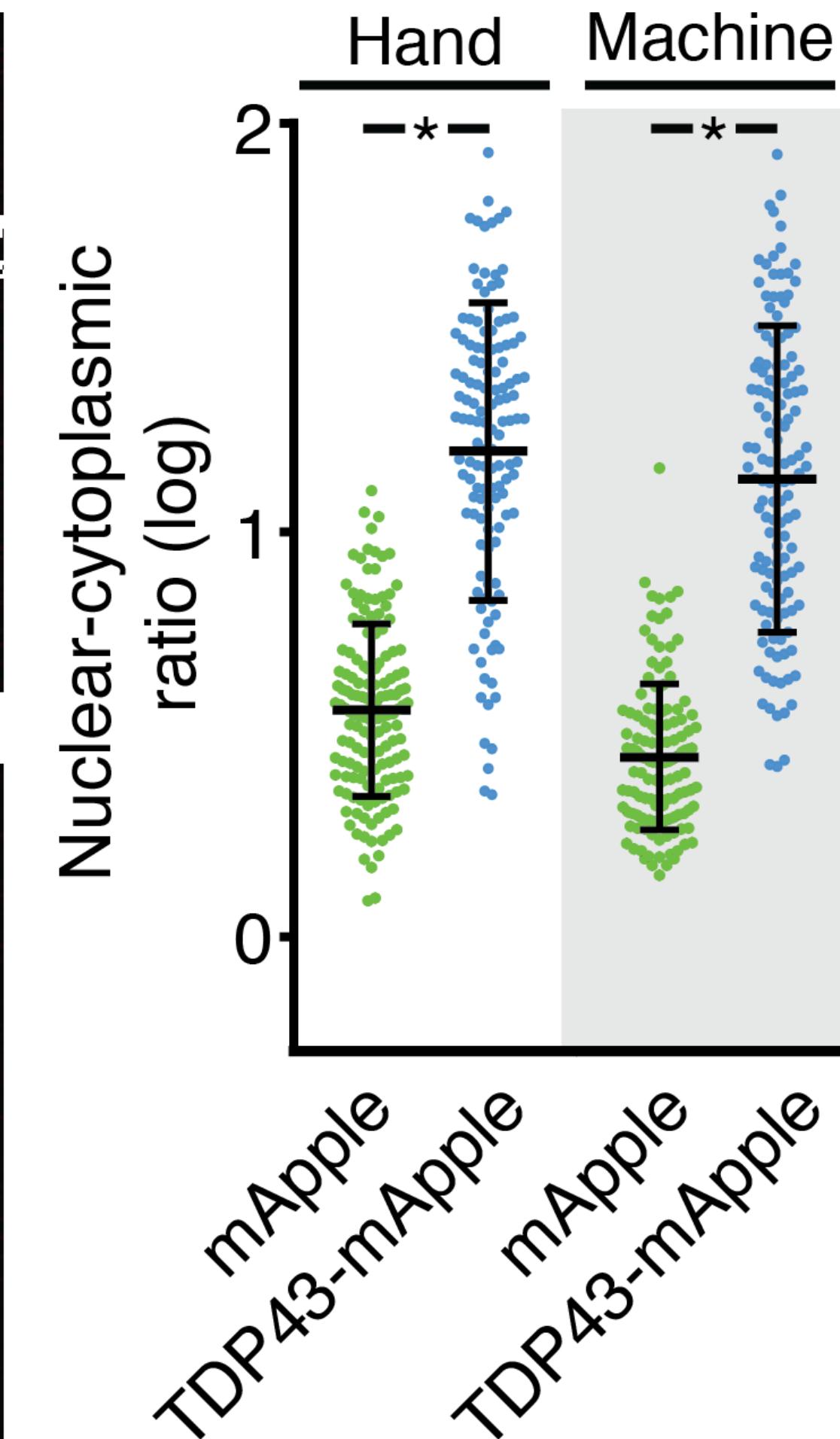
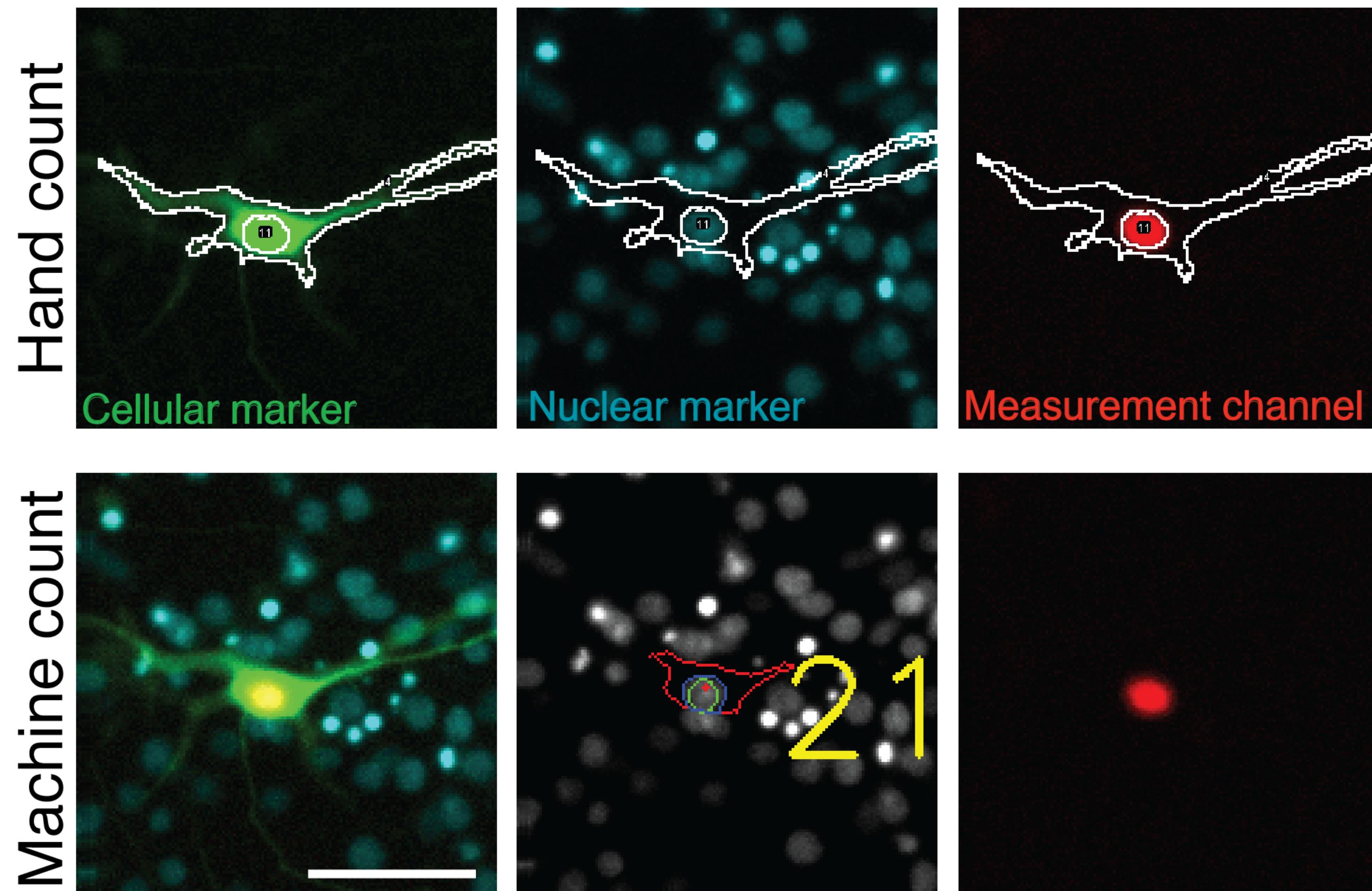


# Factors governing TDP43 localization

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# Automated detection of protein localization

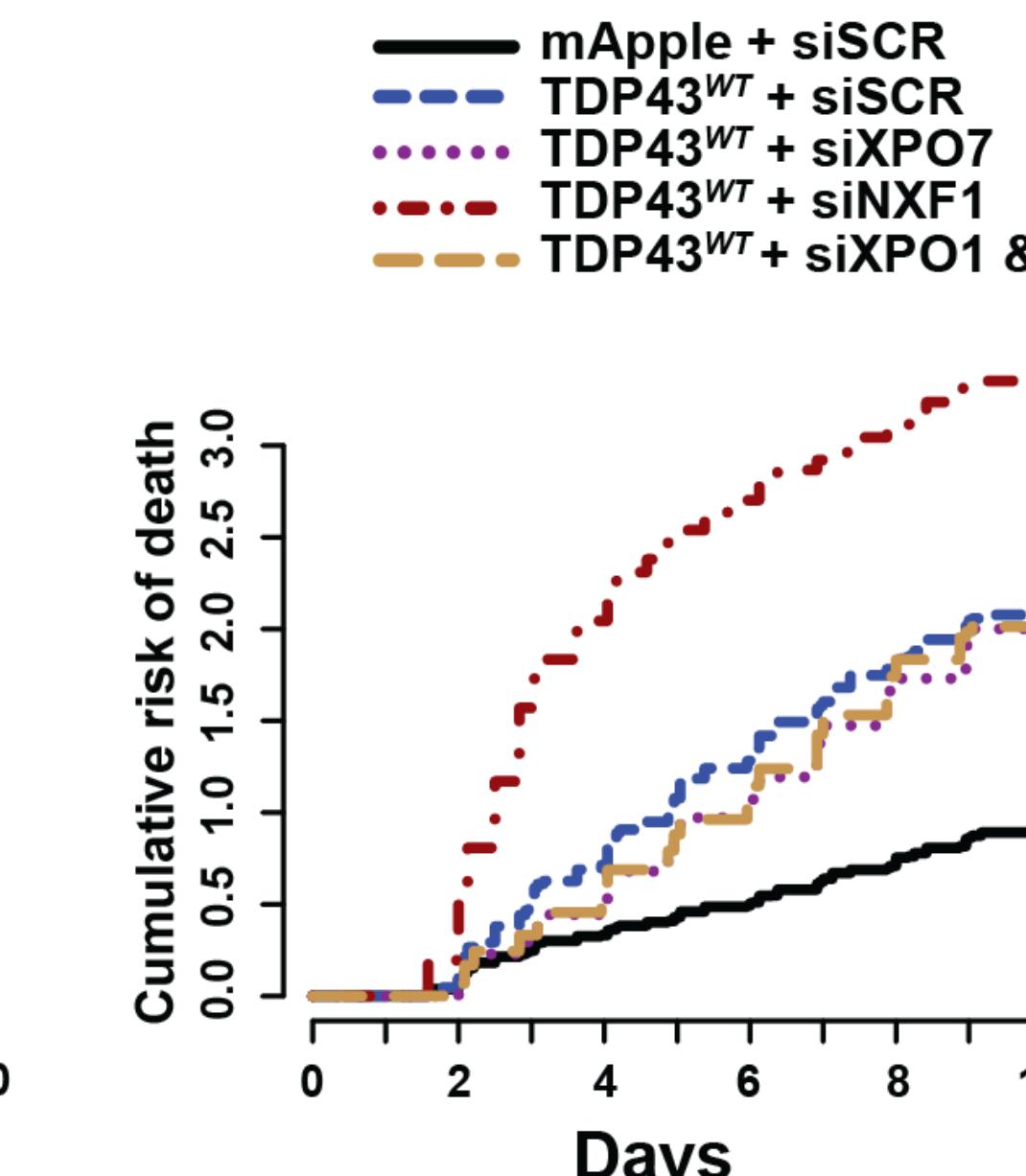
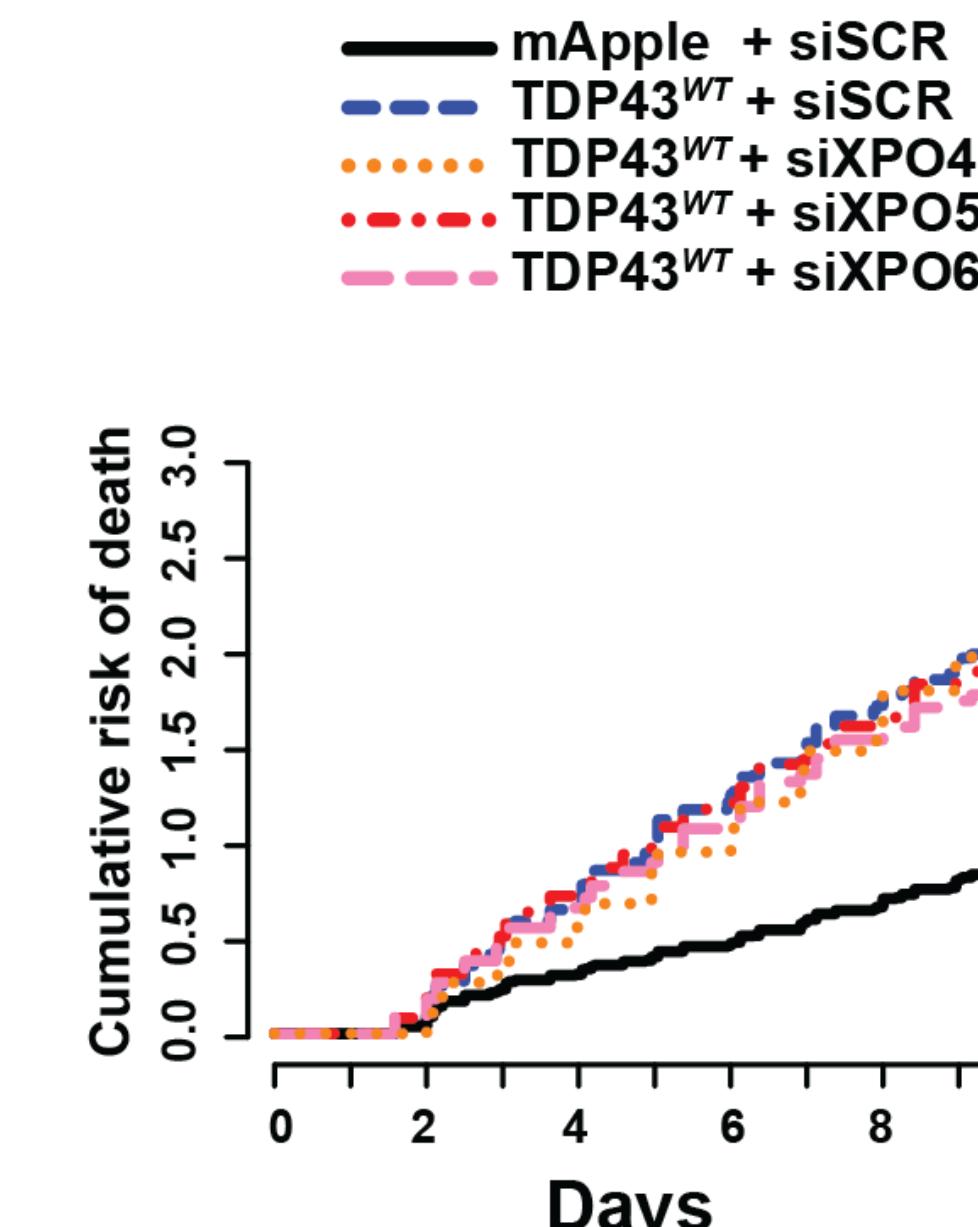
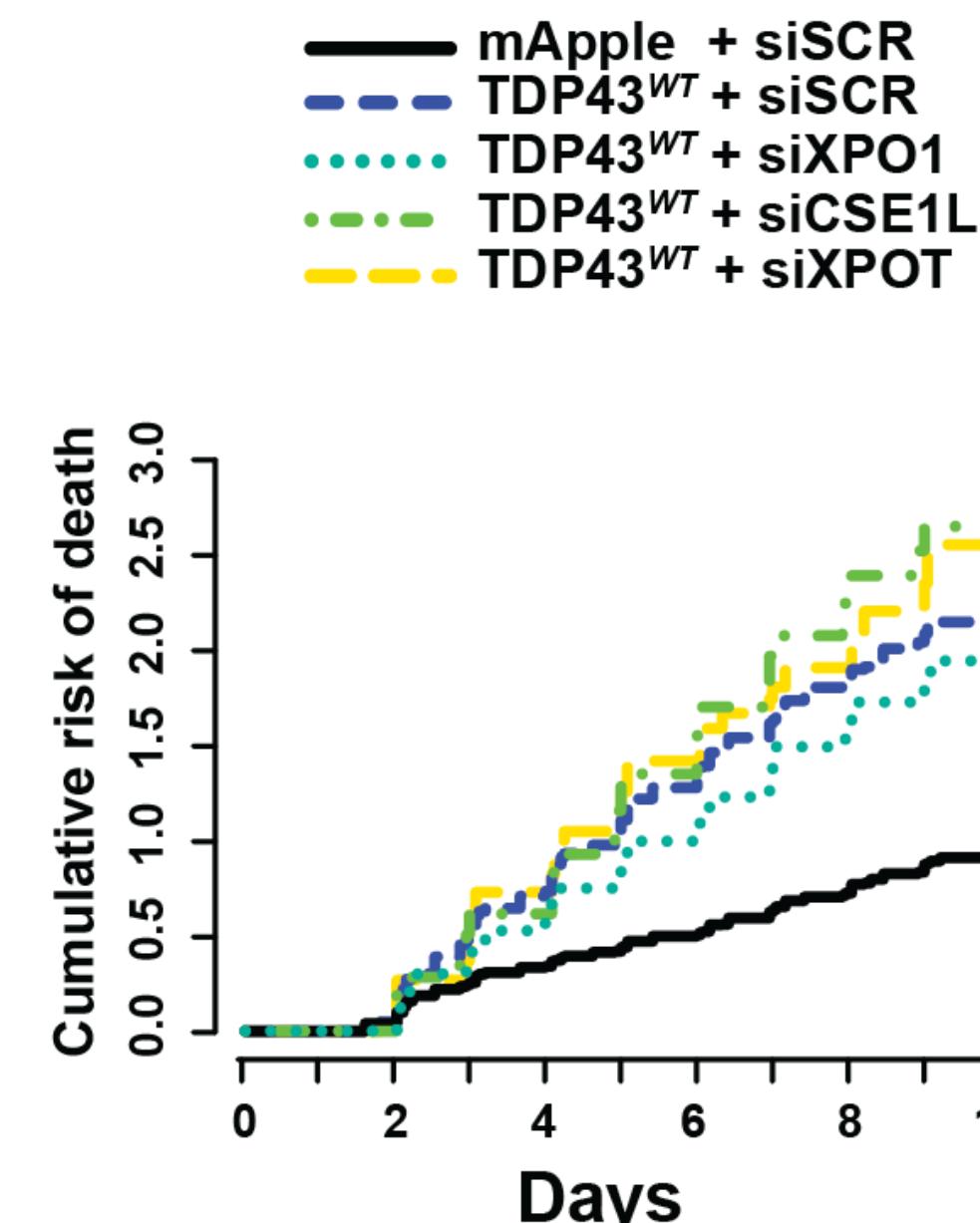


# XPO7 is a TDP43 exporter

Protein	Description		
<b>XPO1</b>	Exportin 1		
<b>CSE1L</b>	Chromosome Segregation 1 Like		
<b>XPO4</b>	Exportin 4		
<b>XPO5</b>	Exportin 5		
<b>XPO6</b>	Exportin 6		
<b>XPO7</b>	Exportin 7		
<b>XPOT</b>	Exportin T		
<b>NXF1</b>	Nuclear RNA export factor 1		

	mApple + siSCR	TDP43 <sup>WT</sup> + siSCR	TDP43 <sup>WT</sup> + siXPO1	TDP43 <sup>WT</sup> + siCSE1L	TDP43 <sup>WT</sup> + siXPOT
	mApple + siSCR	TDP43 <sup>WT</sup> + siSCR	TDP43 <sup>WT</sup> + siXPO4	TDP43 <sup>WT</sup> + siXPO5	TDP43 <sup>WT</sup> + siXPO6
	mApple + siSCR	TDP43 <sup>WT</sup> + siSCR	TDP43 <sup>WT</sup> + siXPO7	TDP43 <sup>WT</sup> + siNXF1	TDP43 <sup>WT</sup> + siXPO1 & 7



# XPO7 is a TDP43 exporter

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Published Online: 10 May, 2018 | Supp Info: <http://doi.org/10.1083/jcb.201712013>  
Downloaded from [jcb.rupress.org](http://jcb.rupress.org) on May 21, 2018



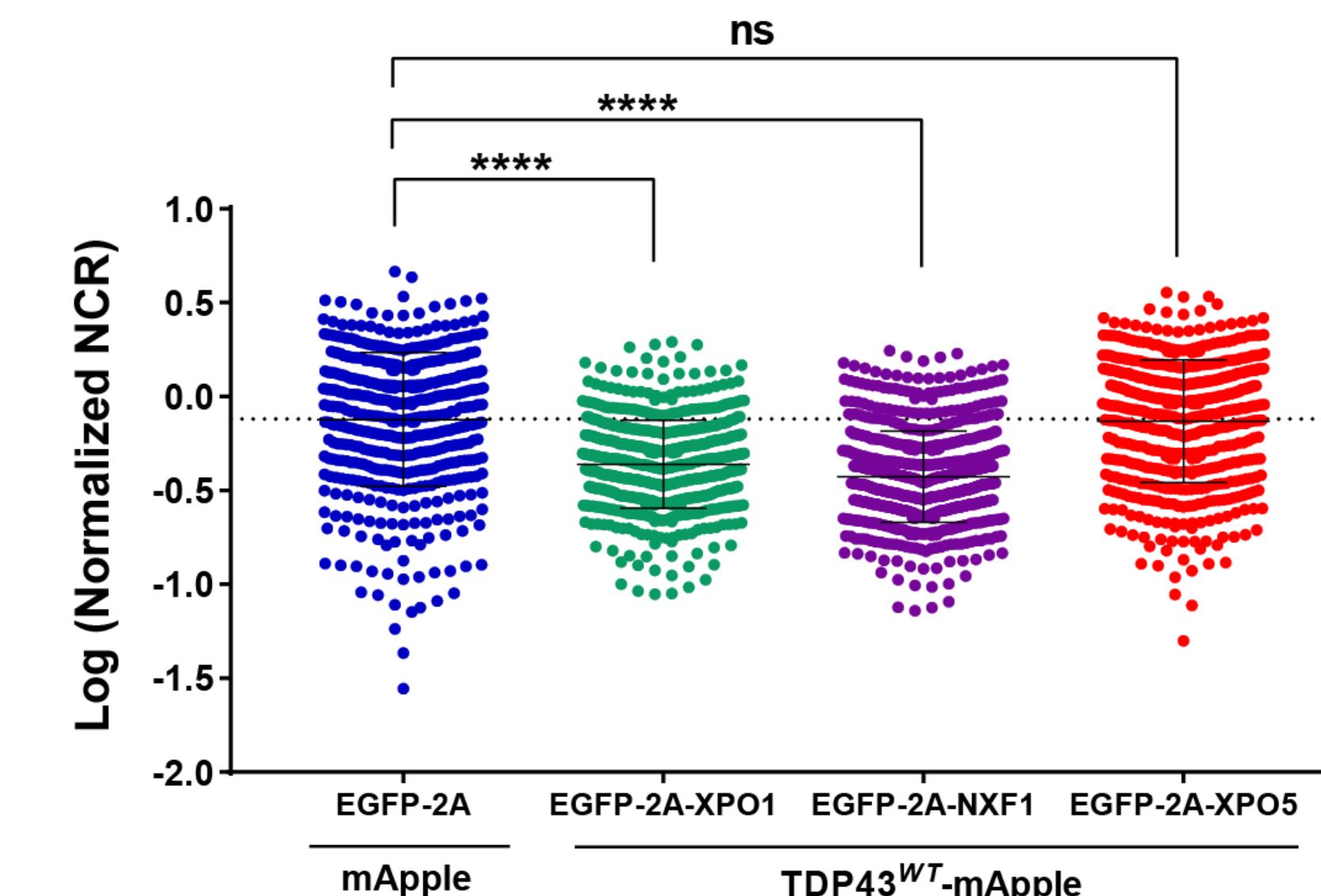
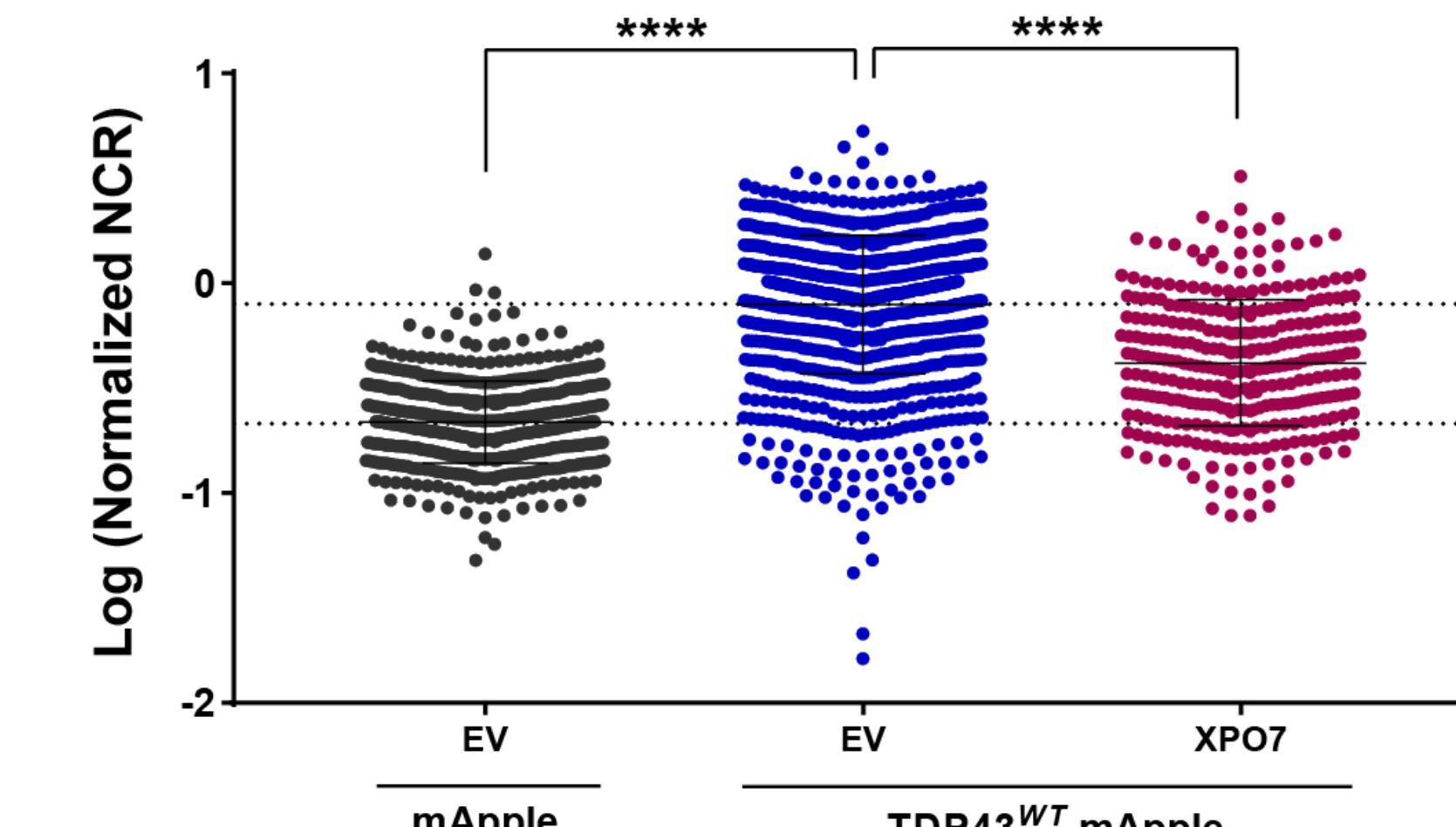
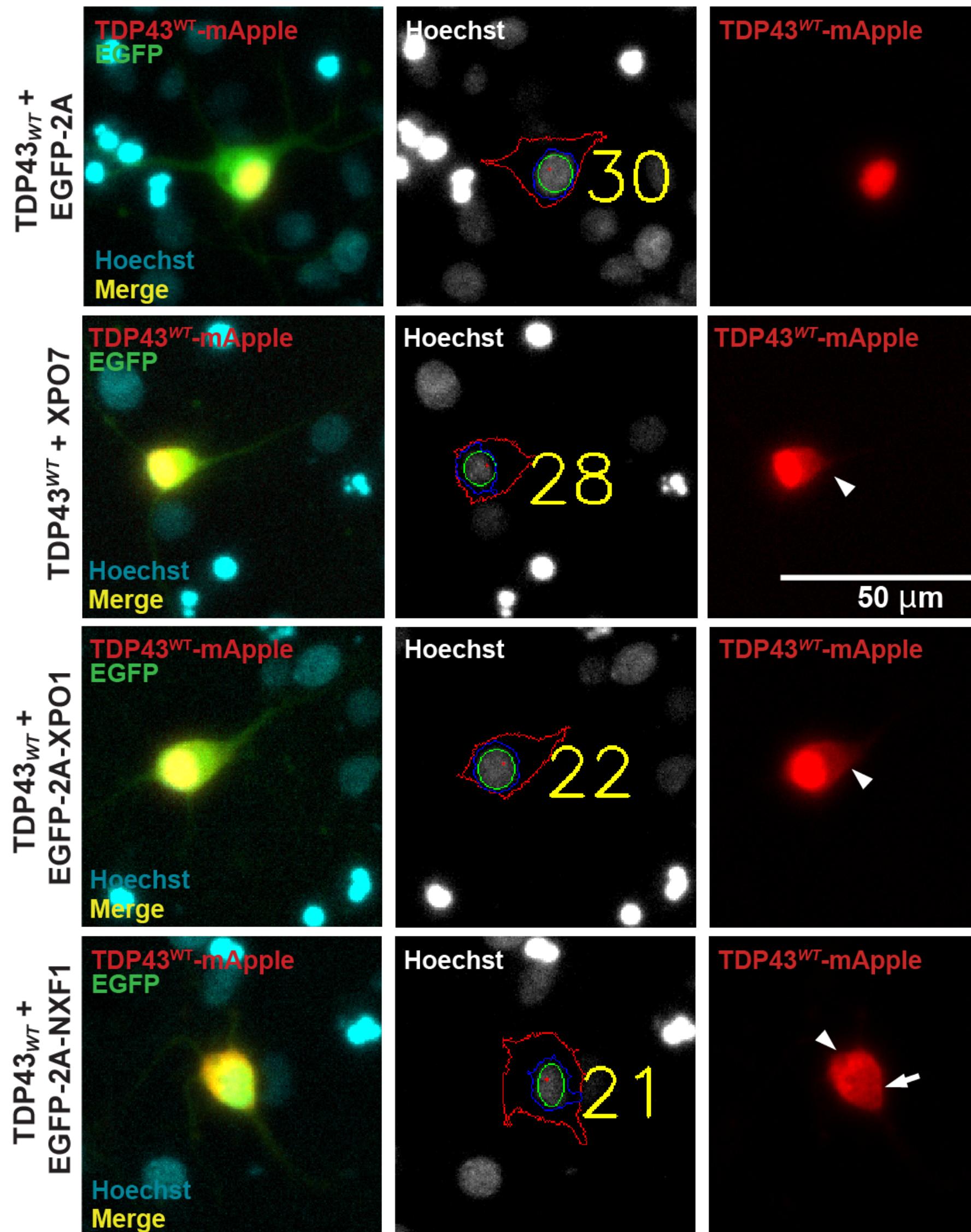
## REPORT

# Xpo7 is a broad-spectrum exportin and a nuclear import receptor

Metin Aksu<sup>1\*</sup> , Tino Pleiner<sup>1\*</sup> , Samir Karaca<sup>2</sup>, Christin Kappert<sup>1</sup> , Heinz-Jürgen Dehne<sup>1</sup>, Katharina Seibel<sup>1</sup> , Henning Urlaub<sup>2,3</sup>, Markus T. Bohnsack<sup>4</sup> , and Dirk Görlich<sup>1</sup>

Protein name	Category	Exportin Score	Importin Score	Enrichment from input (Xpo7+Ran)	Enrichment from input (Xpo7)	Simplified Localisation UniProt	Molecular weight [kDa]
TAR DNA-binding protein 43 (TDP-43)	Export	4.3	-5.1	22.8	0.9	Nucleus	44.5

# XPO7, XPO1 and NXF1 are all sufficient to export TDP43



# Determinants of neurodegeneration

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aggregation

abundance

localization

clearance

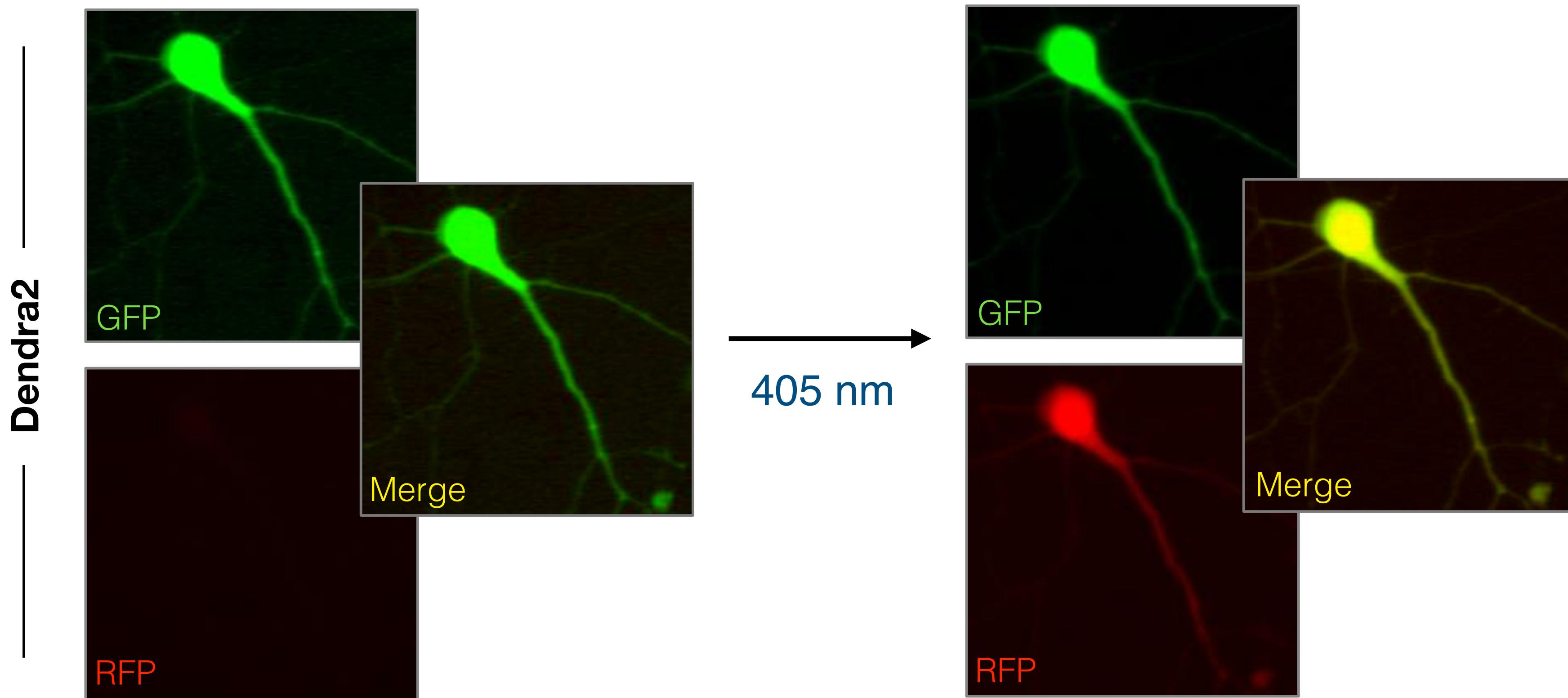
catabolic pathways

**TDP43**

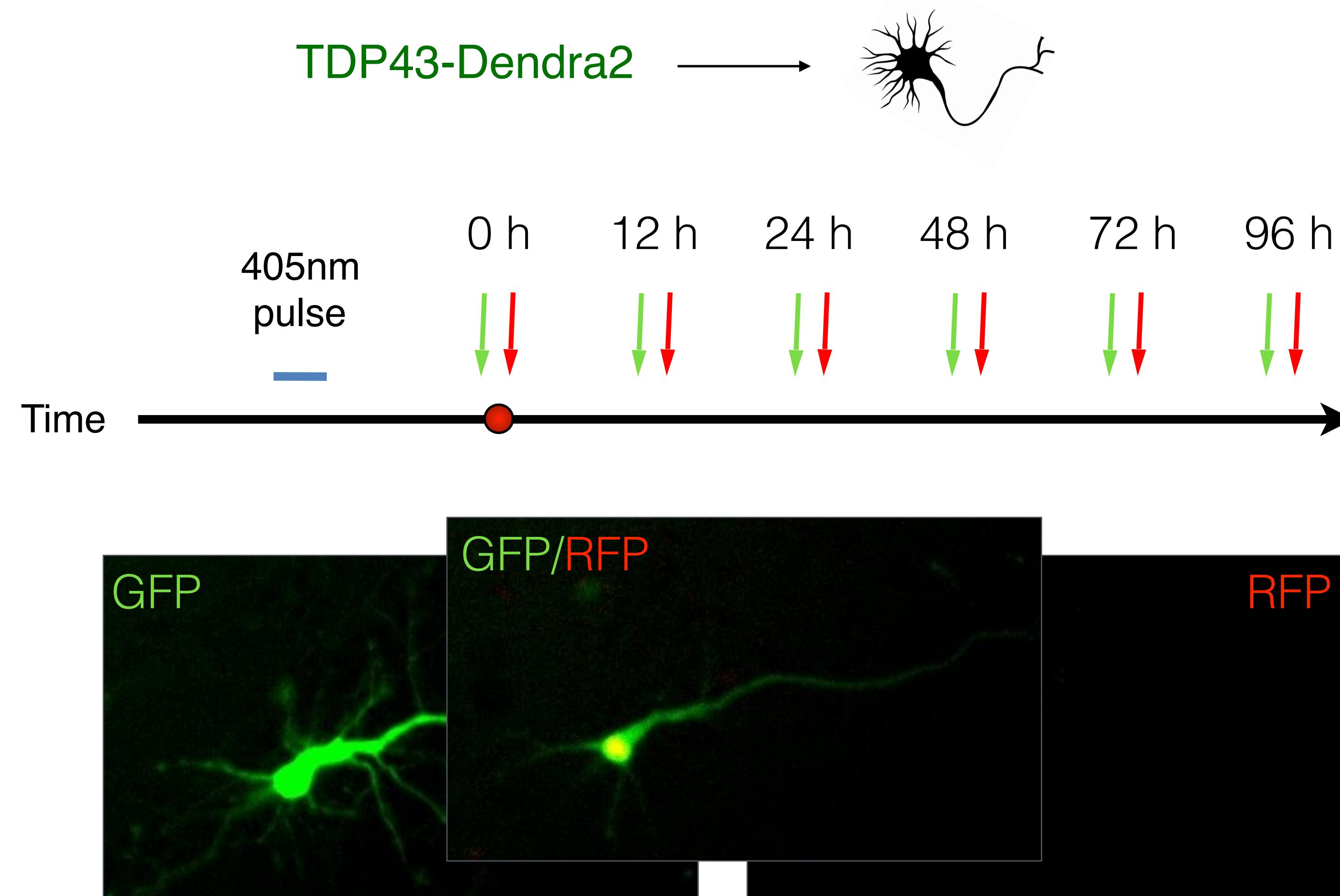
catabolic pathways

# Dendra2 labeling for estimations of protein clearance *in situ*

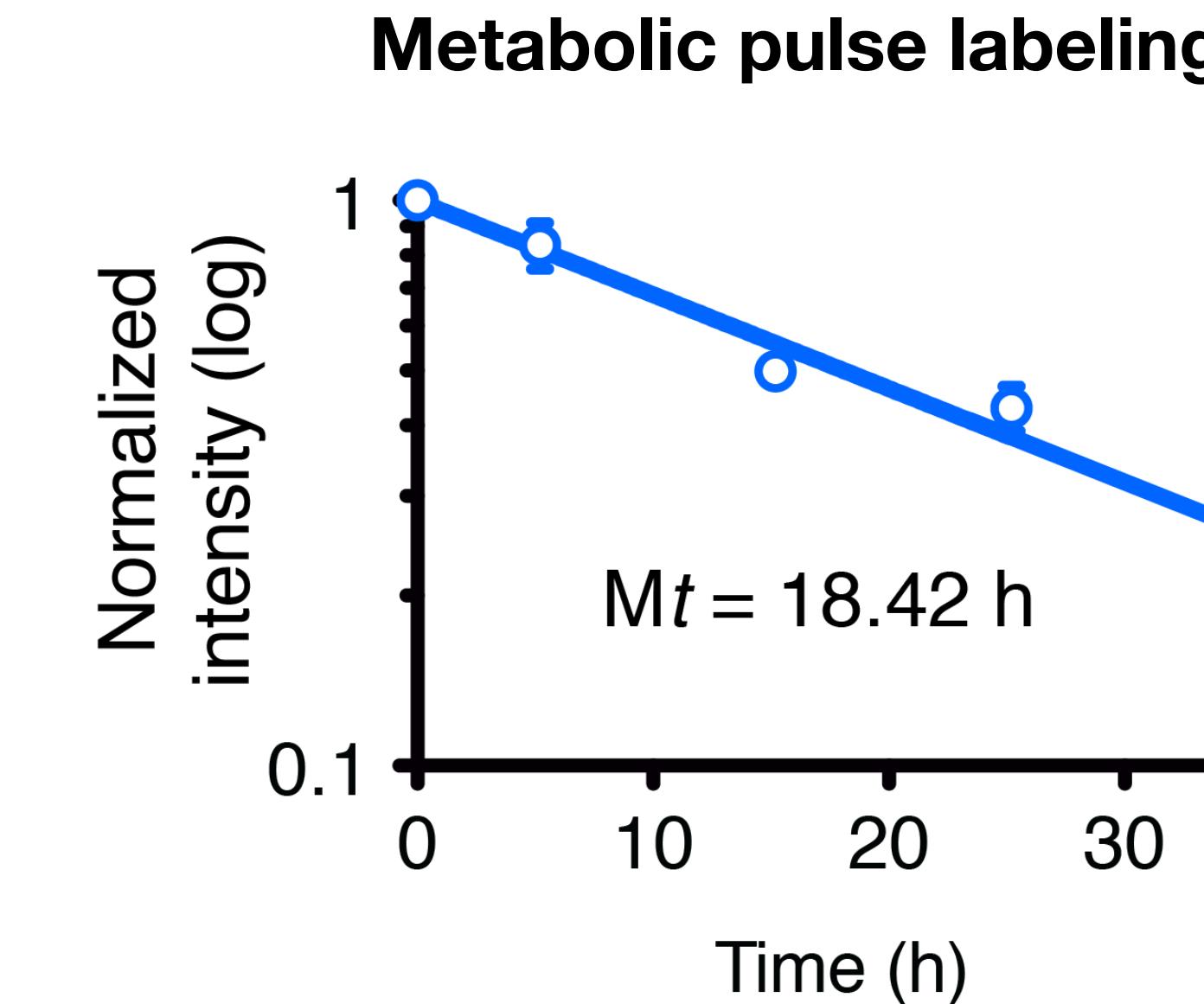
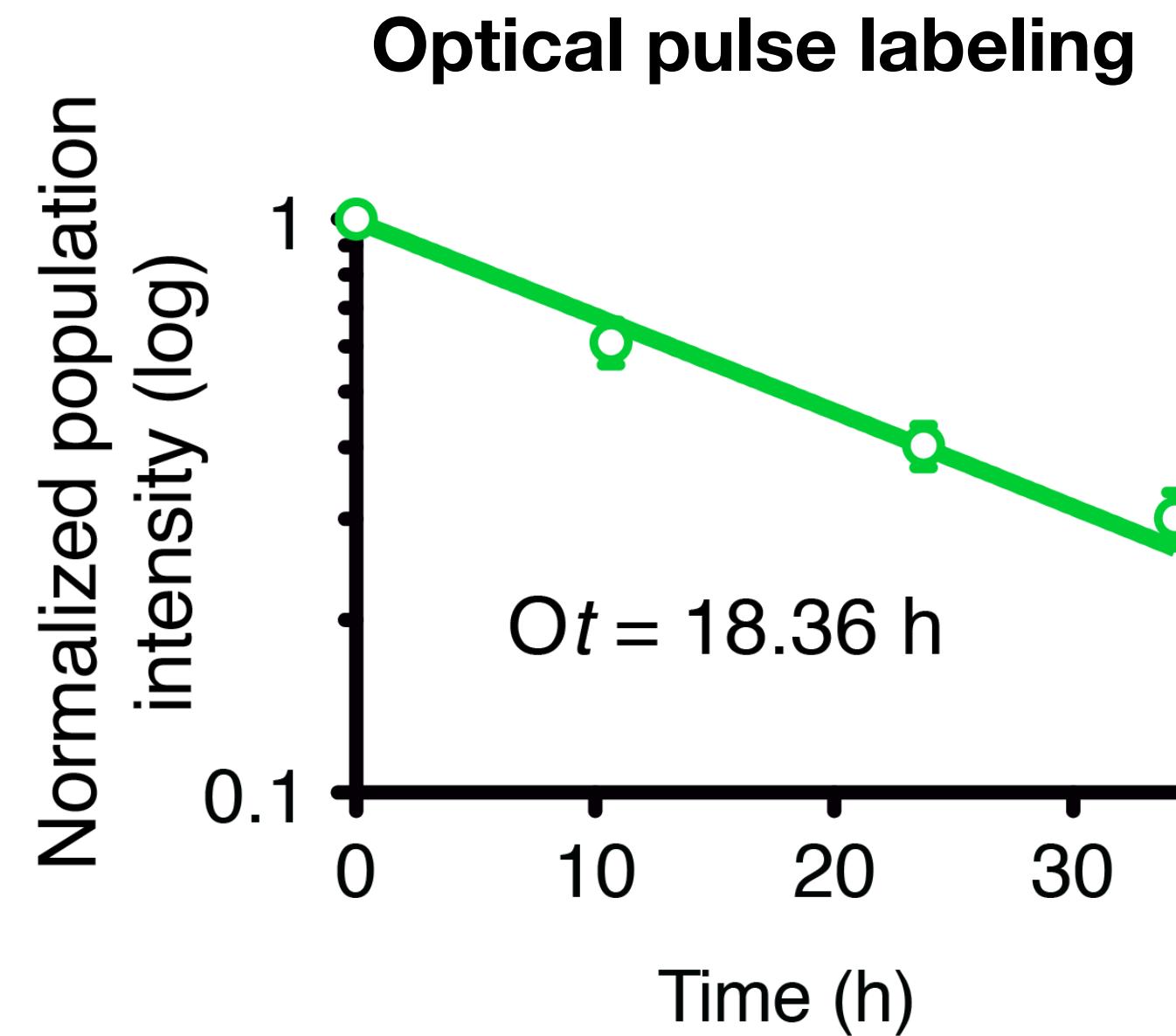
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# Tracking TDP43 clearance by optical pulse labeling



# Optical pulse labeling is an accurate means of tracking protein turnover



## Effect of disease associated mutations in TDP43, MATR3, Htt

Barmada et al. *Nature Chem Biol.* (2014)

Tsvetkov et al. *Nature Chem Biol.* (2013)

Malik et al. *eLife* (2018)

Flores et al. *Cell Reports* (2019)

## Effect of autophagy/proteasome manipulation on protein turnover

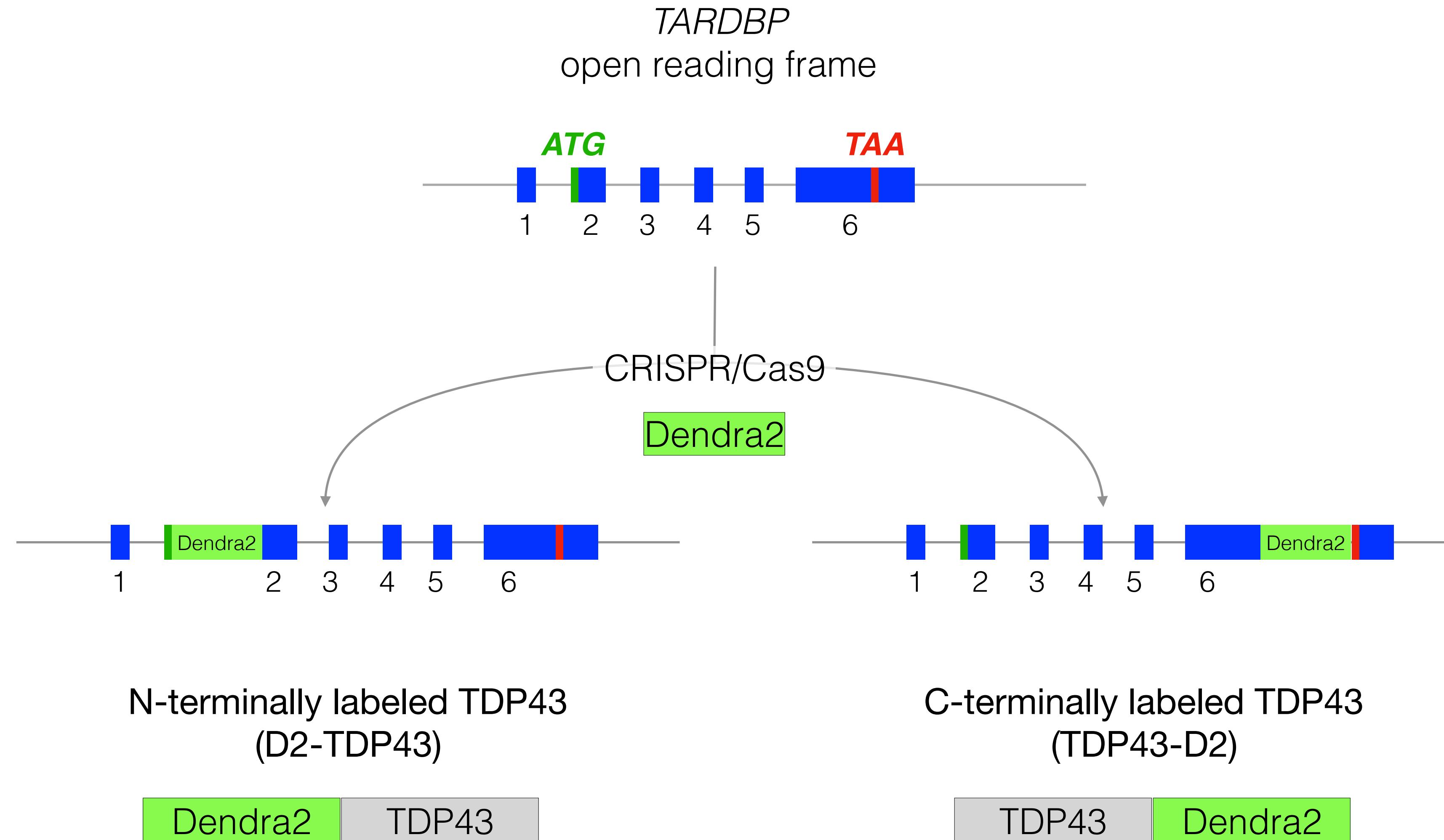
Barmada et al. *Nature Chem Biol.* (2014)

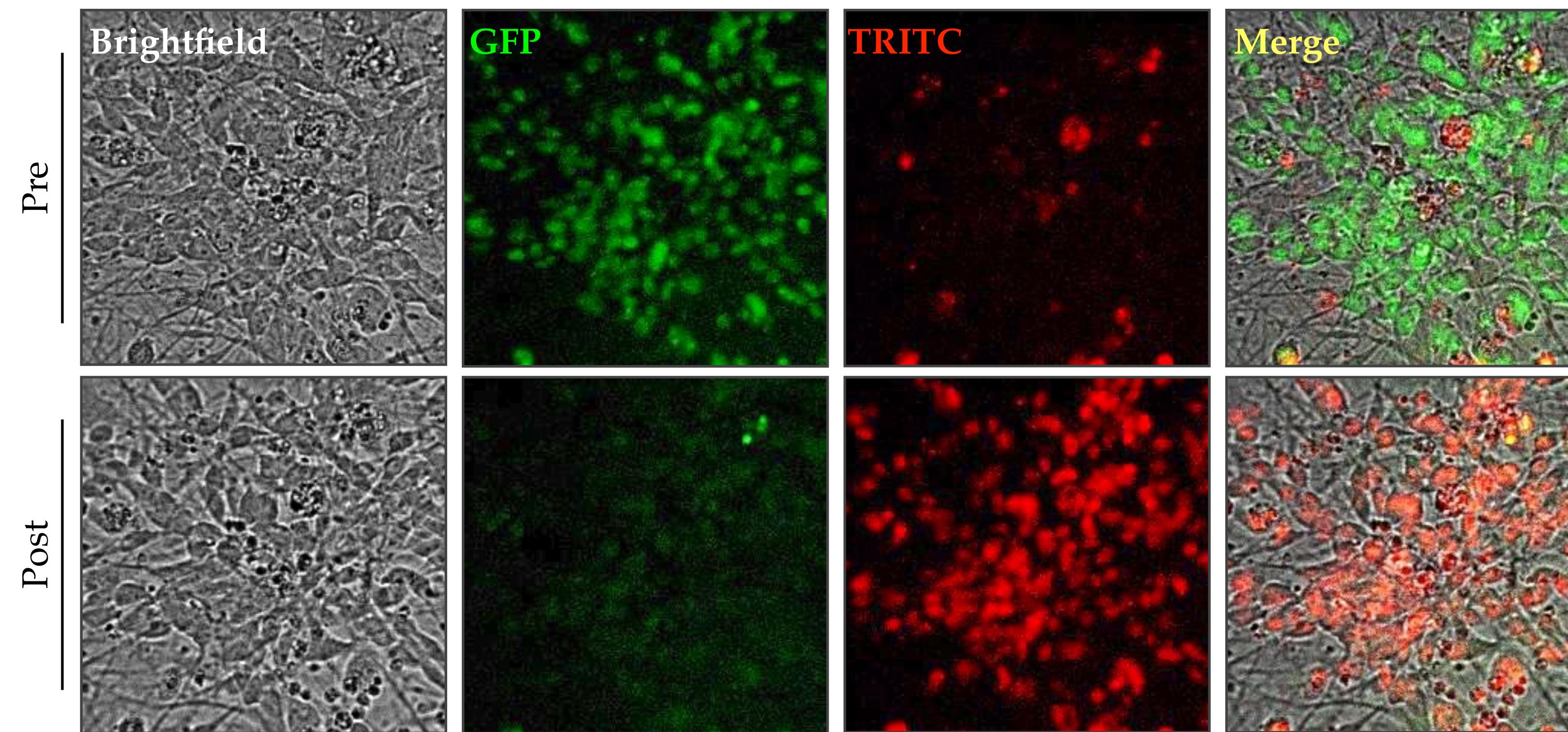
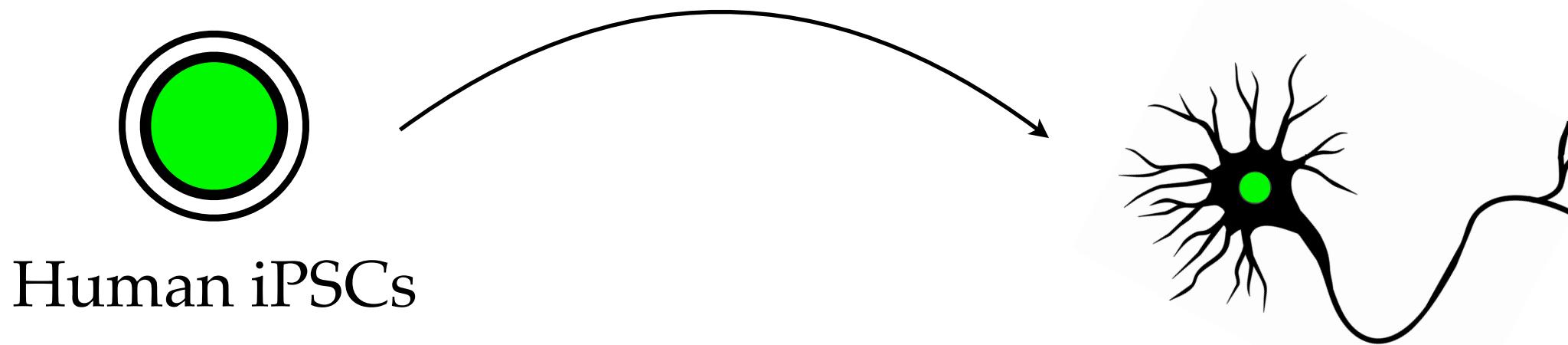
Gupta et al. *eNeuro* (2017)

Al-Ramahi et al. *eLife* (2017)

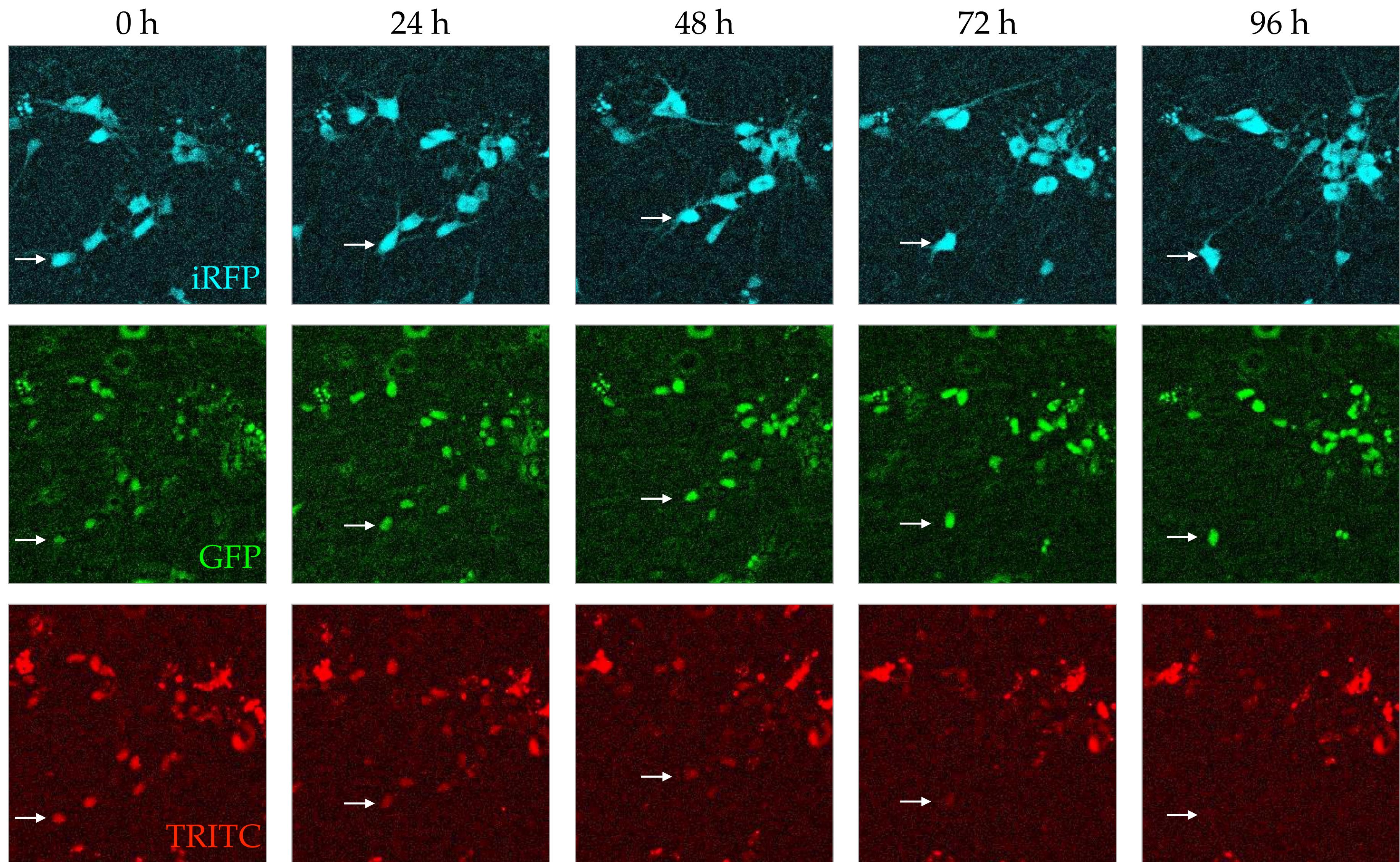
Safren et al. *under review*

# Labeling endogenous TDP43





Elizabeth Tank, Nate Safren, Kaitlin Weskamp



Elizabeth Tank, Nate Safren, Kaitlin Weskamp

# Modulating neurodegeneration

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## Pharmacologic

Genetic

siRNA/shRNA  
overexpression  
antisense oligonucleotides (ASOs)  
locked nucleic acids (LNAs)  
CRISPR/Cas9

Armakola et al. *Nature Genetics* (2012)

Barmada et al. *Nature Chem Biol.* (2014)

Barmada et al. *PNAS* (2015)

Green et al. *Nature Comm.* (2017)

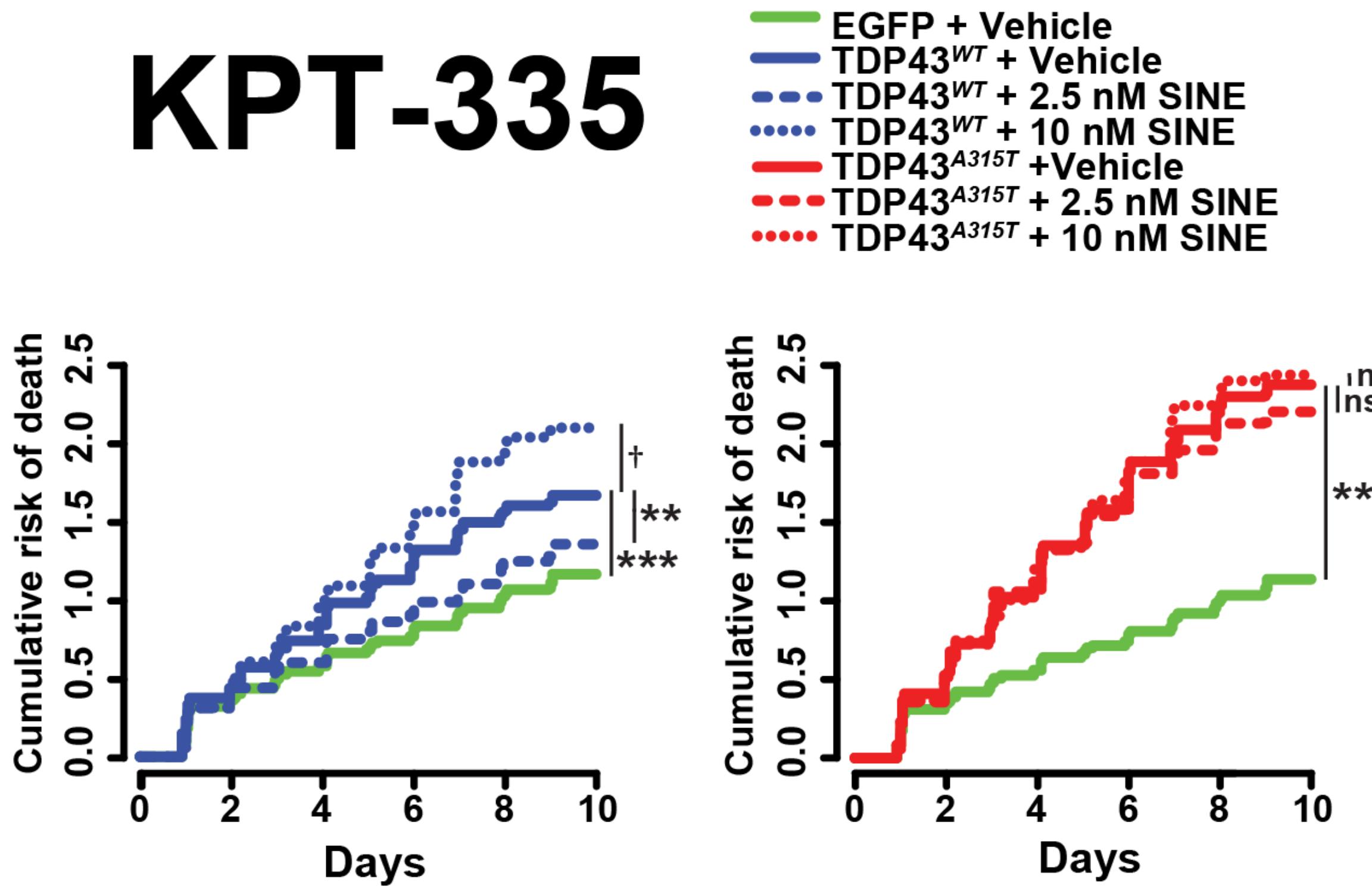
Archbold et al. *Sci. Reports* (2018)

Linsalata et al. *EMBO Reports* (2019)

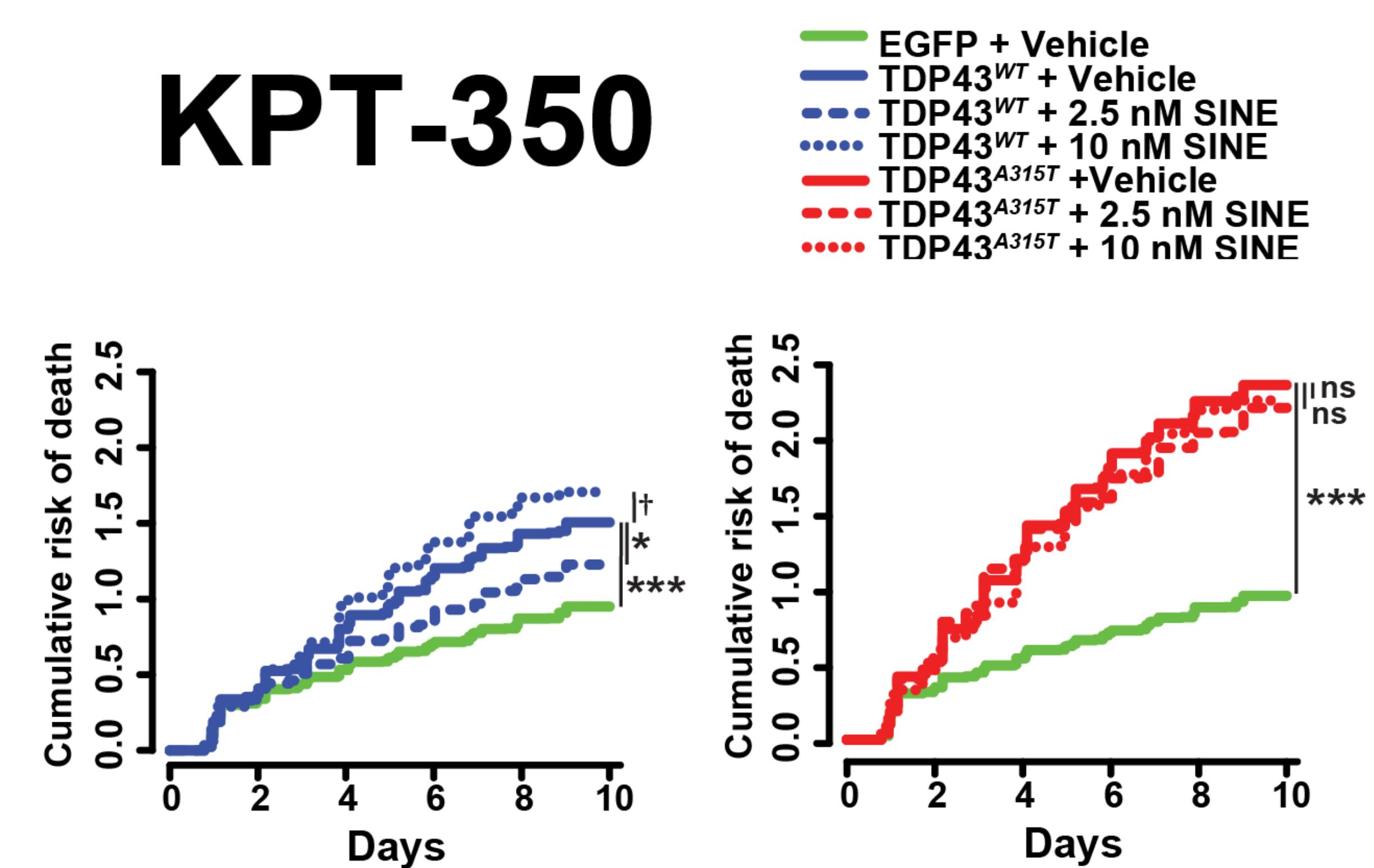
Safren et al. *under review*

# XPO1 inhibition extends neuronal survival in ALS/FTLD-TDP models

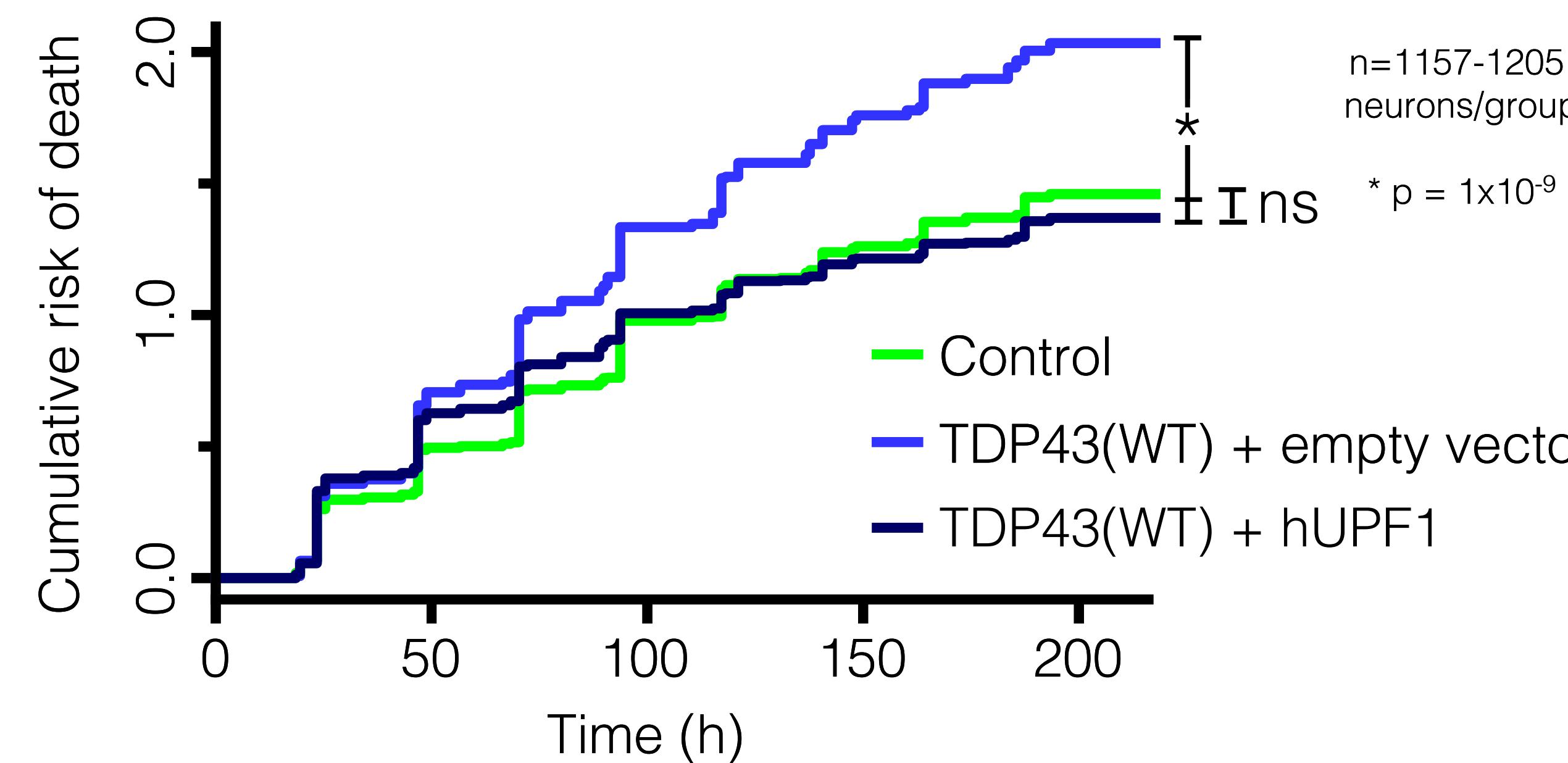
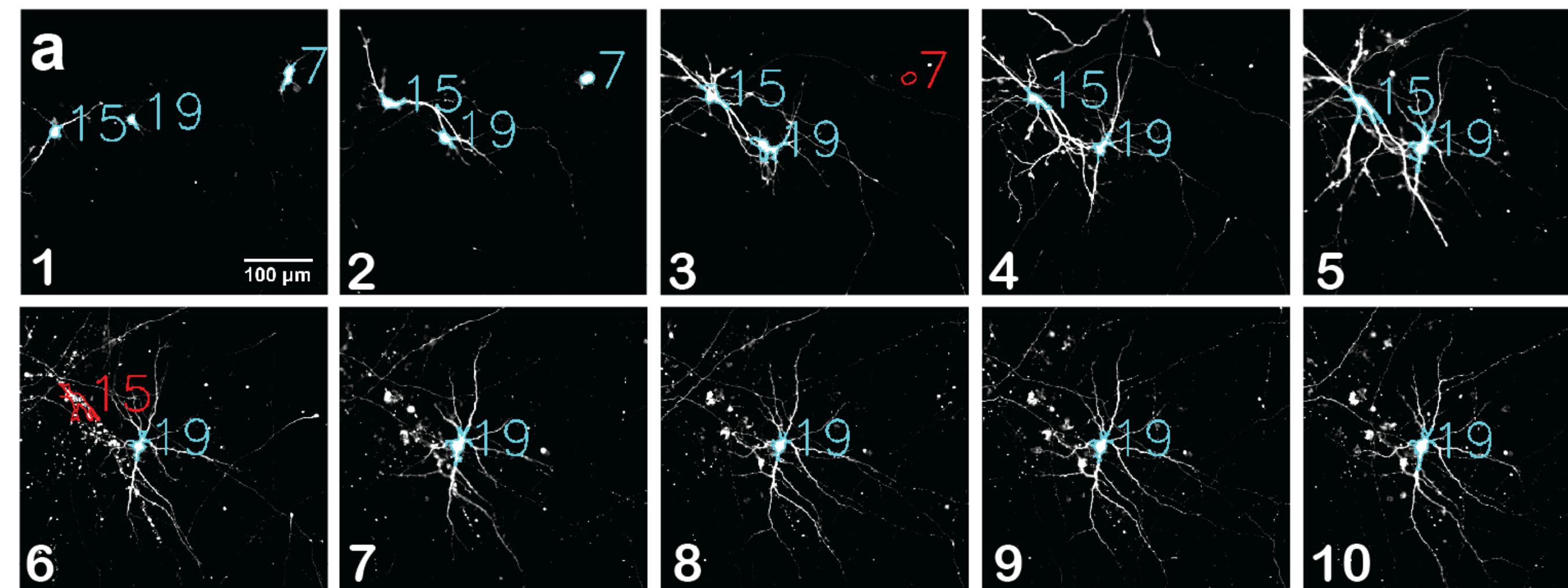
**KPT-335**



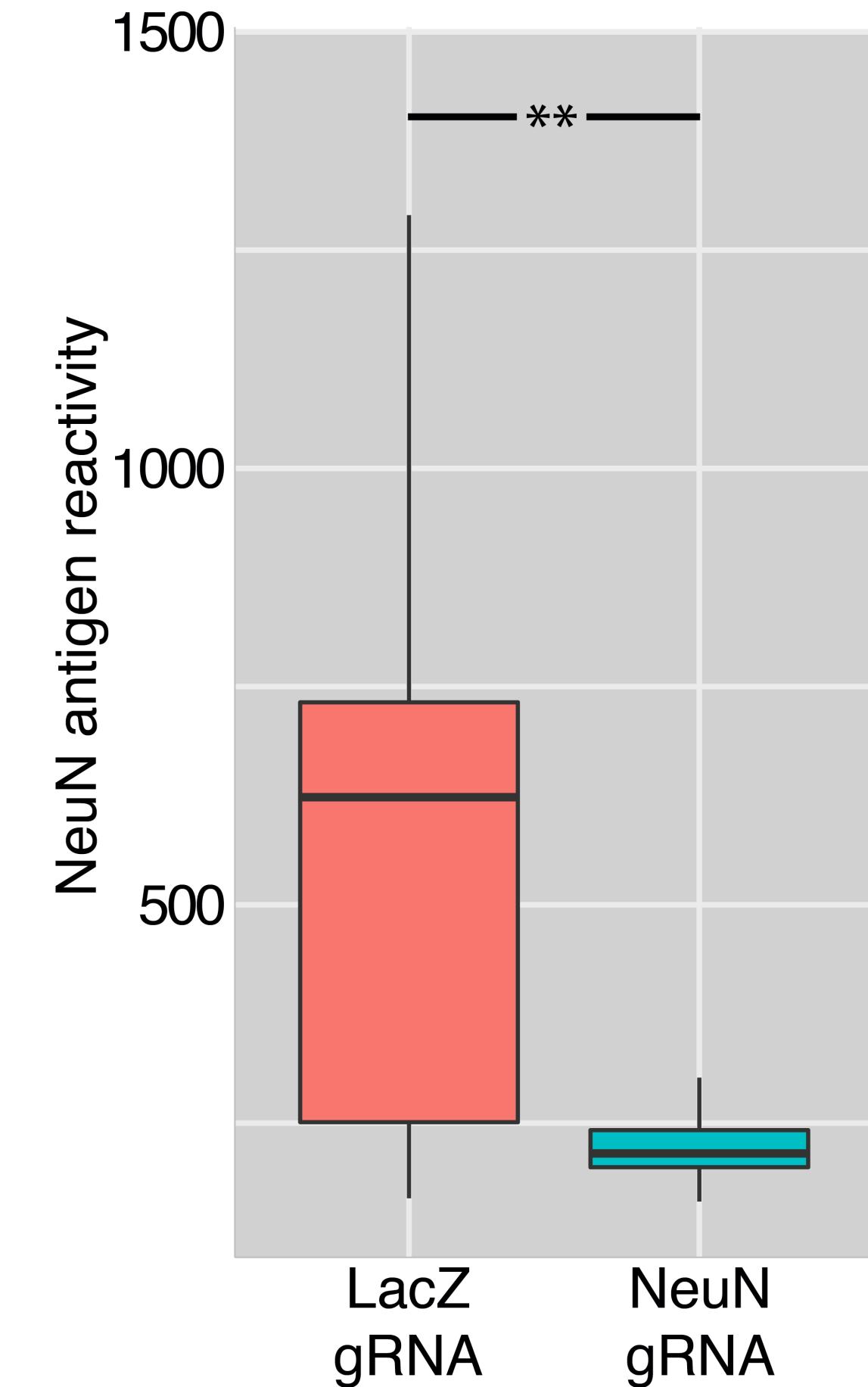
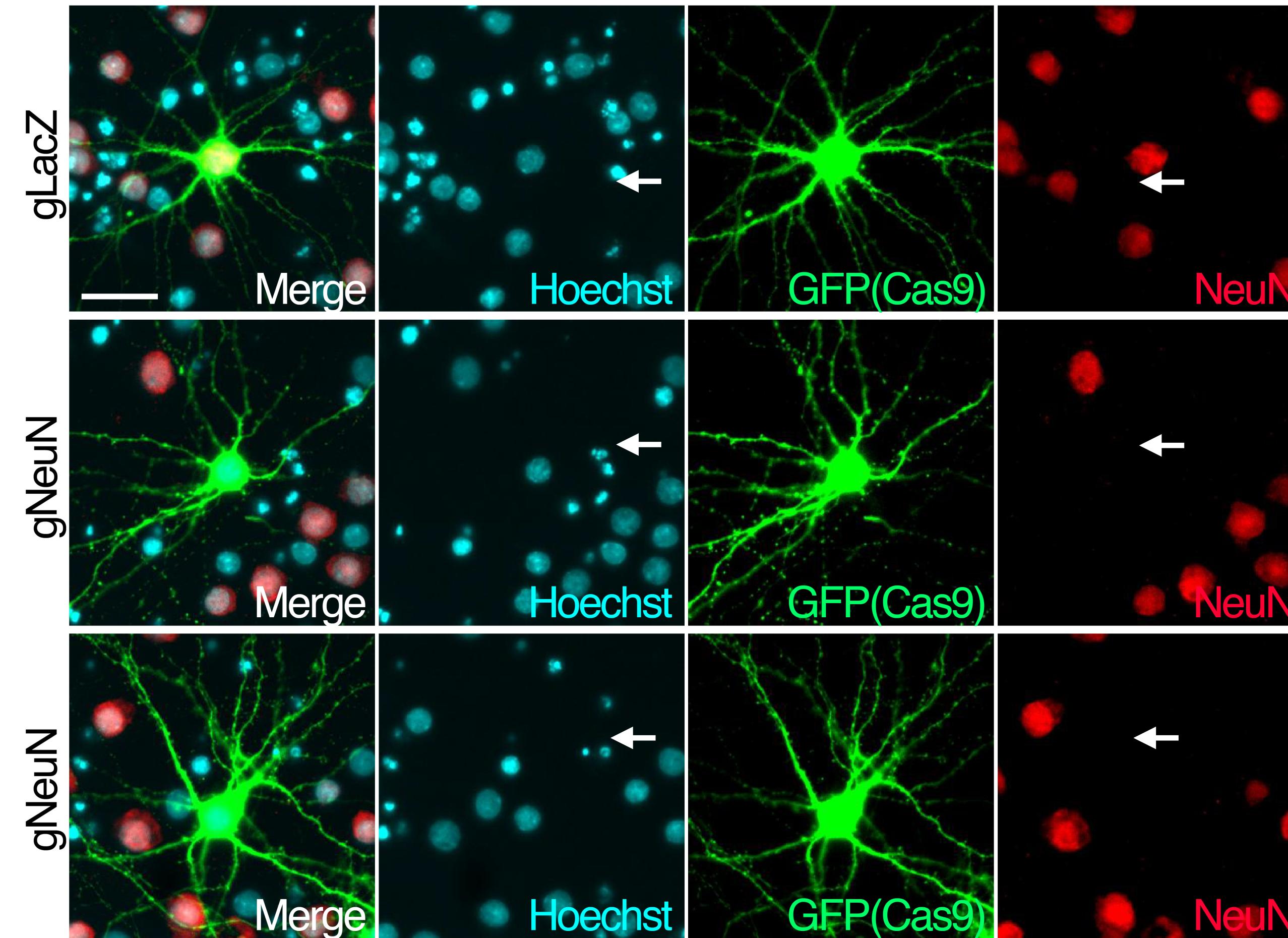
**KPT-350**



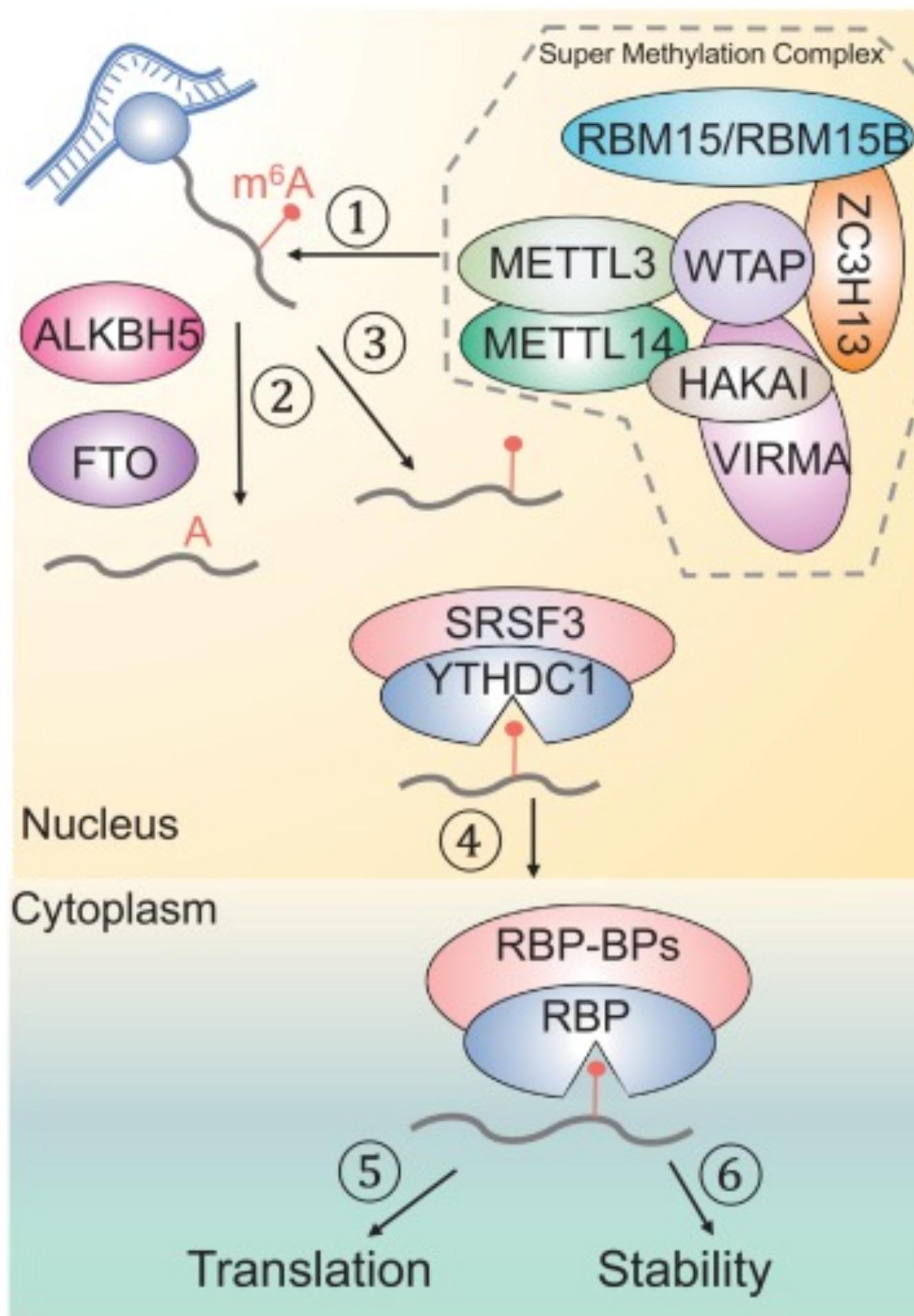
# The NMD factor UPF1 is protective in ALS/FTLD-TDP models



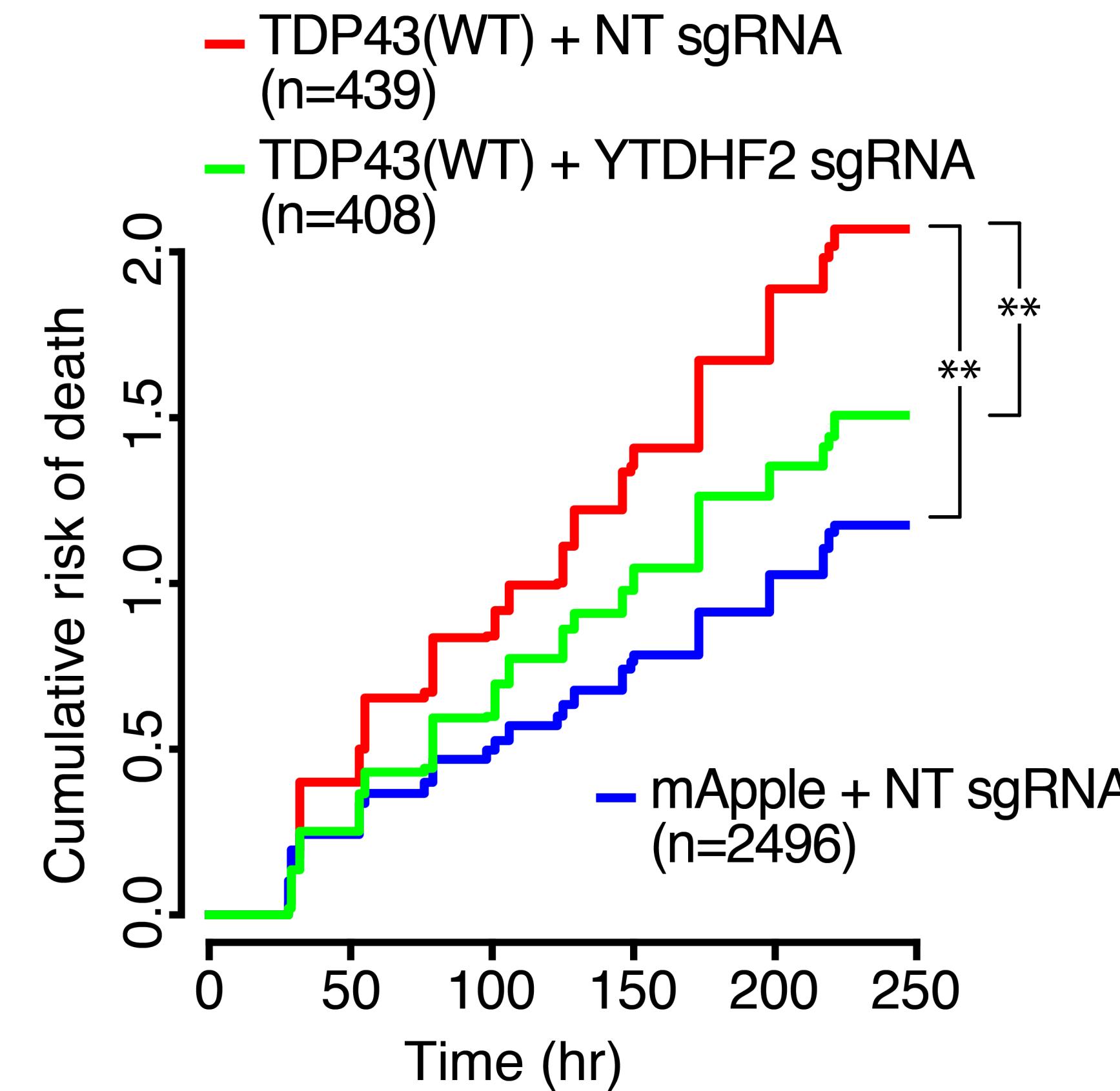
# Single-cell CRISPR/Cas9 knockout



# A small scale CRISPR/Cas9 screen for modifiers of TDP43 related toxicity



Zhang et al. *Pharmacol Ther.* (2018)



# Summary

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Automated microscopy for prospective analyses of neurodegeneration

Determinants of neurodegeneration

Genetic and pharmacological modifiers of neurodegeneration

# Hyperexcitability in ALS

LE JOURNAL CANADIEN DES SCIENCES NEUROLOGIQUES

## Cortical Excitability in Amyotrophic Lateral Sclerosis: A Clue to Pathogenesis

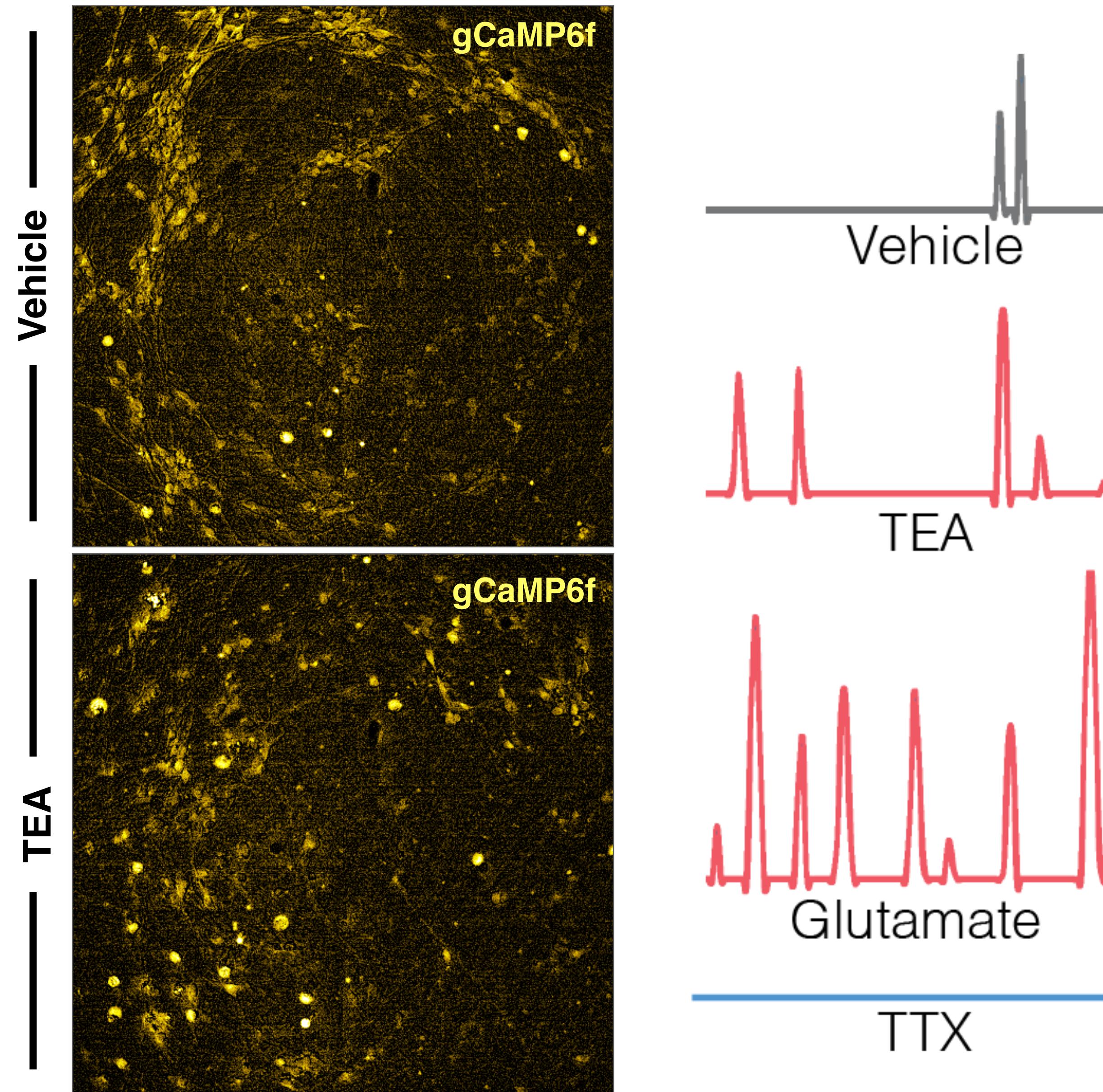
Andrew Eisen, Bhanu Pant and Heather Stewart

**ABSTRACT:** Motor evoked potentials (MEPs) were recorded from selected non-wasted, non-denervated hand muscles in 40 patients with Amyotrophic Lateral Sclerosis (ALS) with both upper and lower motor neuron signs. In most the compound muscle action potential (CMAP) of the target muscle was normal. Compared to the control group, cortical threshold in ALS varied considerably and there was a significant ( $r^2 = 0.702$ ) inverse, exponential, correlation between cortical threshold and MEP/CMAP ratio. There was a linear correlation between threshold and disease duration ( $r^2 = 0.66$ ) so that early in the disease threshold was normal and later the motor cortex could not be stimulated. It is suggested that early in ALS normal threshold reflects glutamate-induced hyper-excitability of the corticomotoneuron. The findings lend support to the hypothesis that ALS is primarily a disease of the corticomotoneuron.

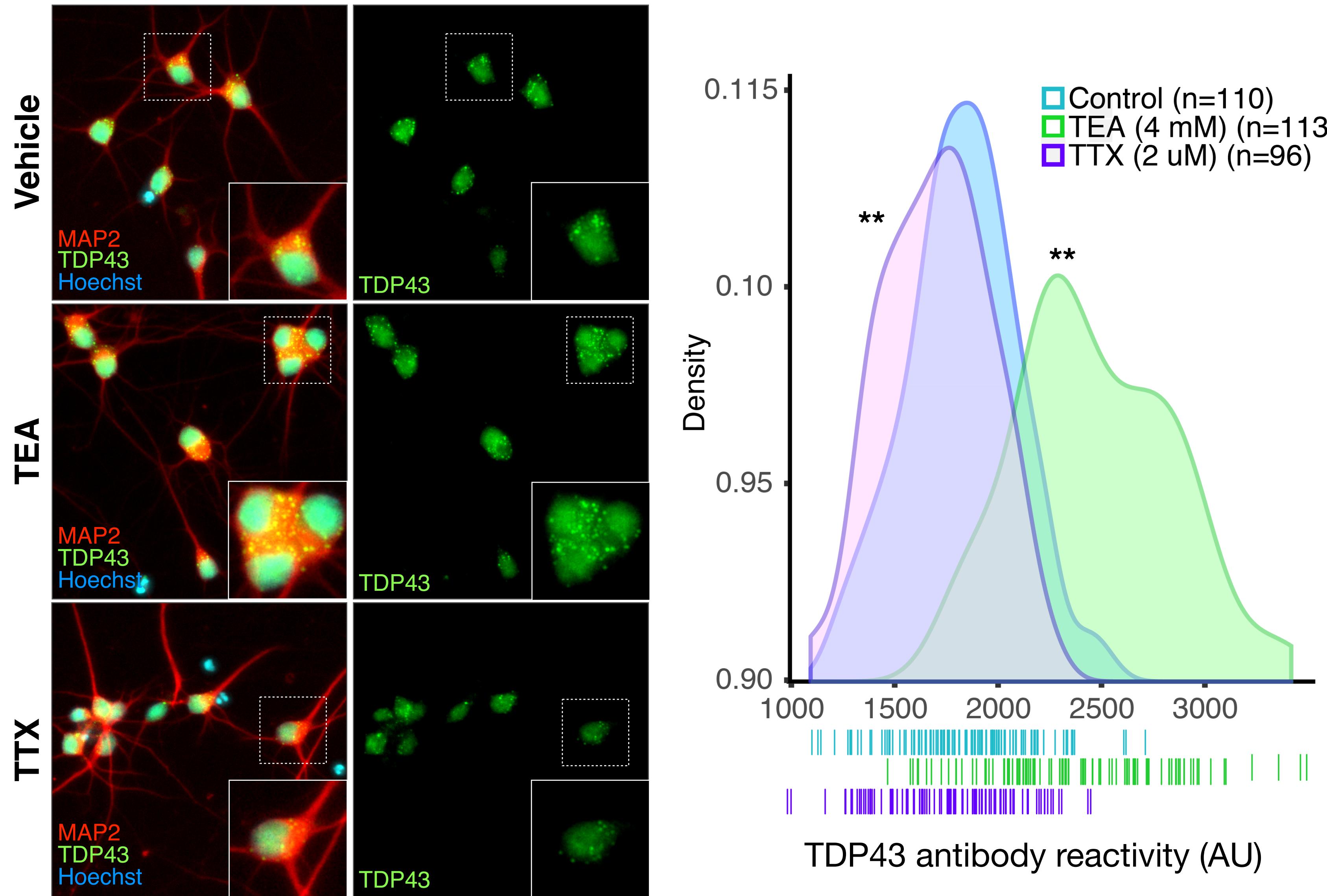
**RÉSUMÉ:** **Excitabilité corticale dans la sclérose latérale amyotrophique: un indice de sa pathogenèse.** Nous avons enregistré les potentiels évoqués moteurs (PEM) de muscles de la main non-atrophiés et non-dénervés chez 40 patients atteints de sclérose latérale amyotrophique (SLA) ayant des signes d'atteinte du neurone moteur central et périphérique. Chez la plupart, le potentiel d'action musculaire composé (PAMC) du muscle cible était normal. Comparé au groupe contrôle, le seuil cortical variait considérablement dans la SLA et il existait une corrélation inverse significative ( $r^2 = 0.702$ ), exponentielle, entre le seuil cortical et le rapport PEM/PAMC. Il existait une corrélation linéaire entre le seuil et la durée de la maladie ( $r^2 = 0.66$ ), de telle sorte que le seuil était normal au début de la maladie et par la suite, le cortex moteur ne pouvait plus être stimulé. Nous suggérons que, tôt dans la SLA, le seuil normal reflète l'hyperexcitabilité du neurone moteur cortical induite par le glutamate. Ces observations supportent l'hypothèse que la SLA est avant tout une maladie du neurone moteur cortical.

*Can. J. Neurol. Sci.* 1993; 20: 11-16

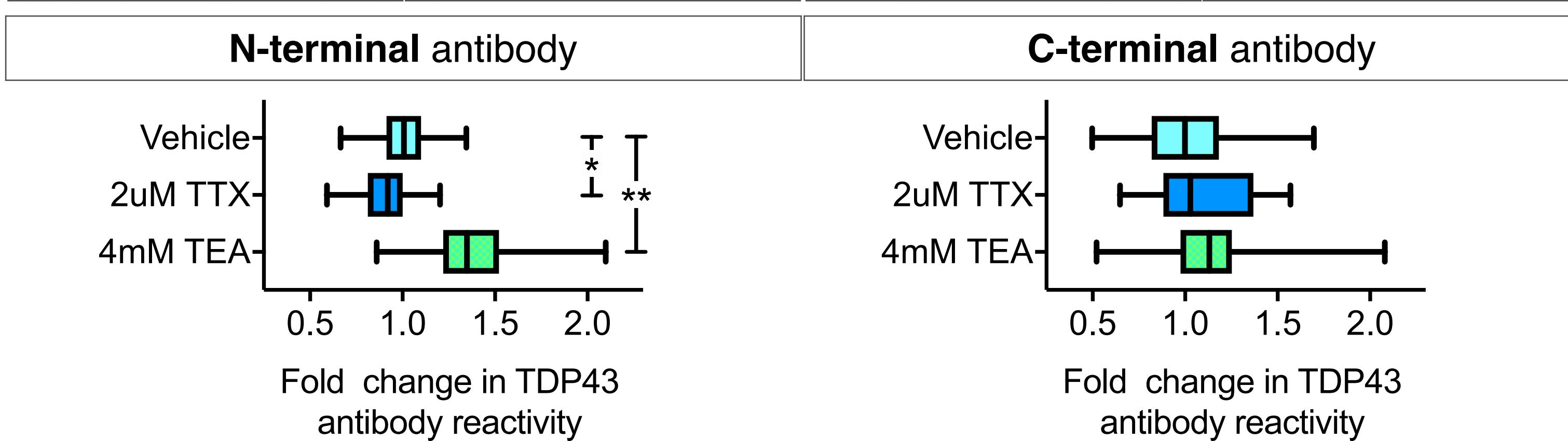
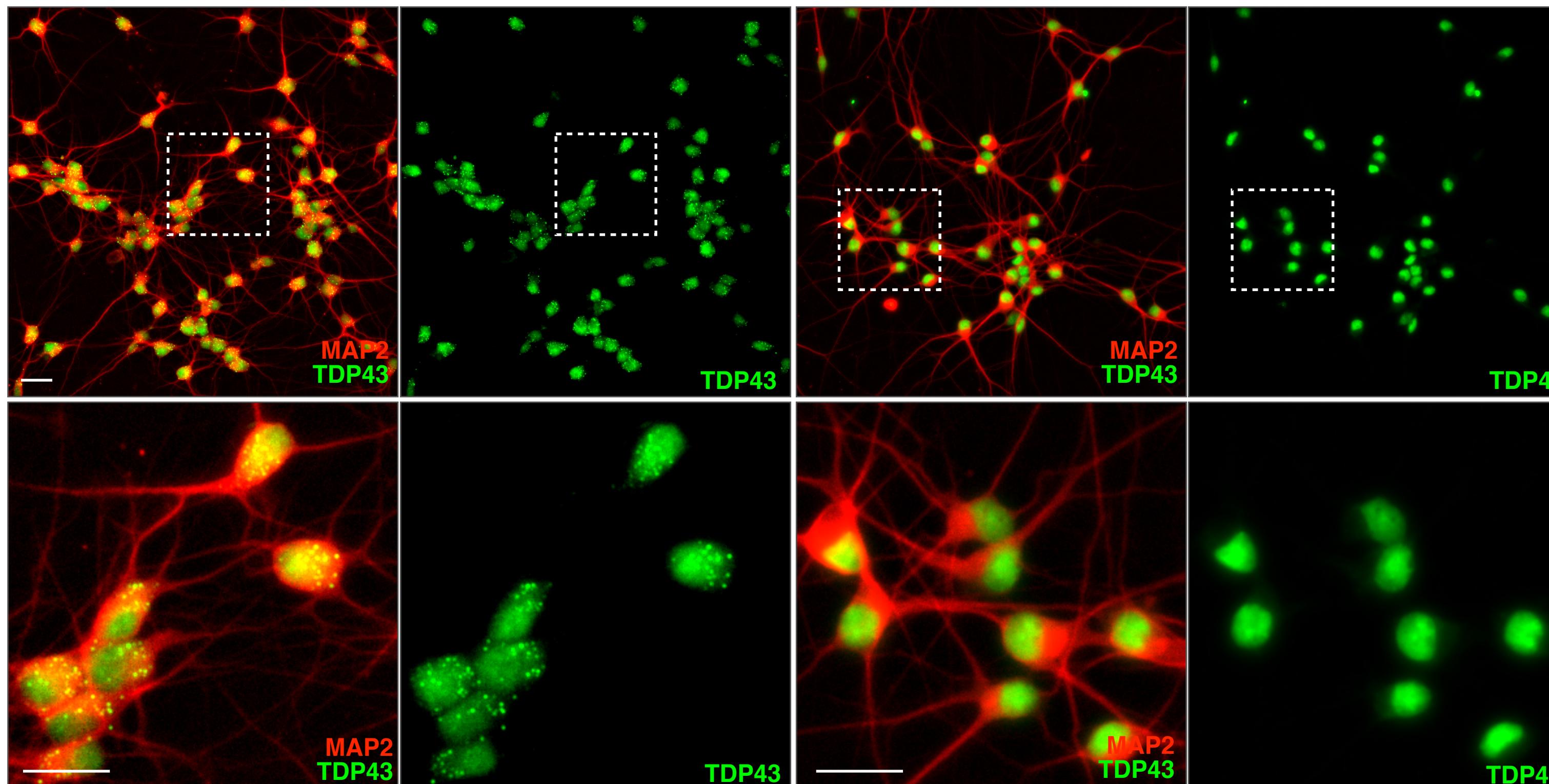
# Modulating activity in iPSC-derived neurons



# Activity-related TDP43 abundance in iPSC-derived neurons

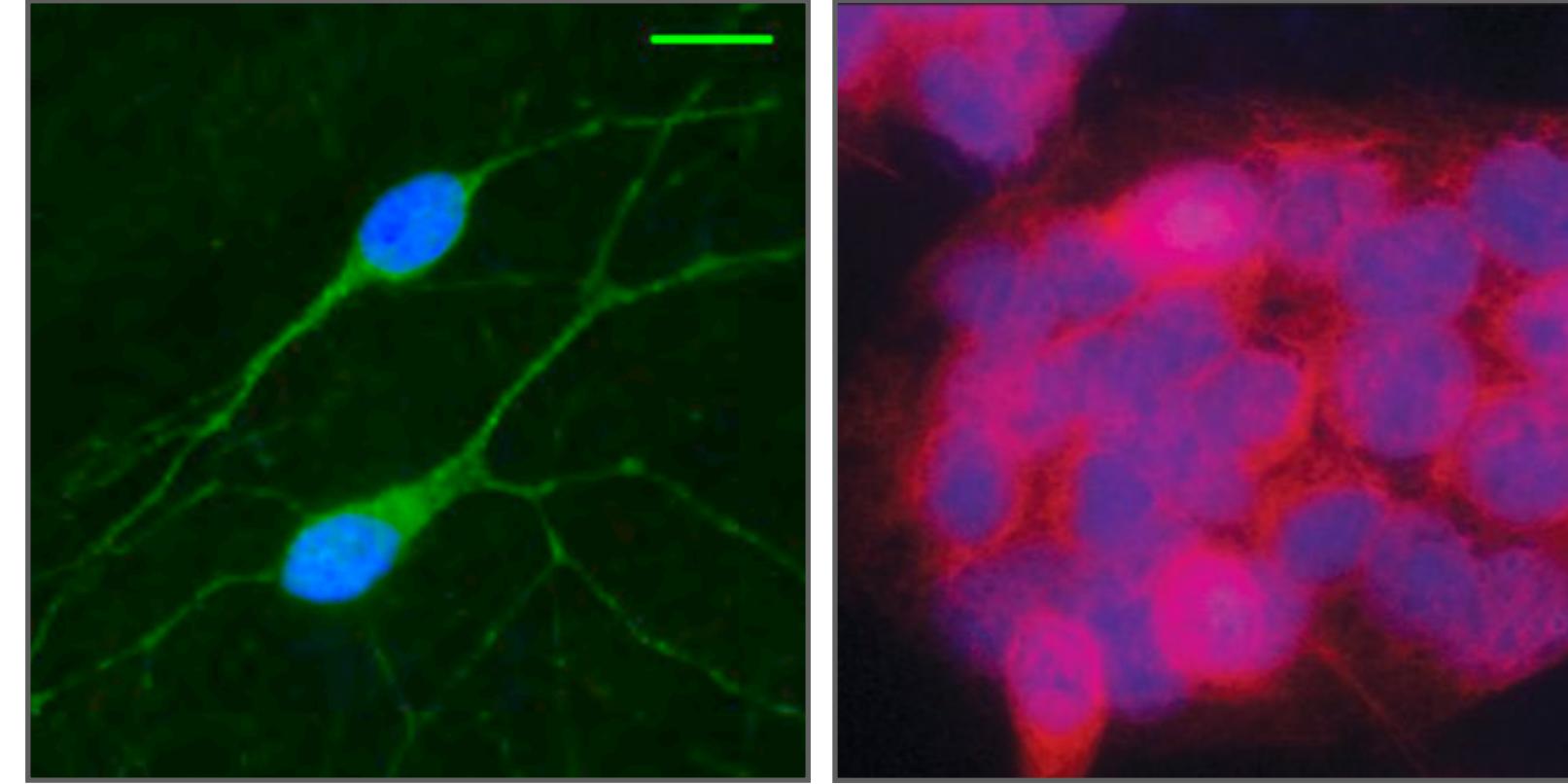


# Activity-related TDP43 abundance in iPSC-derived neurons



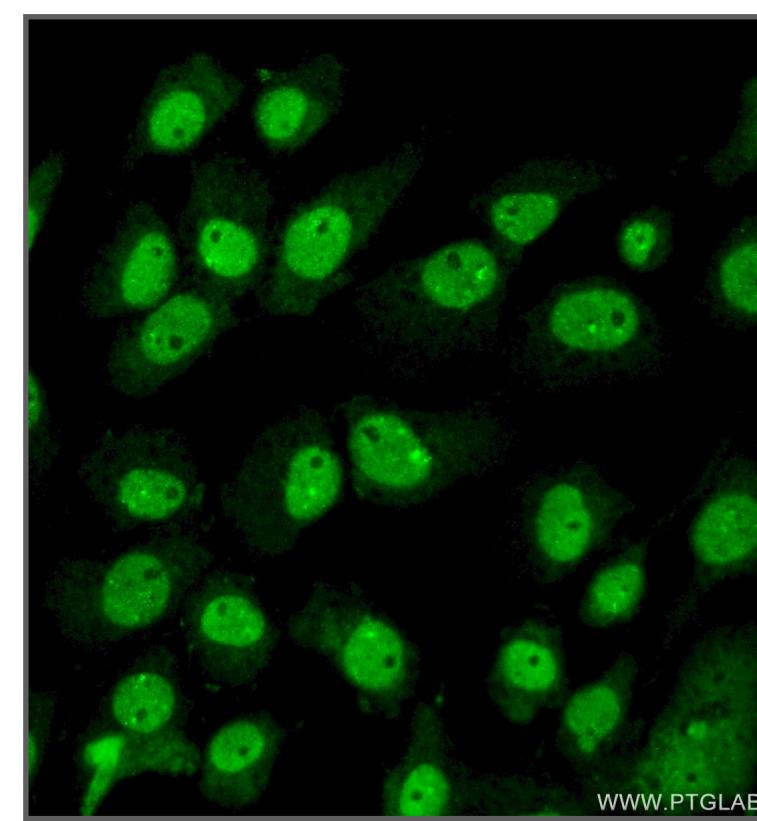
# Variability in TDP43 staining patterns

Pinarbasi et al., Sci Rep (2018)

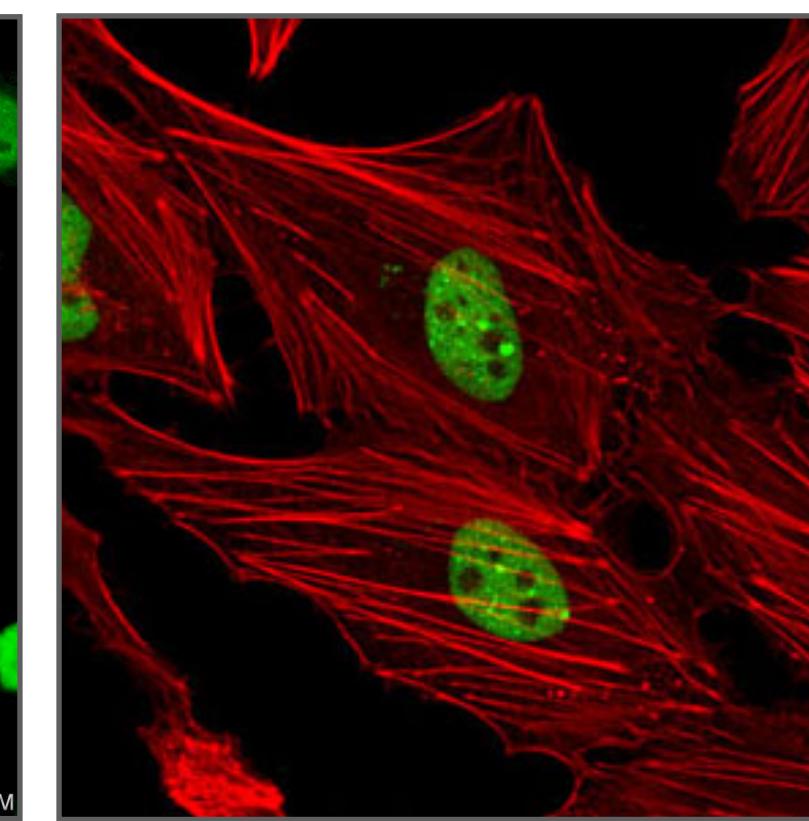


anti-Nterm TDP43  
Proteintech

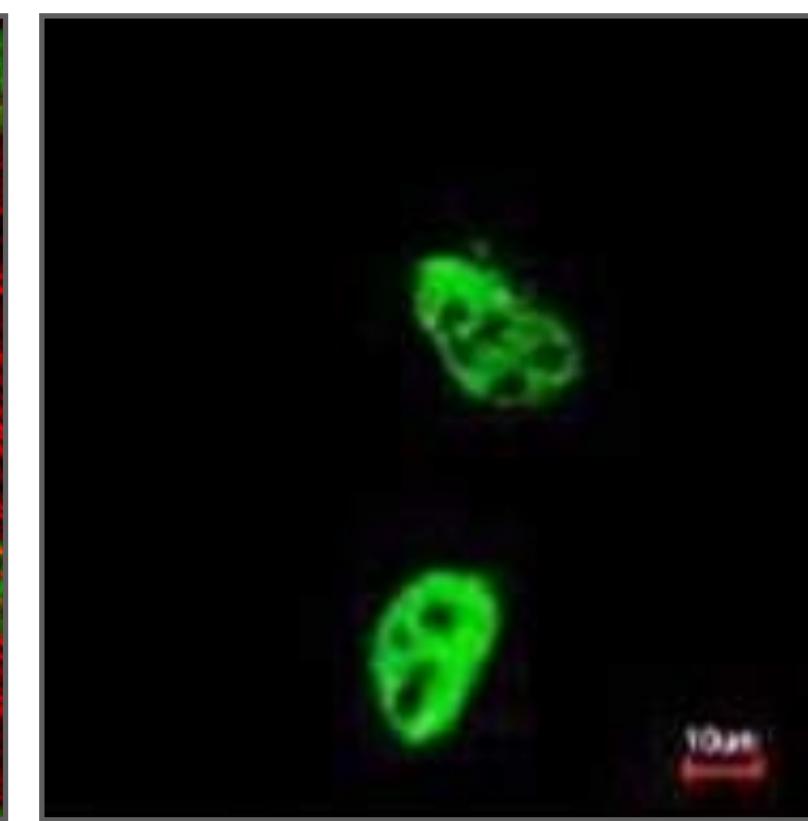
anti-Nterm TDP43  
Sigma Aldrich



anti-Cterm TDP43  
Proteintech

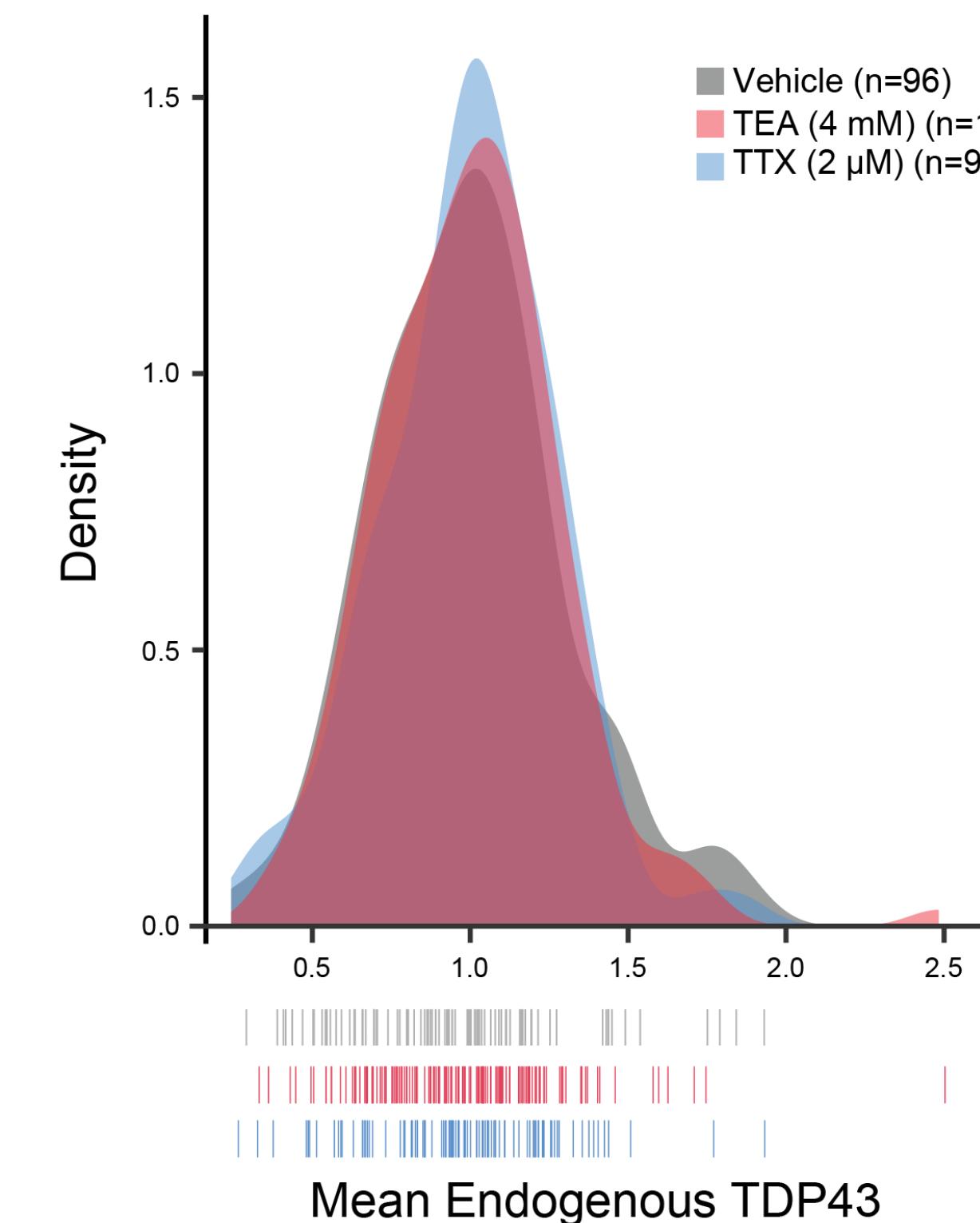
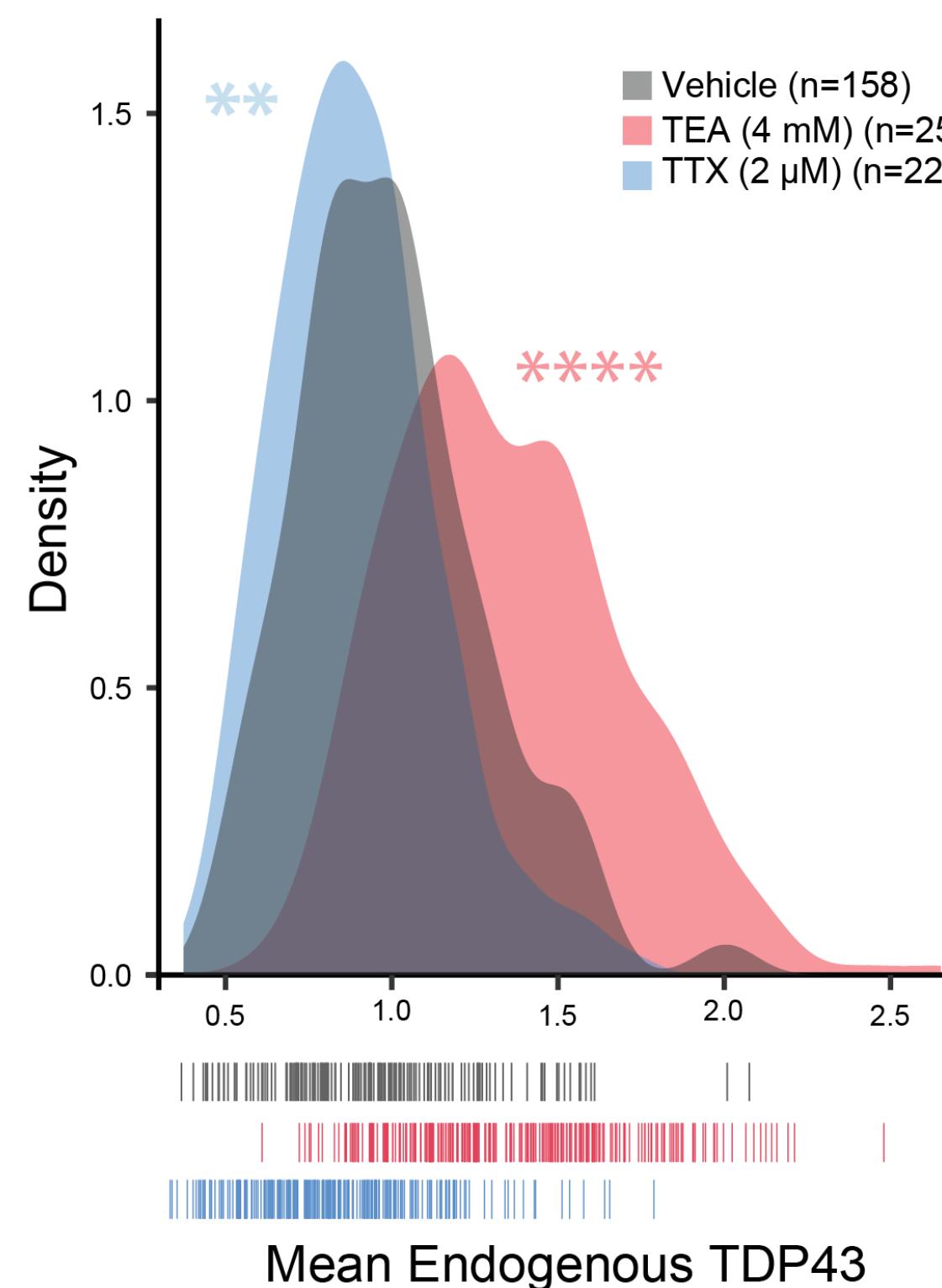
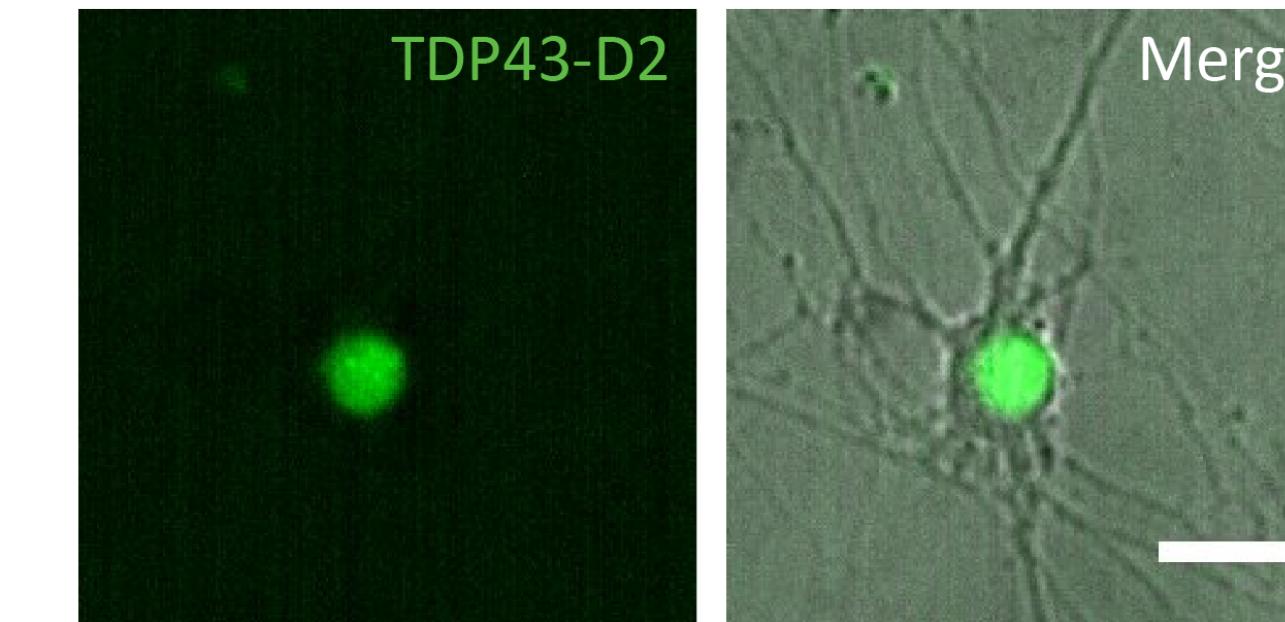
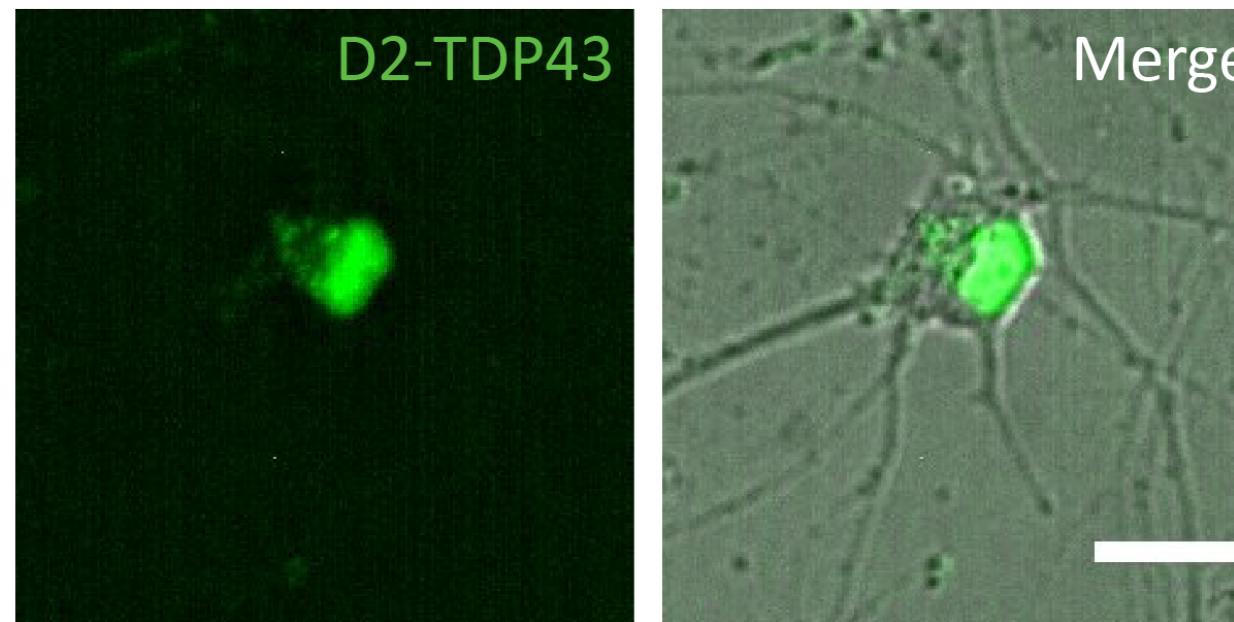


anti-Cterm TDP43  
Cell Signaling



anti-Cterm TDP43  
Thermo Fisher

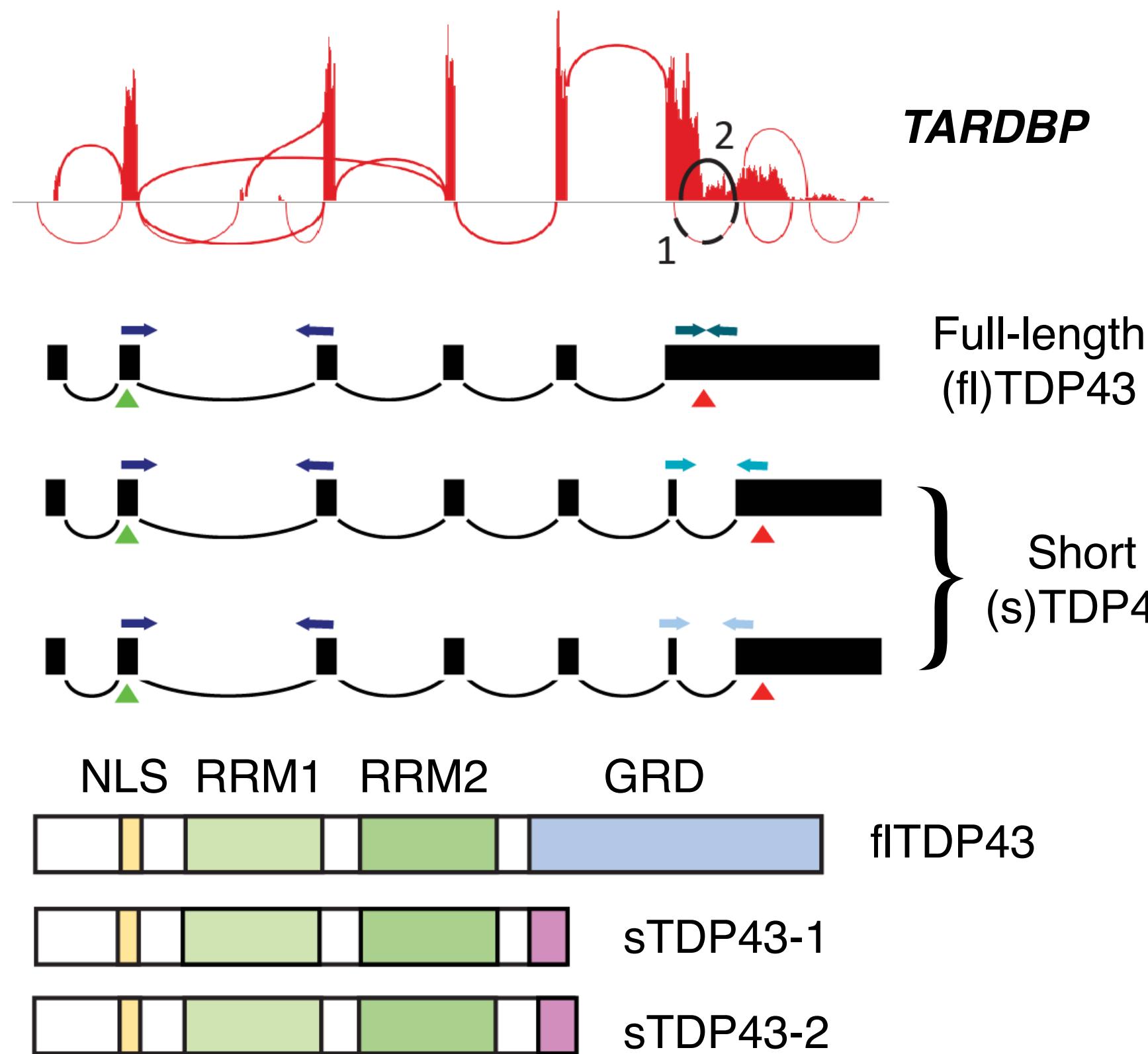
# Activity-related changes in N-terminally labeled TDP43



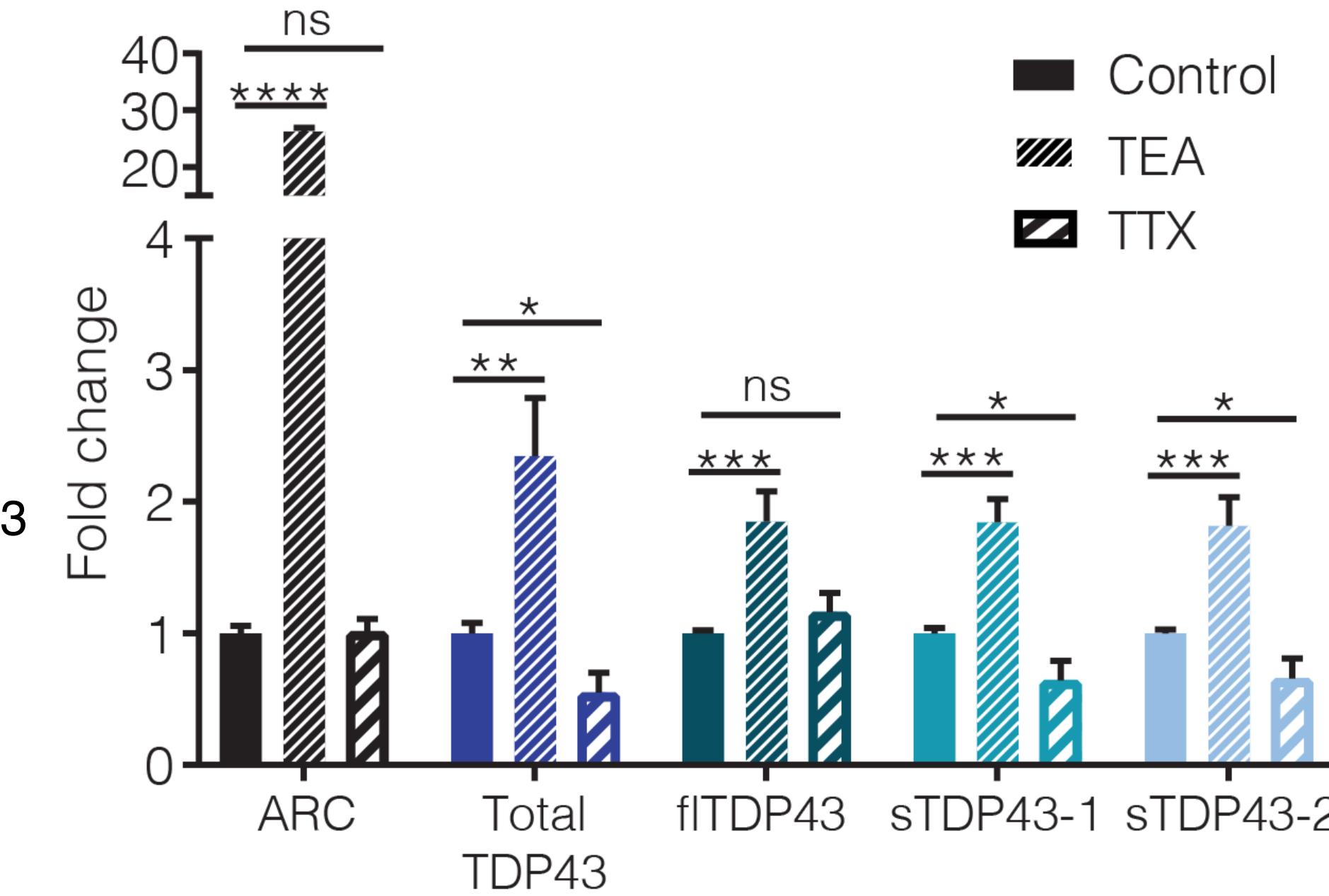
# *TARDBP* splice variants

## HEK293T cells

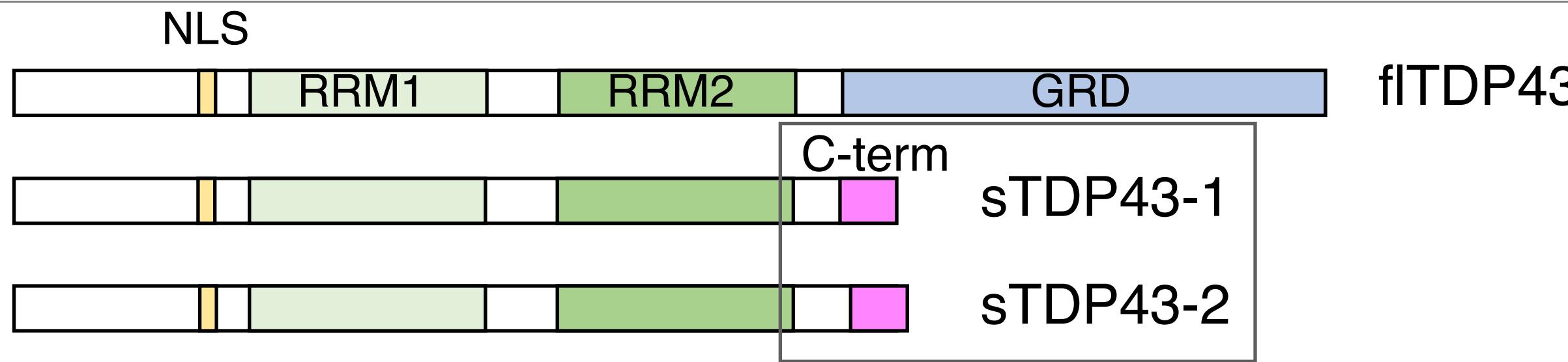
(Flores et al. 2019)



## Human iPSC-derived neurons



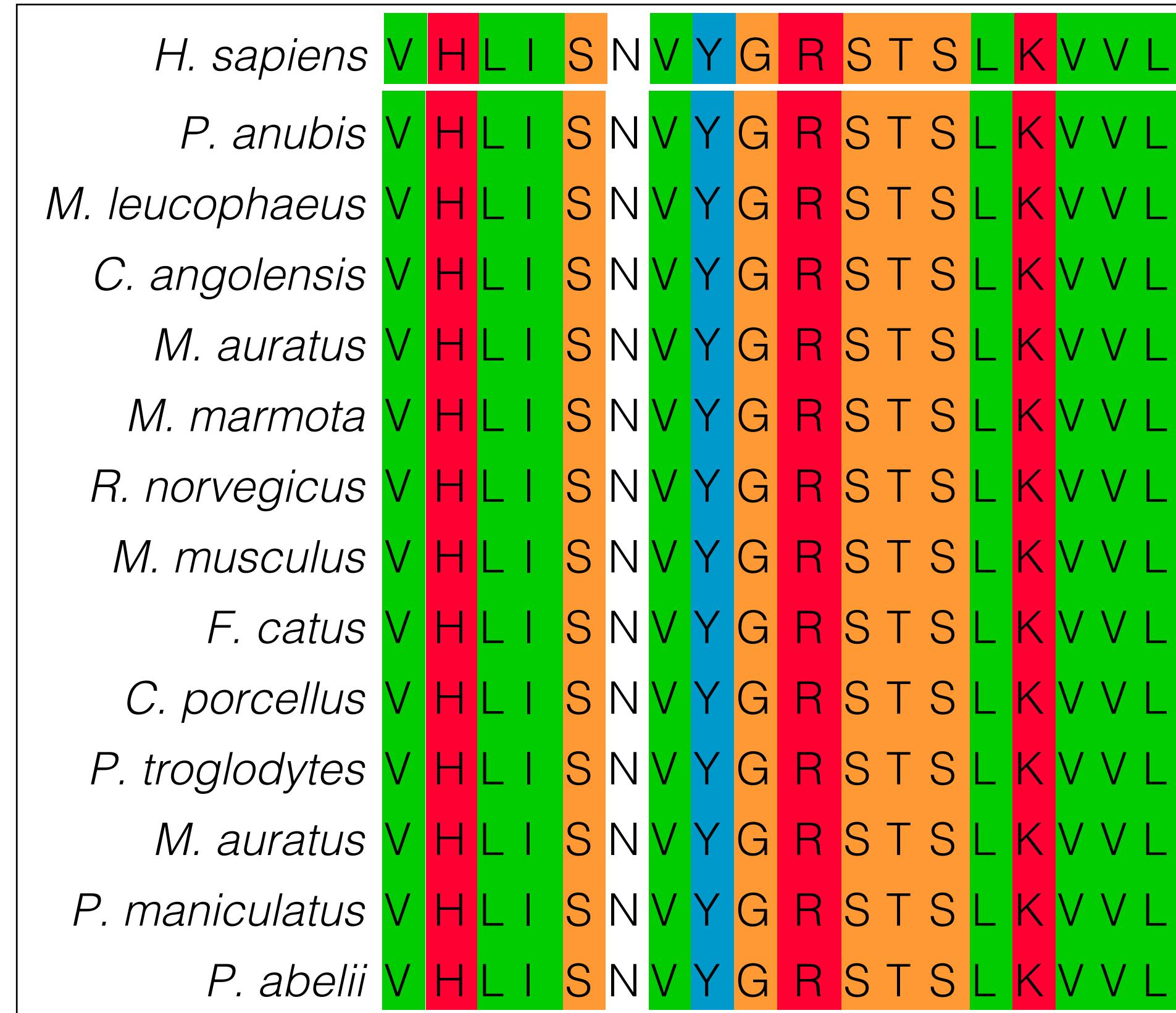
# sTDP43 is highly conserved



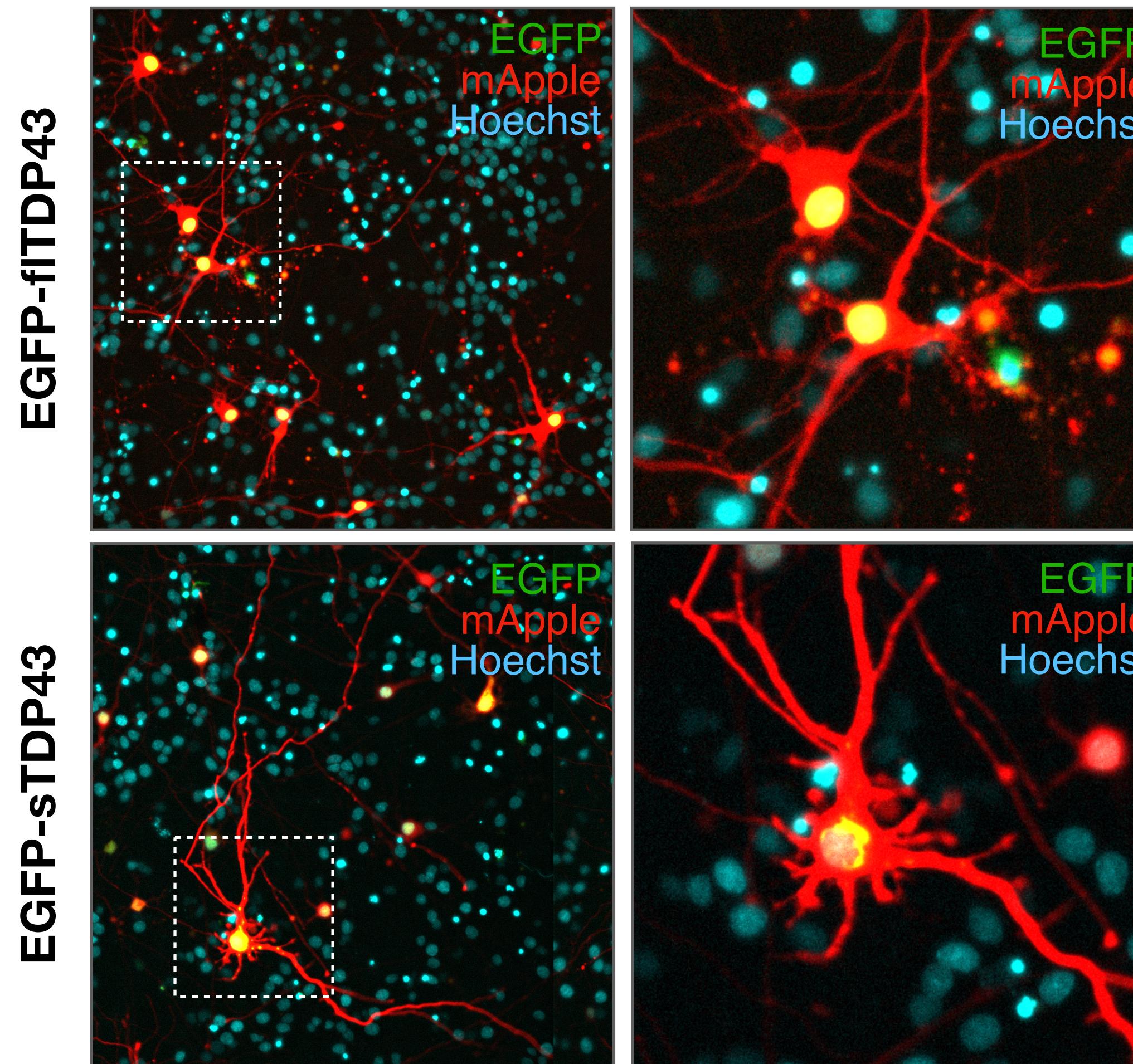
sTDP43 transcript conservation

Species	Isoform 1	Isoform 2
Baboon ( <i>P. anubis</i> )	99%	99%
Drill ( <i>M. leucophaeus</i> )	96%	96%
Colobus ( <i>C. angolensis</i> )	96%	96%
Cat ( <i>F. catus</i> )	96%	96%
Squirrel ( <i>I. tridecemlineatus</i> )	96%	96%
Brown rat ( <i>R. norvegicus</i> )	96%	96%
Prairie vole ( <i>M. ochrogaster</i> )	96%	96%
House mouse ( <i>M. musculus</i> )	96%	96%
Guinea pig ( <i>C. porcellus</i> )	95%	95%
Golden cichlid ( <i>M. auratus</i> )	95%	92%

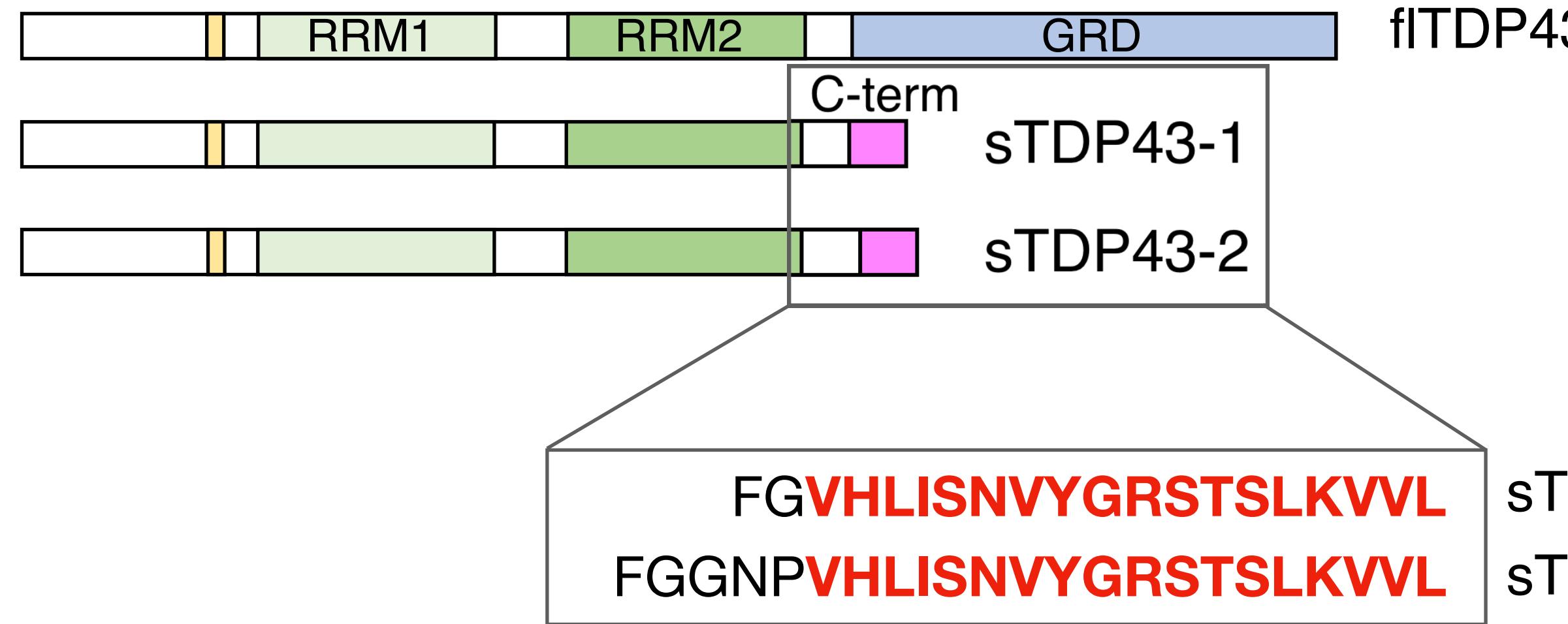
sTDP43 protein (C-terminus) conservation



# Cytoplasmic accumulation of sTDP43



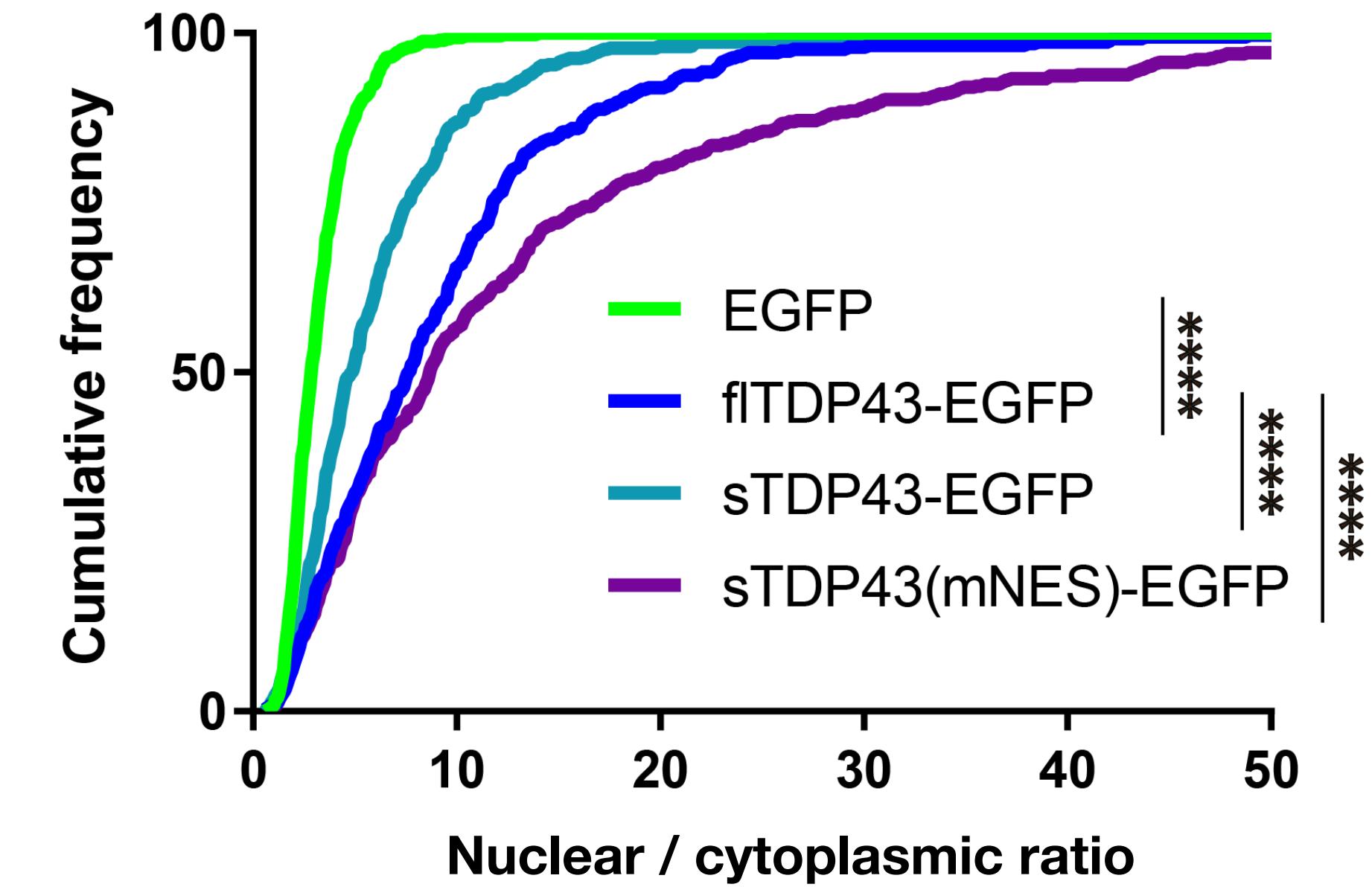
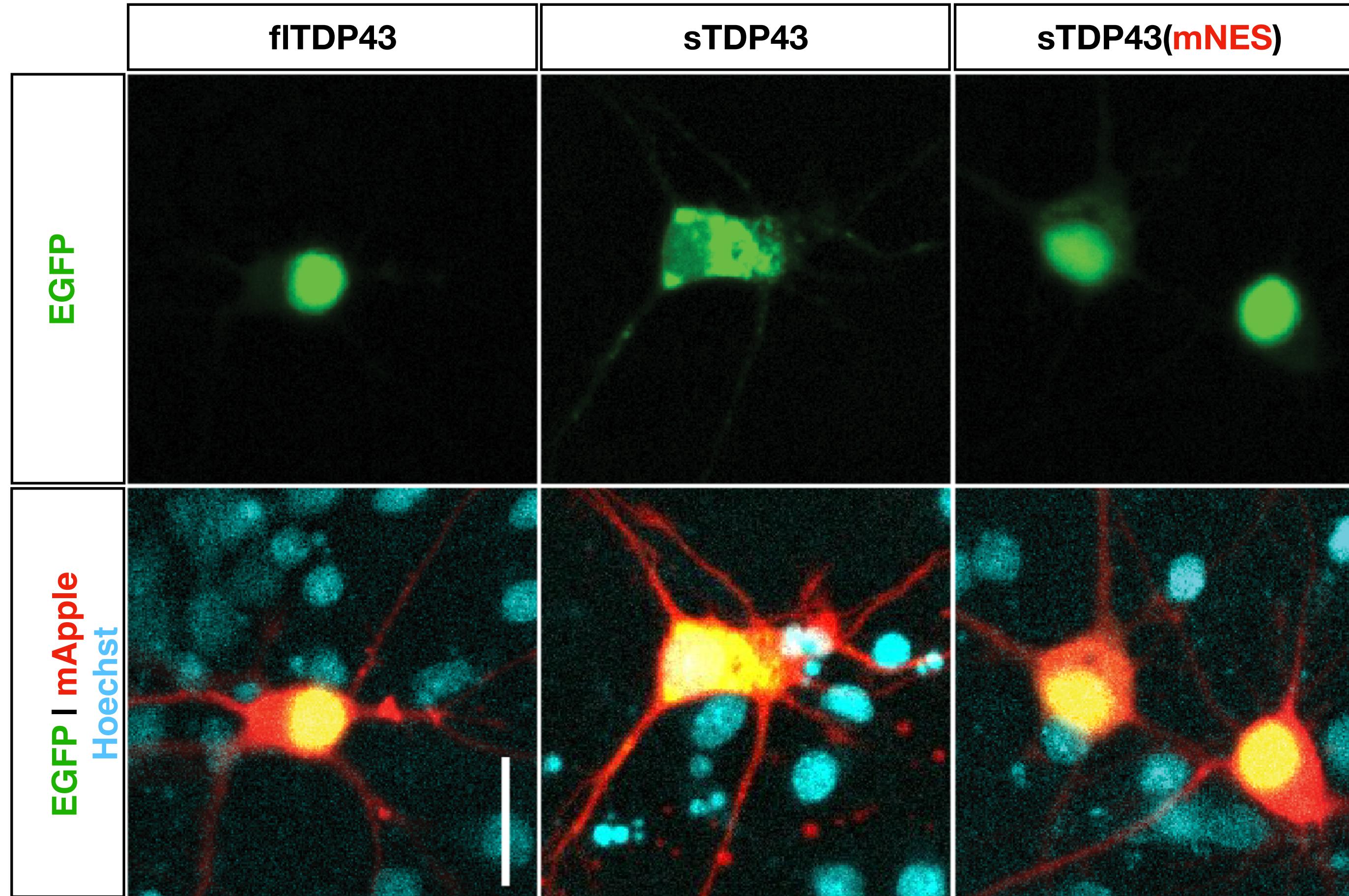
NLS



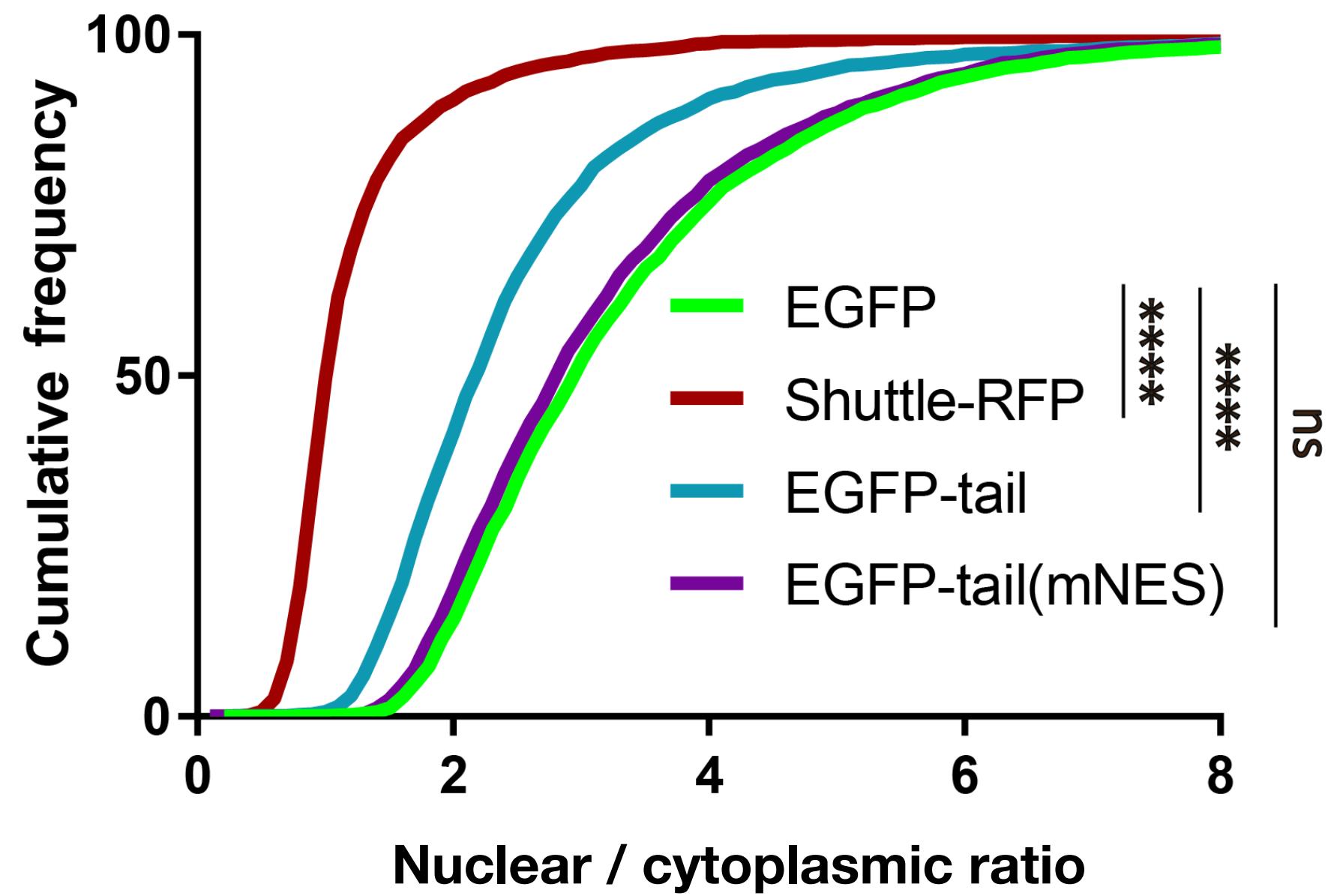
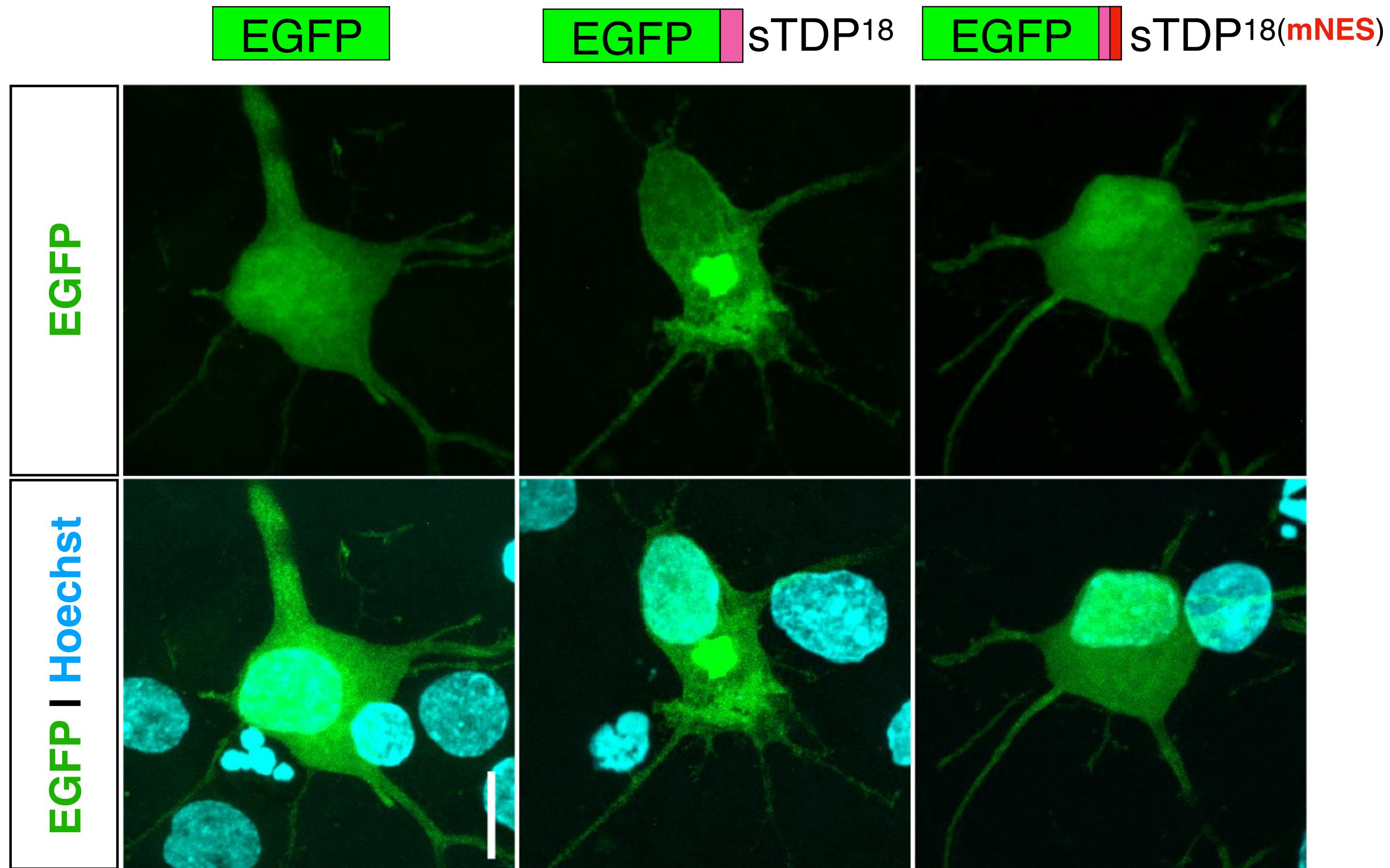
Residue	ANN	HMM	NES	Predicted
F	0.103	0	0	-
G	0.1	0	0	-
V	0.097	0.195	0	-
H	0.081	0.195	0	-
L	0.187	0.231	0	-
I	0.185	0.251	0.002	-
S	0.09	0.251	0	-
N	0.12	0.251	0.003	-
V	0.101	0.266	0.019	-
Y	0.088	0.073	0	-
G	0.093	0.073	0	-
R	0.073	0.073	0	-
S	0.075	0.073	0	-
T	0.082	0.807	0.509	Yes
S	0.105	0.807	0.51	Yes
L	0.085	0.807	0.51	Yes
K	0.069	0.804	0.502	Yes
V	0.072	0.804	0.502	Yes
V	0.068	0.749	0.445	-
L	0.083	0	0	-

Nuclear export  
sequence?

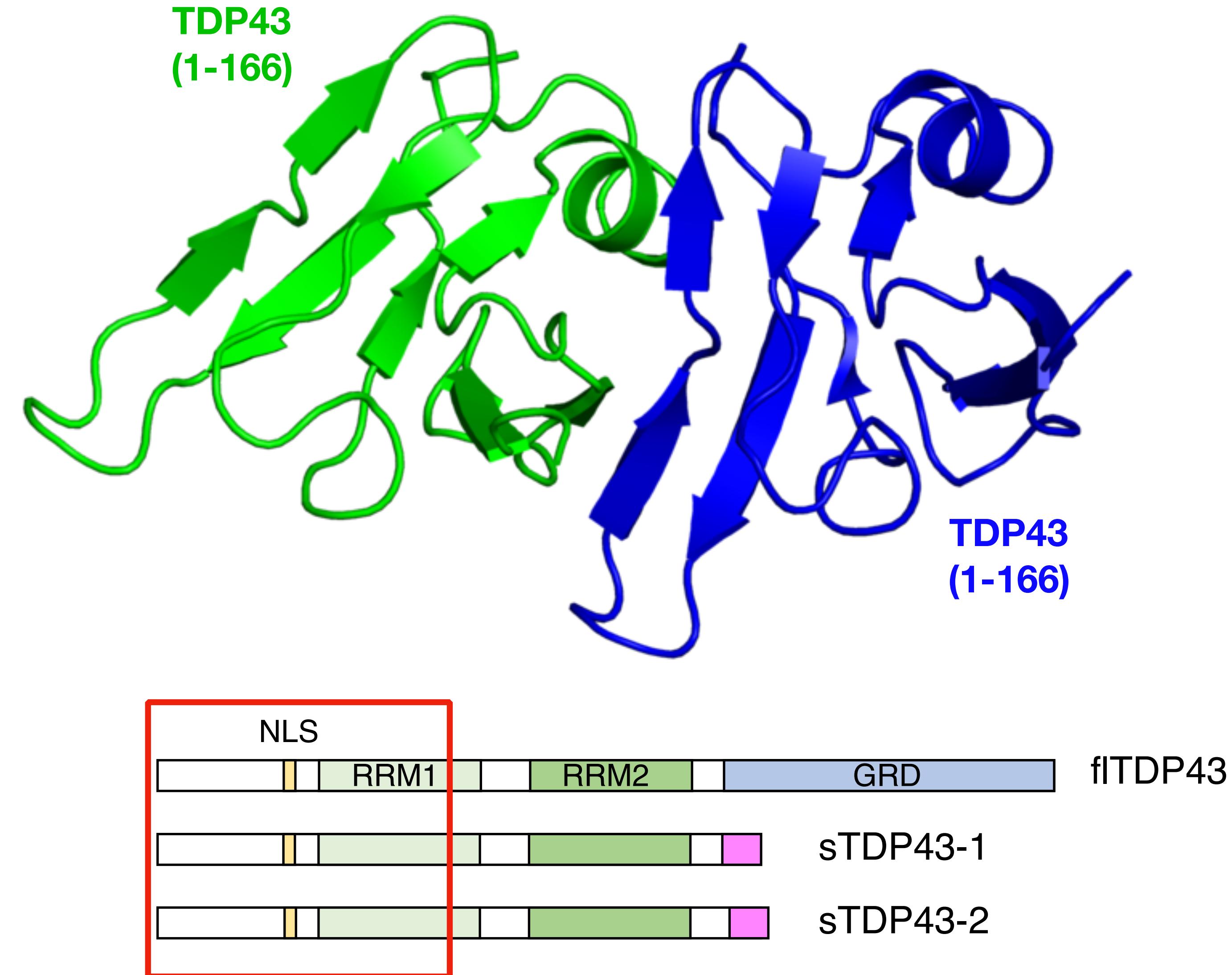
# A putative nuclear export sequence (**NES**)



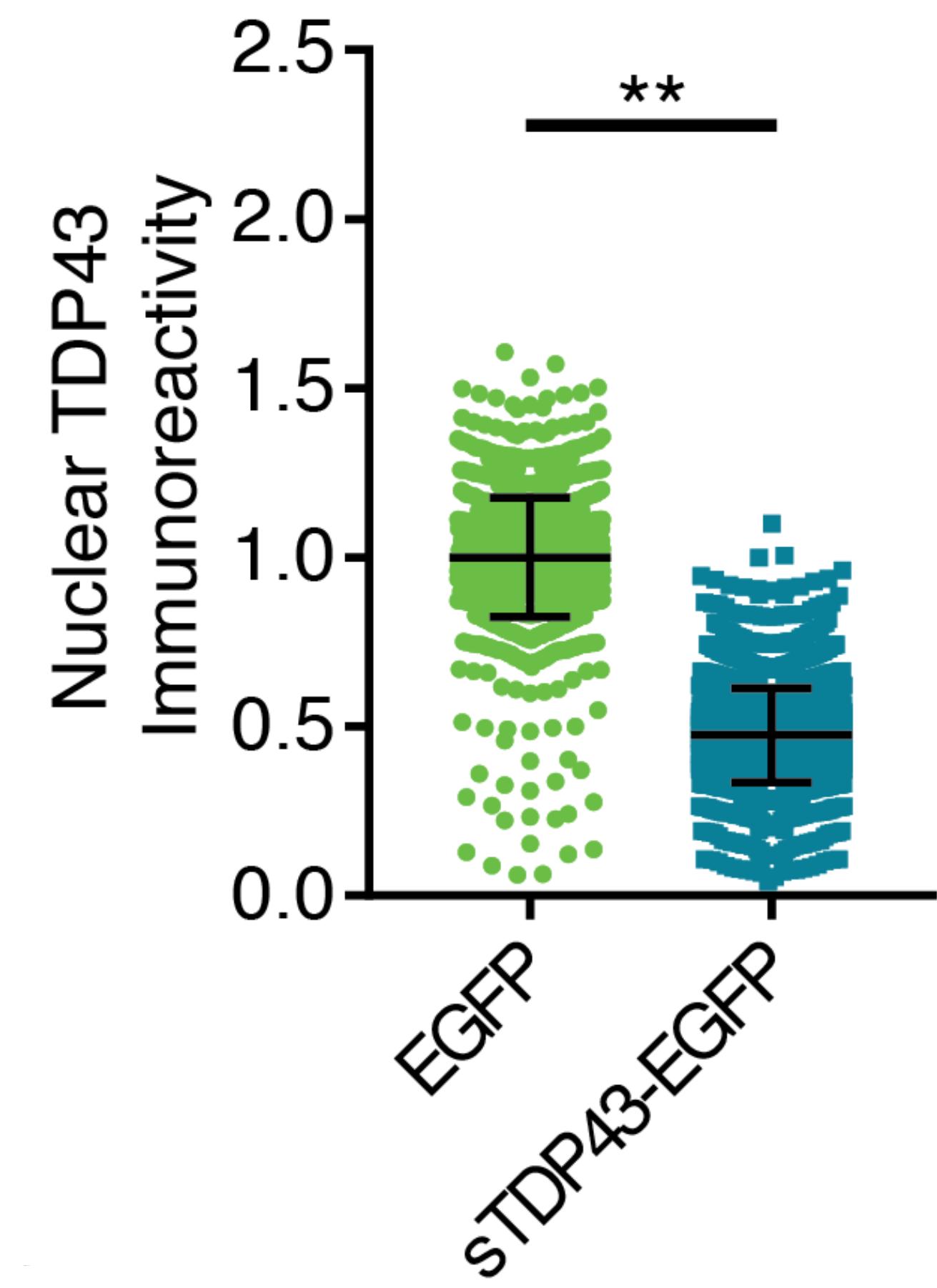
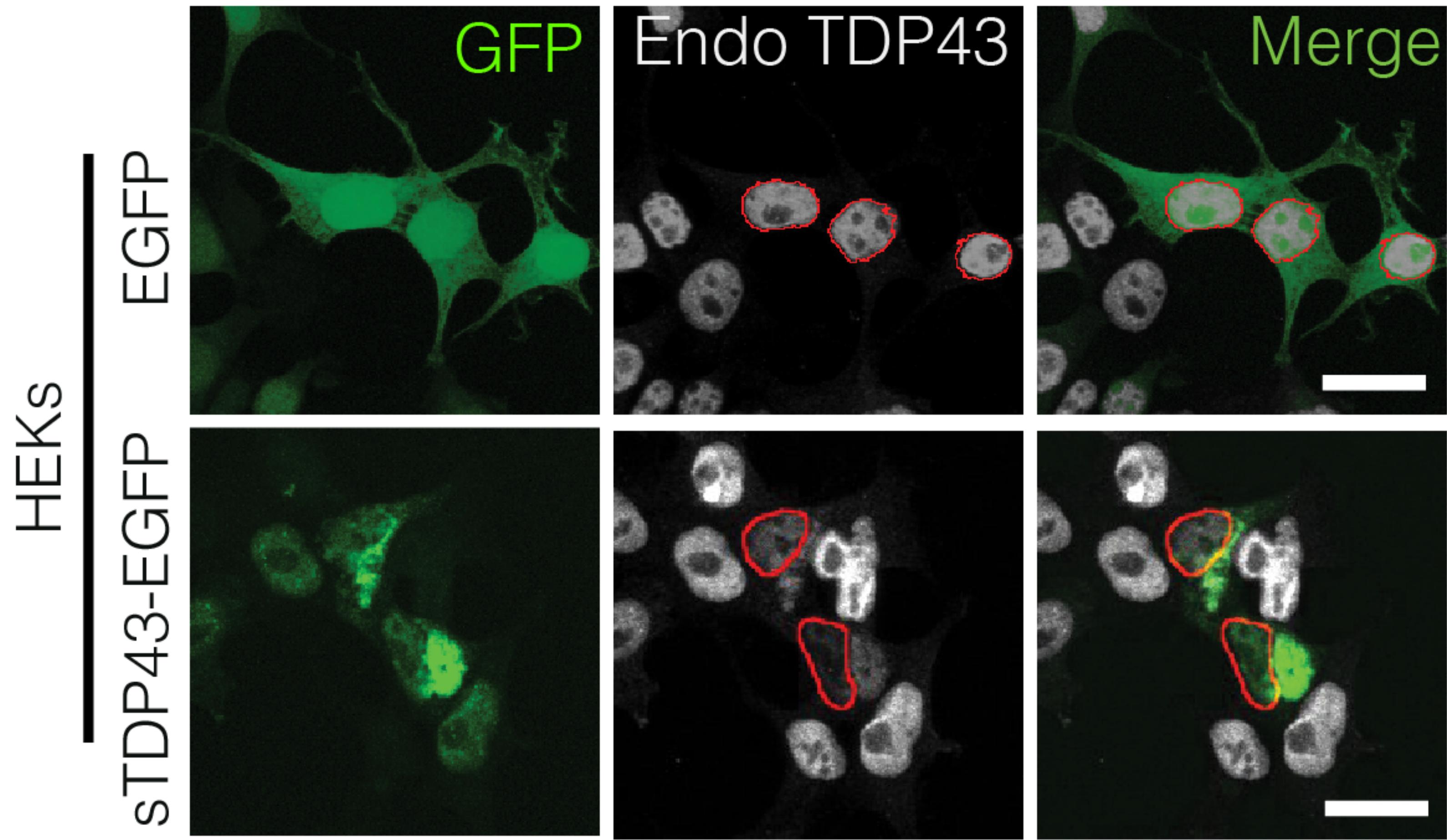
# A putative nuclear export sequence (**NES**)



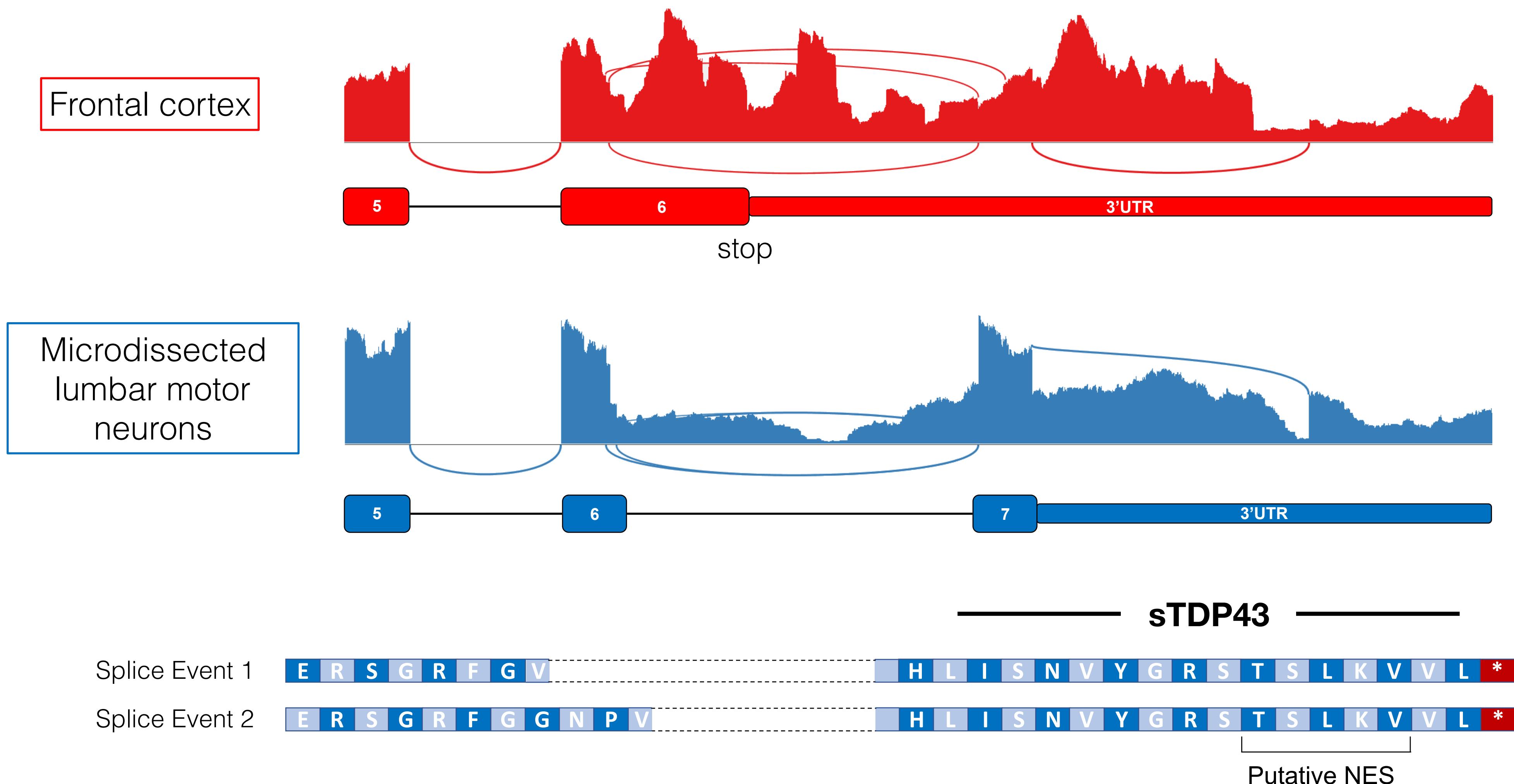
# TDP43 interacts with itself via its N-terminus



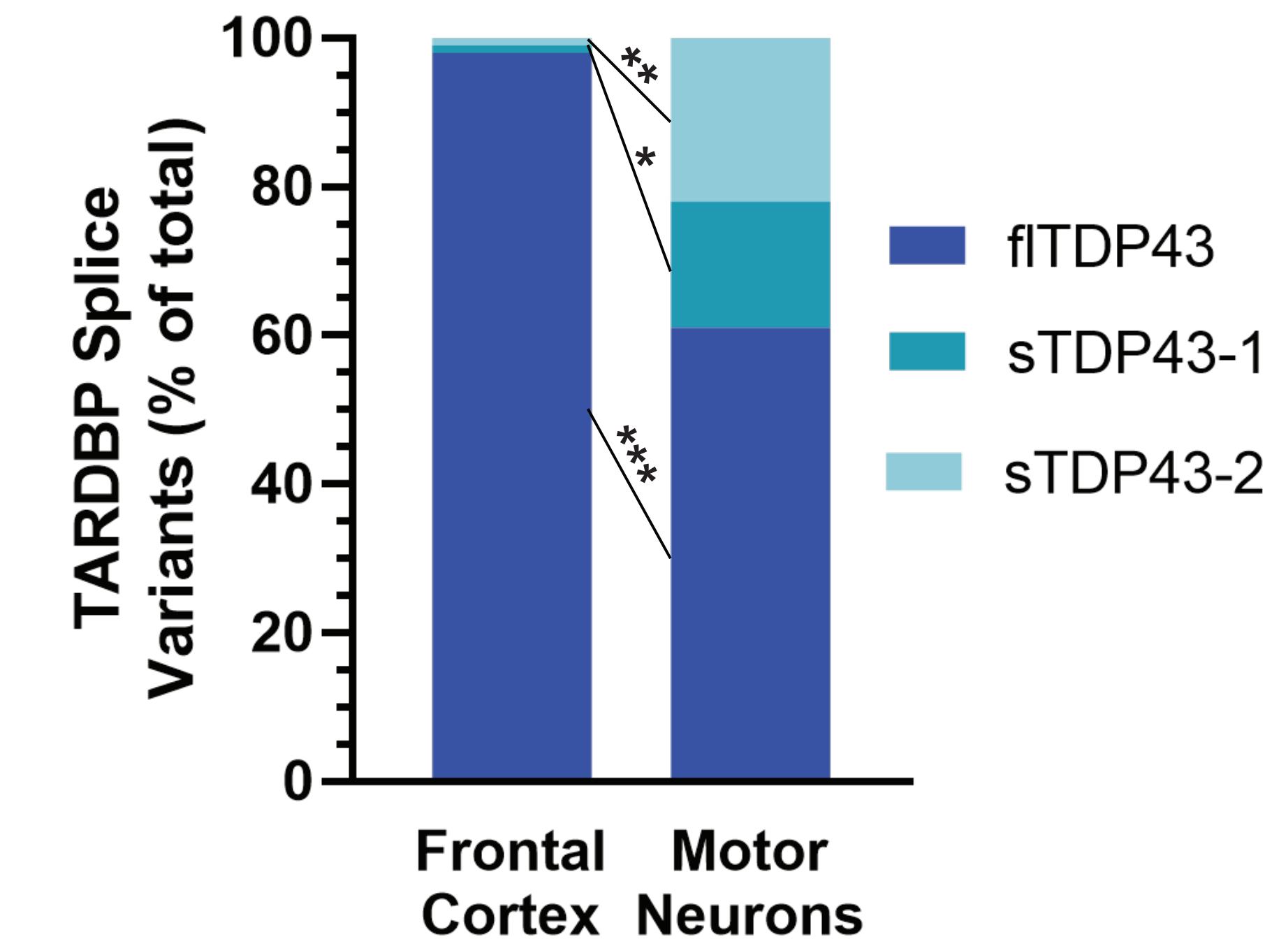
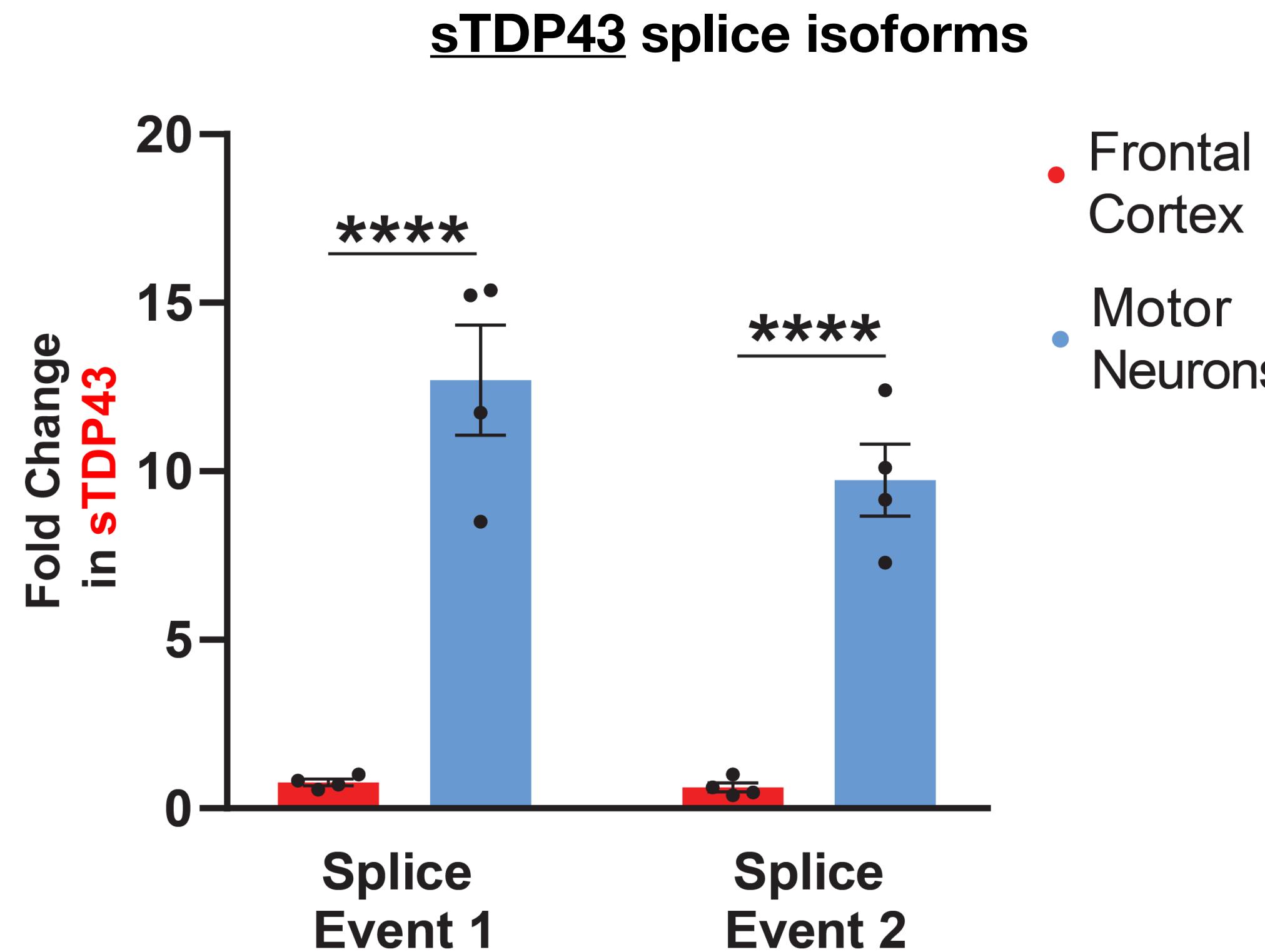
# Sequestration of full-length TDP43 by sTDP43



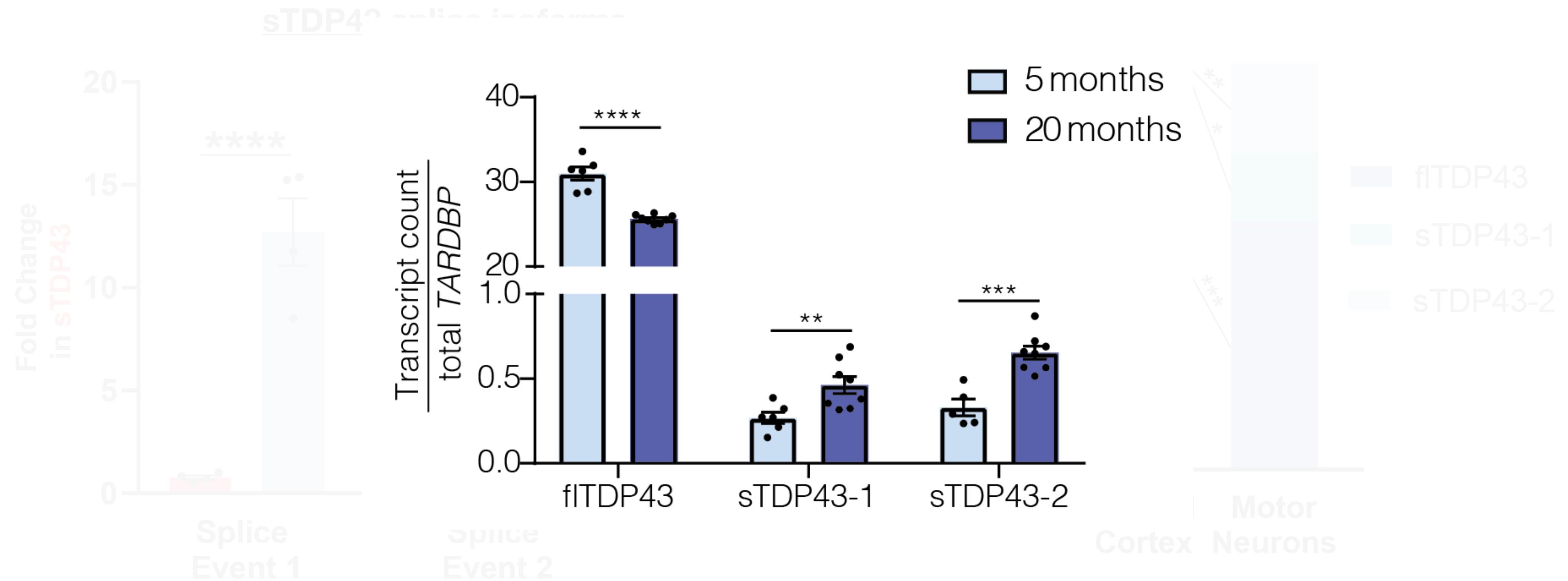
# sTDP43 is upregulated in motor neurons



# sTDP43 is upregulated in motor neurons



# sTDP43 is upregulated in motor neurons ...and with age



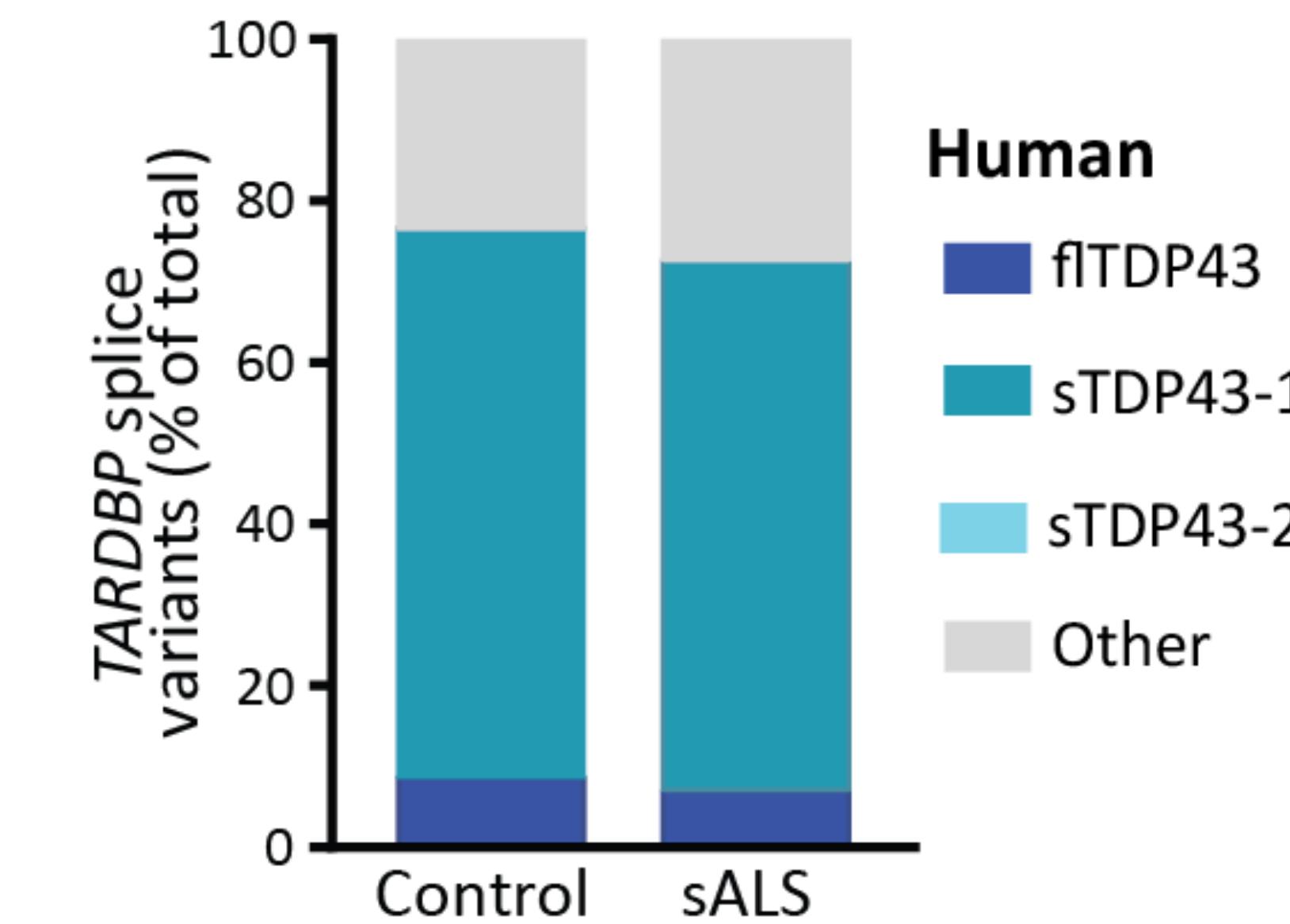
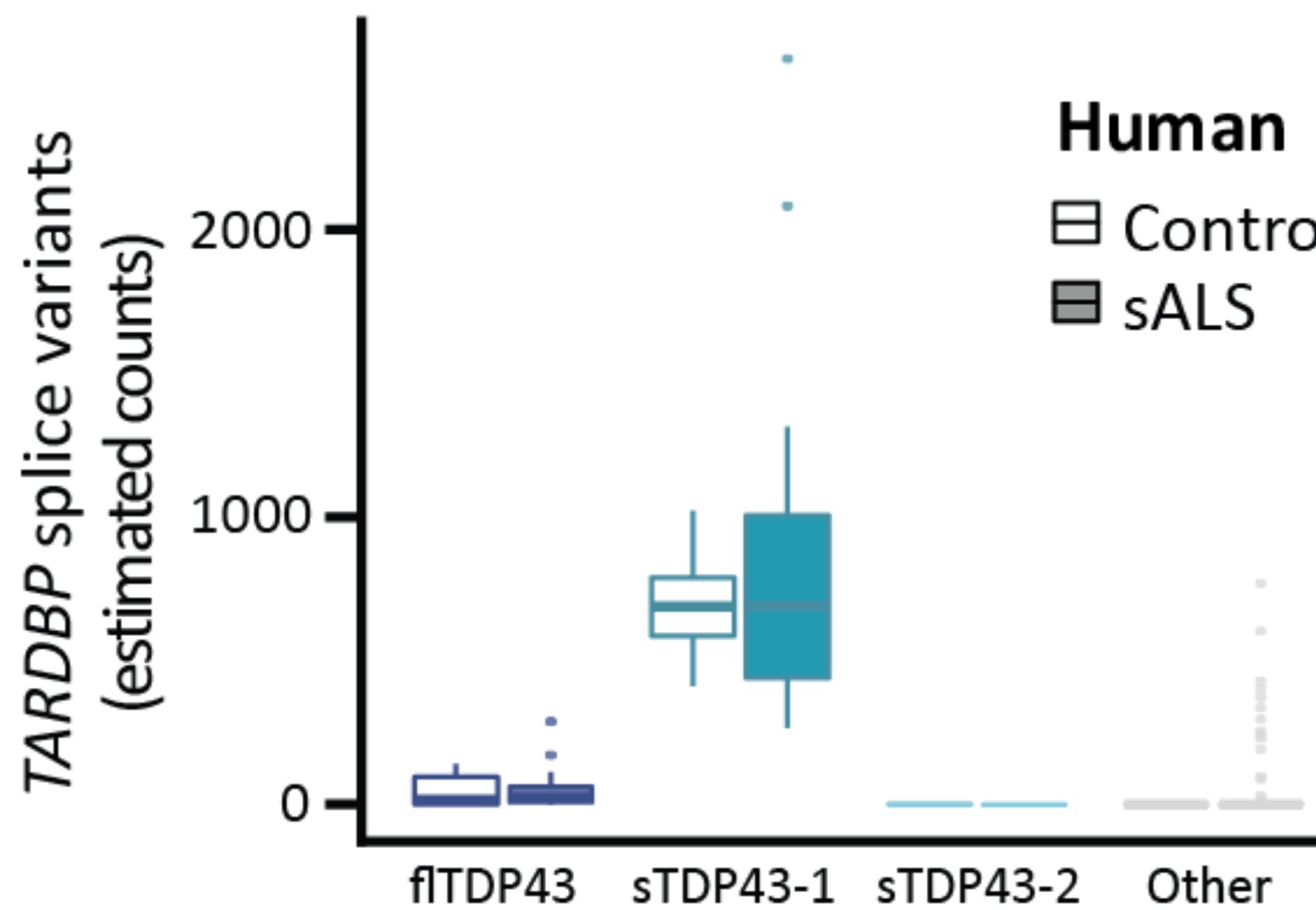
# sTDP43 in human CNS

Krach et al. 2017

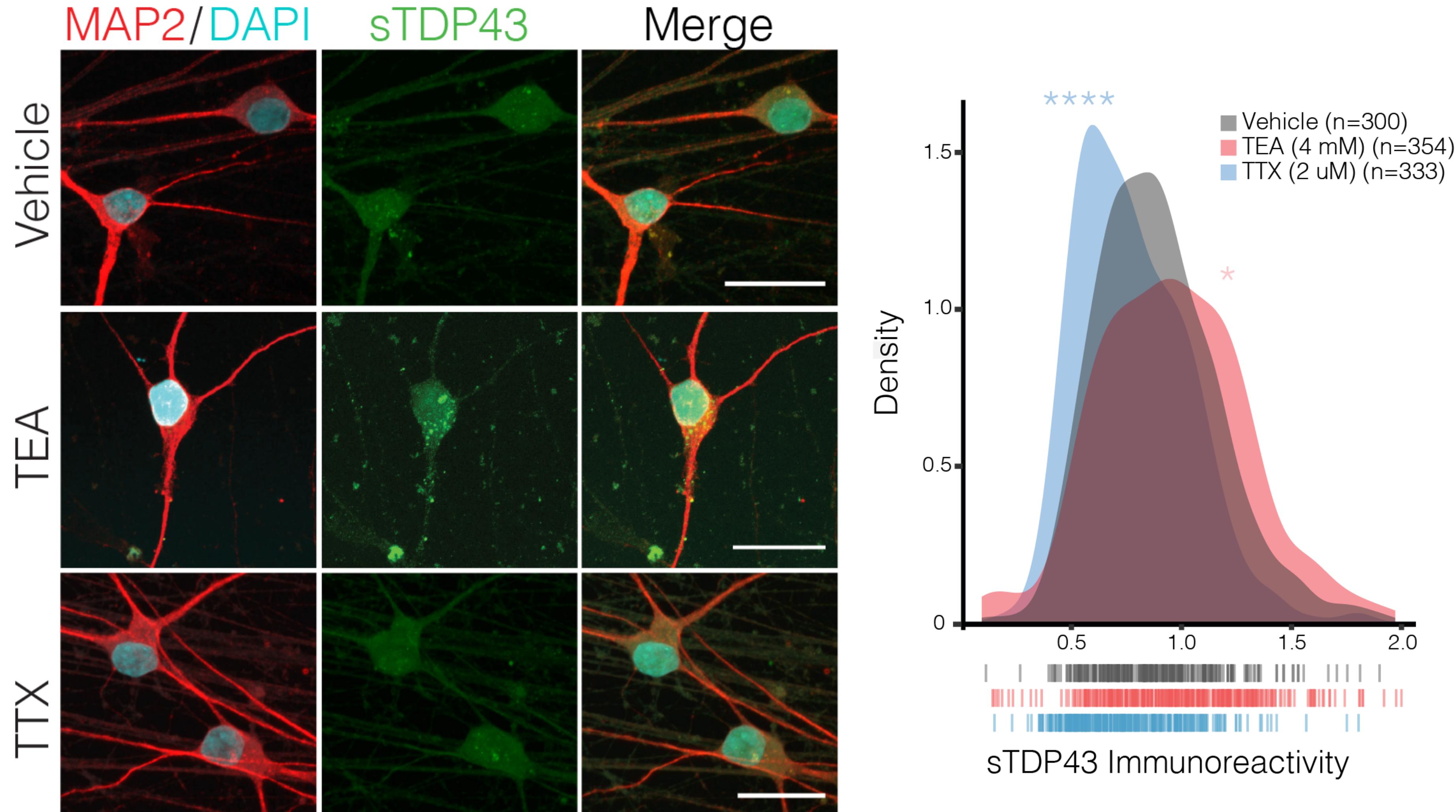
## Transcriptome–pathology correlation identifies interplay between TDP-43 and the expression of its kinase CK1E in sporadic ALS

Florian Krach<sup>1,2,3</sup> · Ranjan Batra<sup>1,2</sup> · Emily C. Wheeler<sup>1</sup> · Anthony Q. Vu<sup>1</sup> · Ruth Wang<sup>1</sup> · Kasey Hutt<sup>1</sup> · Stuart J. Rabin<sup>4</sup> · Michael W. Baughn<sup>2</sup> · Ryan T. Libby<sup>4</sup> · Sandra Diaz-Garcia<sup>2</sup> · Jennifer Stauffer<sup>2</sup> · Elaine Pirie<sup>1,2</sup> · Shahram Saberi<sup>2</sup> · Maria Rodriguez<sup>2</sup> · Assael A. Madrigal<sup>1</sup> · Zacharias Kohl<sup>6</sup> · Beate Winner<sup>3</sup> · Gene W. Yeo<sup>1,5,7</sup> · John Ravits<sup>2</sup> 

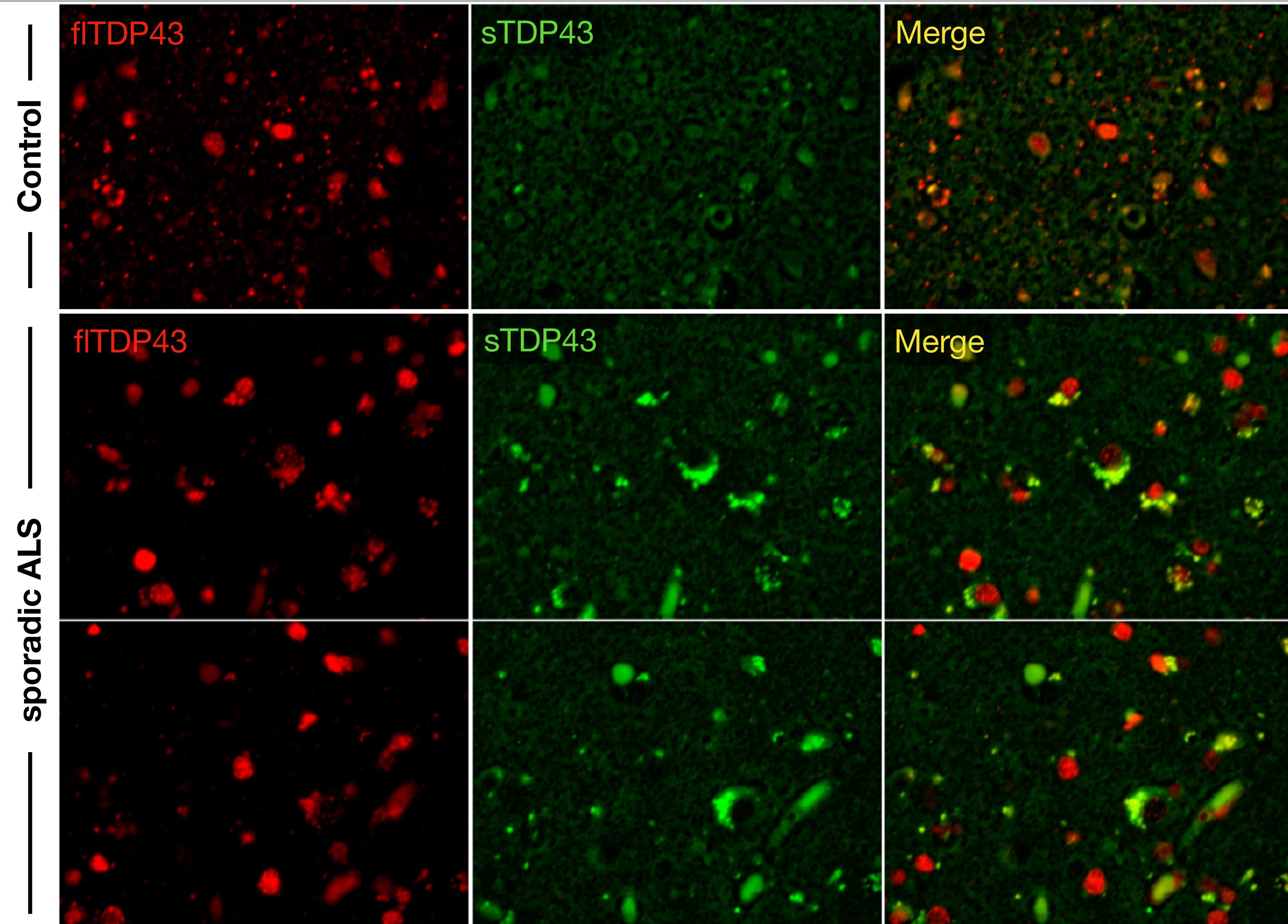
### Laser-capture microdissected spinal neurons



# Detecting endogenous sTDP43 using a specific antibody

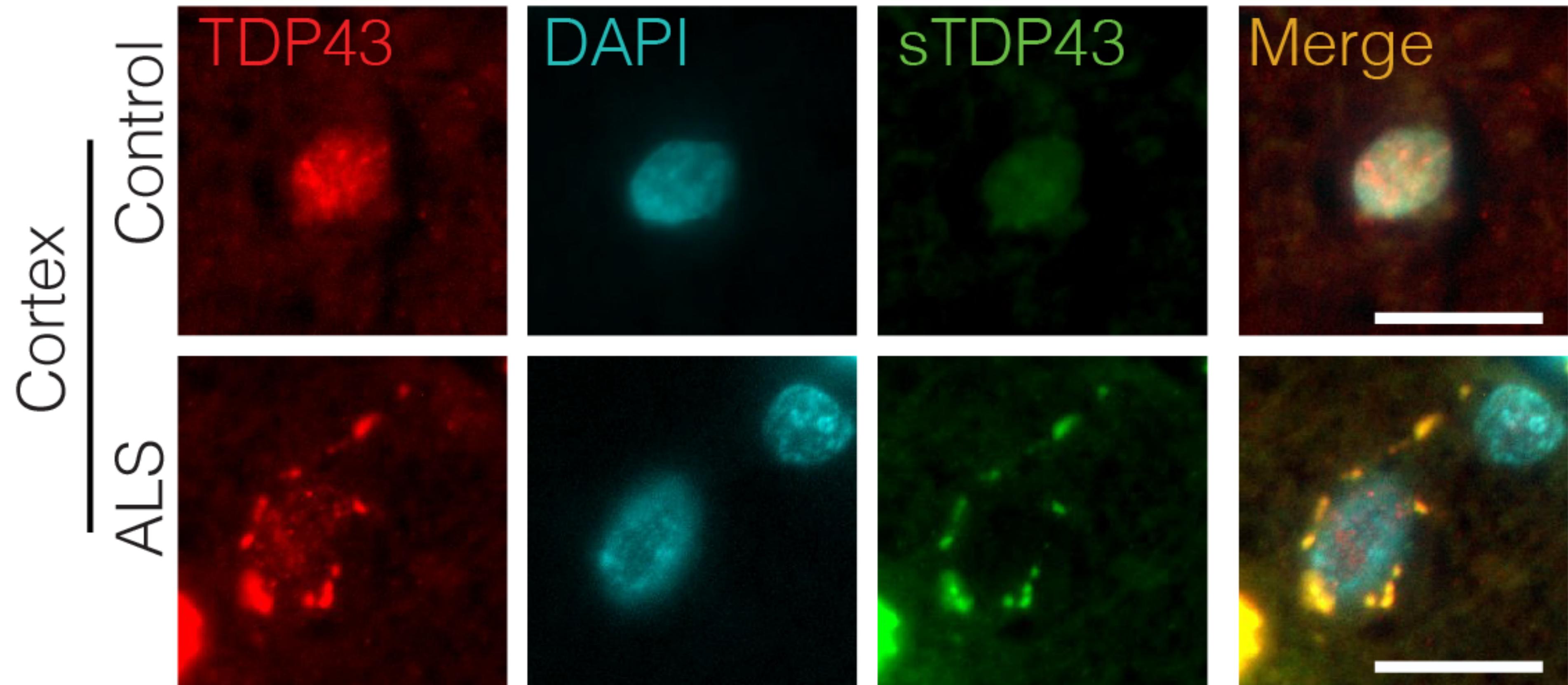


# sTDP43 in ALS



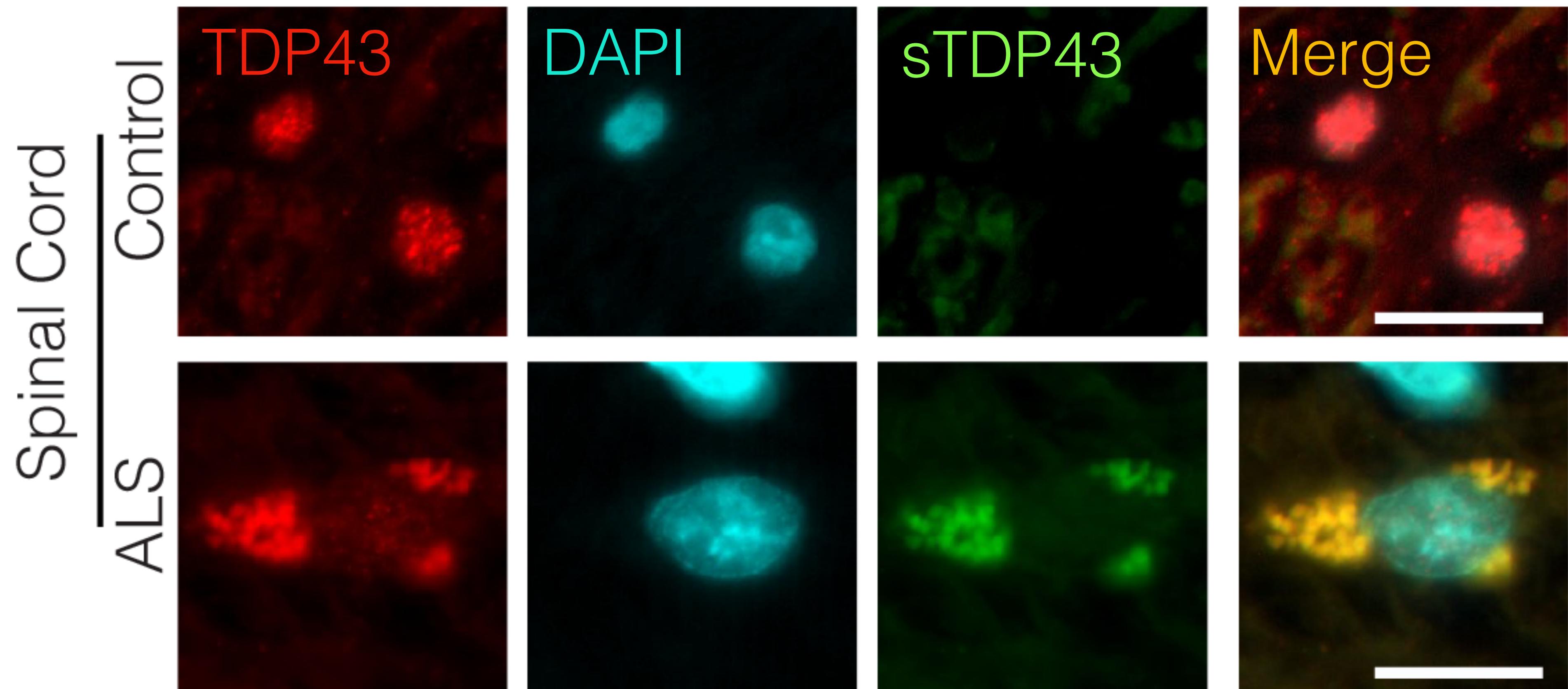
# sTDP43 in ALS

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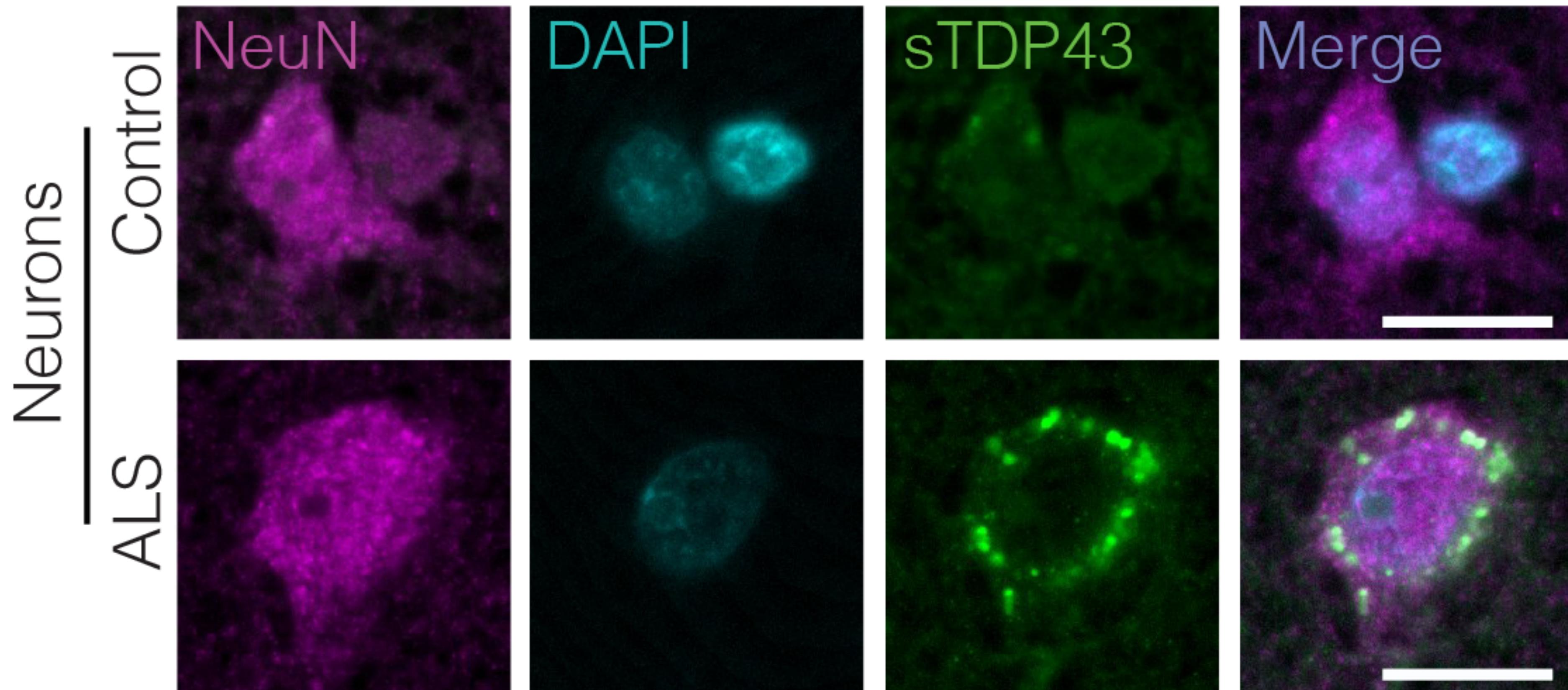
# sTDP43 in ALS

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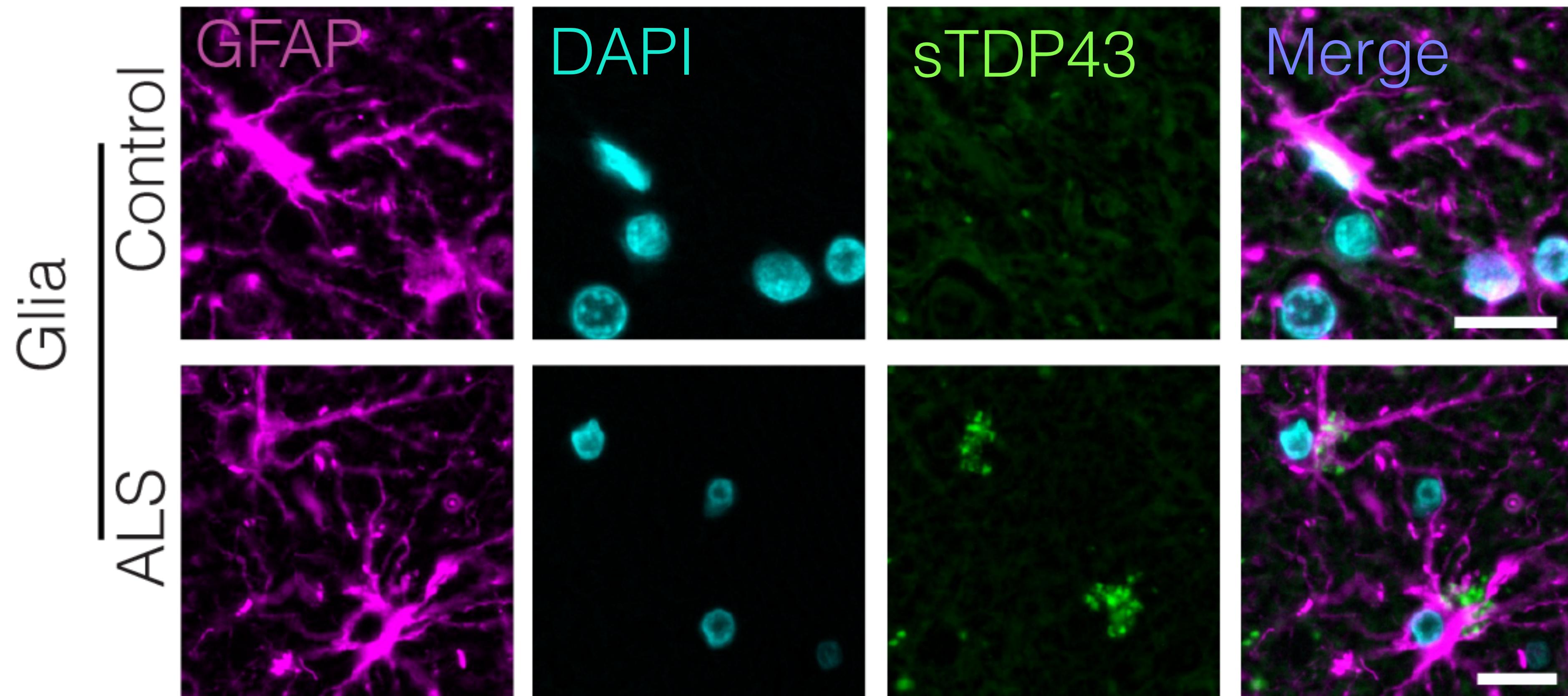
# sTDP43 in ALS

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# sTDP43 in ALS

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Neuronal  
hyperactivity



↑ in lower motor neurons

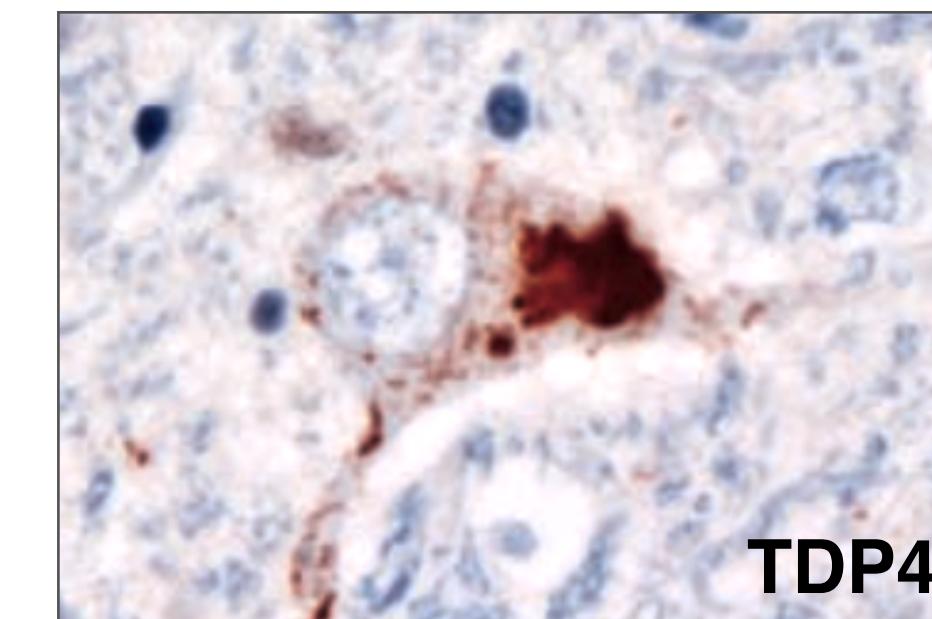
↑ with age

*TARDBP* Regulation?

splicing Upper motor neurons?

sTDP43  
production

Native function?



Hum Mol Genet, 2009, Vol. 18, 2

mRNA stability,  
transport &  
translation?

sTDP43  
cytoplasmic  
accumulation

fITDP43  
nuclear  
exclusion

RNA splicing &  
stability?  
RNP dynamics?

Neurodegeneration

[www.barmadalab.com](http://www.barmadalab.com)

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Xingli Li  
Roberto Miguez  
**Kaitlin Weskamp**  
Nathaniel Safran  
Ahmed Malik  
Michael McMillan  
**Nico Gomez**  
Jason Chua  
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Erb Family Foundation

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**Robert Packard Center**

**Ann Arbor Active Against ALS**

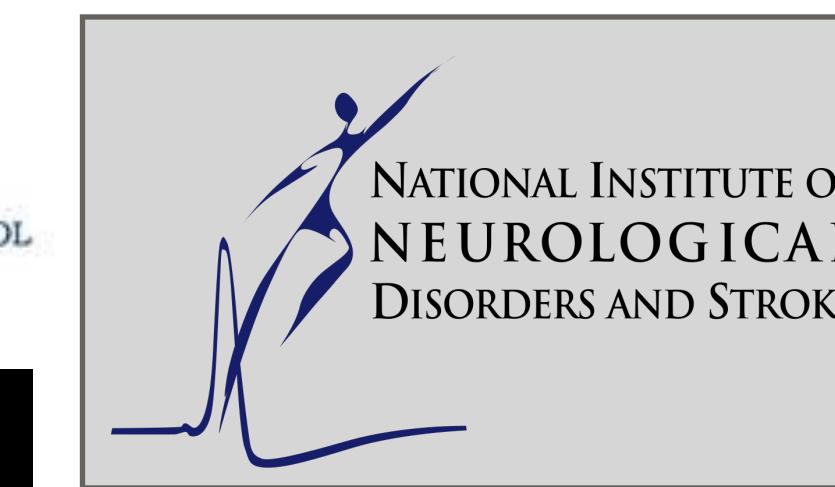
**Angela Dobson, Lyndon Welch & family**

ALS Therapy Alliance



**PACKARD CENTER**

*ALS Research at Johns Hopkins*



**ACTIVE AGAINST ALS**