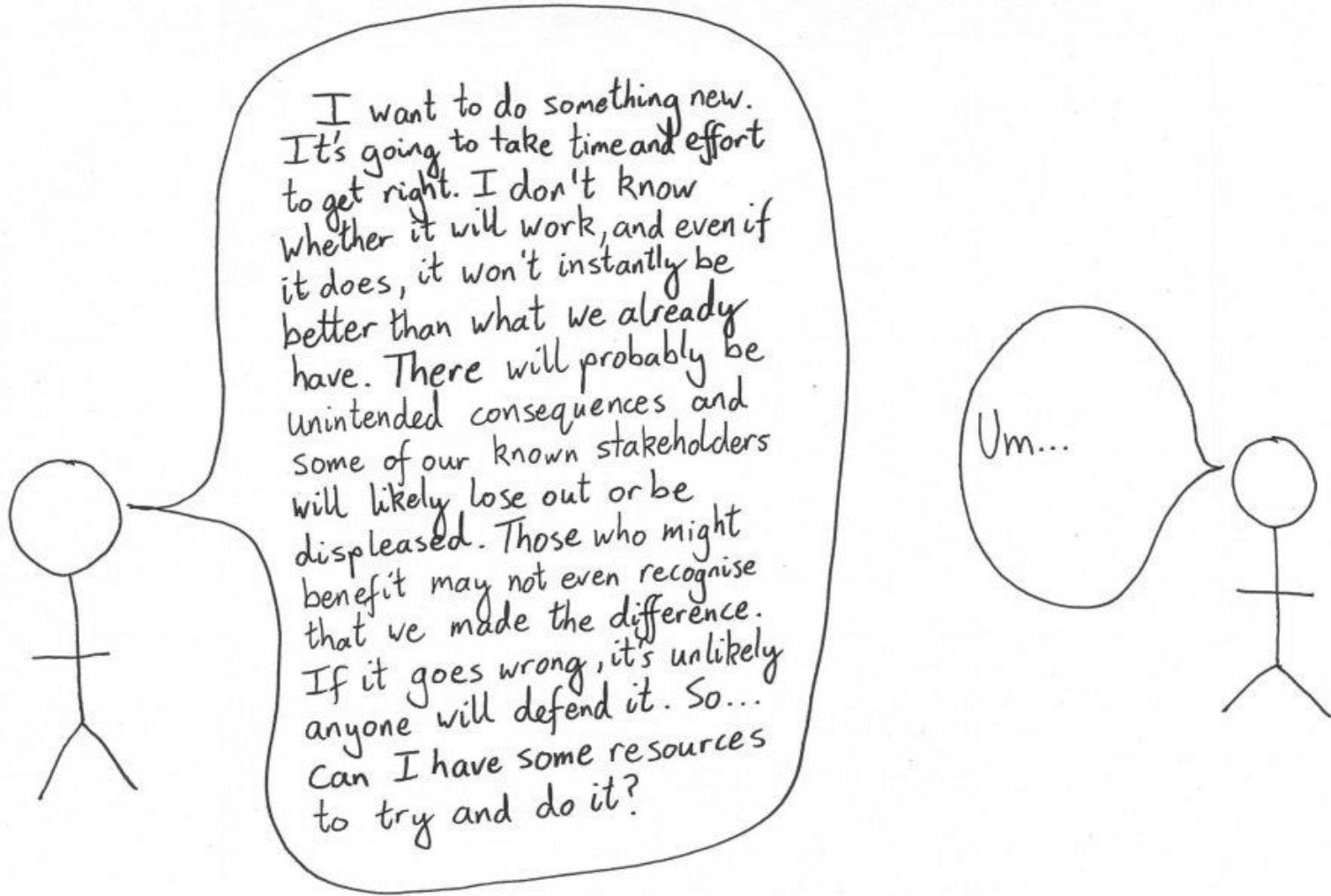


A Neurogenomics Story: Searching for a Cure to ALS and Neurodegenerative Disease



Model Organisms

- High degree of conservation (structure & function)
- Facilitate understanding of normal and pathogenic processes
- Key to most medical breakthroughs in the past century
- Drug testing



Model Organisms

- Purebred mice and rats
- Zebrafish
- Drosophila
- *C. elegans*
- Xenopus Levis
- Sea Urchin
- Dogs, cats
- monkeys, chimpanzees↑



<http://www.nature.com/nature/journal/v491/n7422/full/491031a.html>

Amyotrophic Lateral Sclerosis (ALS) aka Lou Gehrig's disease

- Progressive, fatal neurodegenerative disease
- Upper and lower motor neurons degeneration in the brain & spinal cord
- ~50% of ALS patients demonstrate mild to moderate cognitive or behavioral symptoms
- ~20% meet the clinical criteria for frontotemporal dementia (FTD) → interestingly, seemingly unrelated to motor symptoms
- Hallmark pathological feature: nuclear depletion and cytoplasmic accumulation of the TAR DNA-binding protein 43 (TDP-43) in degenerating neurons. usually present in homeostatic levels
- No cure, riluzole (glutamate release inhibitor) extends life by 2-3 months; and edaravone, a free radical scavenger that slows ALS progression in early disease.
- Disease onset (median age 50y) → death 2-4 years

ALS Genetic Basis

- ~10% of ALS patients are defined as having **familial ALS** typically inherited as a **highly penetrant, dominant trait**.
- The remaining 90% of cases are classified as **sporadic** because they lack a clear family history, although this term does not preclude a genetic contribution in these cases.

ALS Genetic Basis

Currently, **20 genes** are implicated in ALS pathogenesis, largely falling into three functional categories:

- The first group influences **cellular proteostasis** and **protein quality control**.
- The second group plays various **roles in the life cycle of RNAs**, affecting **RNA processing**, **assembly into granules**, and **intracellular transport**.
- The third group play roles in **cytoskeletal dynamics** within the motor neuron axon and distal terminal.

ALS Genetic Basis

- ALS-causing mutations are typically **missense** substitutions.
- Two ALS genes (CHCHD10 and C9ORF72) **defy this simplified classification or have less clear function.**
→ not a missense, and not any of the 3 categories, but are of the most common familial ALS.
- Notable exception of **C9ORF72**, in which ALS is caused by a **massive expansion of an intronic hexanucleotide repeat (healthy controls: 2-23 repeats; ALS: hundreds/thousands!).**
→ gene is no longer able to function

Most common familial cause of ALS: Autosomal dominant inheritance of expanded GGGGCC hexanucleotide repeats in the first intron of the C9orf72 gene → Thus, repeat expansion (NRE) in C9orf72 (C9-ALS) is the leading known cause of ALS → treatment target!

C9ORF72 Drosophila model of ALS

- Drosophila model of C9ORF72 associated ALS/FTD expressing 8 or 58 G4C2 repeats
 - ↪ # of repeats
 - ↪ 1 or 2 chromosomes
- Demonstrating length- and dosage-dependent degeneration phenotypes:



??? audio quality
is not good.

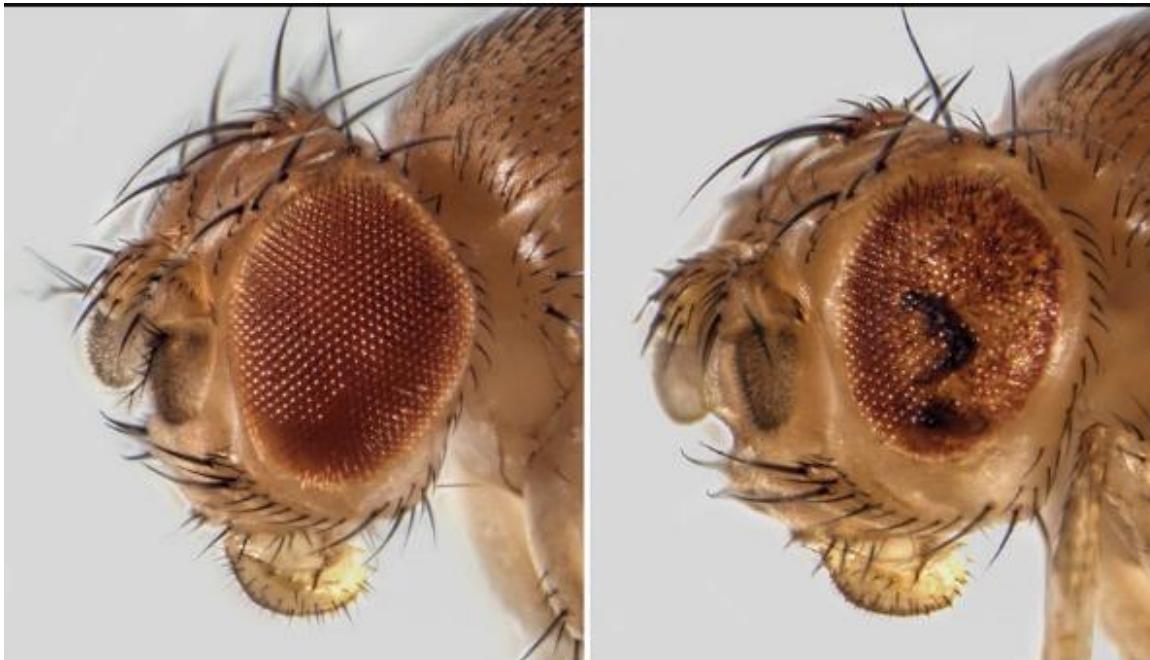
doi:10.1038/nature14974

dipeptide repeat

- Both G4C2 repeat-expanded RNA and DPRs from the sense strand were expressed in flies expressing (G4C2)58! Translation of expanded C9ORF72 transcripts produces five different poly-dipeptide repeats (DPRs): poly-Gly-Ala (poly(GA)) and poly-Gly-Arg (poly(GR)) from sense transcripts, poly-Pro-Ala (poly(PA)) and poly-Pro-Arg (poly(PR)) from antisense transcripts, and poly-Gly-Pro (poly(GP)) from both sense and antisense transcripts

A Window into the soul of ALS

Since familial is only ~10% of ALS, this only addresses ~4%.



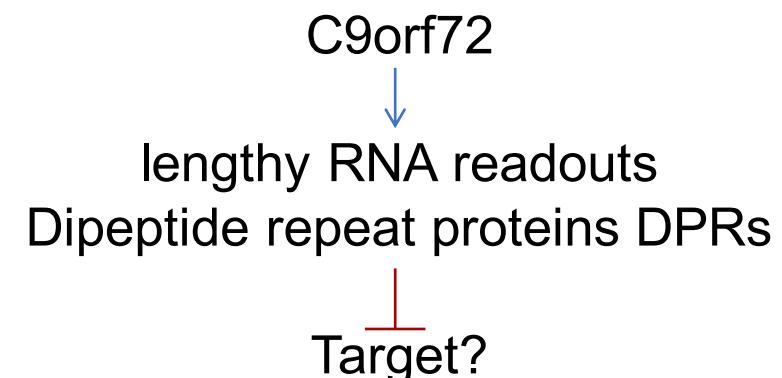
To halt brain diseases, drugs take aim at protein traffic jams that kill neurons

By Ellie Dolgin | Wed, 16 Jan 2019

The normal compound fly eye (left) is marred by cell death in a strain (right) with a mutation causing amyotrophic lateral sclerosis. KIRSTIN MAULDING

The flies carry a mutation found in ~40% of ALS patients who have a family history of the disease

By measuring the extent of damage to each insect's eyes, researchers can quickly gauge whether a drug, genetic modification, or some other therapeutic intervention helps stop neuronal loss.



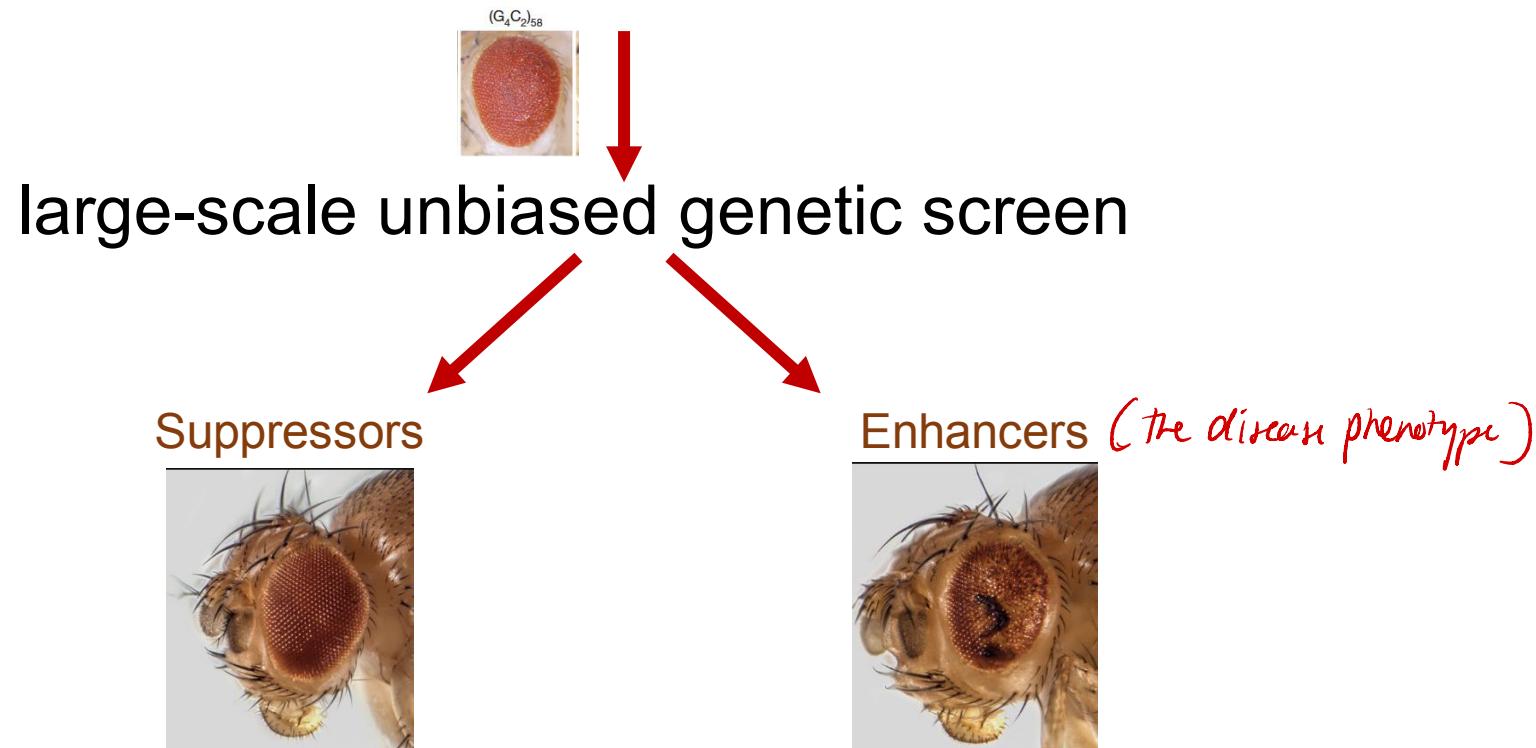
Look for modifiers of neurodegeneration

Solve the ALS mystery

What kills neurons
and ultimately, the
patient?

Solve the ALS mystery

(G₄C₂)₅₈-expressing flies



Identify modifiers of G4C2 repeat-mediated toxicity

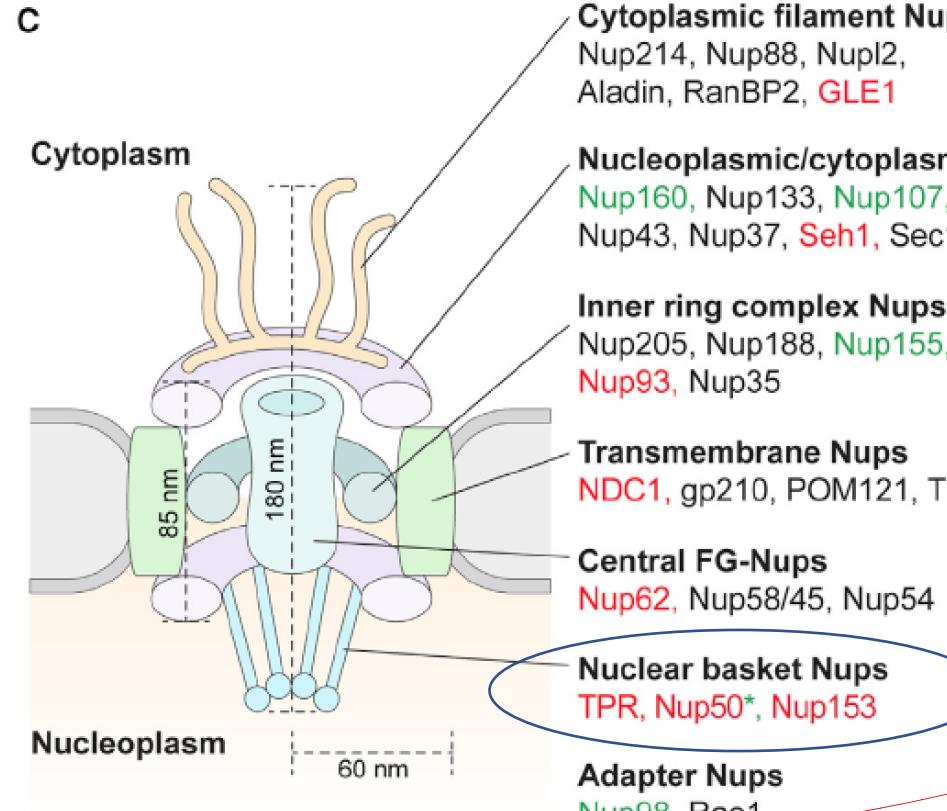
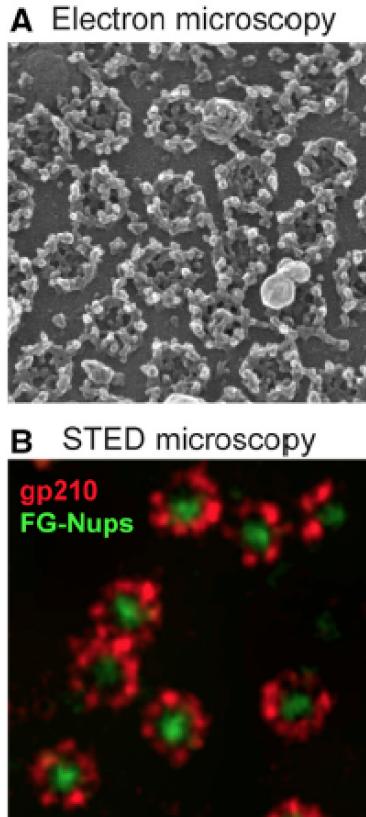
Solve the ALS mystery

One screen identified two genes:

- **Nup50** (Drosophila orthologs of human NUP50) whose loss strongly **enhanced** neurodegeneration in (G4C2)58-expressing flies
- **Ref1** (Drosophila orthologs of human ALYREF), whose loss strongly **suppressed** neurodegeneration in (G4C2)58-expressing flies

Understanding the role of Nup50

gateway between nucleus and cytoplasm.



Kim & Taylor Neuron 96, 285-297 (2017)

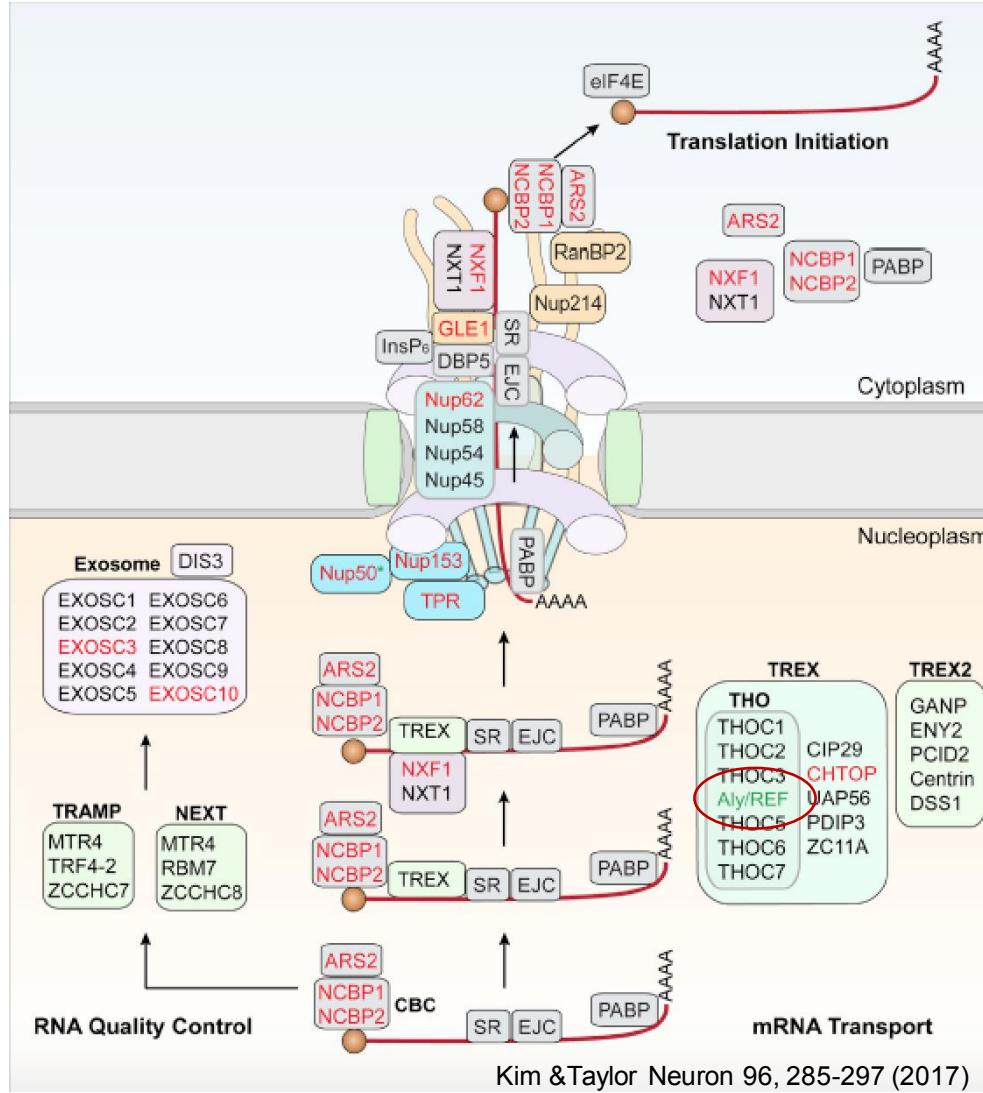
NUP50 plays a critical role in promoting the **nuclear import** of proteins through the nuclear pore complex (NPC) by interacting with importin α and Ran

so reducing import seems to be associated with neurodegeneration.



found in NPC, particularly the nuclear basket.

Understanding the role of Ref1



Export factor (in humans, ALYREF) is a component of the TREX complex.

TREX complex associates with the 5' cap of mRNAs and facilitates delivery of mRNPs to NXF1 (Nuclear RNA Export Factor 1), which then mediates mRNP export through the NPC

Specifically, Ref1 **prevents** RNA degradation in the nucleus

↓
so ↑ RNA degradation
is associated w/ the suppression
of neurodegeneration

The Emergence of Nucleocytoplasmic Transport Defects in C9 ALS-FTD

Further dissection of these two pathways:

- Identification of 18 strong genetic modifiers of neurodegeneration in (G4C2)58-expressing flies
- **All encode components of the NPC and nucleocytoplasmic transport machineries!**
- G4C2-expressing flies exhibited nuclear envelope abnormalities and nuclear RNA retention in a G4C2 length- and dose-dependent manner
 - ↳ the eye phenotype is a window into all the things going wrong @ the cellular level.
import and export are both going wrong.
- Mirroring the results from the Drosophila model, nuclear retention of RNA was also demonstrated in human cells and induced pluripotent stem cell (iPSC)-derived cortical neurons from patients with C9 ALS-FTD!!!

Toxicity—What is the root cause?

Mediated directly by the toxicity of
repeat-expanded RNA?

Or

Mediated by **dipeptide repeats**
(DPRs) derived from these RNAs?

Yeast based large scale screen

Focus specifically on DPR-mediated toxicity

Engineered yeast cells expressing each of the five DPR species produced by Repeat-associated non-ATG (RAN) translation of expanded G4C2 repeats in C9 ALS-FTD patients

want to see to what extent each one affecting the cells.

Arginine-containing DPRs (poly(GR) and poly(PR)) - highly cytotoxic!

Large-scale, unbiased genetic screen to identify **modifiers of poly(PR)-associated toxicity in yeast**

what leads to variety (?)
gt

when a transcript gets so long that it folds in on itself

2 of the 5 seems to be the ones that are causing most of the problem

↓
11 genes that regulate nucleocytoplasmic transport

Taken together:

Strong evidence that impairment of

nucleo-cytoplasmic transport (NCT)

is an important driver of toxicity in

simple genetic models of ***C9 ALS-FTD***

Relevant to Human ALS-FTD?

- Similar defects in patient-derived neurons
- Associated histopathological defects in patient-derived CNS tissue.



Impairment of NCT is also a contributor to the initiation or progression of ALS-FTD in human patients!

Toxicity – root cause?

How might DPRs
produce a defect in
NCT?

Search for interactors:

Affinity purification to identify protein interactors of each of the five DPRs produced in C9 ALS-FTD patients

(poly(GR) and poly(PR))

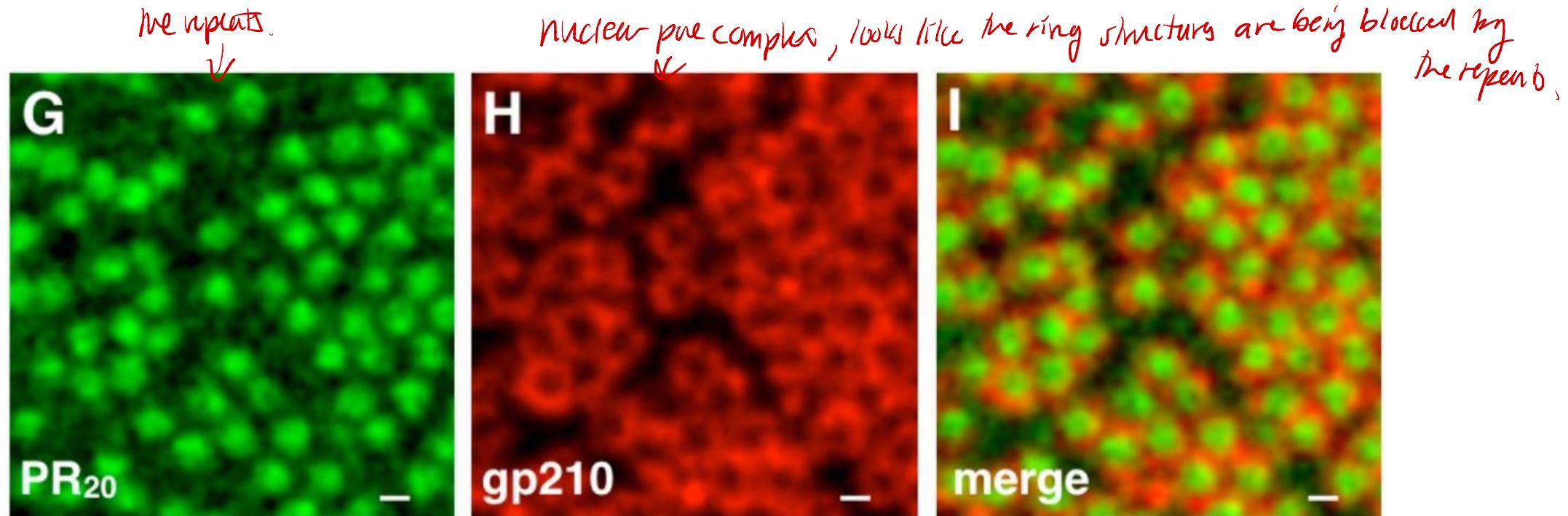


- RNA-binding proteins (e.g., hnRNPA1, hnRNPA2B1, FUS, and TDP-43)
- Nuclear pore components
- Components of membrane-less organelles (e.g., nucleolus and stress granules)

Instead of just associated
↑

Chemical cross-linking approach to capture direct interactors – showed consistent results

DPRs Produced by Mutant C9ORF72 Directly Plug the Nuclear Pore!



Shi et al., PNAS 114 (7) E1111-E1117 (2017)

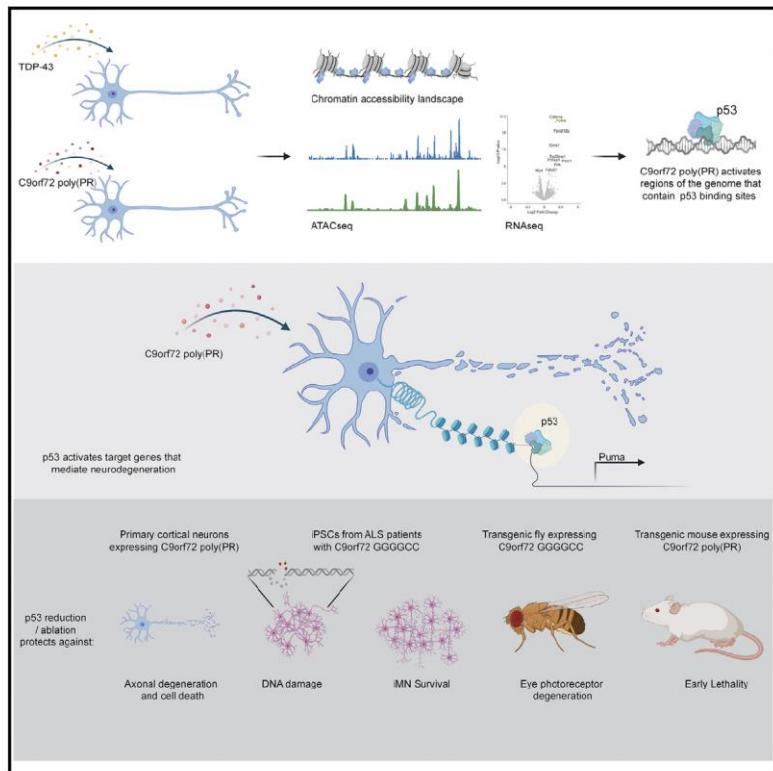
Accumulation in the central channel of the nuclear pore



Defect in nuclear transport of RNA and protein

p53 is a central regulator driving neurodegeneration caused by C9orf72 poly(PR)

Graphical Abstract



Authors

Maya Maor-Nof, Zohar Shipony,
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In Brief

C9orf72 mutations associated with ALS and FTD activate a specific transcriptional program that converges on p53 to drive neurodegeneration in multiple C9orf72 models.

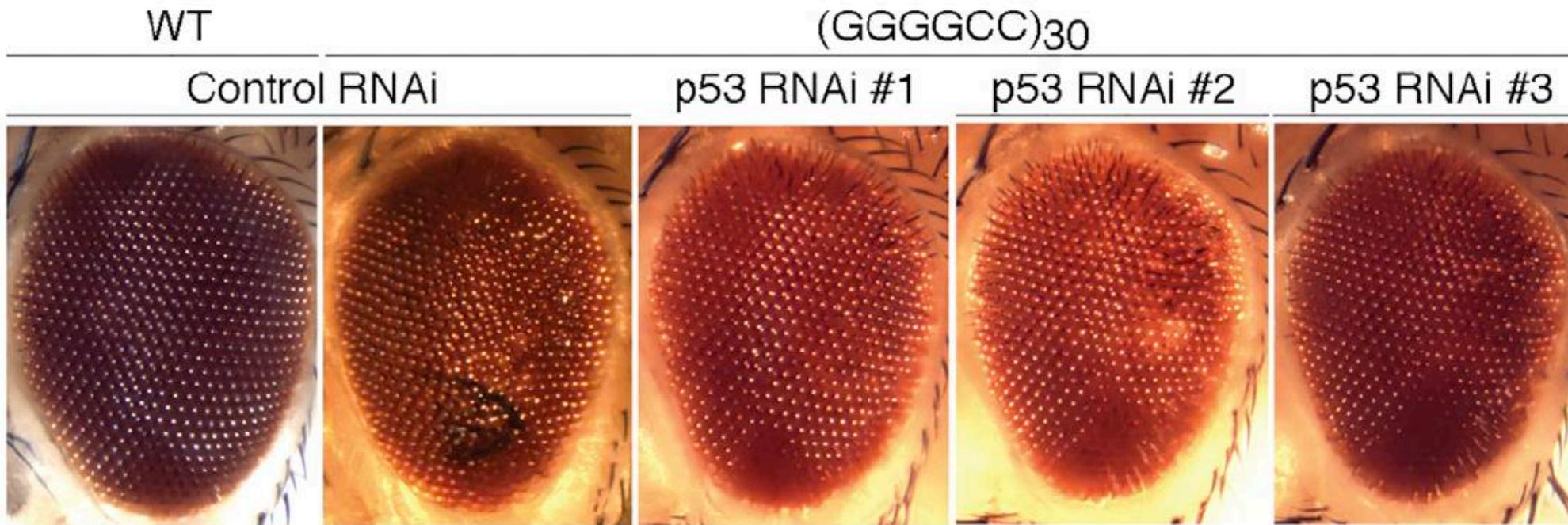
Highlights

- ATAC-seq reveals a chromatin accessibility signature activated by C9orf72 poly(PR)
- C9orf72 poly(PR) leads to p53 stabilization and transcription of p53 target genes
- p53 ablation protects against neurodegeneration in multiple C9orf72 models

Maor-Nof et al., 2021, Cell 184, 689–708
February 4, 2021 © 2020 Elsevier Inc.
<https://doi.org/10.1016/j.cell.2020.12.025>

p53 reduction modifies neurodegeneration in C9-ALS models

knocking down p53 improves the phenotype.



What about the RNA?

Massive, so they fold in on themselves.

Basic rational: stable, G4C2 repeat-containing RNA species that accumulate in patient tissues might sequester RNA-binding proteins and deplete their function

→ saves time + money.

Targeted modifier screen in Drosophila (focused on genes encoding proteins reported to physically interact with G4C2 repeat-containing RNAs in vitro)

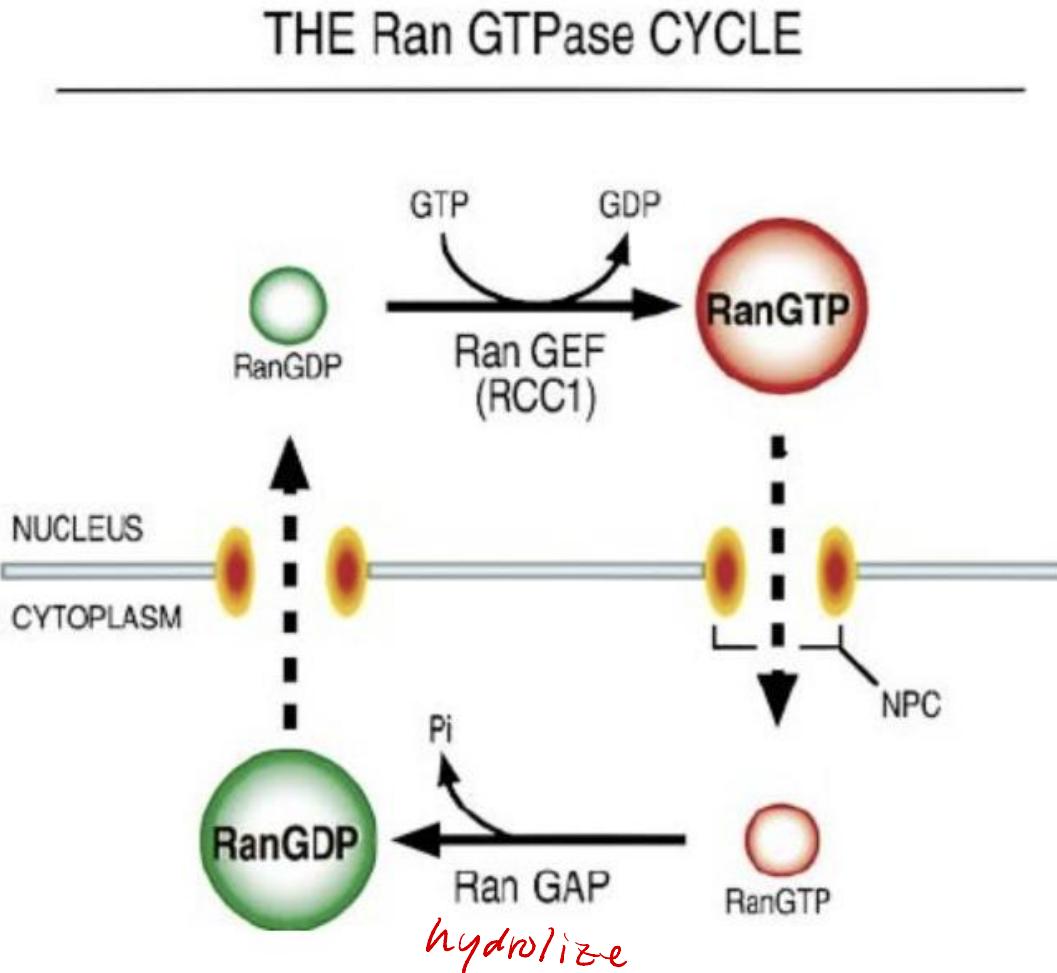
Identified a **dominant gain-of-function allele of RanGAP** that suppressed neurodegeneration in flies expressing expanded G4C2 repeats.

→ a protein that is also involved with nuclear transcript.

RanGAP, a major regulator of nuclear transport!

RanGAP is a major regulator of NCT

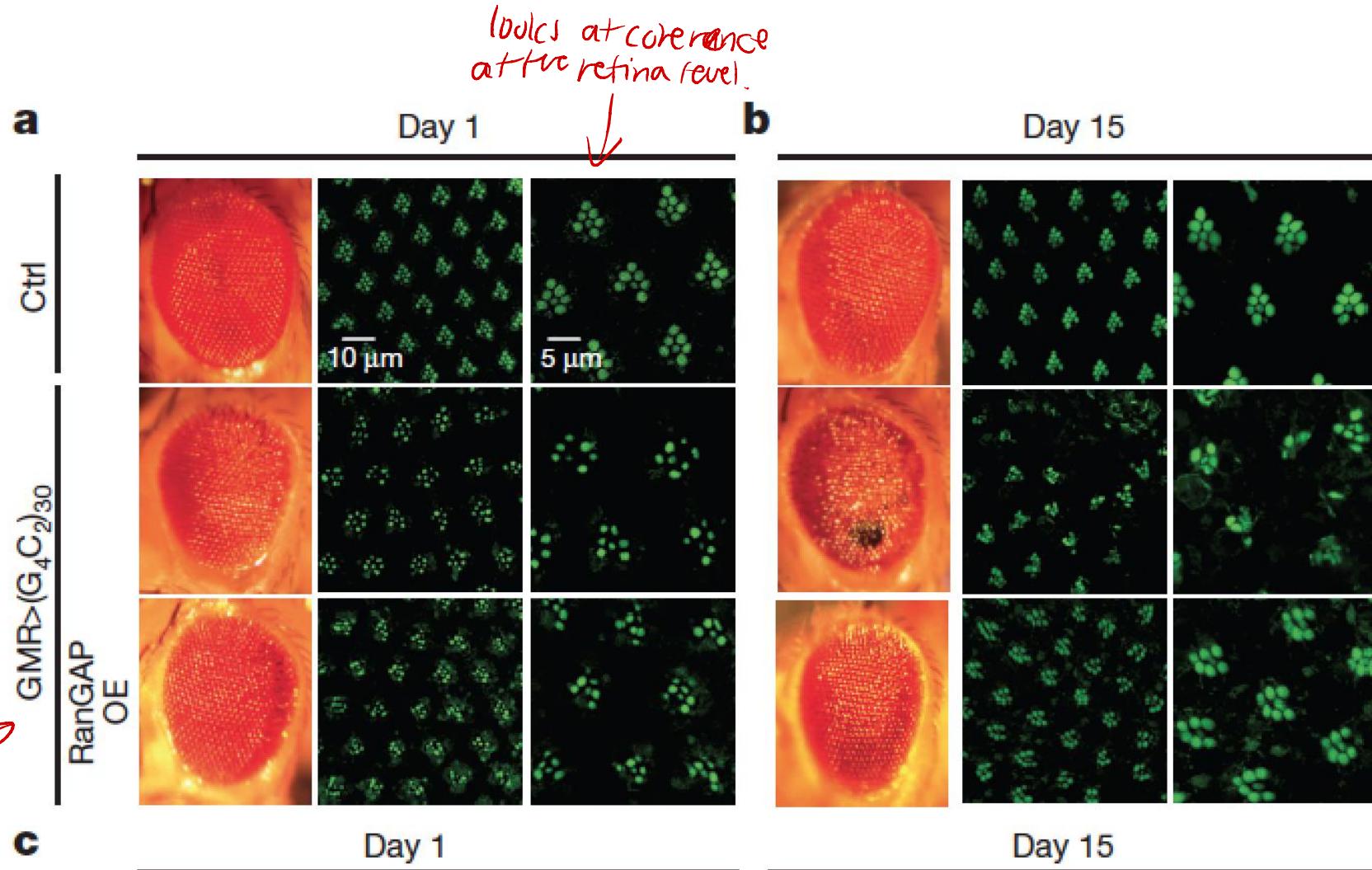
- Stimulates hydrolysis of RanGTPase in cytoplasm, favouring importin-mediated nuclear import of proteins
- RanGAP is **inhibited by the GGGGCC repeat**
- RanGAP inhibition disrupts the nuclear import of NLS containing proteins in fly & iPSN models of C9-ALS.



so if RanGAP doesn't work, we don't have the same level of nuclear transport + content.

coherence
gets worse over time
gives on.

by overexpressing
RanGAP, you can
restore some of normal
phenotype.

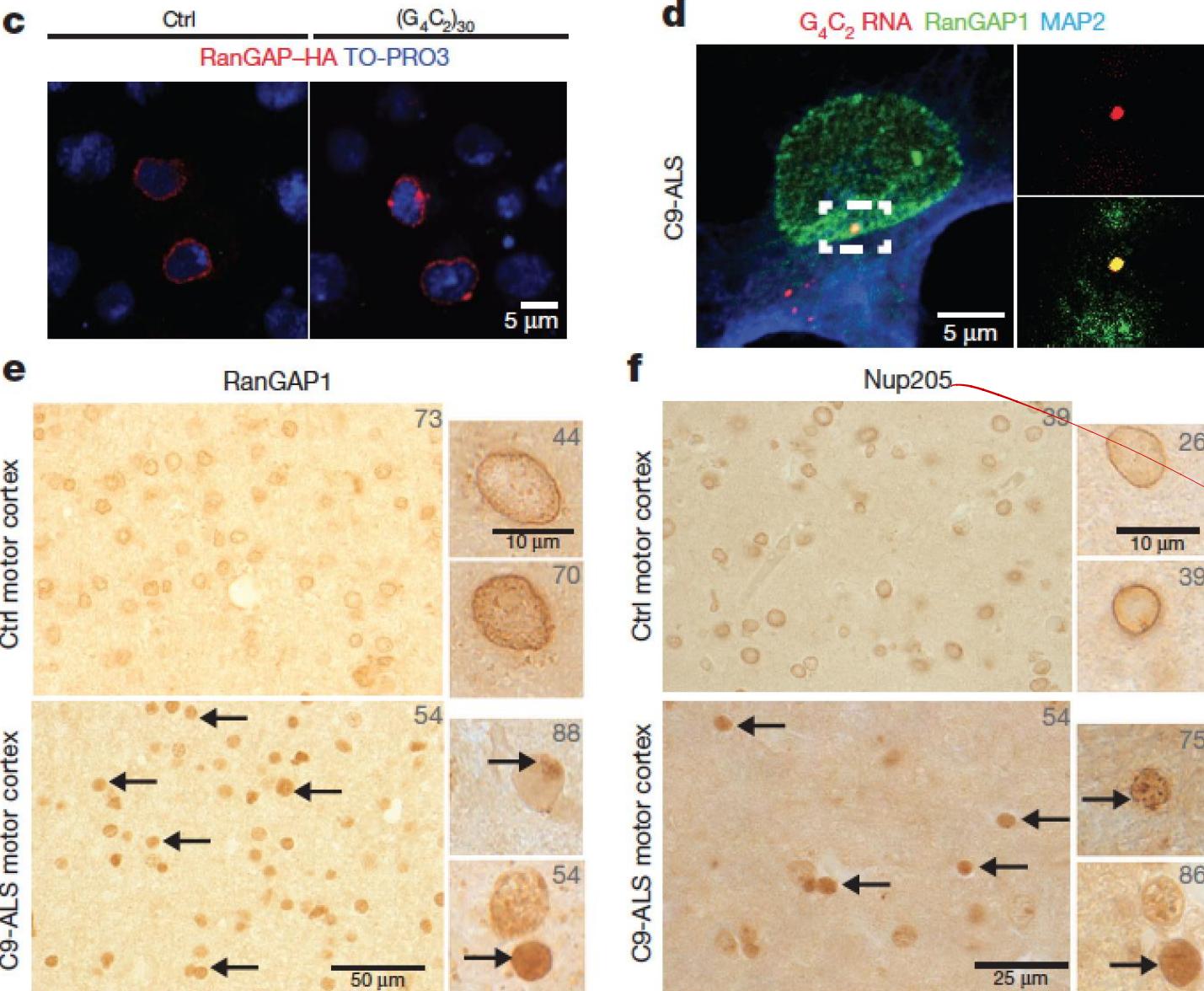


RanGAP, a major regulator of NCT

RanGAP1 and two NPC components, Nup205 and Nup107, abnormally localized to nuclear aggregates

RanGAP (red)
surrounding the
nucleus (blue)

Zeroing
Down



Zhang et al. Nature 525, 56–61 (2015)

clumps of RANGAP,
and overlaps of clumps of
G₄C₂RNA and RanGAP.
Patient derived iPSC-derived neurons

RNA is likely
sequestering the RanGAP
away from where it
should be located

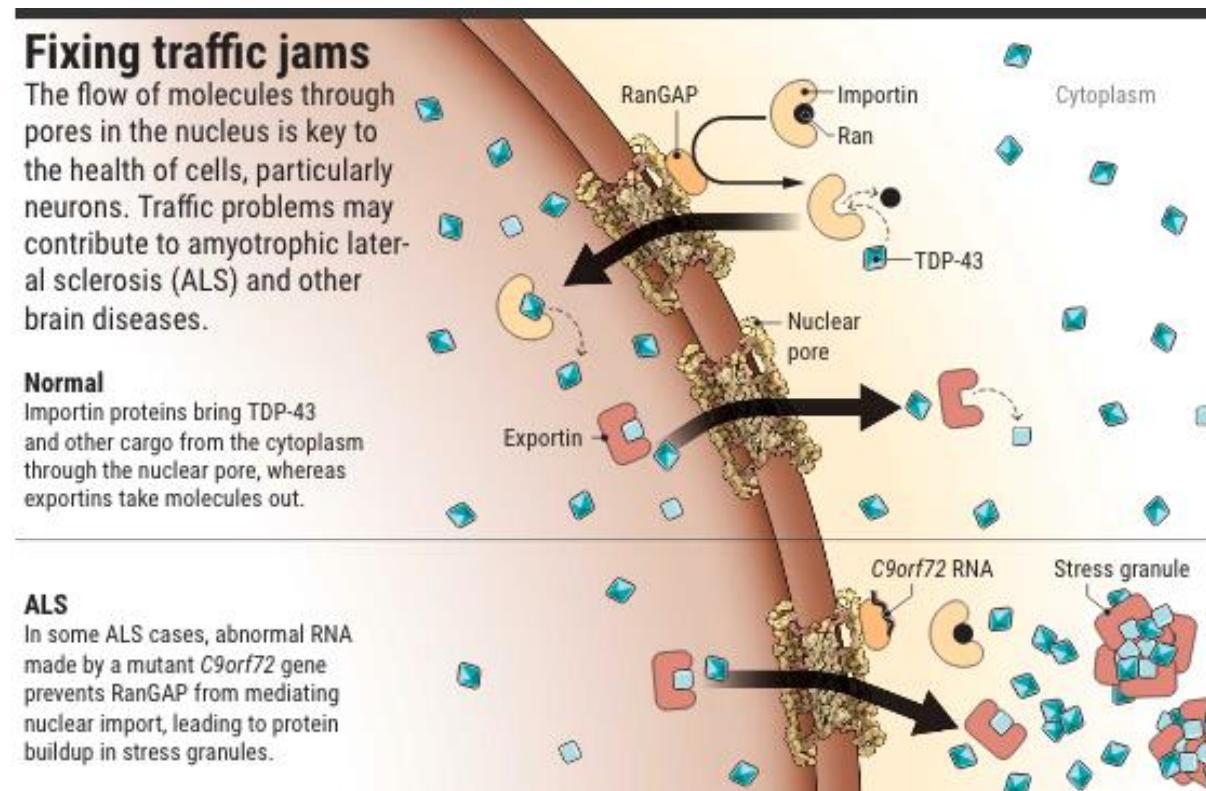
also happening
to other nuclear
protein, so RNA might not

Cortical tissue from
C9ORF72 ALS patients

be specific to RanGAP
but rather ??

Dysfunctional Trafficking Across the Nuclear Divide

Identified a transport protein, RanGAP, as key to neurodegeneration

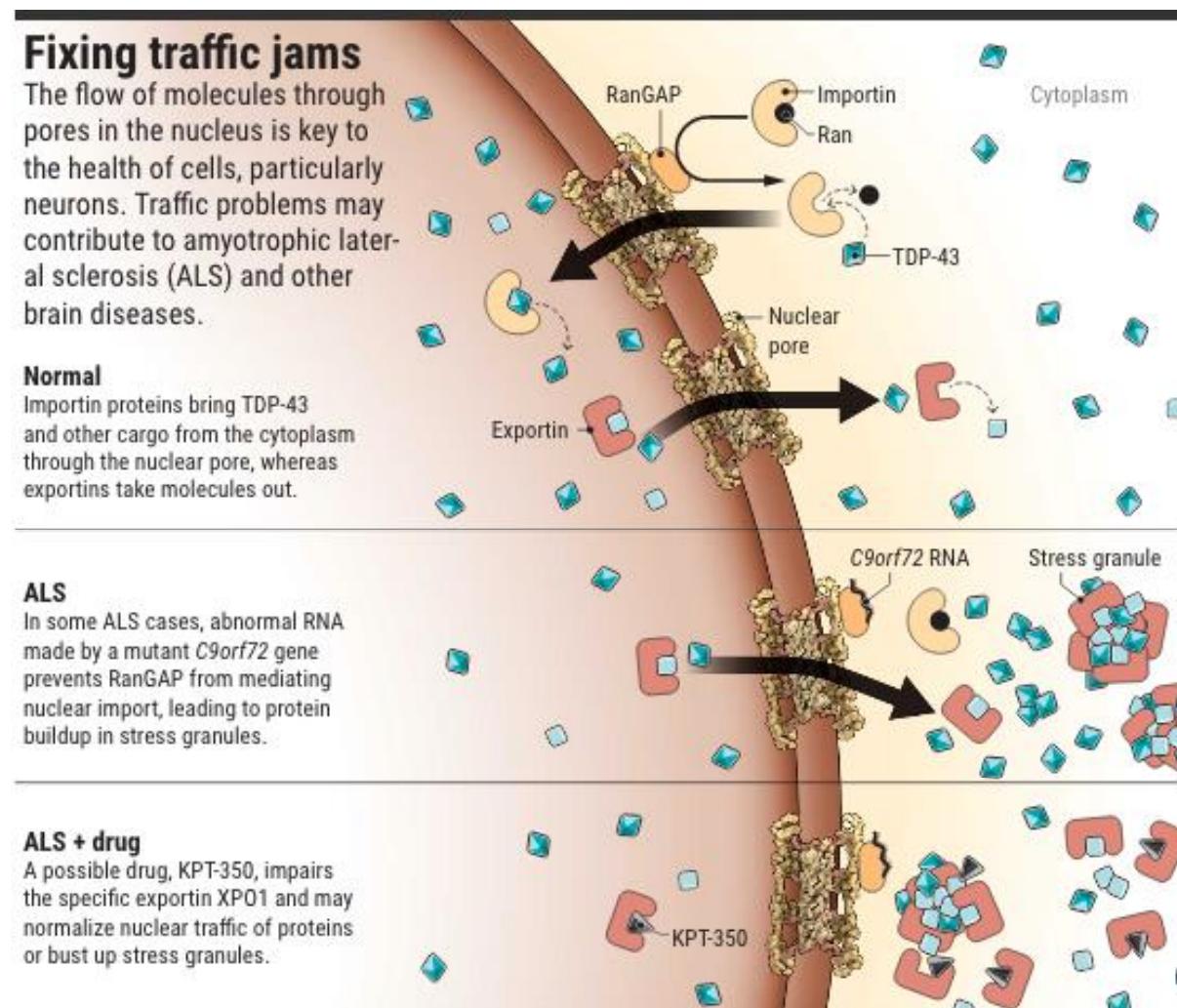


Devising strategies to restore NCT function

Basic rational: Since impairment of RanGAP favors cytoplasmic localization of nuclear proteins, **compounds that block nuclear export** may rescue deficits caused by disruption of nuclear import

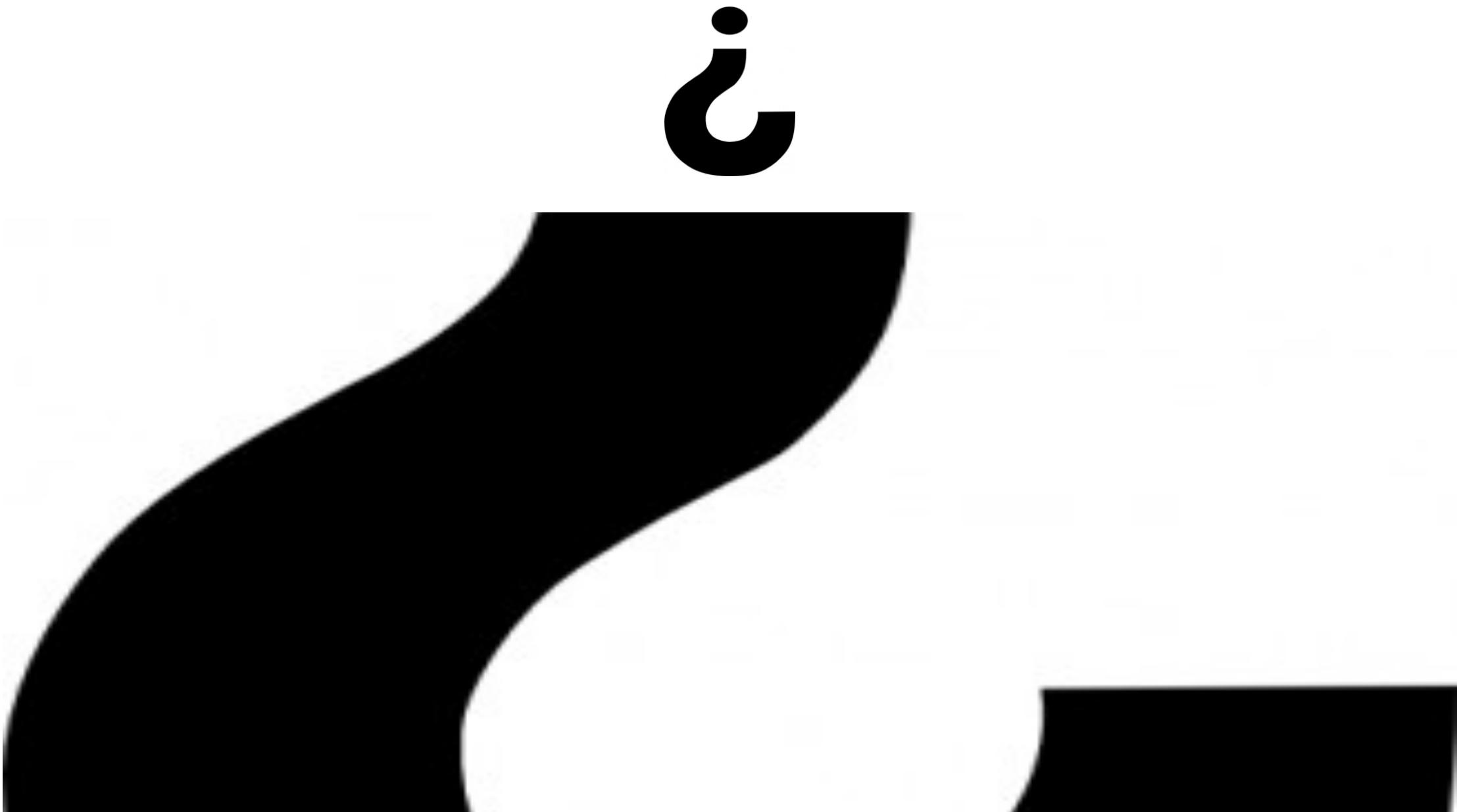
Selective inhibitors of nuclear export (**SINE compounds**), specifically inhibit Exportin-1 (XPO1), a protein that exports (nuclear export signal (NES))-containing proteins from the nucleus into the cytoplasm.

Dysfunctional Trafficking Across the Nuclear Divide



Karyopharm Therapeutics

Selective Inhibitors of Nuclear Export (SINE compounds)



Wait a second...

The exact mechanism of action of KPT-350 is still unclear

Stress granules in ALS or FTD persist and turn sticky

Components of the nuclear transport machinery - including importers, exporters, and parts of the nuclear pore itself - also get tangled up in those aggregates

XPO1 may play a role in stress granules

KPT-350 may serve primarily as a stress granule buster!

Stress granules – a promising therapeutic target

Accumulation of nuclear transport proteins within stress granules (SGs) in response to cellular stress may be a common pathogenic mechanism in multiple neurodegenerative diseases including C9-ALS



so this finding is not just applicable to ALS!!

SGs are a promising therapeutic target upstream of NCT disruption

Other approaches to break up stress granules are being developed as an indirect way of restoring healthy nuclear transport

NCT Defects → Broad Implications For Neurodegenerative Diseases

ALS and Huntington's are quite connected.

Neuron
Article



Mutant Huntingtin Disrupts the Nuclear Pore Complex

Jonathan C. Grima,^{1,2,3} J. Gavin Daigle,^{2,3} Nicolas Arbez,^{1,5} Kathleen C. Cunningham,^{3,4} Ke Zhang,^{2,3} Joseph Ochaba,⁷ Charlene Geater,⁷ Eva Morozko,⁷ Jennifer Stocksdale,⁷ Jenna C. Glatzer,^{2,3} Jacqueline T. Pham,^{2,4} Ishrat Ahmed,¹ Qi Peng,¹ Harsh Wadhwa,³ Olga Pletnikova,⁶ Juan C. Troncoso,^{3,6} Wenzhen Duan,^{1,4,5} Solomon H. Snyder,^{1,5} Laura P.W. Ranum,⁸ Leslie M. Thompson,⁷ Thomas E. Lloyd,^{1,2,3,4} Christopher A. Ross,^{1,5} and Jeffrey D. Rothstein^{1,2,3,4,9,*}

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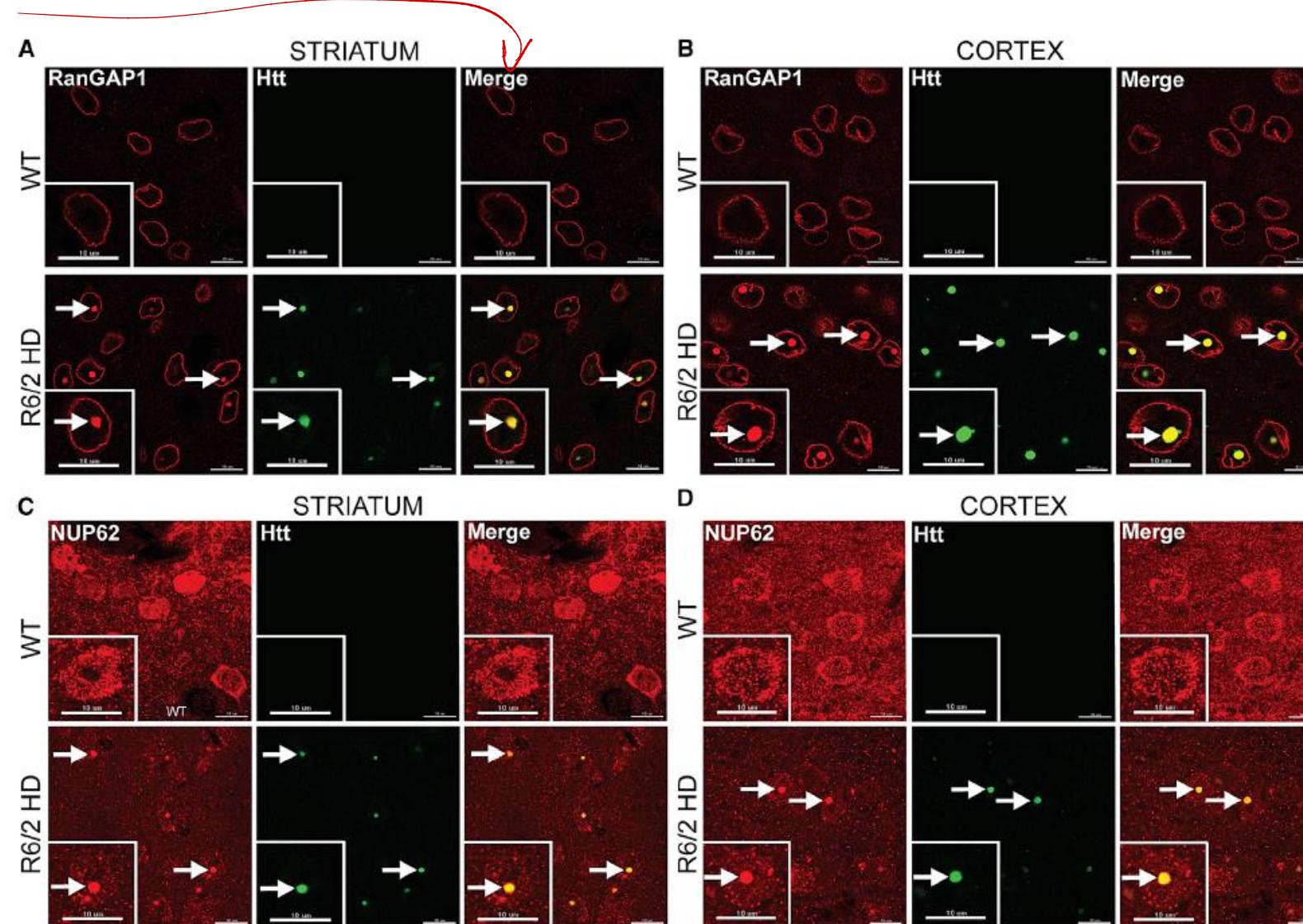
⁹Lead Contact

*Correspondence: jrothstein@jhmi.edu

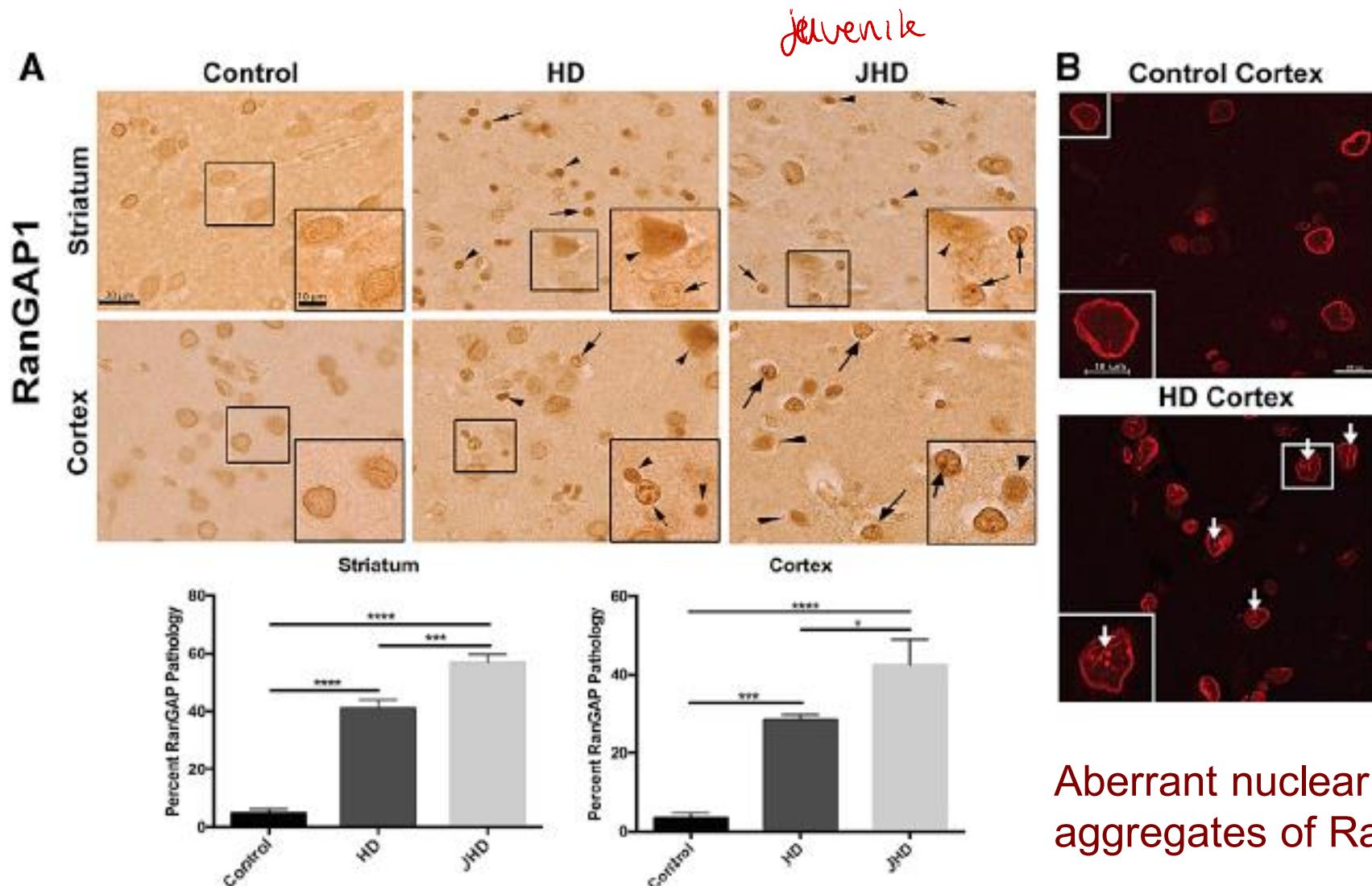
<http://dx.doi.org/10.1016/j.neuron.2017.03.023>

NUPs Aggregate and Co-localize with mHtt in HD Mouse Model

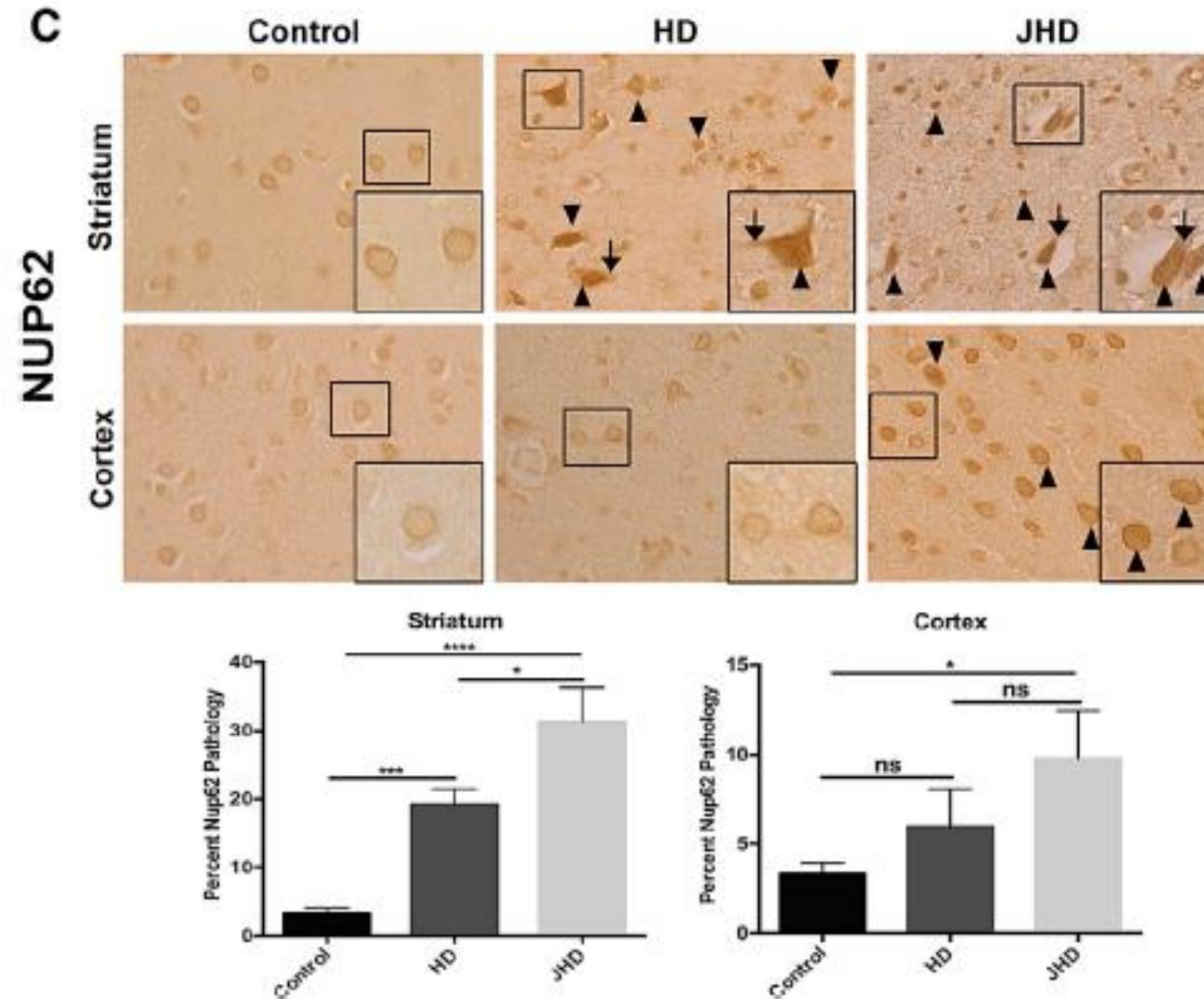
We see overlap
of aggregates in
RanGAP1 and
huntington



NUP Pathology in Human HD & JHD Brain Tissue



NUP Pathology in Human HD & JHD Brain Tissue

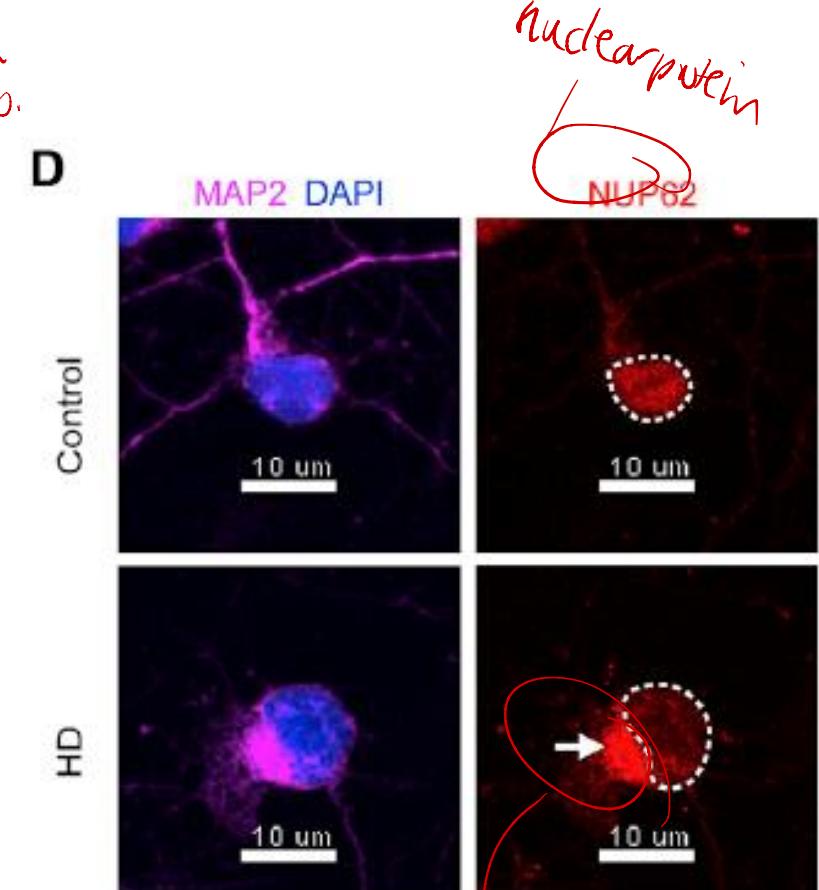
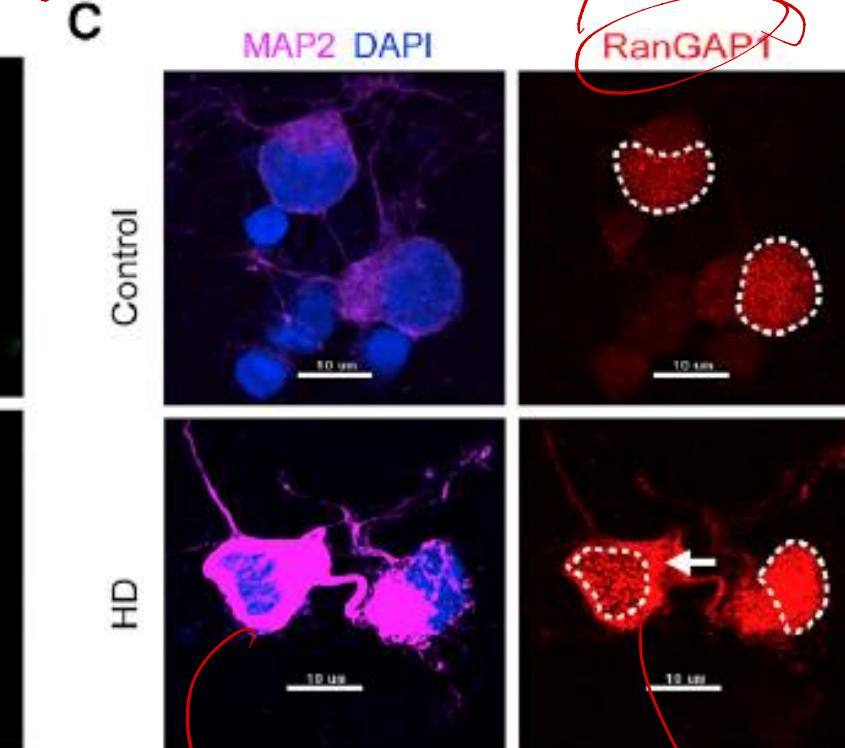
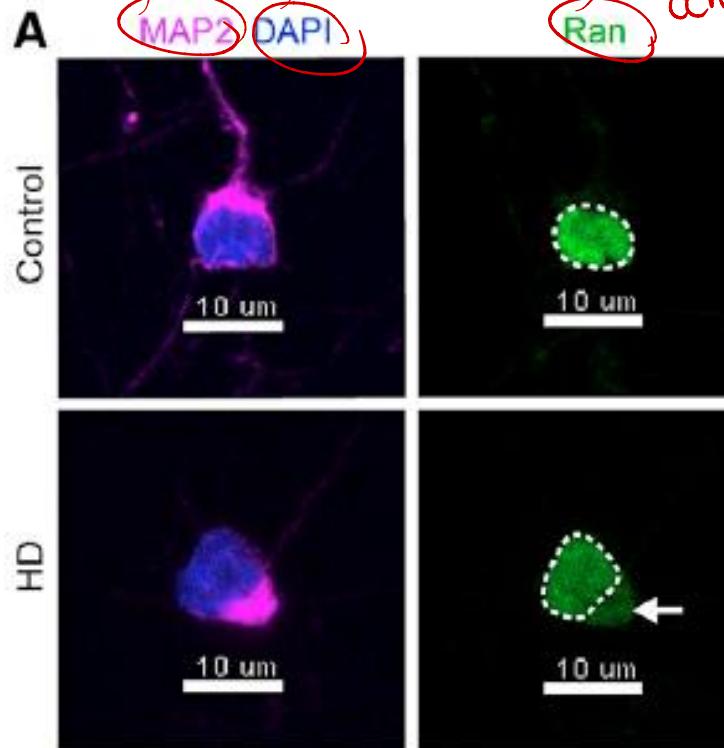


Nucleocytoplasmic Transport Defects in Human HD iPSNs

cytoskeleton/cytoplasm

nucleus.

should typically
be localized
center of nucle

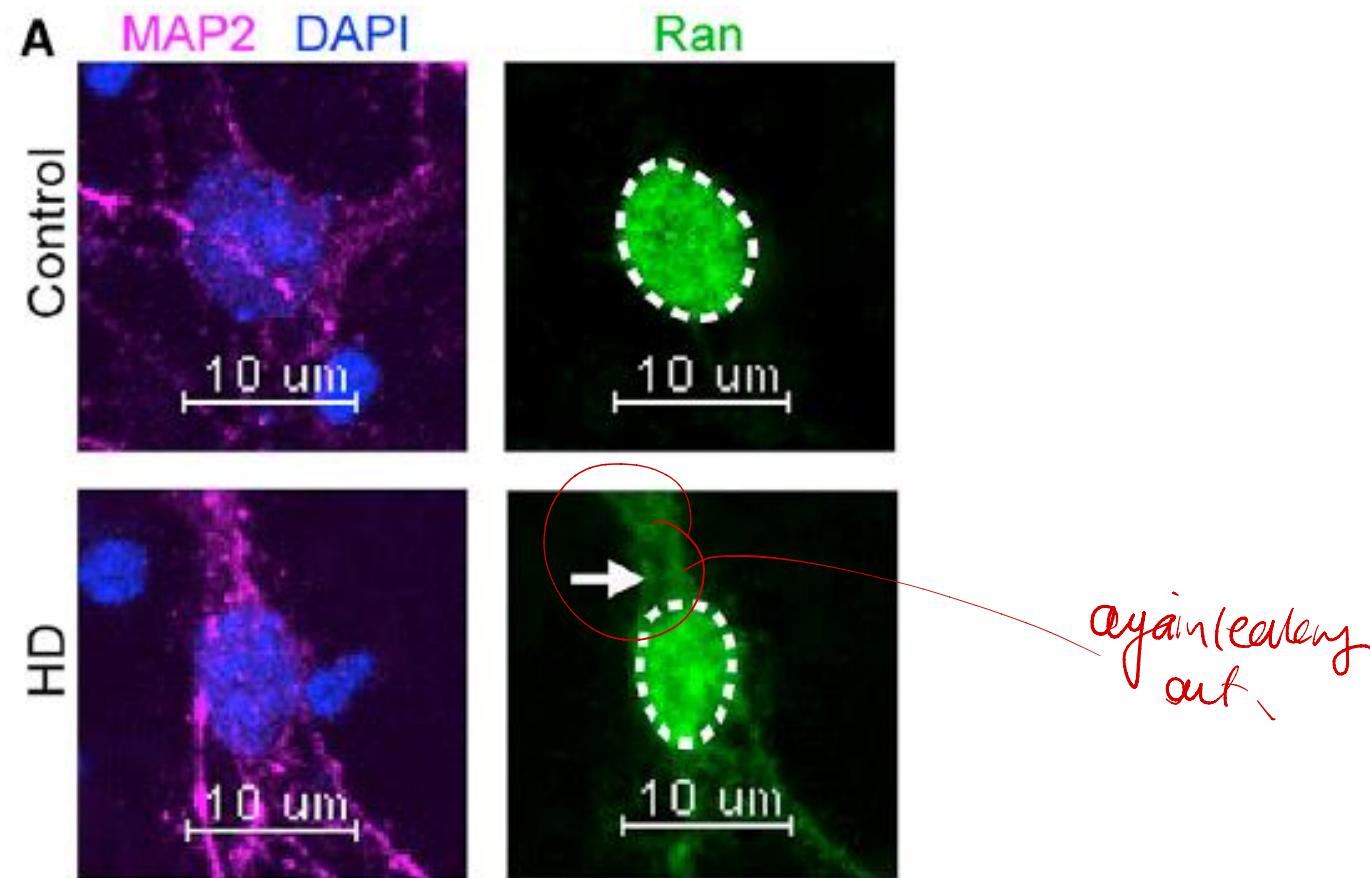


seems to be
leaking (nuclear membrane is damaged)

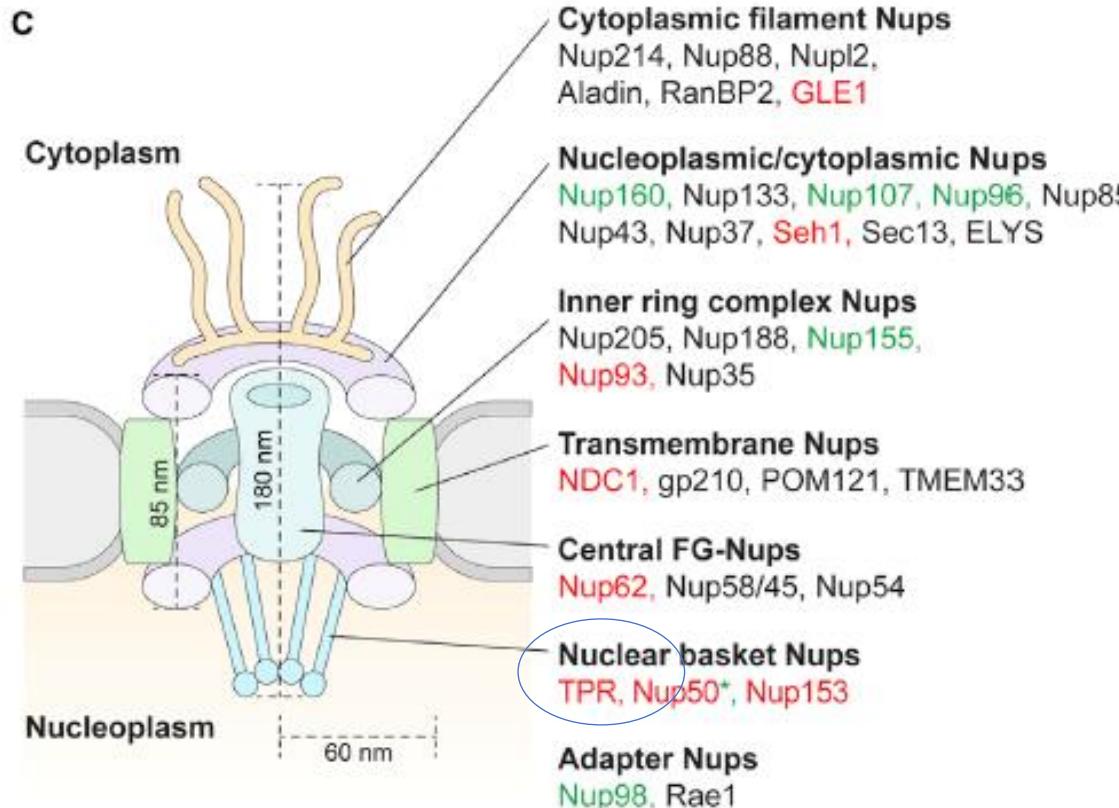
RanGAP1 now localized outside
of the nucleus.

localizes primarily
to the cytoplasm.

Nucleocytoplasmic Transport Defects in Primary Neurons Transfected with Full-Length mHTT



The N-terminal Htt Fragment Directly Interacts with TPR



Htt-TPR interaction is inhibited by the presence of
a polyglutamine expanded repeat and Htt
aggregation



Decreasing Htt export to the cytoplasm and
increasing nuclear accumulation of mutant Htt

Similar results reported
in spinocerebellar ataxia type 7.

NUCLEOCYTOPLASMIC TRANSPORT BROAD IMPLICATIONS IN NEURODEGENERATIVE DISEASES

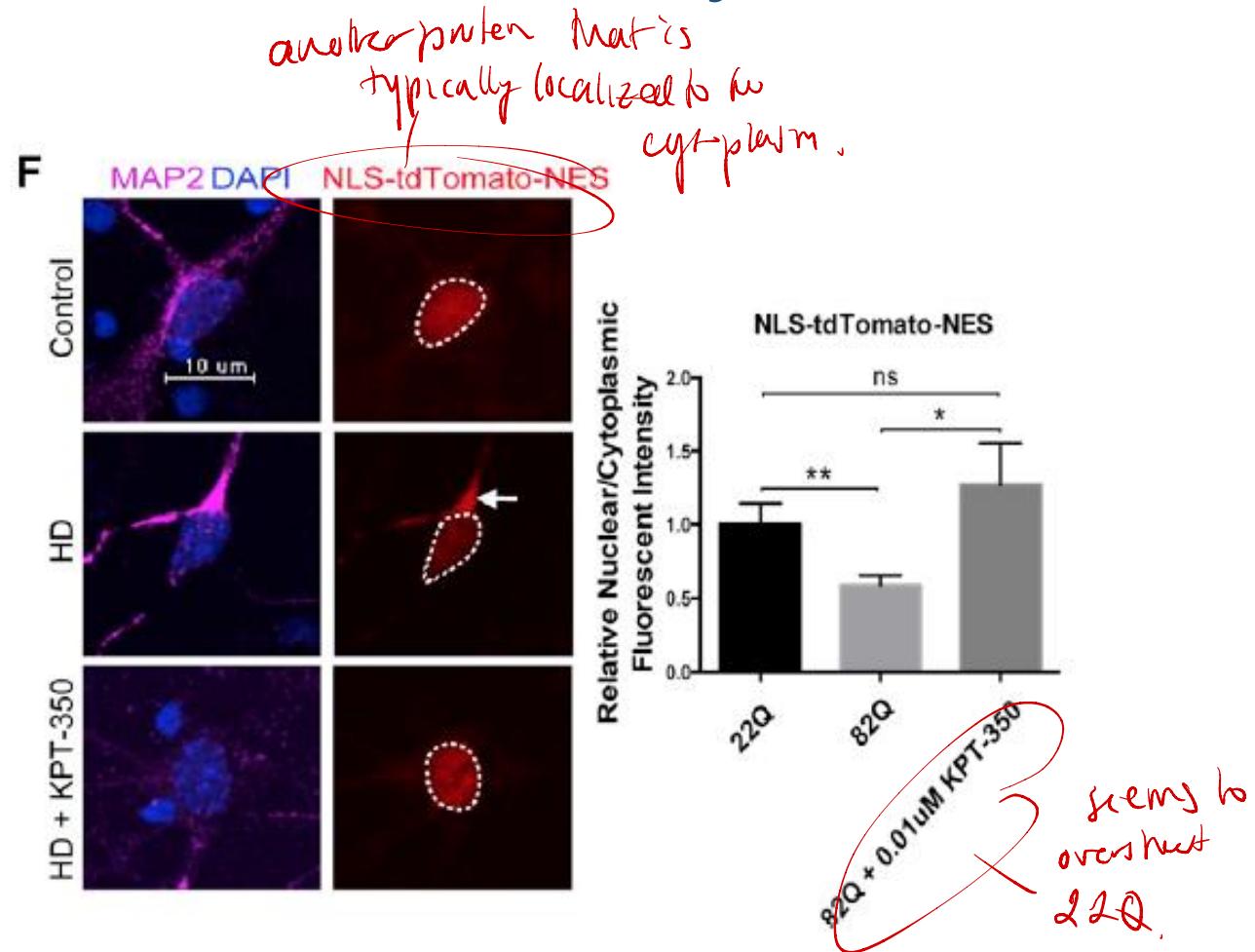
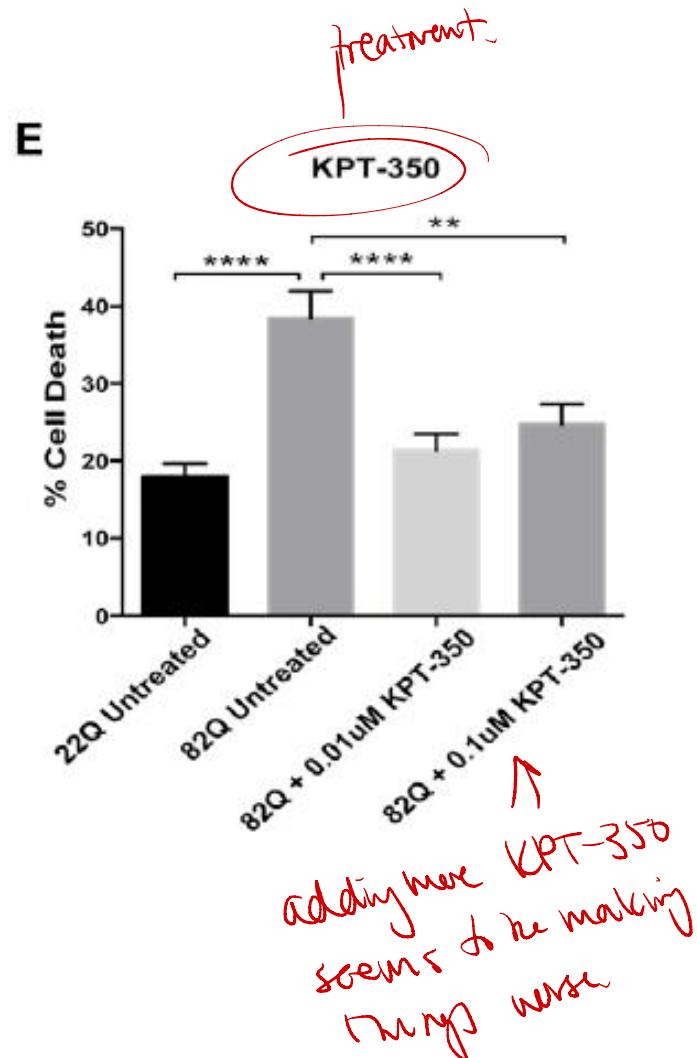
- Mouse
- Fly
- iPSC-derived neurons (human patient-derived)
- Primary neurons transfected with full-length mHTT
- Post-mortem human tissues

All revealed highly abnormal NPC pathology in cells expressing mutant Htt/or HD-RAN translation products

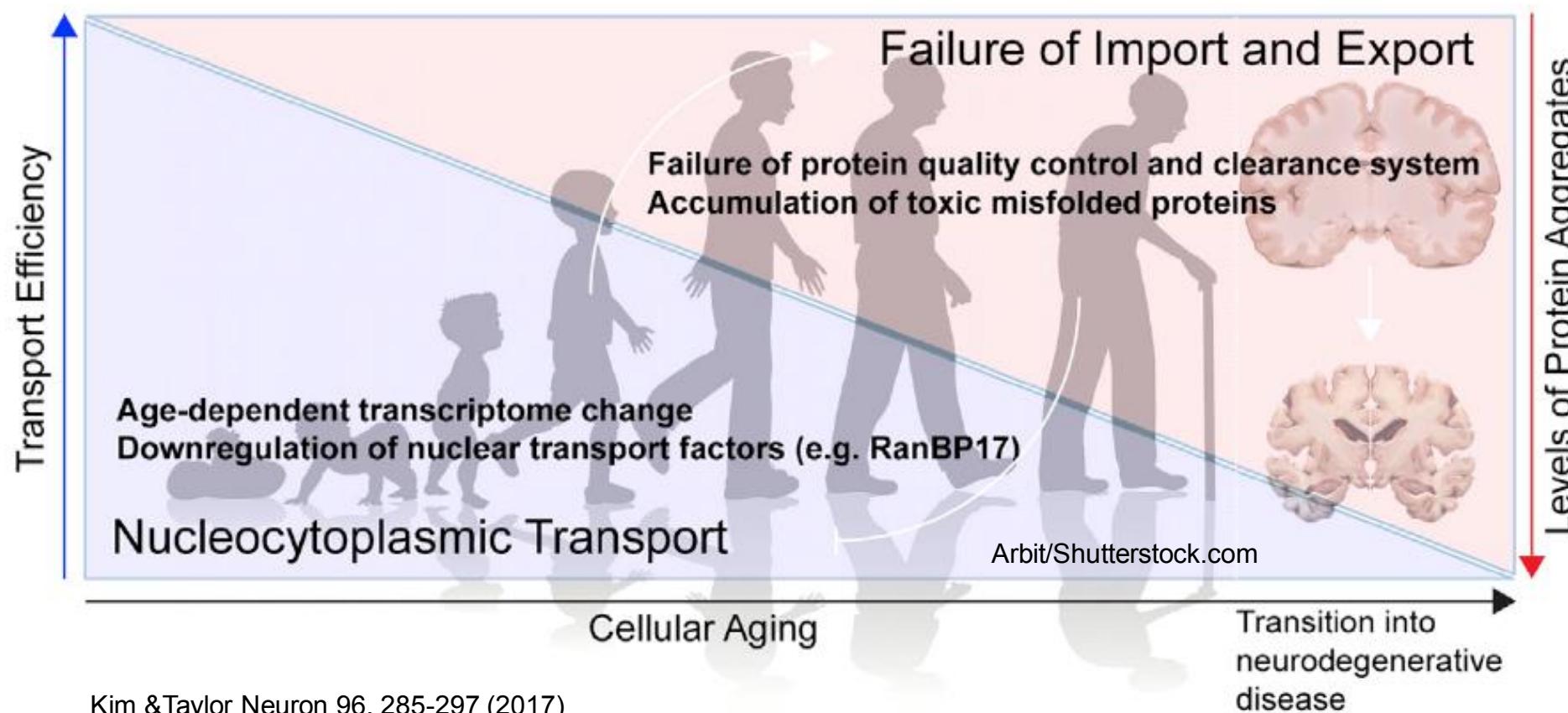


Defects in mRNA export

Pharmacological Rescue of Nucleocytoplasmic Transport Defects and Neurotoxicity in HD



Cerebral Aging and Nucleocytoplasmic Transport



LOST IN TRANSPORTATION: NUCLEOCYTOPLASMIC TRANSPORT DEFECTS IN NEURODEGENERATIVE DISEASES

- Frontotemporal Dementia (FTD)
- Alzheimer
- Huntington
- Spinocerebellar Ataxia
- Age-related neurodegeneration

KPT-350- Neuroprotective effects across a range of human cell, fly, and rodent models of ALS, Huntington, MS & other brain diseases!

Does it get better?

- Most cases of ALS are of unknown origin (sporadic ALS)
- The nuclear pore complex is known to be involved in familial ALS
- Can it also play a role in sporadic ALS?

BIOMEDICINE

Repairing the nuclear pore in ALS

Sci. Transl. Med. **13**, eabe1923 (2021).

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

AMYOTROPHIC LATERAL SCLEROSIS

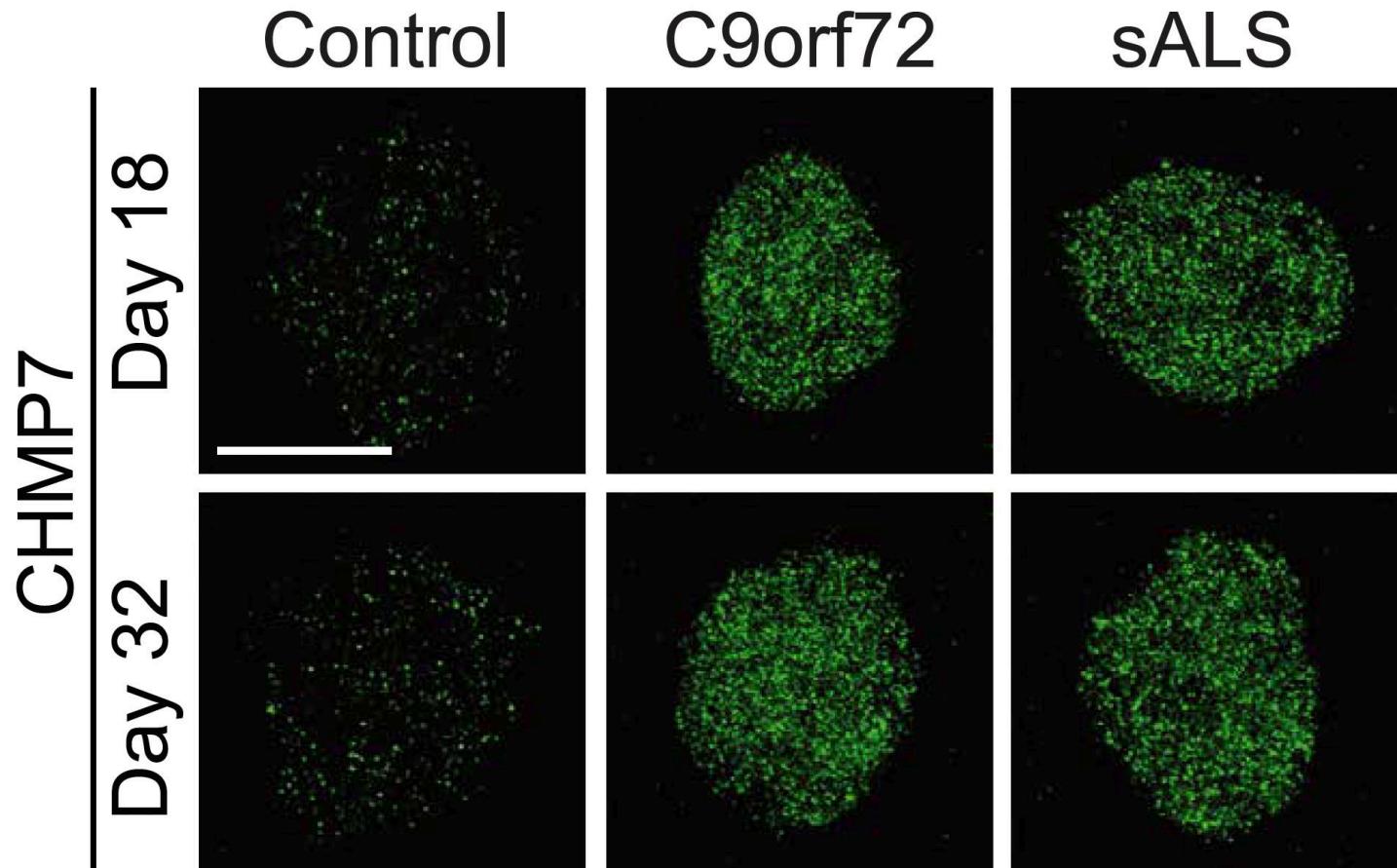
Nuclear accumulation of CHMP7 initiates nuclear pore complex injury and subsequent TDP-43 dysfunction in sporadic and familial ALS

Alyssa N. Coyne^{1,2*}, Victoria Baskerville¹, Benjamin L. Zaepfel³, Dennis W. Dickson⁴, Frank Rigo⁵, Frank Bennett⁵, C. Patrick Lusk⁶, Jeffrey D. Rothstein^{1,2*}

Hypothesized that Nup alterations and the consequential loss of NPC function may lie upstream of TDP-43 dysfunction and mislocalization widely observed in ALS, FTD, and related neurodegenerative diseases.

CHMP7 - a critical mediator of NPC quality control

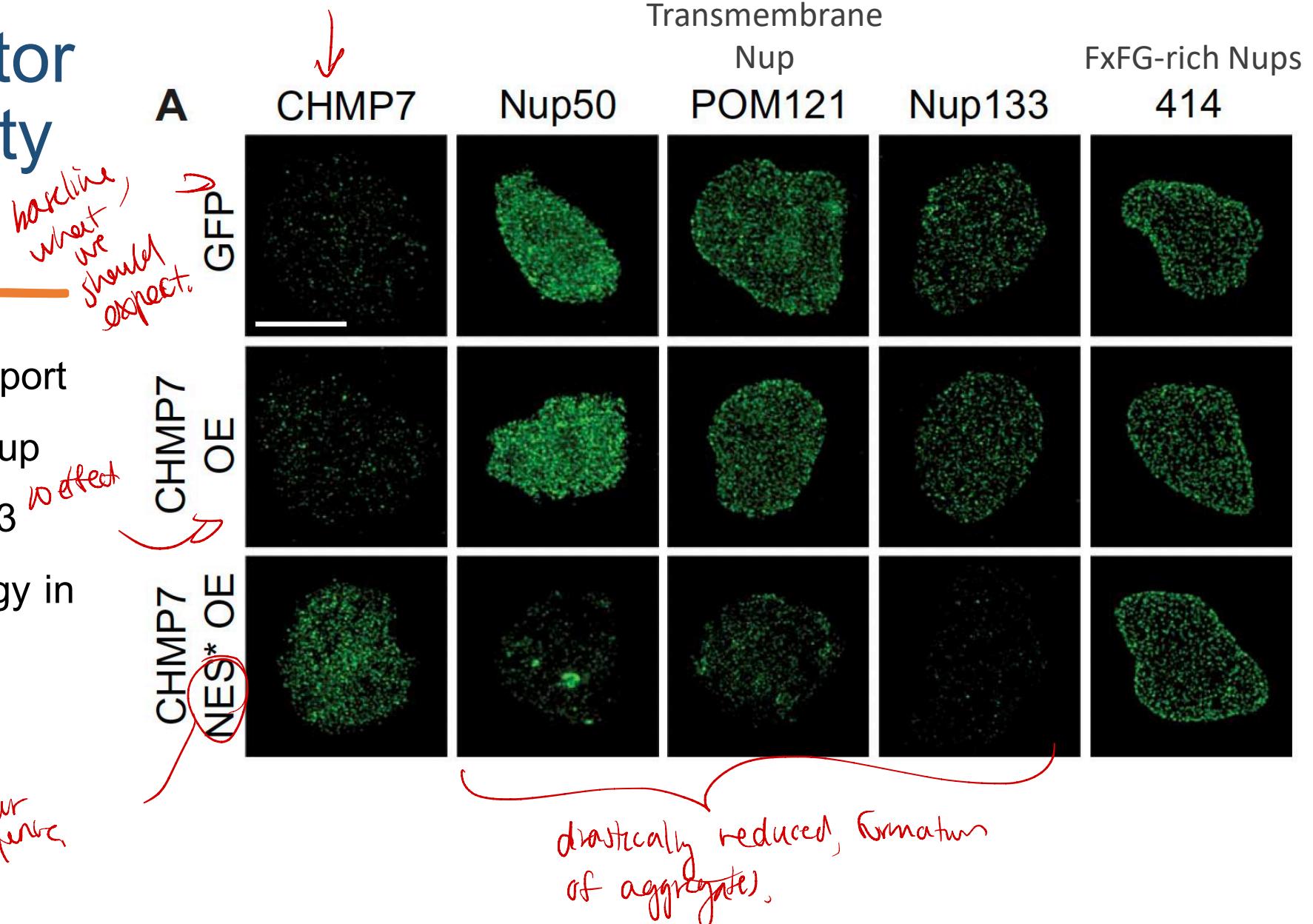
CHMP7- increased in nuclei of C9orf72 and sporadic ALS iPSC - derived spinal neurons (iPSNs) and postmortem human motor cortex before the emergence of Nup alterations!



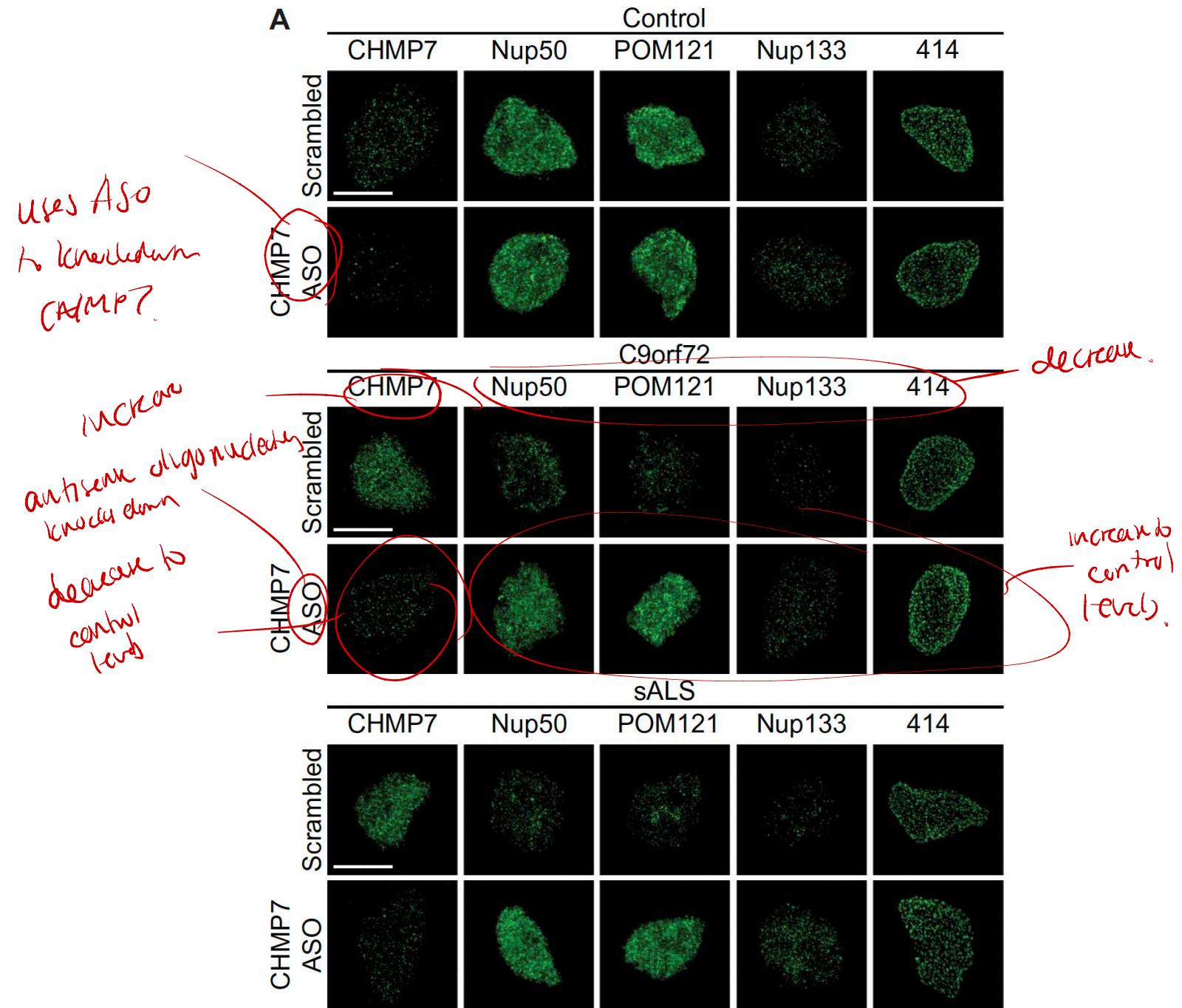
CHMP7 - a critical mediator of NPC quality control

Inhibiting the nuclear export of CHMP7 triggered Nup reduction and TDP-43 dysfunction and pathology in human neurons.

Reduced # of CHMP7 mat caught out of nucleus.
Without nuclear export specific



ASO-mediated knockdown of CHMP7 restores the nuclear expression of specific Nups



Bottom line:

Supports a role for altered CHMP7-mediated Nup homeostasis as a prominent initiating pathological mechanism for familial and sporadic ALS

→ highlights the potential for CHMP7 as therapeutic target!

