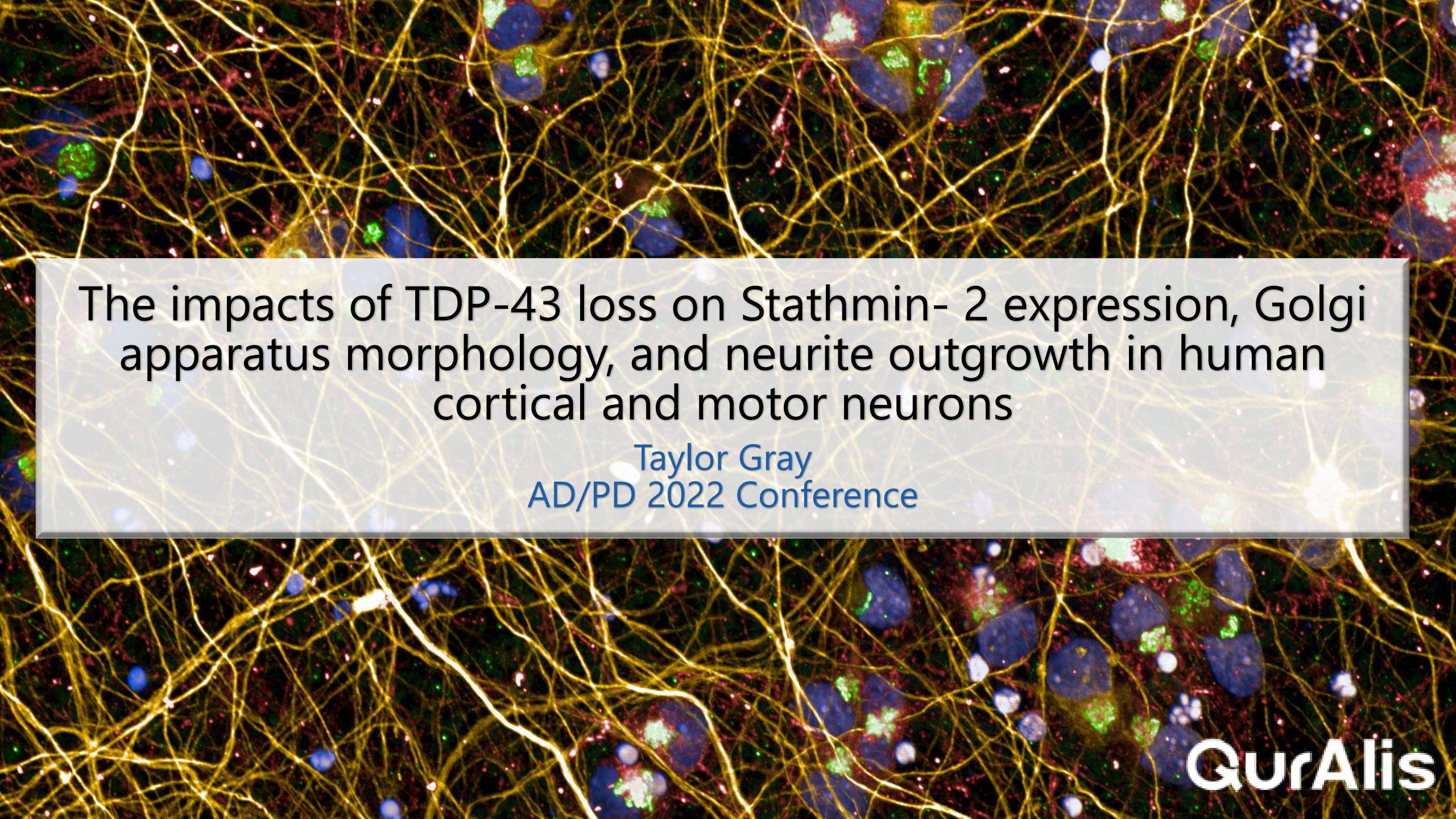


The impacts of TDP-43 loss on Stathmin- 2 expression, Golgi apparatus morphology, and neurite outgrowth in human cortical and motor neurons

Taylor Gray

AD/PD 2022 Conference

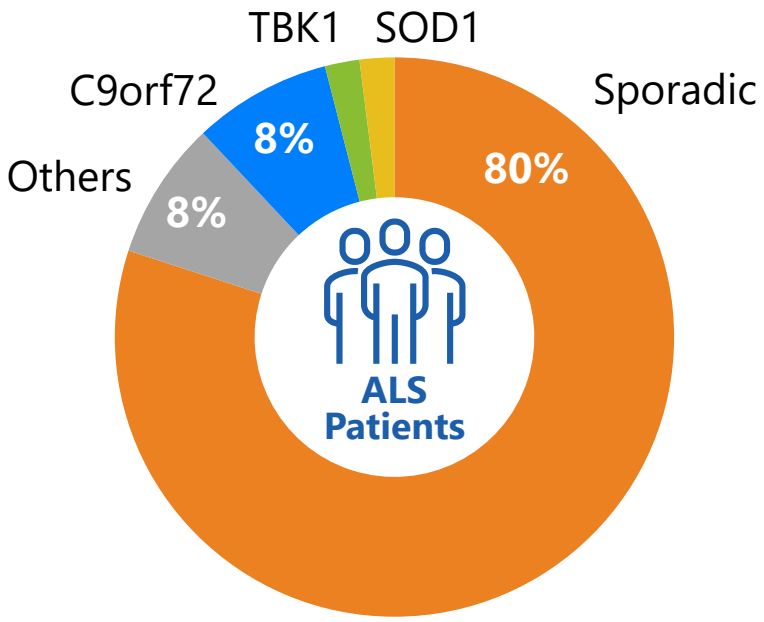
GurAlis

Why QurAlis can succeed in ALS & other neurodegenerative diseases

- Past companies applied "one-drug fits all" approach
- Lacked understanding of "disease-drivers"

- QurAlis is applying precision oncology-like approaches in neuroscience
- QurAlis' unique understanding of disease and biomarkers lead to identification of sub-groups of patients for the right therapy

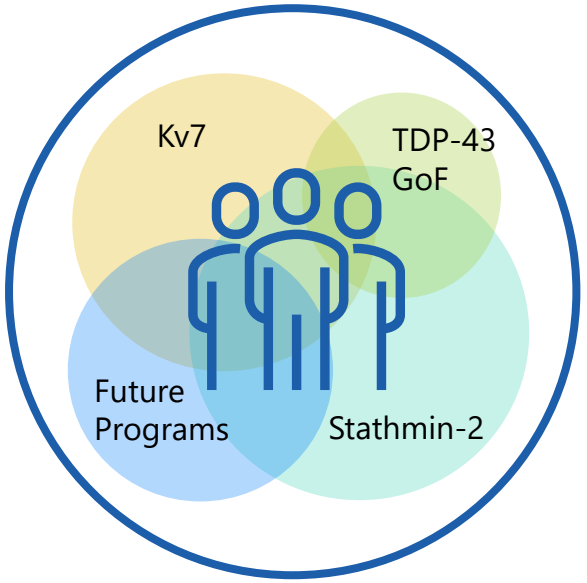
Applying Precision Medicine Approaches Initially in ALS



Understand
Genetic Drivers in Sub-Forms of ALS

Design
Precision Medicines & Biomarkers

Deliver
Precision Therapies to Patients



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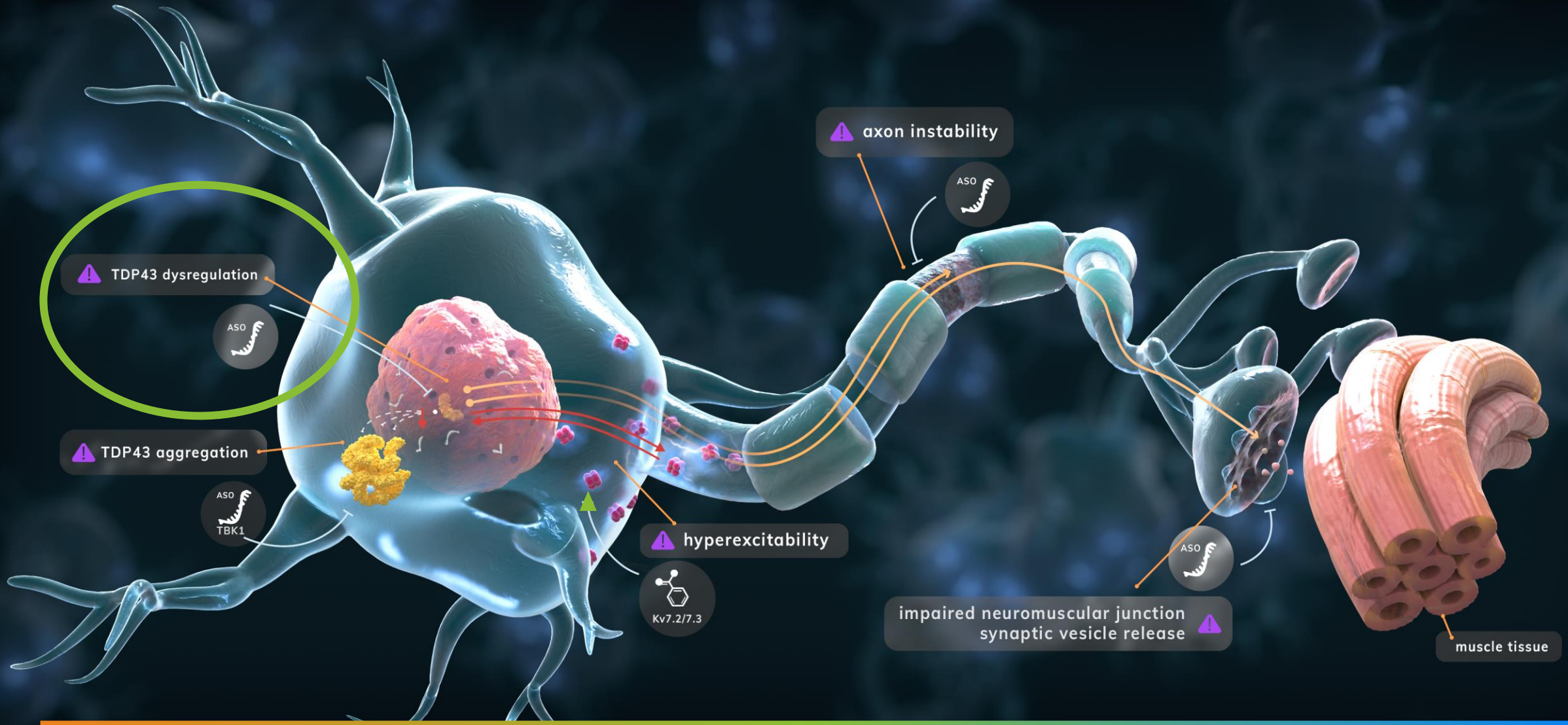
Genetics of ALS uncovers major disease drivers linked to TDP-43

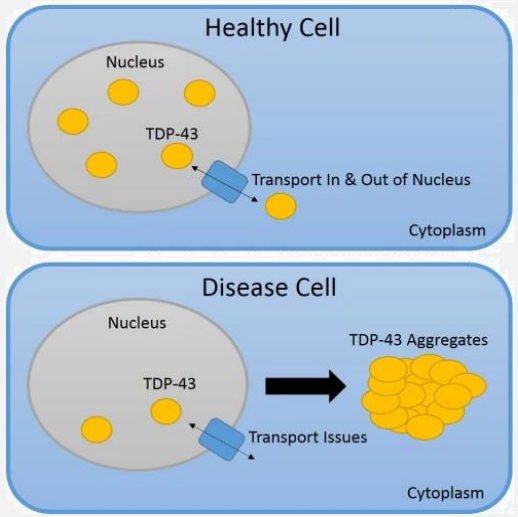


Only company with multiple programs against TDP-43 proteinopathy affecting ~90% ALS, ~50% FTD, ~30% AD and ~7% PD

Stathmin-2: A genetic target for the sporadic ALS & FTD population

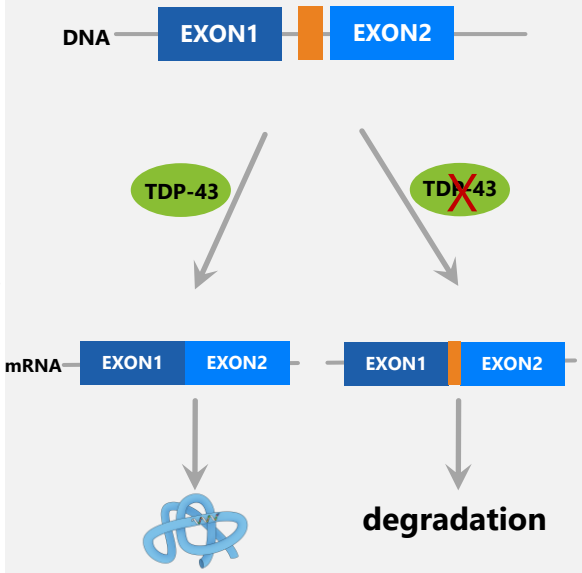
QurAlis Therapeutic Strategy

In ALS motor neurons TDP-43 leaves the nucleus



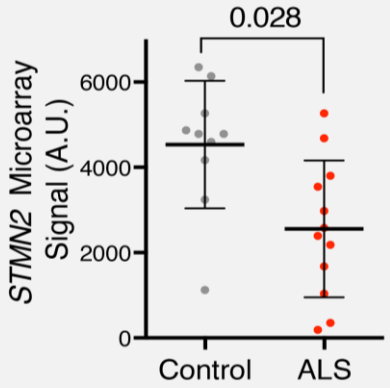
QurAlis niche

Loss of TDP-43 controlled cryptic exon splicing



TDP-43 regulation of *STMN2*

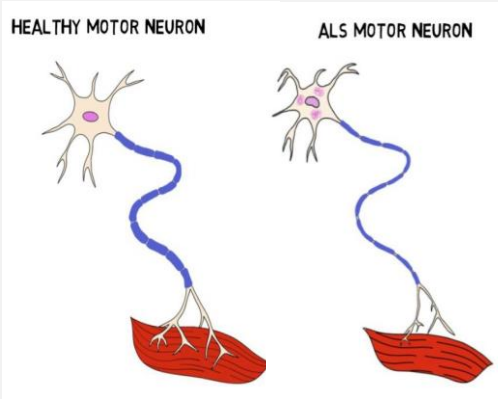
Loss of full length *STMN2* gene



Rabin et al., 2009

Cryptic splicing-ASO approach to restore *STMN2*

Axonal degeneration and impaired repair



Rescue of axonal stability and repair

Nat. Neurosci. Feb 2019, Eggan ALS One 2020, Li et al., 2009, Morii et al., 2006, Shin et al., 2012, Shin et al., 2014, Xu et al., 2010,

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STMN2 (Stathmin-2) is an ALS/FTD gene – loss of *STMN2* leads to neurodegeneration

- Most consistently decreased gene in all sporadic ALS patient data sets
- Mice k/o studies show progressive neurodegenerative phenotype
- Most significantly regulated gene by TDP-43 exclusively in humans
- Rescue of *STMN2* in presence of TDP-43 pathology restores neurogenerative phenotypes
  - Neuronal processes
  - Golgi transport

Internal data; Rabin et.al, 2009; Highley et.al., 2014; D'Erchia et. Al., 2017; Eggan (ALS One 2020); Nat. Neurosci. Feb 2019; J Clin Invest. 2020;130(11):5677-5680/Microtubule Regulator Connects TDP-43 to Axonal Dysfunction | ALZFORUM

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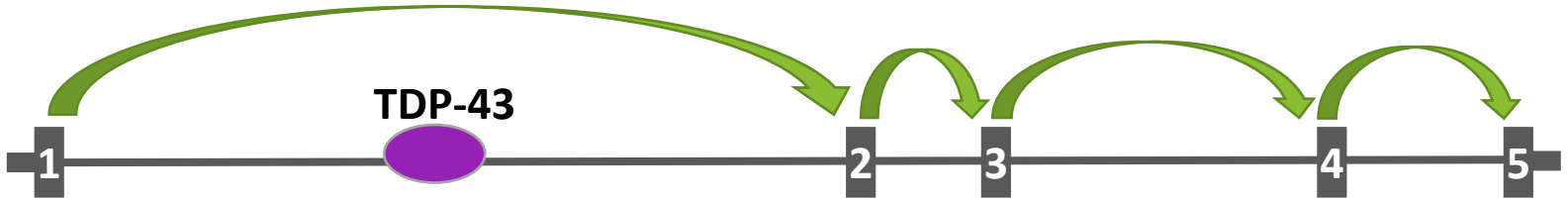
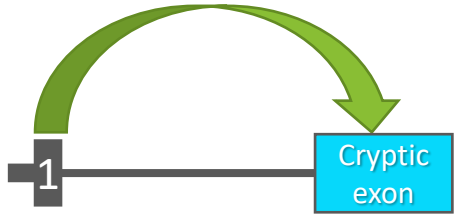
Stathmin-2 program: background on splicing assay

TDP-43 binding regulates *STMN2* splicing



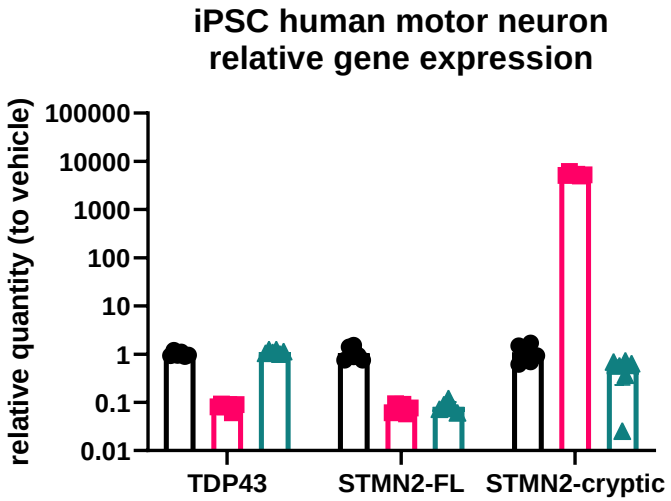
Loss of nuclear TDP-43 causes mis-splicing of *STMN2* into cryptic exon

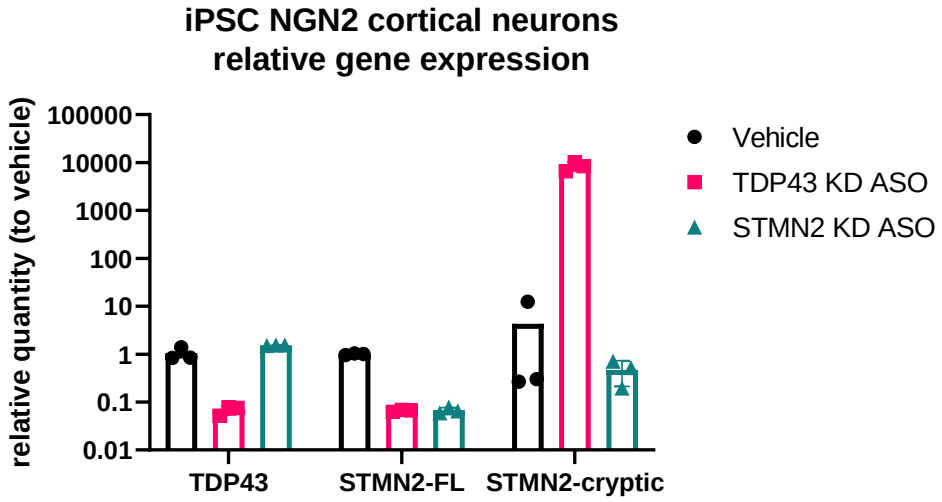


Premature transcript termination
- Transcript degradation
- Loss of functional Stathmin-2 protein

Cellular model for ASO screening

- TDP-43 knockdown using a gapmer ASO
- Loss of full length STMN2 (RNA and protein can be quantified)
- Induction of cryptic exon expression (RNA can be quantified)
- iPSC-derived motor neurons and cortical neurons can both be used for a model for STMN2 mis-splicing





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Loss of TDP-43 results in *STMN2* mis-splicing in iPSC motor neurons

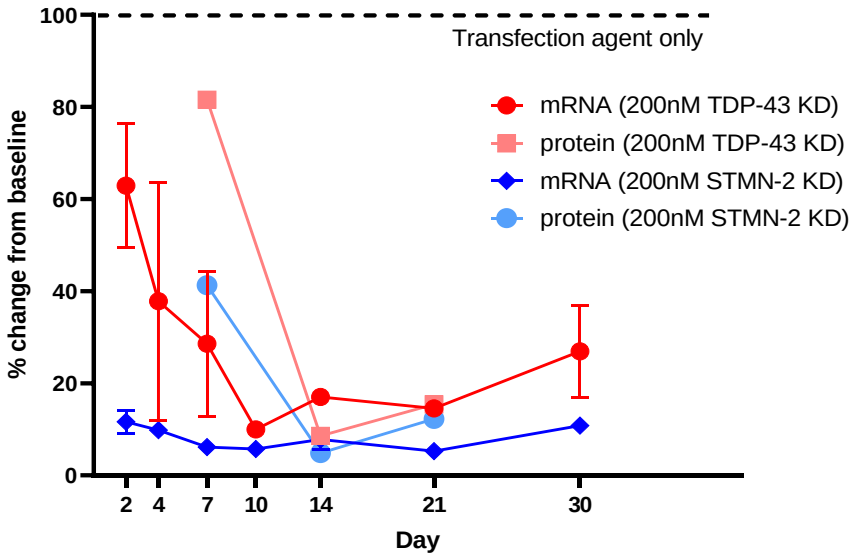
Knockdown with *TARDBP* (TDP-43) gapmer ASO results in:

- ✓Sustained loss of *TARDBP* transcripts and protein
- ✓Sustained loss of *STMN2* transcripts and protein
- ✓Accumulation of *STMN2* cryptic exon transcripts

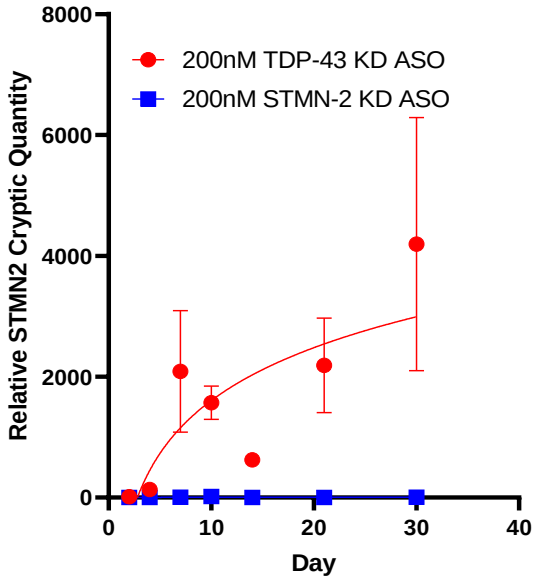
Knockdown with *STMN2* (Stathmin-2) gapmer ASO results in:

- ✓No change in *TARDBP* transcripts and protein
- ✓Sustained loss of *STMN2* transcripts and protein
- ✓No change in *STMN2* cryptic exon transcripts

Stathmin-2 mRNA and protein



Stathmin-2 cryptic exon mRNA



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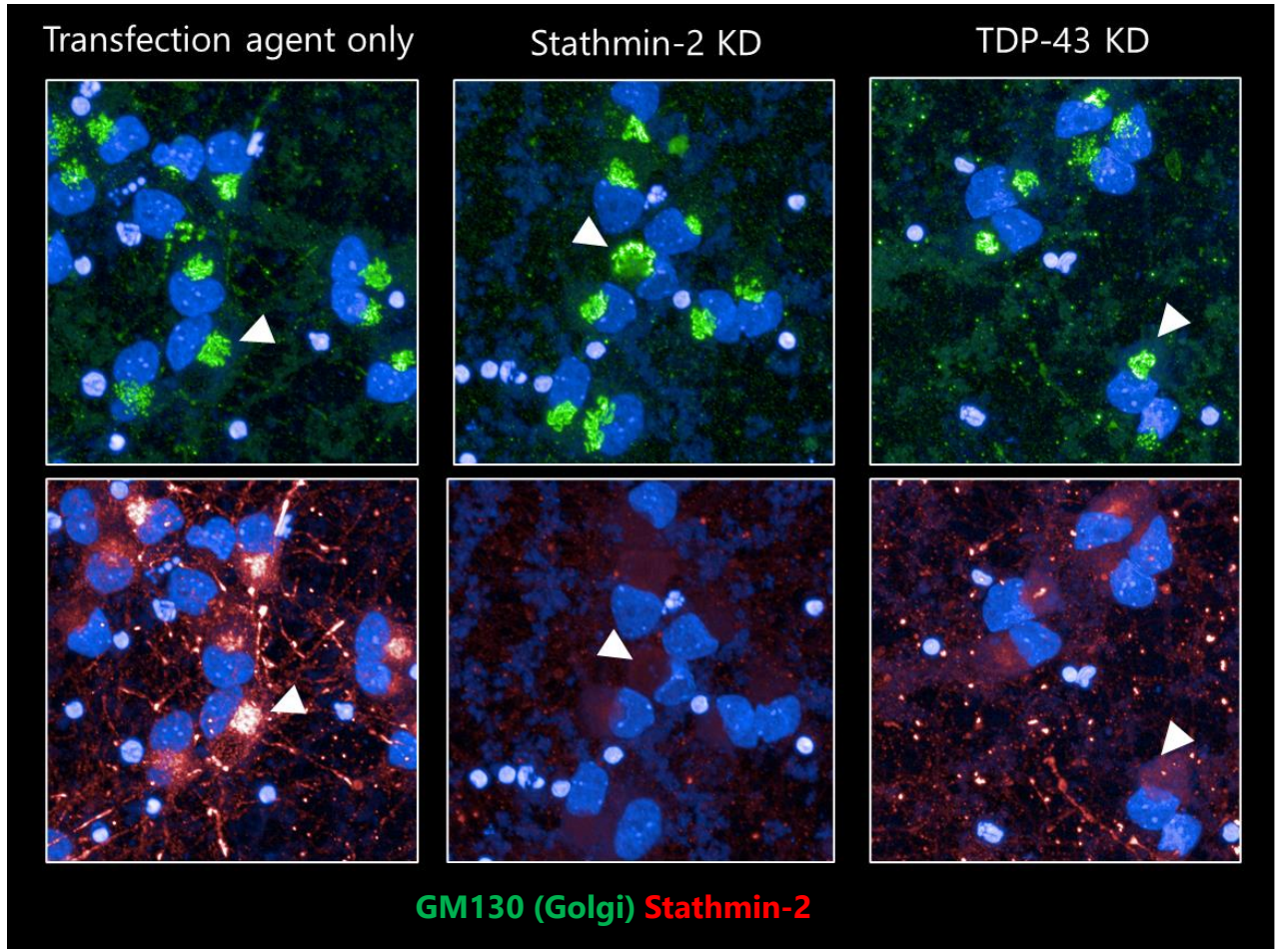
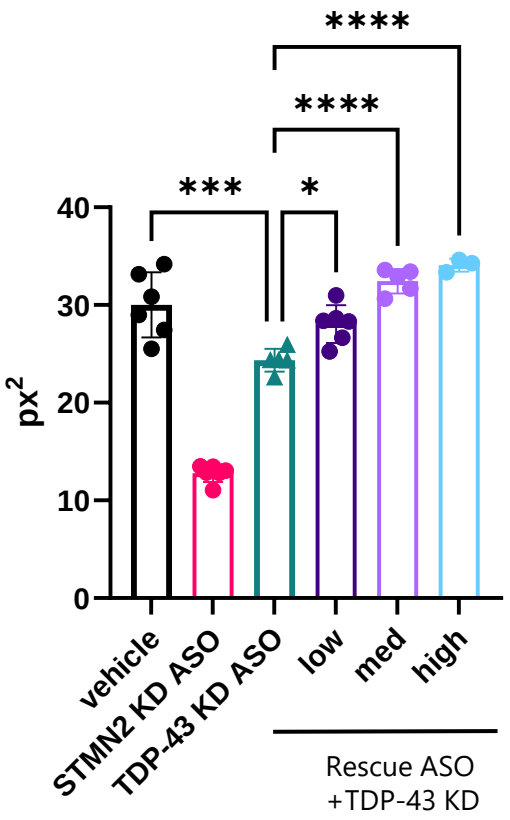
Loss of TDP-43 in iPSC motor and cortical neurons results in loss of Stathmin-2 co-localization to the Golgi apparatus



Area of Stathmin-2 staining in GM130+ Golgi



One-way ANOVA with Dunnett multiple comparison test vs. TDP-43 KD
*p<0.05
**p<0.01
***p<0.001
****p<0.0001

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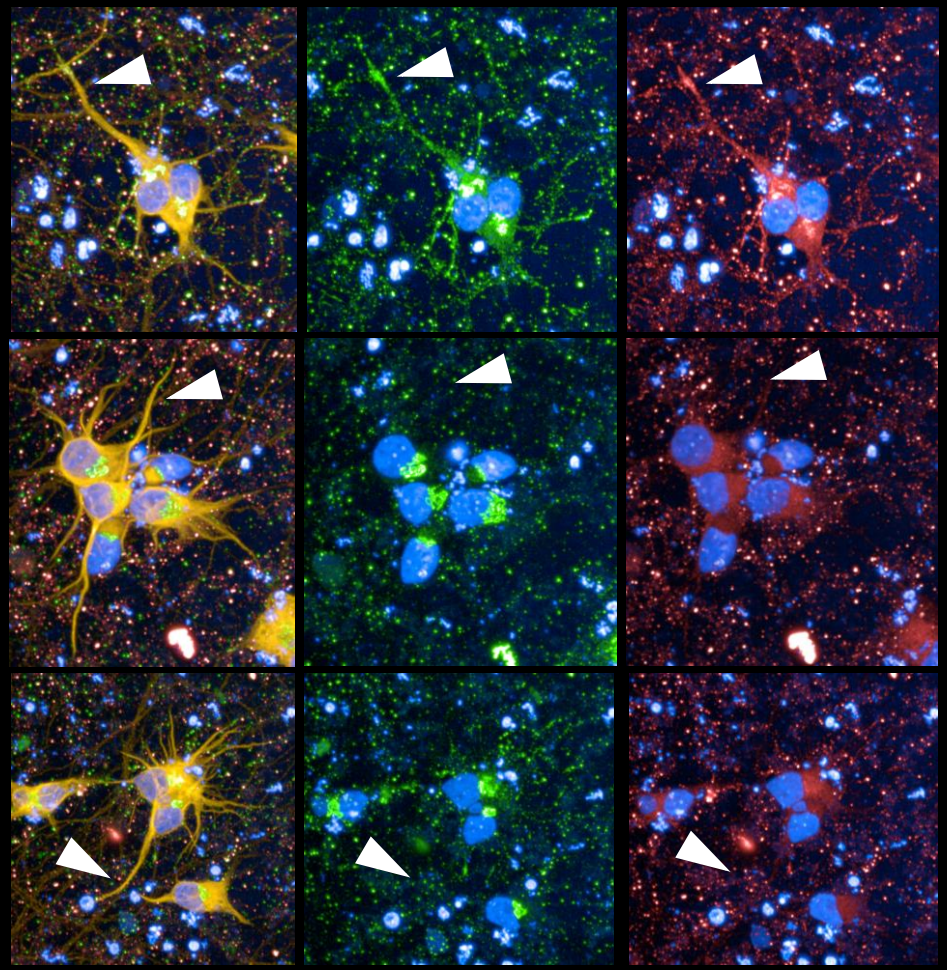
Loss of Stathmin-2 & Golgi outposts in human iPSC neuron dendrites

Motor neurons



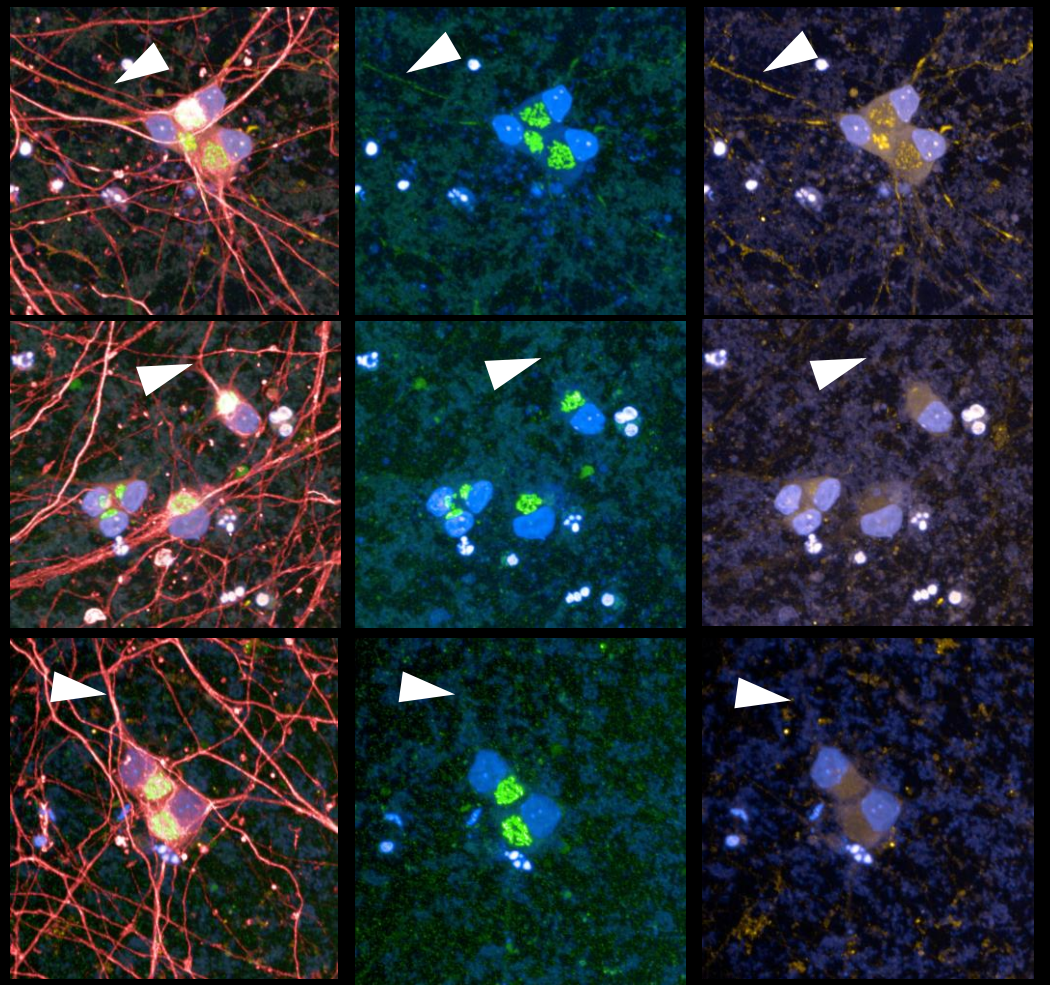
MAP2 GM130 Stathmin-2 | GM130 Stathmin-2 | Stathmin-2

Vehicle

TDP-43 KD ASO

STMN2 KD ASO

Cortical neurons



Stathmin-2 TGN46 NFH | TGN46 | Stathmin-2

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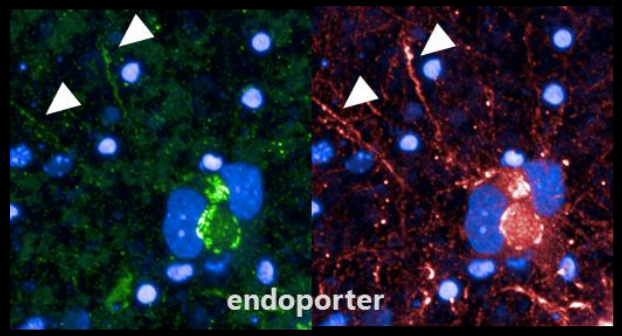
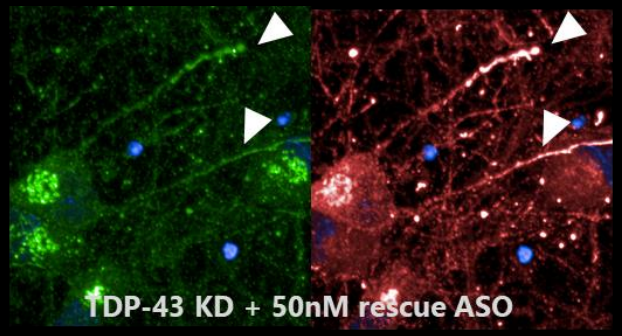
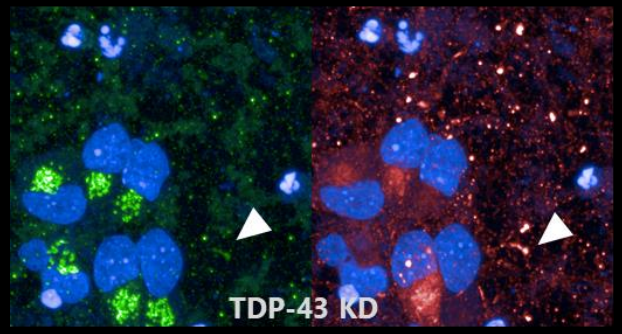
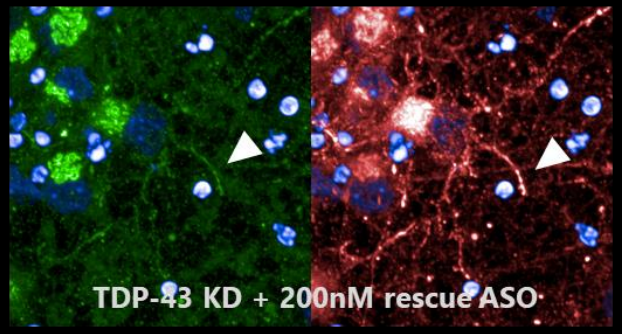
STMN2 splice-switching ASOs significantly rescue Stathmin-2 function in Golgi trafficking at all doses

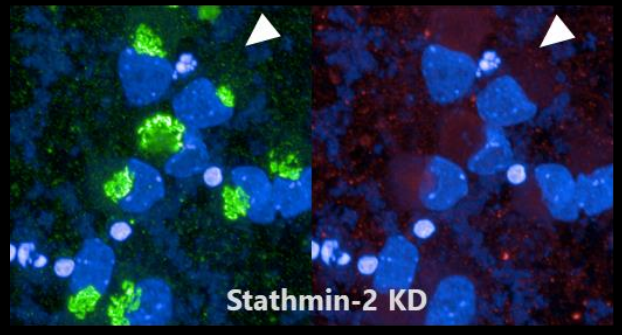
DAPI GM130 (Golgi) Stathmin-2

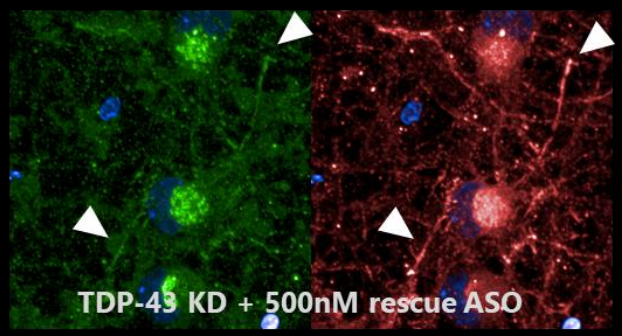




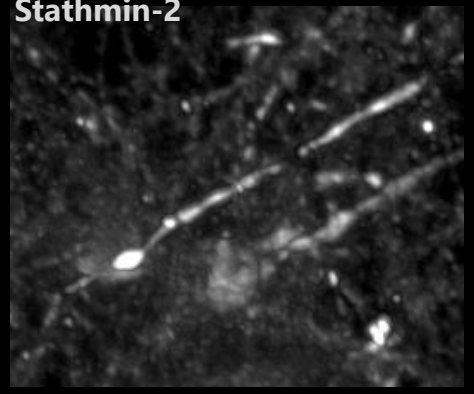


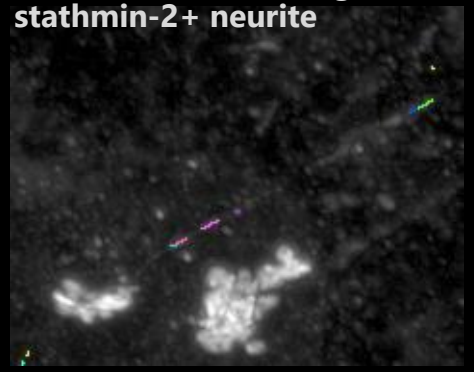


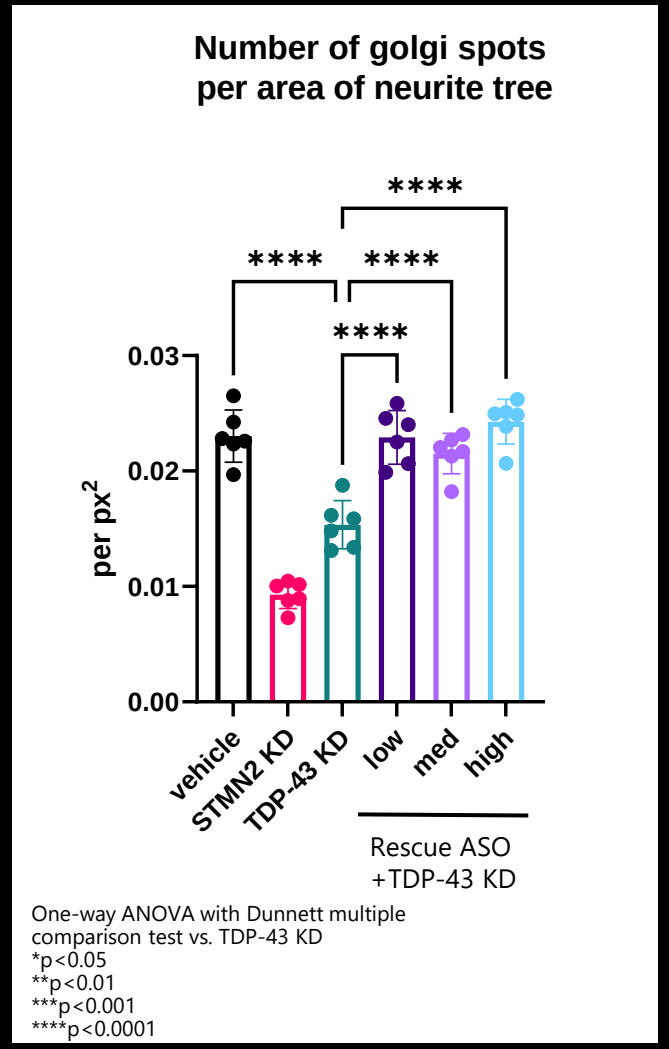




Golgi outpost quantitation in stathmin-2+ neurites







Stathmin-2/TDP-43 in AD, PD and dementias

SCG10 promotes non-amyloidogenic processing of amyloid precursor protein by facilitating its trafficking to the cell surface

Jingjing Wang[1,†], Chunyan Shan[1,†], Wenyuan Cao[1,†], Chen Zhang[1], Junlin Teng[1] and Jianguo Chen[1,2,*]

JCI The Journal of Clinical Investigation

Truncated stathmin-2 is a marker of TDP-43 pathology in frontotemporal dementia

Mercedes Prudencio, … , Pietro Fratta, Leonard Petrucelli

J Clin Invest. 2020. https://doi.org/10.1172/JCI139741.

TDP43-positive intraneuronal inclusions in a patient with motor neuron disease and Parkinson's disease

Jean-Baptiste Chanson [1], Andoni Echaniz-Laguna, Thomas Vogel, Michel Mohr, Aurélien Benoilid, Georges Kaltenbach, Michèle Kiesmann

Affiliations + expand

PMID: 20197650 DOI: 10.1159/000273591

frontiers in Molecular Neuroscience

Front Mol Neurosci. 2020; 13: 26. PMCID: PMC7059763

Published online 2020 Feb 28. doi: 10.3389/fnmol.2020.00026 PMID: 32180703

TDP-43: From Alzheimer's Disease to Limbic-Predominant Age-Related TDP-43 Encephalopathy

Wendi Huang,[1,†] Yongjian Zhou,[2,†] Lin Tu,[3] Zhisheng Ba,[3] Juan Huang,[4] Nanqu Huang,[3] and Yong Luo[3,*]

Brain Pathology ISSN 1015–6305

RESEARCH ARTICLE

TDP-43 pathology in Alzheimer's disease, dementia with Lewy bodies and ageing

Kirsty E. McAleese; Lauren Walker; Daniel Erskine; Alan J. Thomas; Ian G. McKeith; Johannes Attems

Institute of Neuroscience, Newcastle University, Newcastle Upon Tyne, UK.

TDP-43 is deposited in the Guam parkinsonism–dementia complex brains FREE

Masato Hasegawa, Tetsuaki Arai, Haruhiko Akiyama, Takashi Nonaka, Hiroshi Mori, Tomoyo Hashimoto, Mineo Yamazaki, Kiyomitsu Oyanagi

Author Notes

Brain, Volume 130, Issue 5, May 2007, Pages 1386–1394, https://doi.org/10.1093/brain/awm065

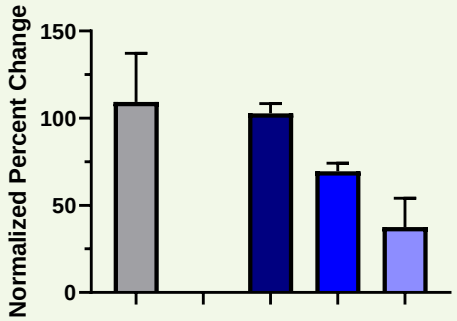
Published: 17 April 2007 **Article history** ▾

QurAlis

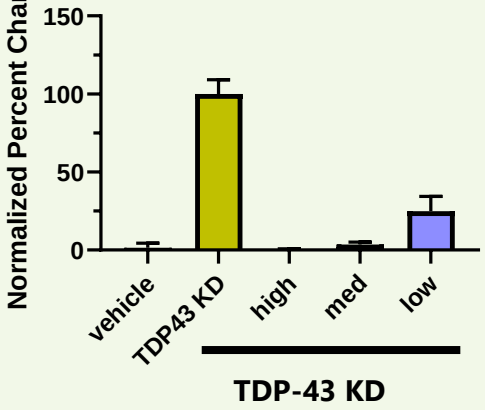
QurAlis's STMN2 splice-switching ASOs potently rescue Stathmin-2 pathology induced by TDP-43

Potency

***STMN2* FL mRNA**

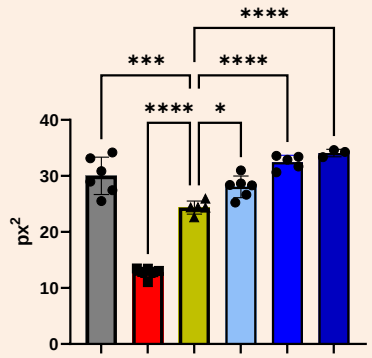


***STMN2* cryptic mRNA**

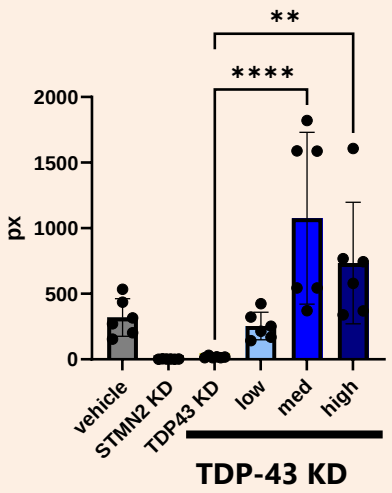


Phenotype

Stahmin-2 rescued in Golgi Apparatus



Stathmin-2 neurite rescue



TDP-43 LOF leads impacts *STMN2*

Decreased *STMN2* mRNA expression and increased *STMN2* cryptic splicing in human motor neurons

Loss of *STMN2* leads to functional deficit in neurons

Decreases in Stathmin-2 protein expression in the Golgi apparatus/dendritic outposts and decreased neurite length of Stathmin-2 positive neurites

Stathmin-2 is an attractive target for a therapeutic intervention in ALS, FTD, AD, and PD

Rescue of Stathmin-2 in presence of TDP-43 pathology restores neurogenerative phenotypes

***STMN2* splice-switching ASOs are a promising therapeutic for ALS and FTD cases with TDP43 pathology**

Restores normal *STMN2* splicing

Rescues stathmin-2 Golgi localization and neurite length

Precision medicine Stathmin-2 biomarkers required for clinical validation

****Check out QurAlis's biomarker talk by Sandy Hinckley!**

One-way ANOVA with Dunnett multiple comparison test vs. TDP-43 KD
*p<0.05
**p<0.01
***p<0.001
****p<0.0001

QurAlis

Acknowledgments

QurAlis Research and Discovery Team

Yasmin Hamwi

JJ Bussgang

Marisa Kamelgarn

Sandy Hinckley

Dan Elbaum

Kasper Roet