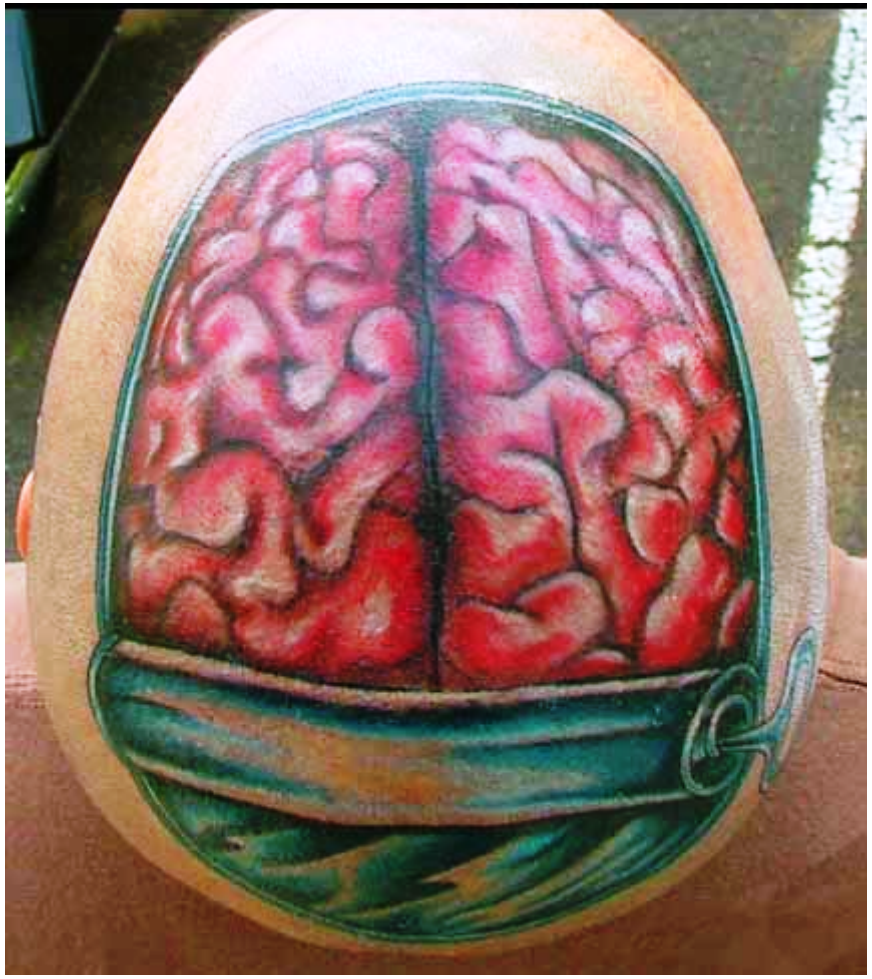
Lab Meeting: 9/17/2010

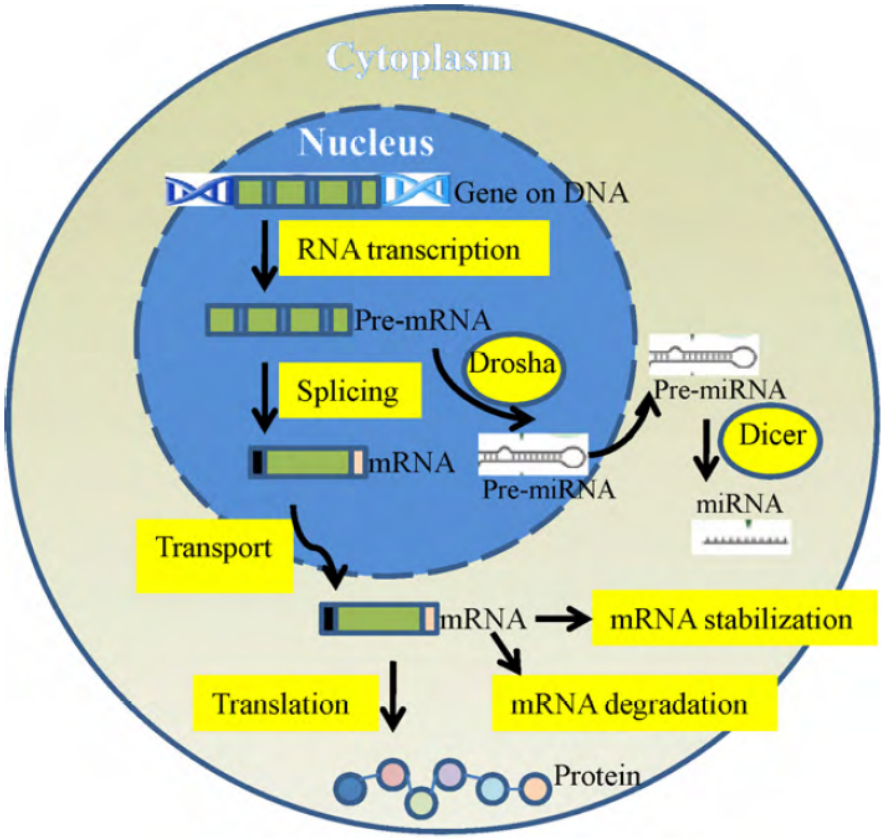


Background

- ALS affects nerves of the CNS controlling muscle movement.
- Prevalence 1:100,000
  - 10% genetic:
    - SOD1 accounts for 2% of cases

Background

- TDP-43
  - Critical for RNA processing
  - Important for development
  - Highly conserved AA between species
  - Major component of neuronal inclusions of neurodegenerative disorders



Warraich et al, 2010

TDP-43 and ALS

- 2006: TDP-43 found to be a major component of pathological ubiquinatin-positive inclusions in both ALS and Frontal Temporal Lobar Degeneration (FTLD)

Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS

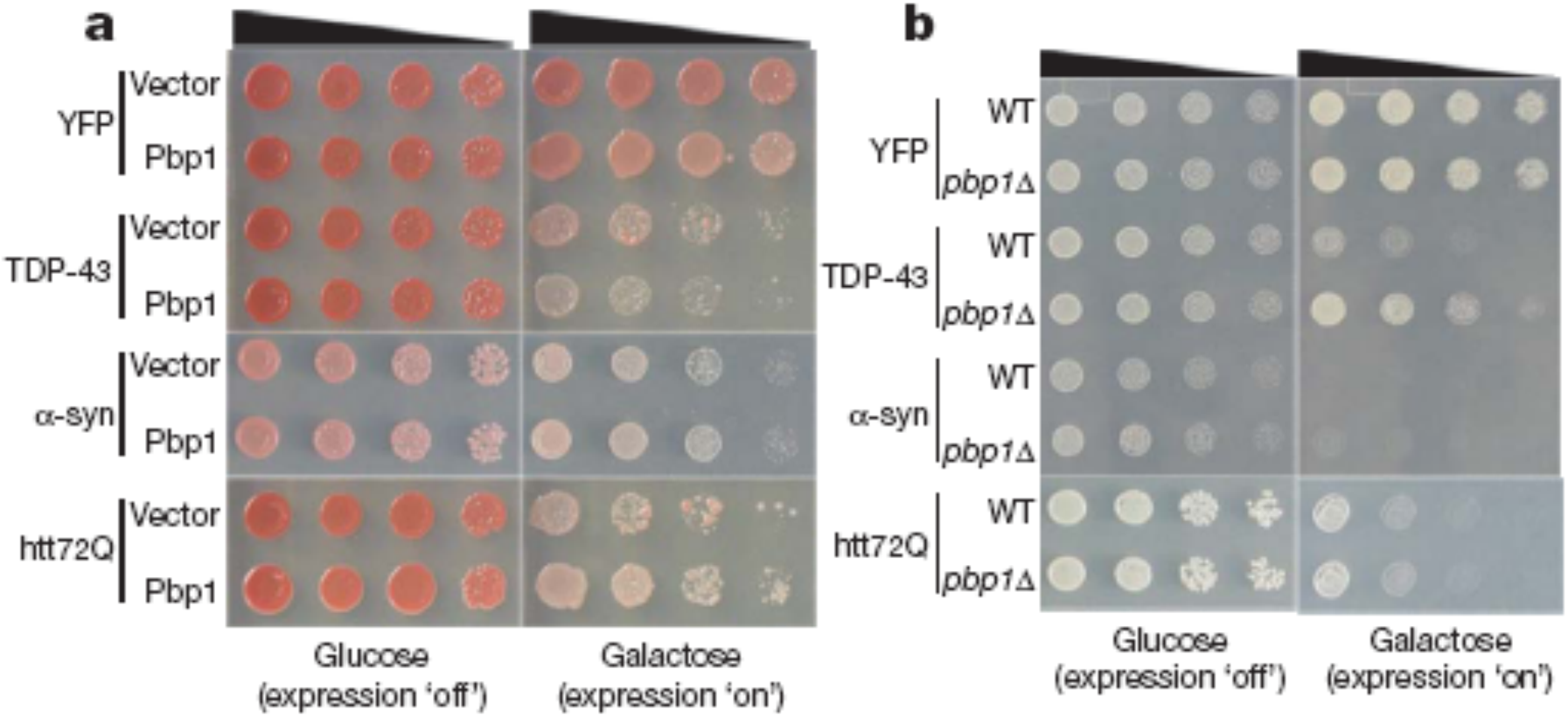
Modifiers of TDP-43

- Yeast screen:
  - FLEXGene plasmid (5,500 genes) transformed into yeast expressing TDP-43
    - +/- regulation
      - 13 genes suppressed TDP-43 toxicity
      - 27 genes enhanced TDP-43 toxicity
    - PBP1 (Pab1-binding protein) enhanced TDP-43 toxicity
      - PBP1 & Pab1 regulate mRNA polyadenylation

Figure 1

Screening for modifiers of TDP-43 in yeast
Yeast Spotting Assay: Expression of TDP-43 with up regulation of Pbp1 enhances toxicity

Screening for modifiers of TDP-43 in yeast
Yeast Spotting Assay: Expression of TDP-43 with down regulation of Pbp1 suppresses toxicity



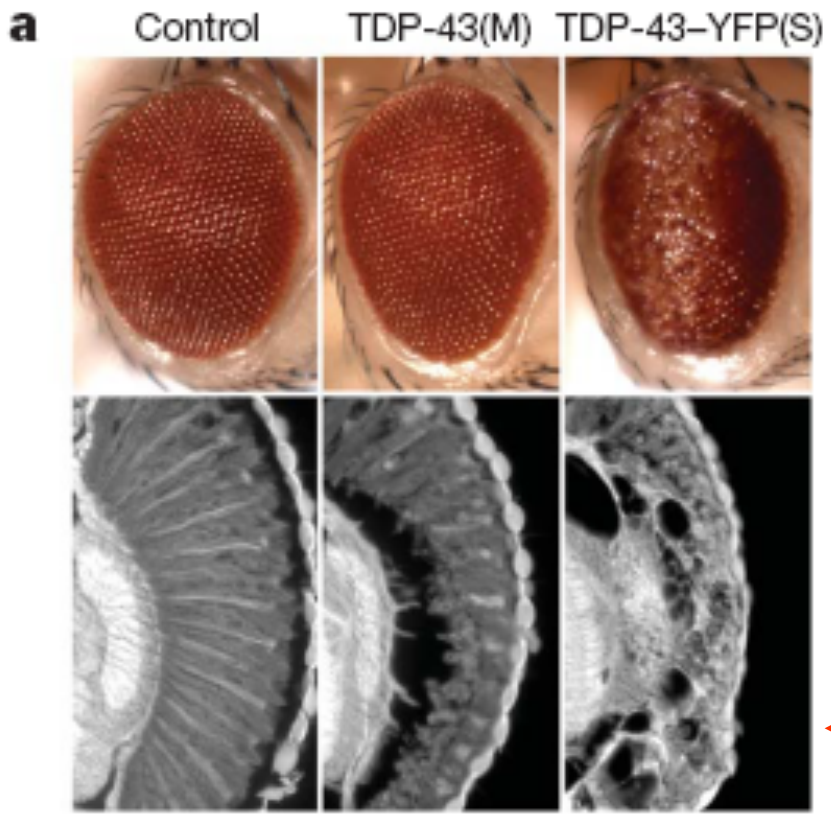
TDP-43 toxicity

- Drosophila model
  - TDP-43 directed:
    - Eye
    - Muscle
    - Nervous system

    - Endogenous Atx2 up regulation and interaction

Figure 2a: TDP-43 toxicity *Drosophila* eye

- hTDP-43 (Control)
- ALS Mutant TDP-43 (Q331K) +/-
  - Moderate expression (TDP-43(M))
  - Strong expression (TDP-43-YFP(S))



What age?

Supplemental 2a

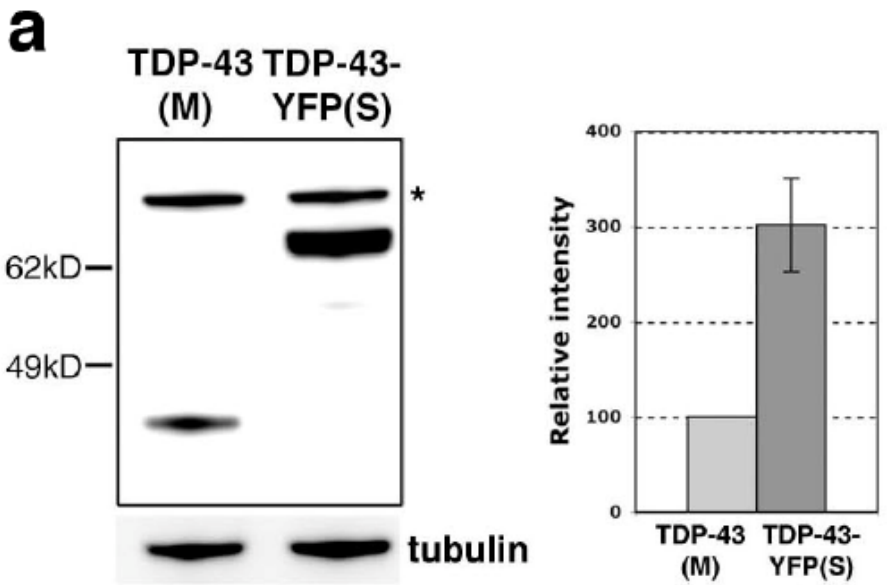
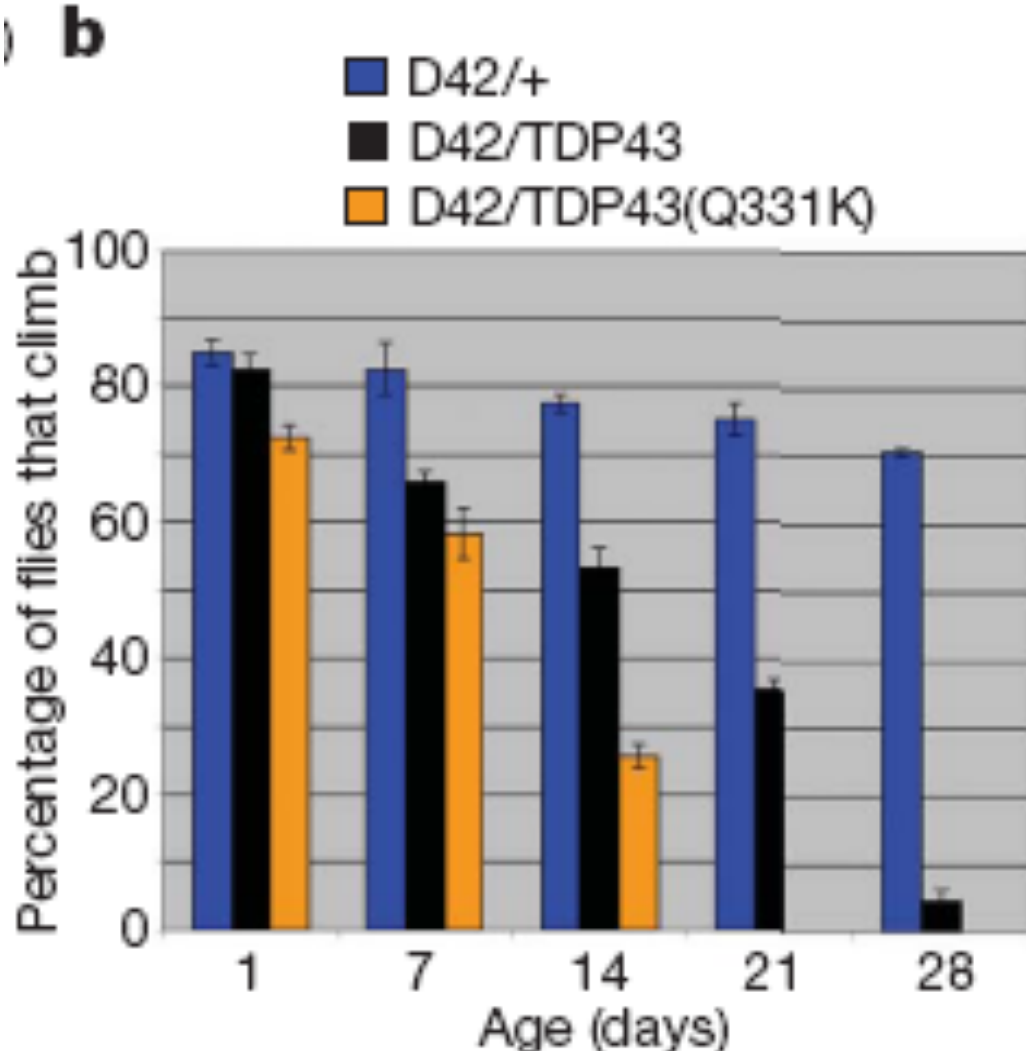


Figure 2b: TDP-43 results in loss of motility *Drosophila* musculature system



Supplemental 2b

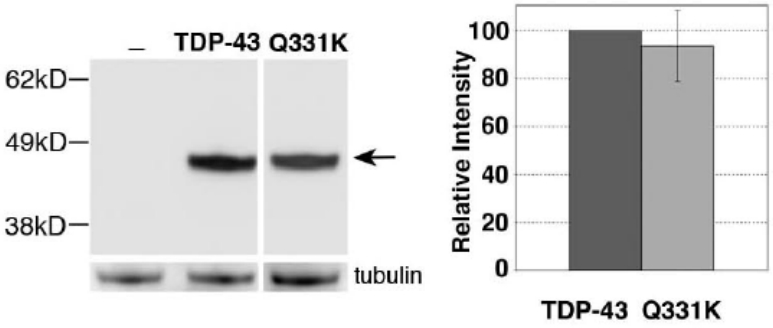
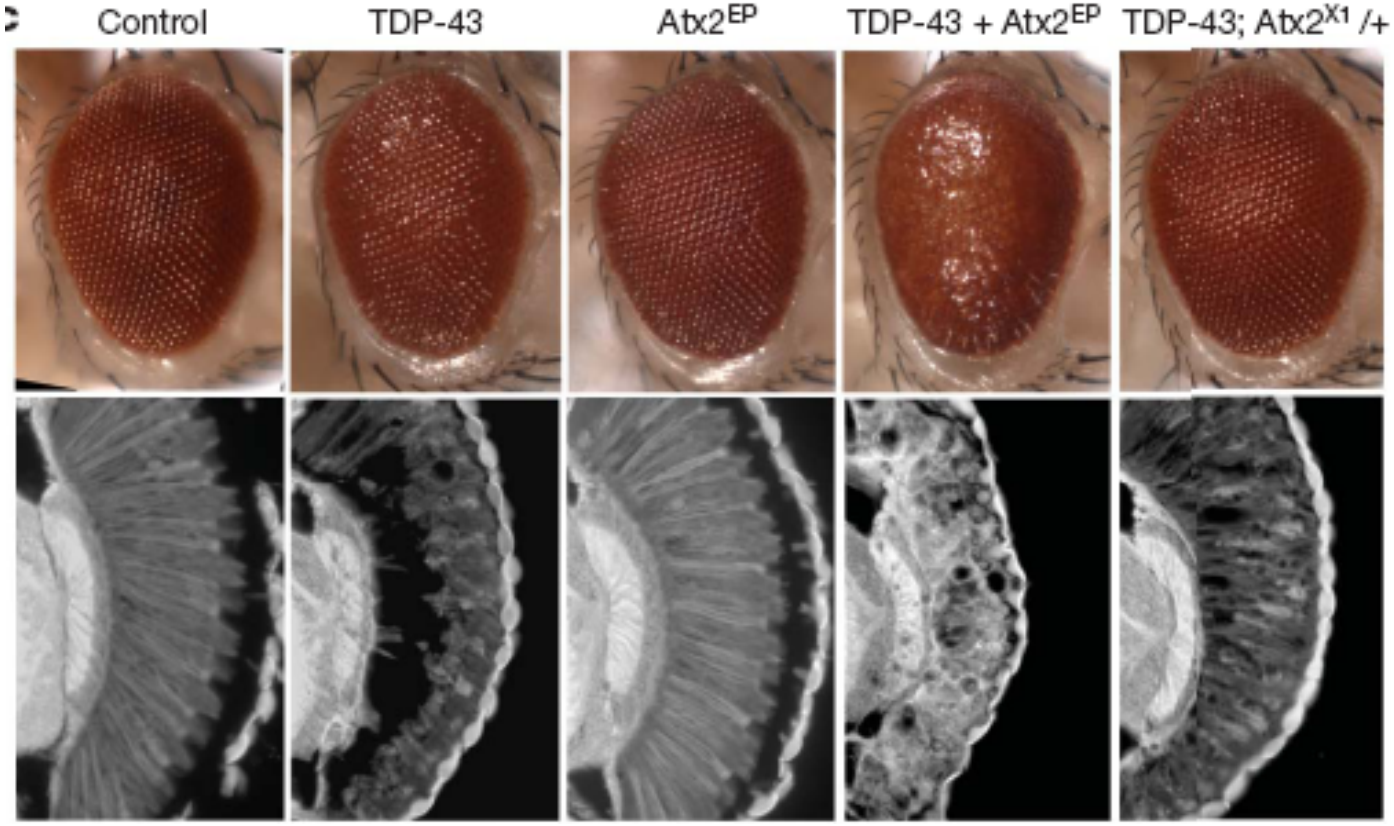


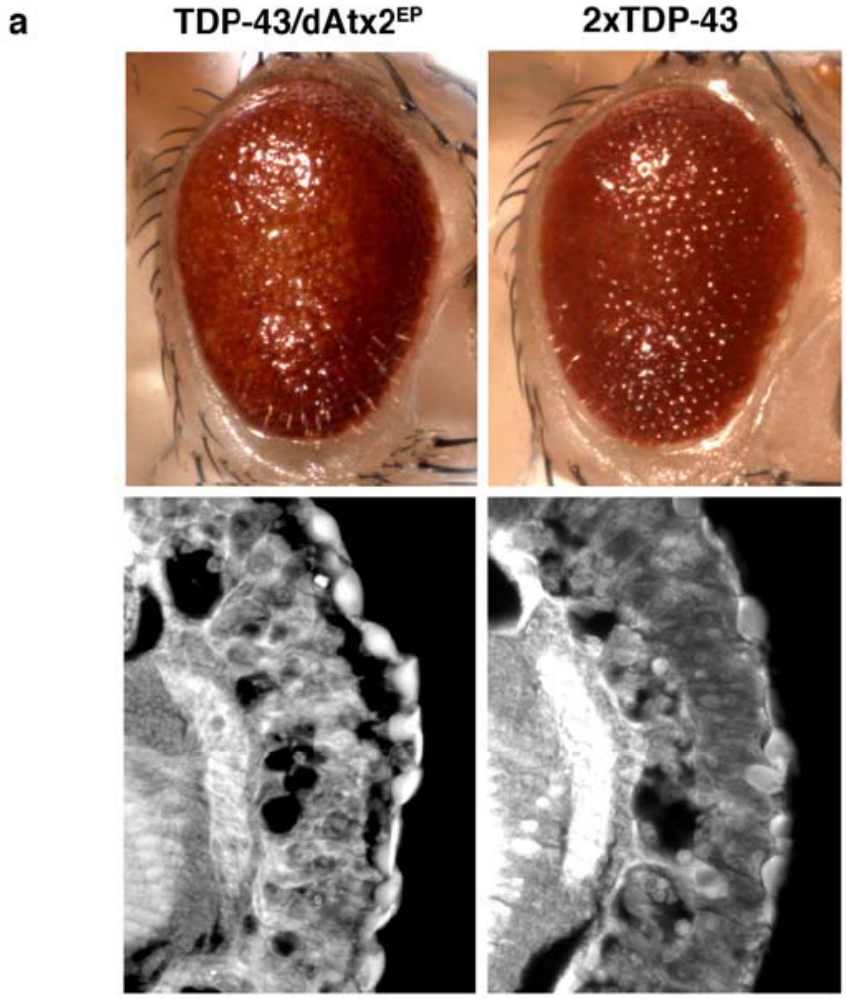
Figure 2c: Atx2 modifies TDP-43 toxicity in *Drosophila* eye

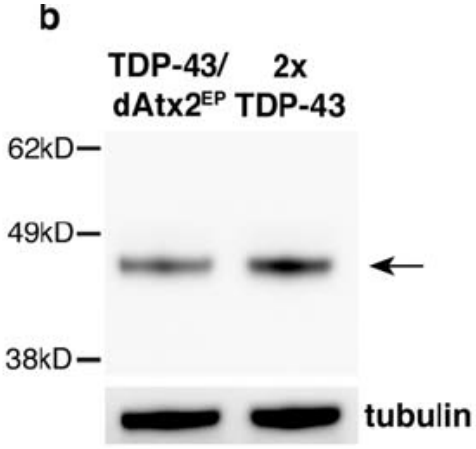
- Up/ down regulation of endogenous Atx2 toxicity with TDP-43

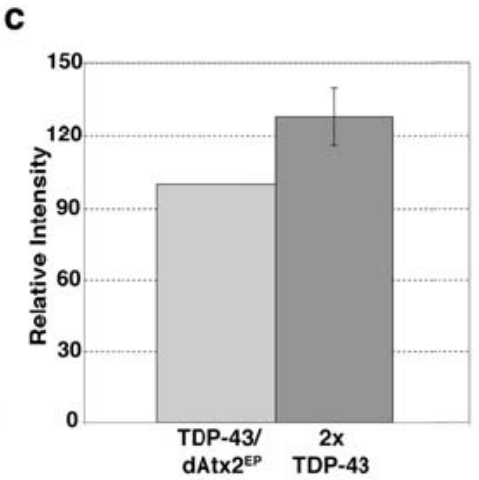


Toxicity not a function of up regulated TDP-43 alone→Atx2 needed

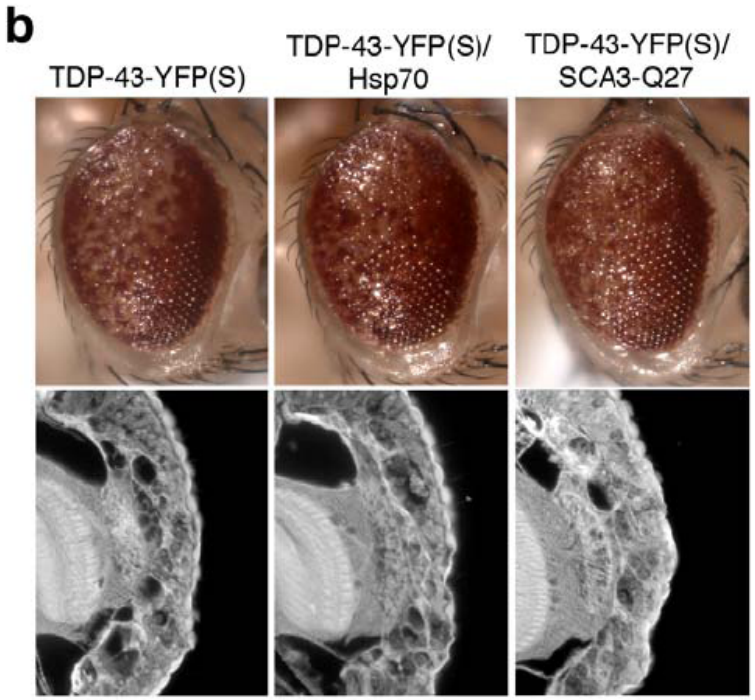
Supplemental Fig 3

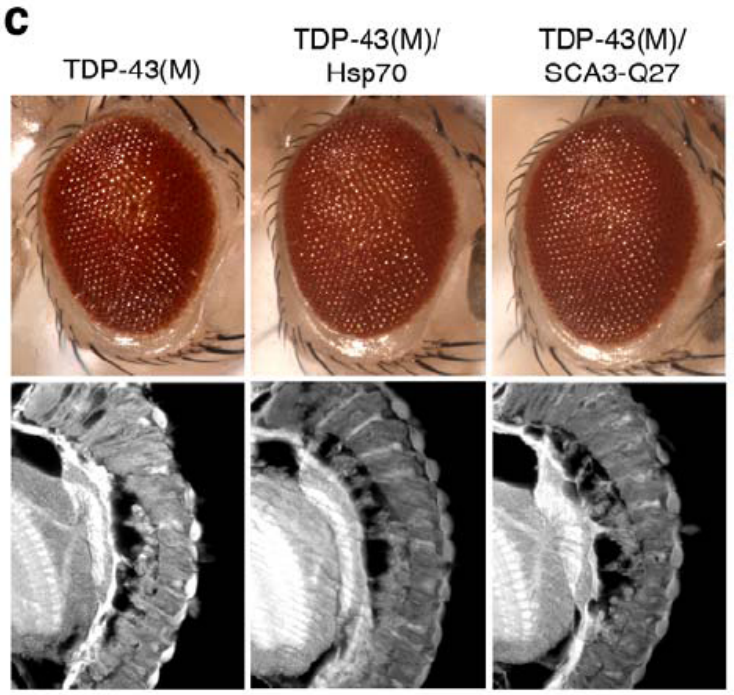






TDP-43 toxicity enhanced specifically by Atx2





Supplemental Fig 4

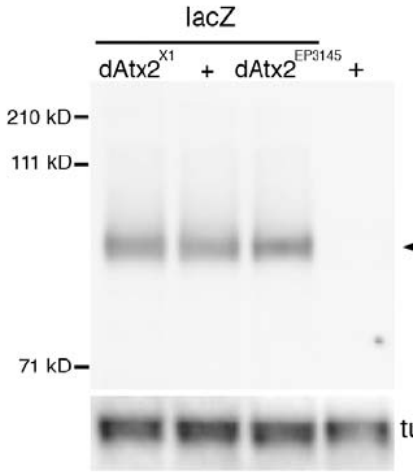


Figure 2d: Atx2 modifies TDP-43 reduced lifespan in *Drosophila* nervous system

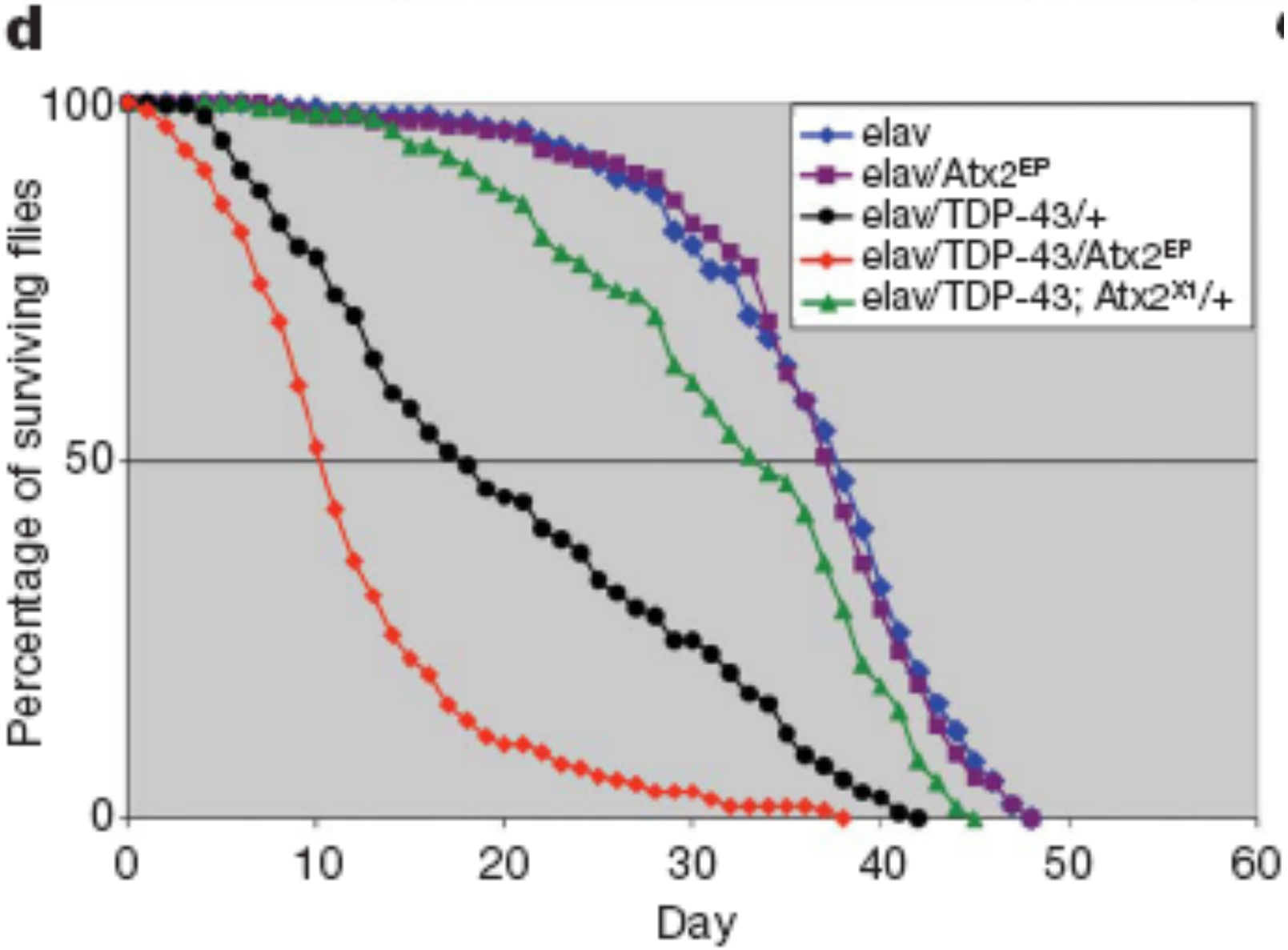
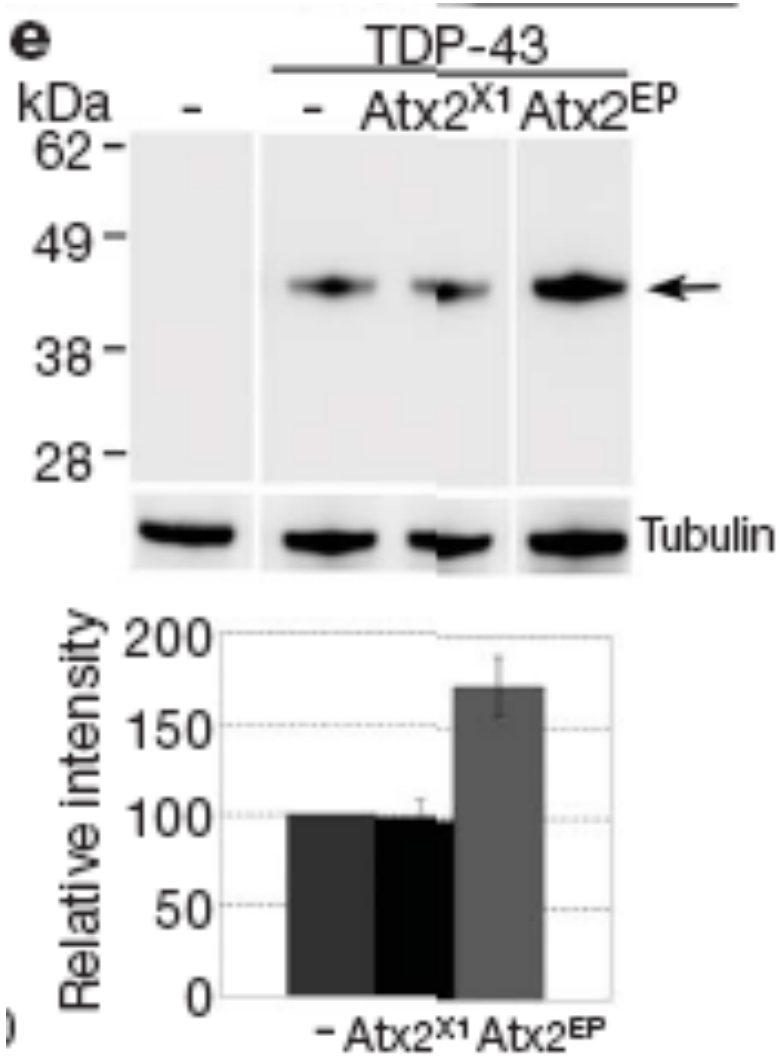


Figure 2e: Atx2 enhances detected TDP-43 protein levels in *Drosophila*

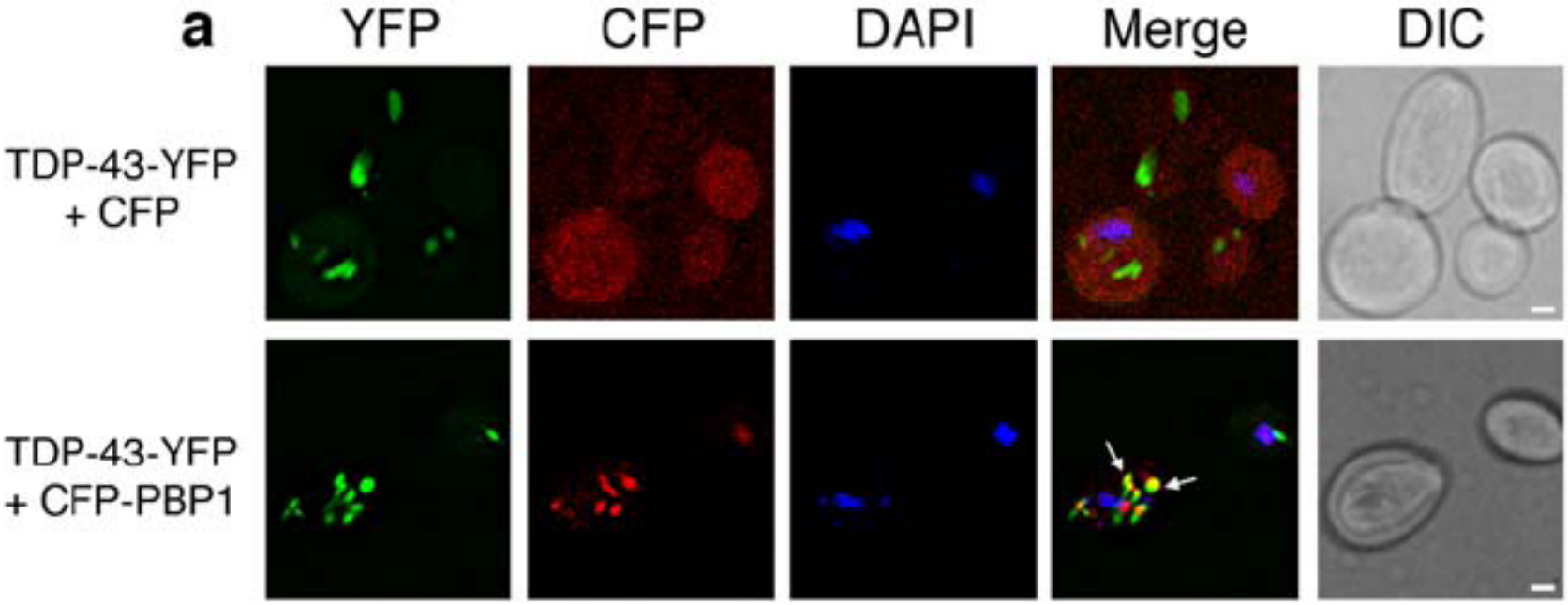


TDP-43 and ATXN2 interactions

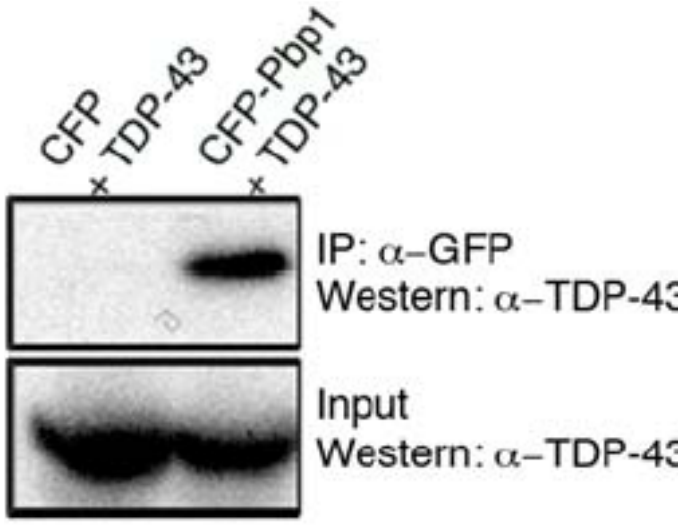
- RNA binding is necessary for TDP-43 toxicity

- Cytoplasmic TDP-43 toxically interacts with ATXN2 with necessity of RNA binding

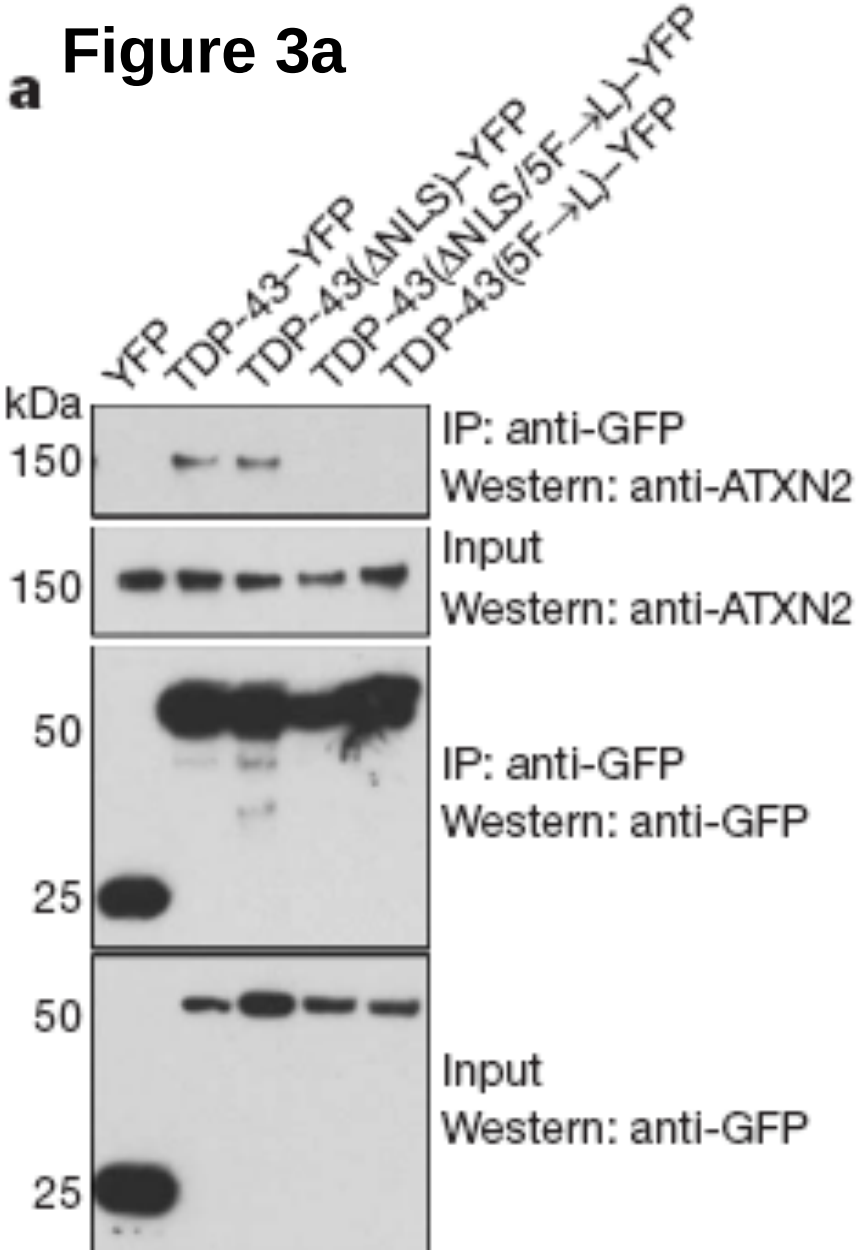
TDP-43 and Atxn2 interactions: yeast



Supplemental Fig 5:



TDP-43 and Atxn2 interactions: human



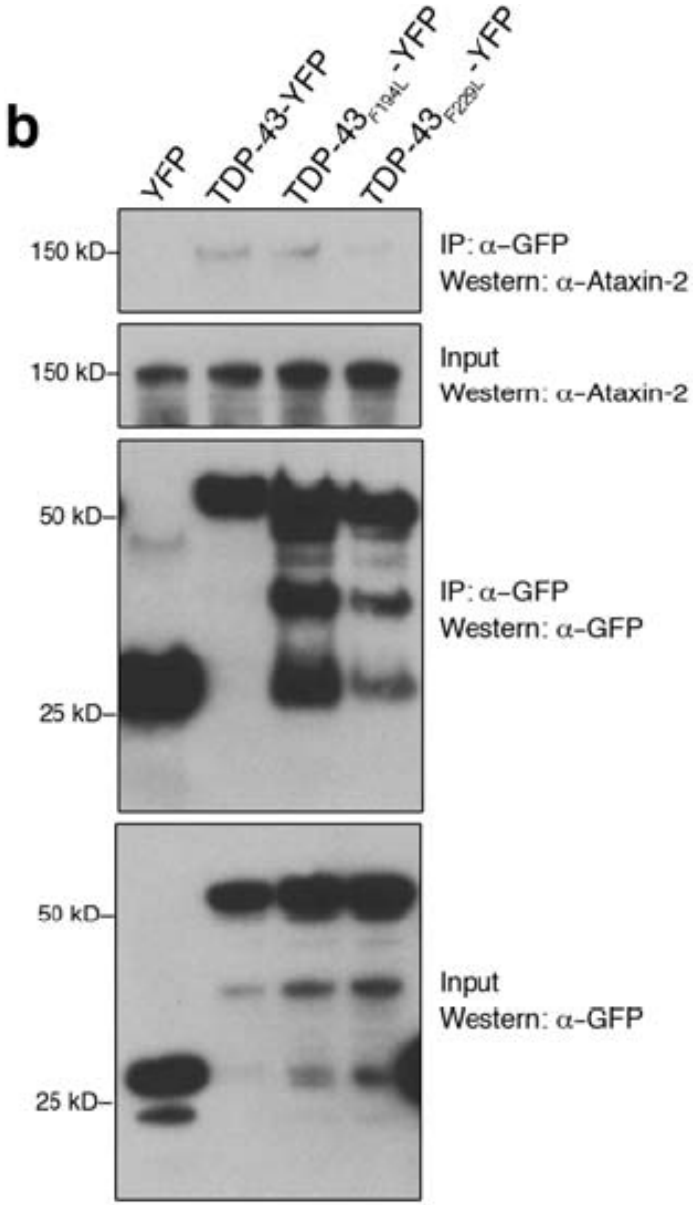
- TDP-43-YFP interacts with ATXN2
- TDP-43-(dNLS)-YFP (cytoplamsic localization) interacts with ATXN2
- TDP-43 disrupted RNA binding sights (5F→L) prevent interaction with ATXN2

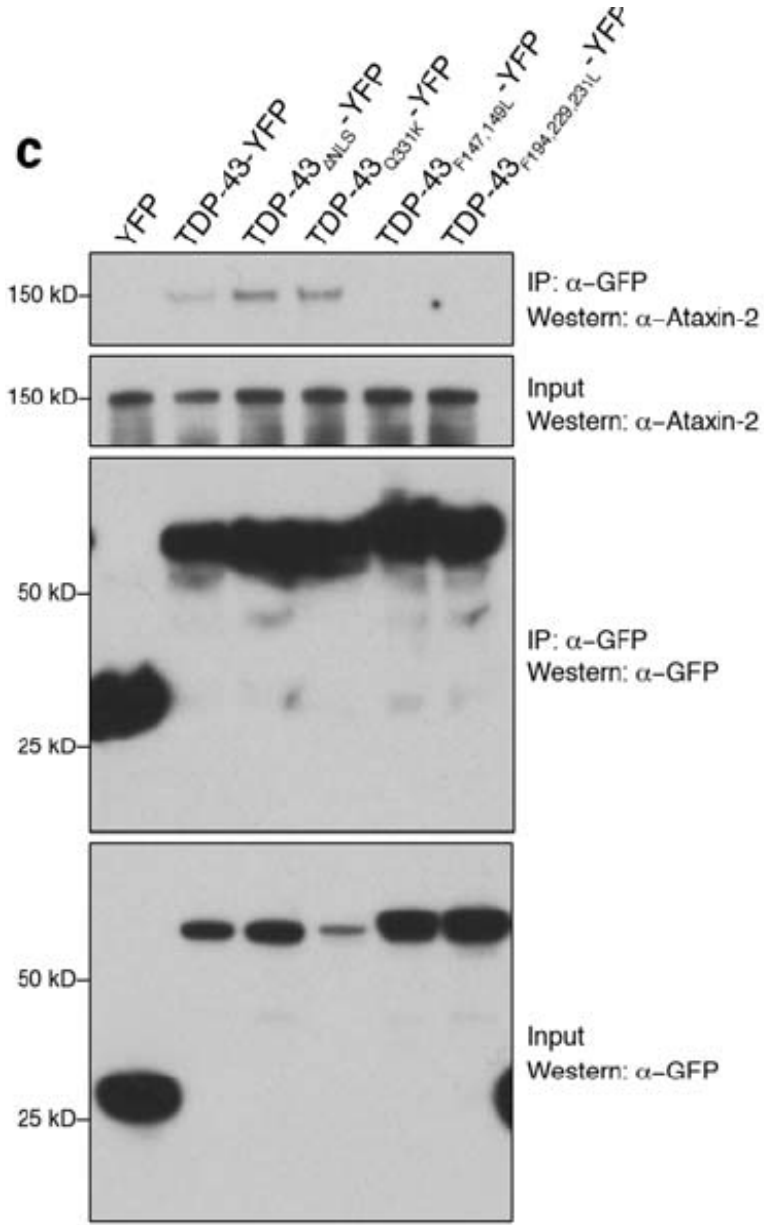
TDP-43 and Atxn2 interactions: human

Supplemental Fig 6

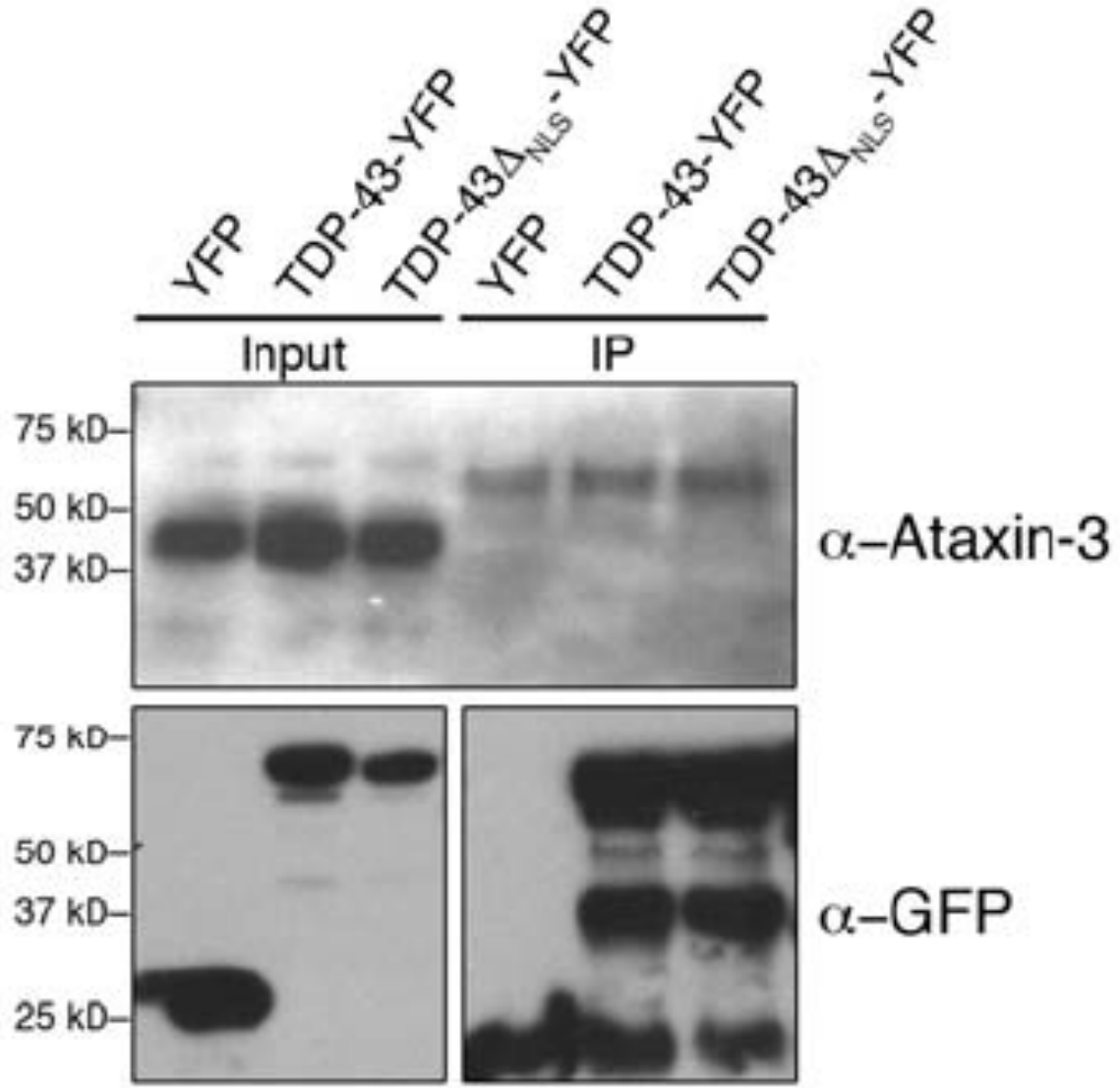
***b –single mutation**

***c-double triple mutation**





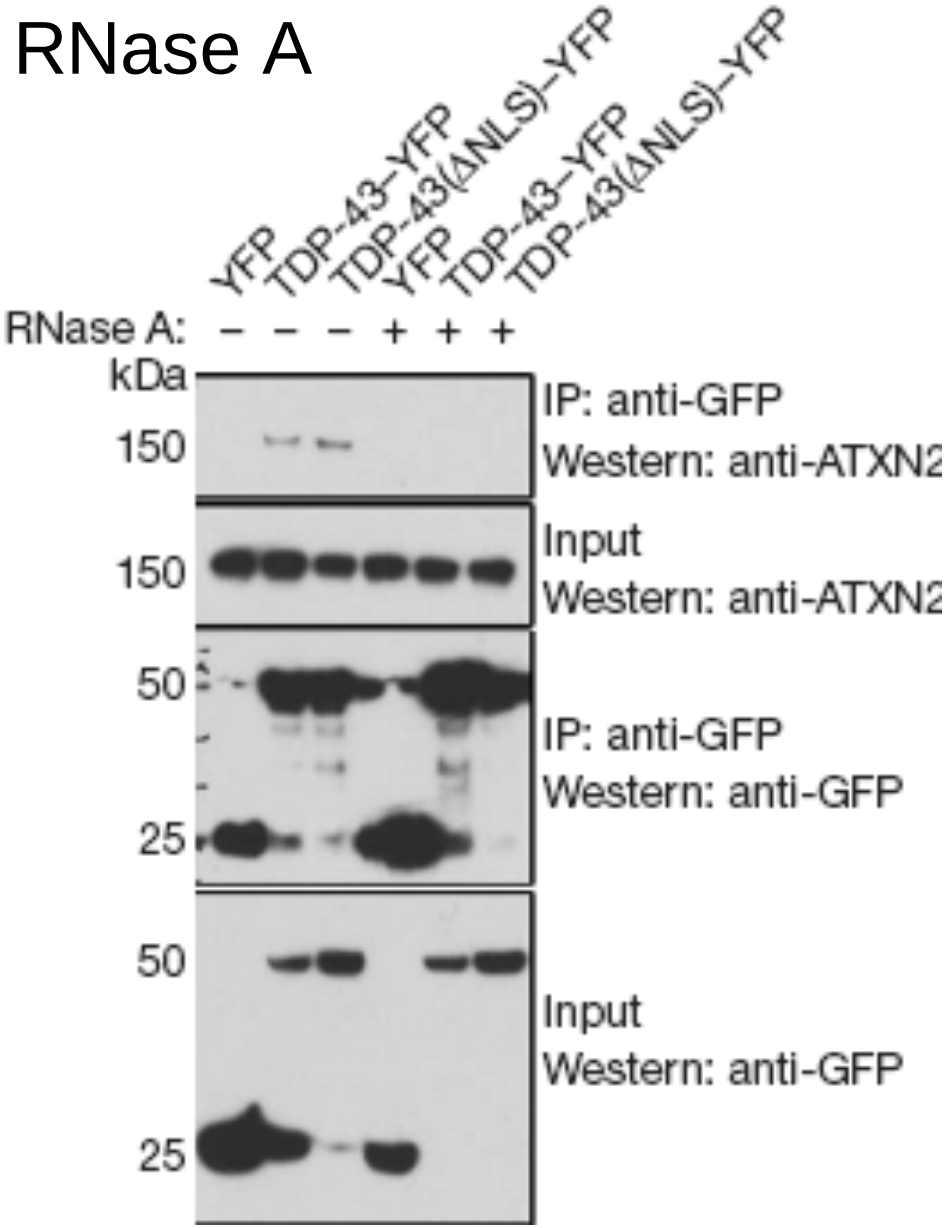
TDP-43 and Atxn2 interactions: human



Supplemental Fig 6a

RNA Fig 3b

Input treated with RNase A



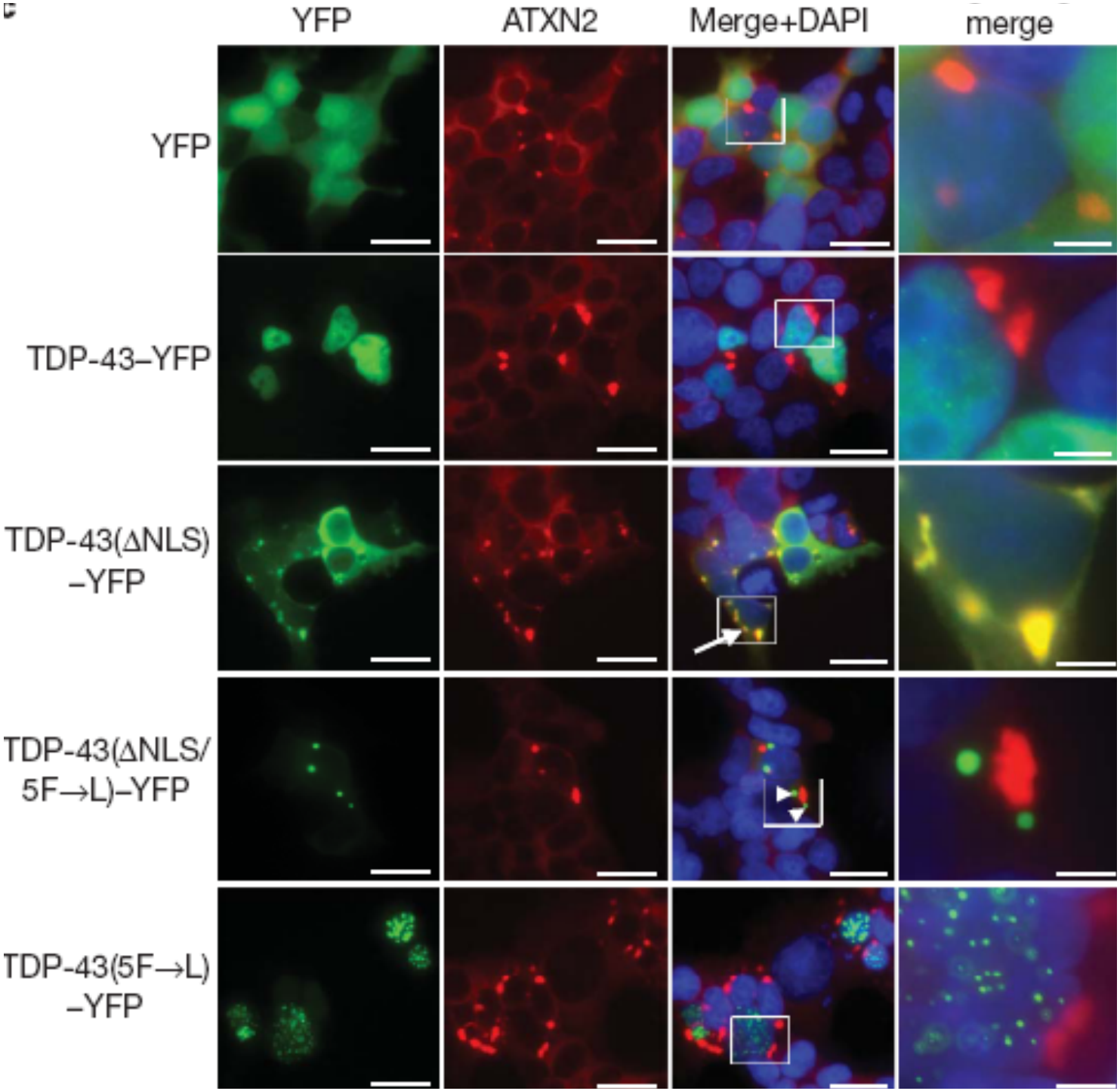
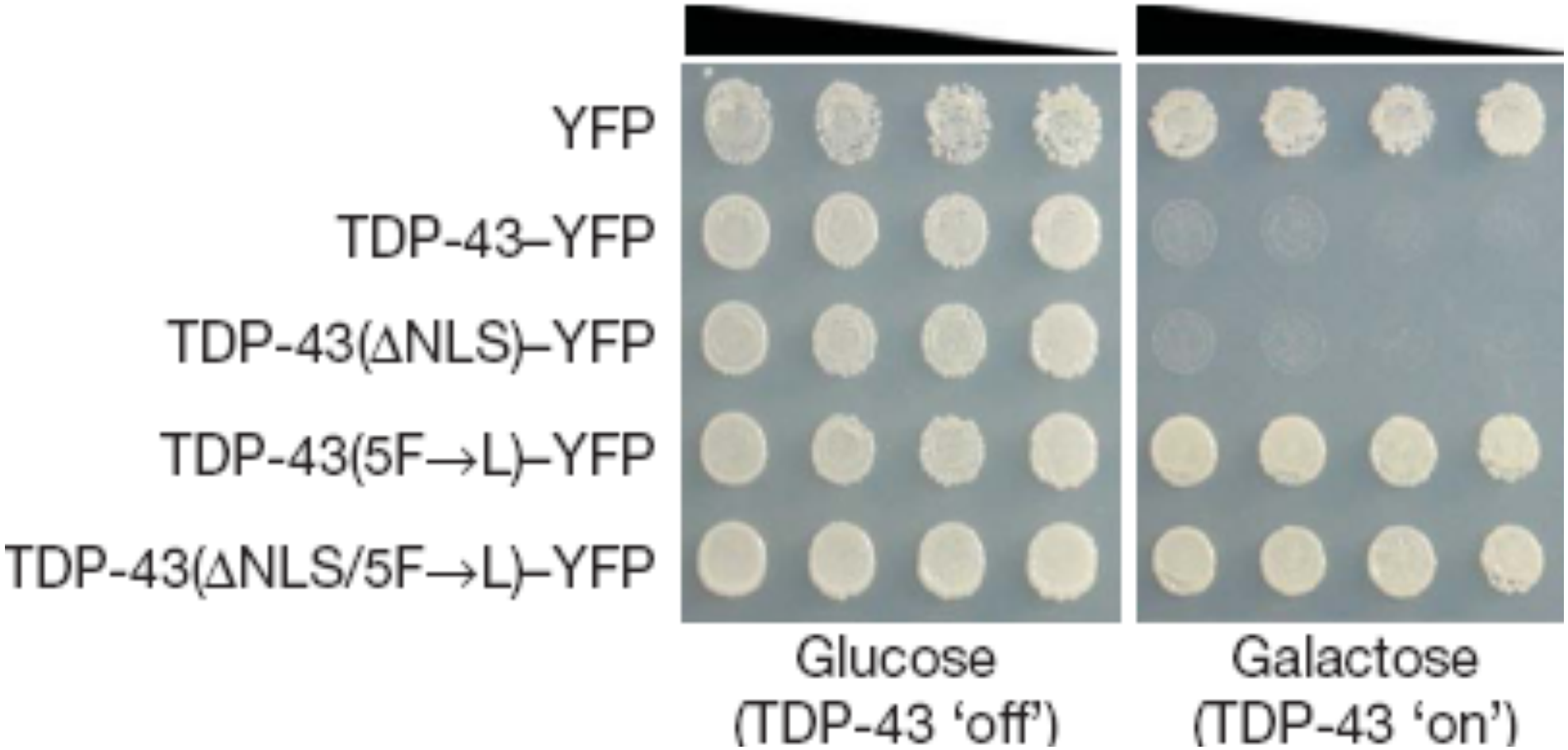


Figure 3c

- HEK 293
- **TDP-43**
- **ATXN2**
- **DAPI**

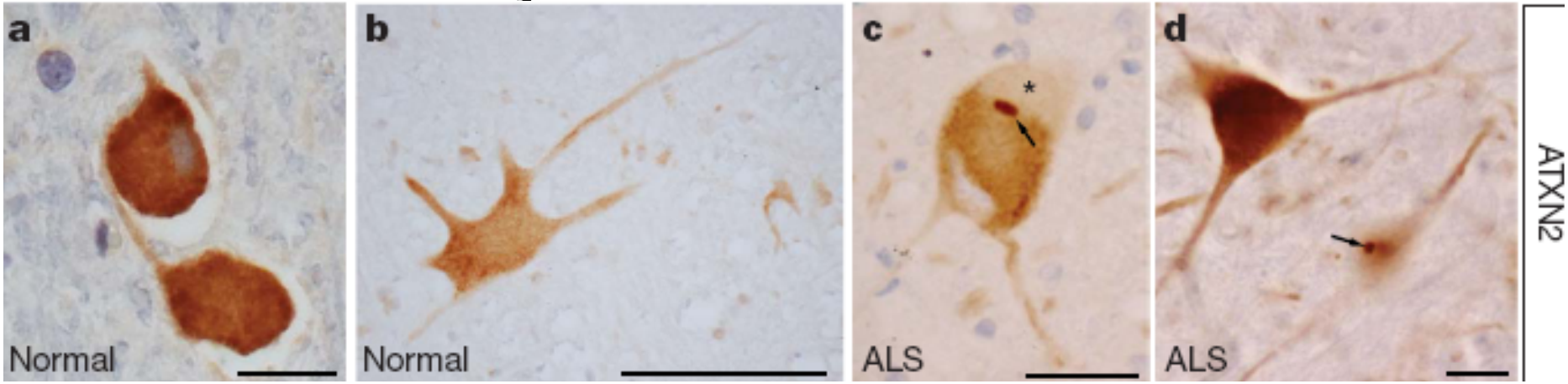
Cytosol TDP-43 is toxic
Mutated RRM prevent TDP-43 toxcity

Figure 3d



ATXN2 disrupted localization in ALS

- Spinal cord neurons from 6 ALS patients and 3 healthy controls



Diagnosis and case no.	Total motor neurons counted	ATXN2 diffuse/ fine granular staining	ATXN2 large accumulations	Percentage of neurons with large accumulations
Normal 1	31	29	2	6.5
Normal 2	55	54	1	1.8
Normal 3	17	16	1	5.9
ALS 1	52	41	10	19.2
ALS 2	40	25	15	37.5
ALS 3	28	24	4	14.3
ALS 4	13	7	6	46.2
ALS 5	61	49	12	19.7
ALS 6	15	11	4	26.7

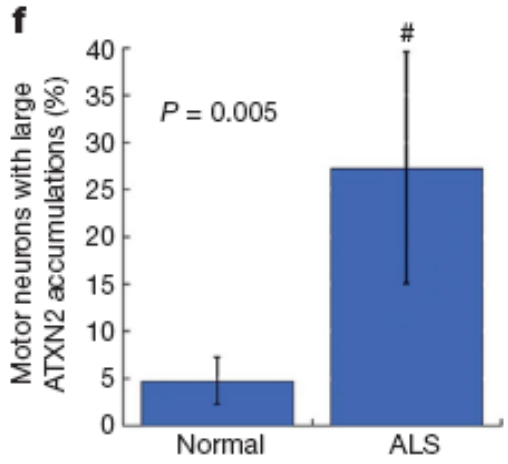
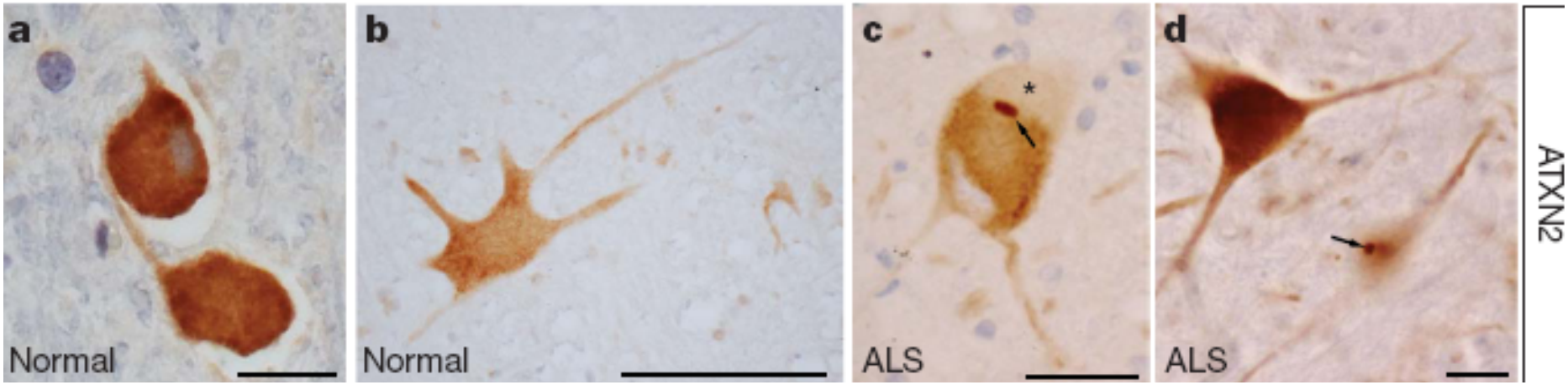
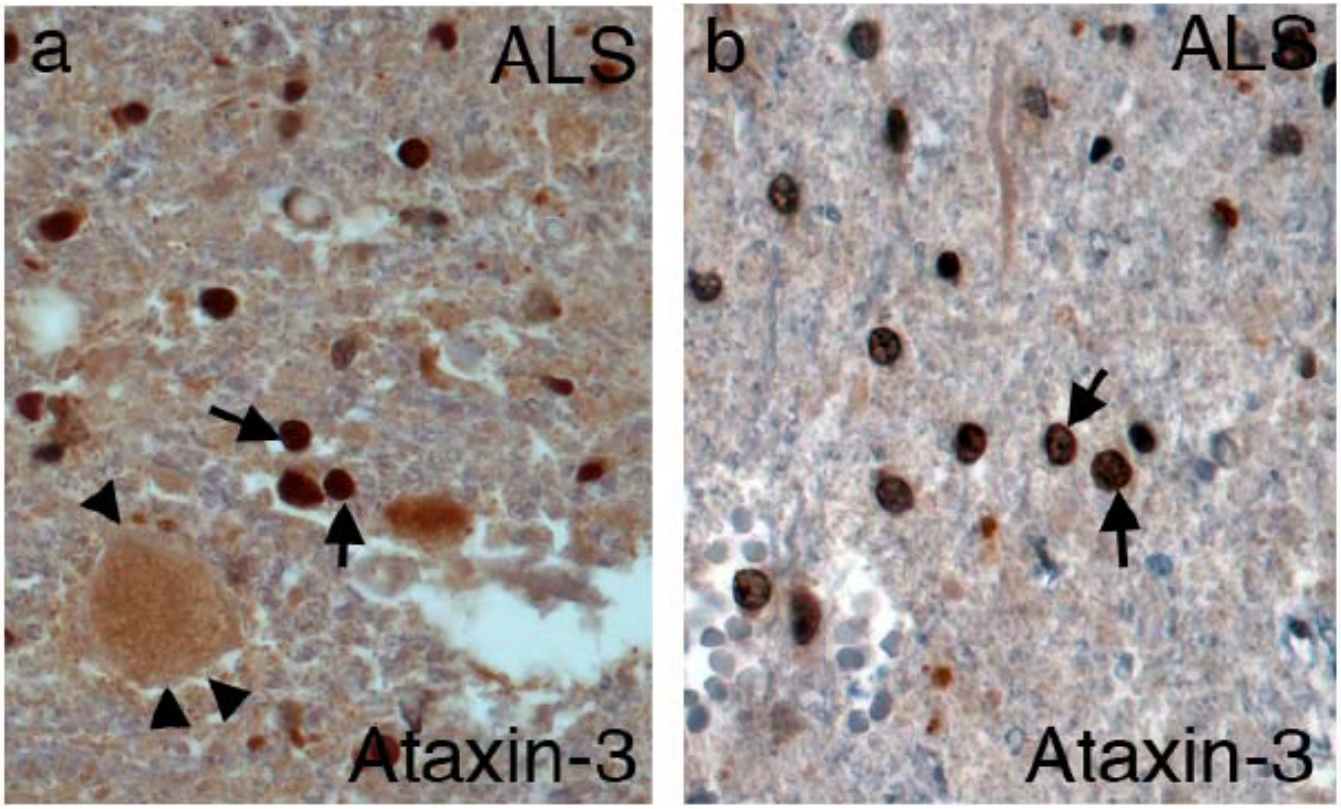


Figure 4



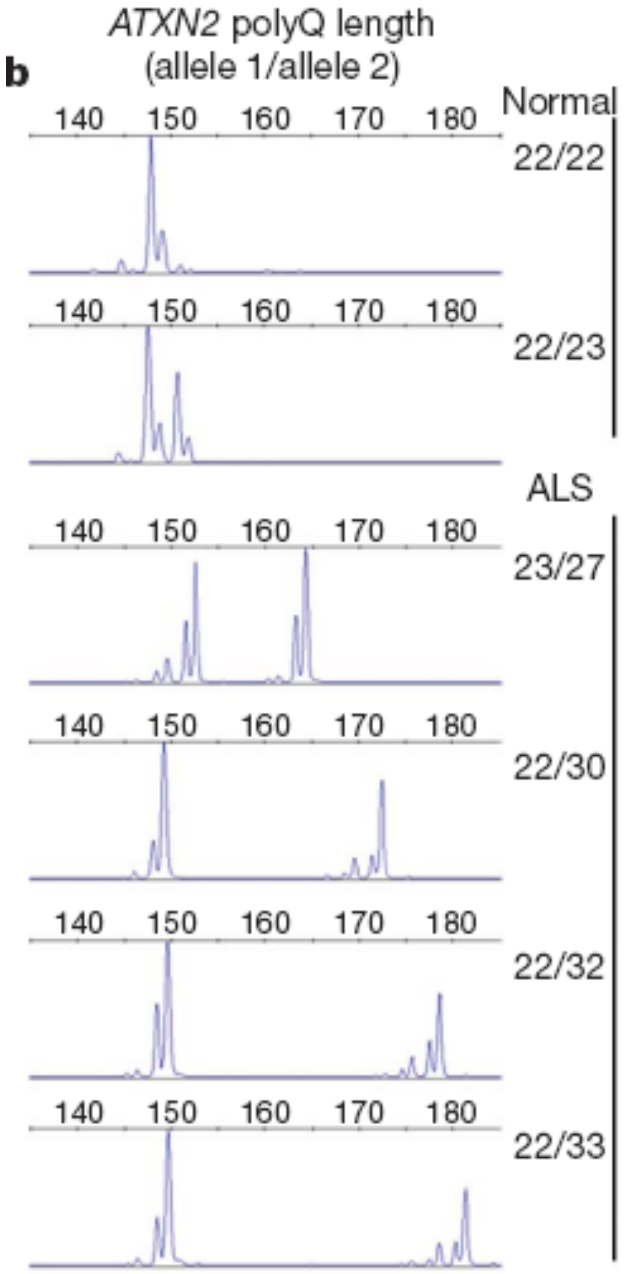


Supplemental Fig 7

ATXN2 PolyQ in ALS

23<Q<34 increase risk of ALS?

- N= 915 ALS
  - 50 of 915 (~5.4) intermediate PolyQ
- N= 980 controls
  - 24 of 980 (~2.4%) intermediate PolyQ

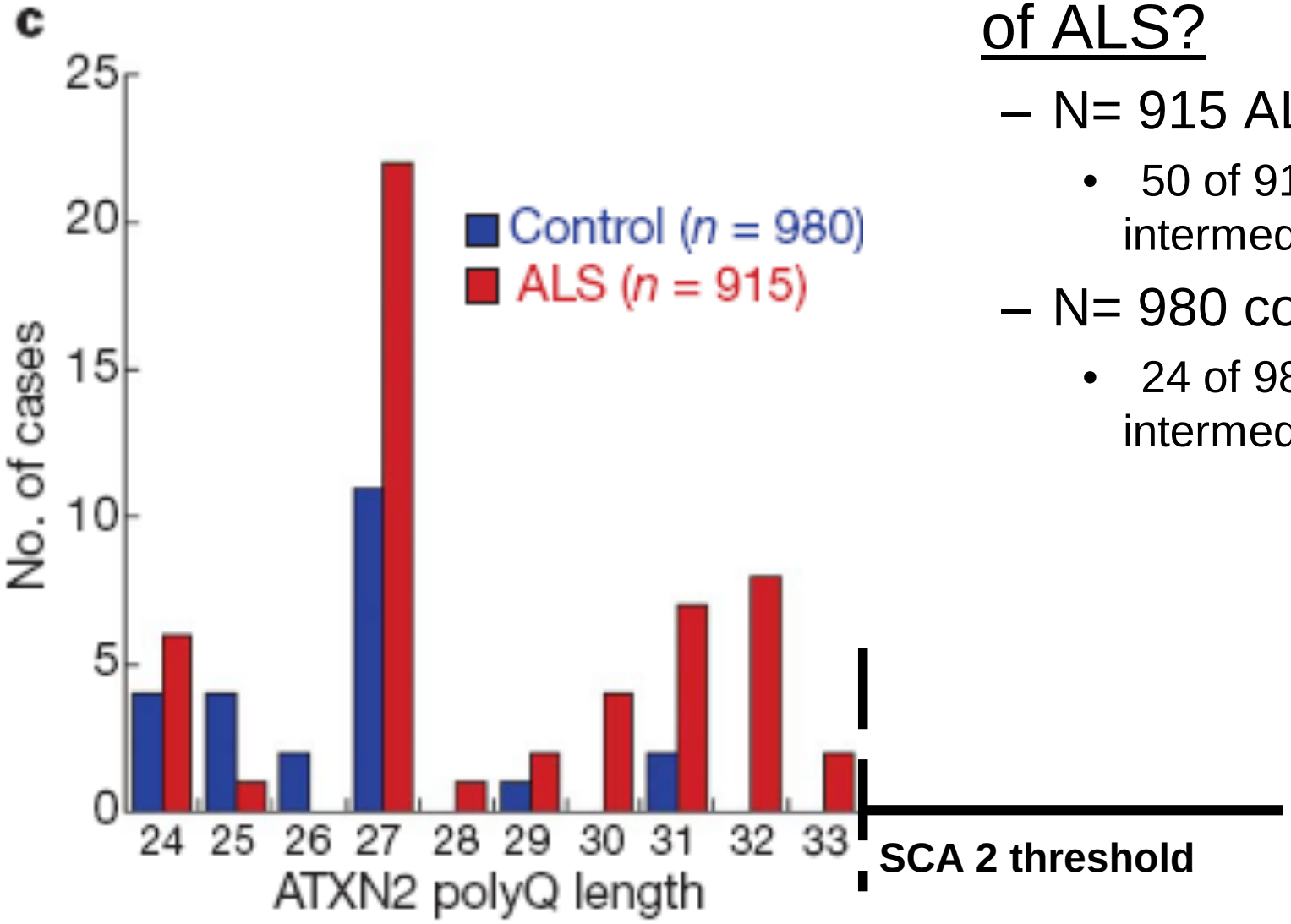


ATXN2 PolyQ in ALS

<u>23<Q<34 increase risk of ALS?</u>

- N= 915 ALS
  - 50 of 915 (~5.4) intermediate PolyQ
- N= 980 controls
  - 24 of 980 (~2.4%) intermediate PolyQ

ATXN2 PolyQ in ALS



<u>23<Q<34 increase risk of ALS?</u>

- N= 915 ALS
  - 50 of 915 (~5.4) intermediate PolyQ
- N= 980 controls
  - 24 of 980 (~2.4%) intermediate PolyQ

ATXN2 PolyQ in ALS

23<Q<34 increase risk of ALS?

- N= 915 ALS
  - 50 of 915 (~5.4) intermediate PolyQ
- N= 980 controls
  - 24 of 980 (~2.4%) intermediate PolyQ

Table 1 | Increased frequency of intermediate-length *ATXN2* polyQ repeat expansions in ALS

Subjects	Total	≤26 repeats	27–33 repeats	Percentage of 27–33 repeats	*P* value	OR (95% CI)
ALS	915	872	43	4.7%	3.6×10^{-5}	2.80 (1.54–5.12)
Neurologically normal	980	966	14	1.4%		

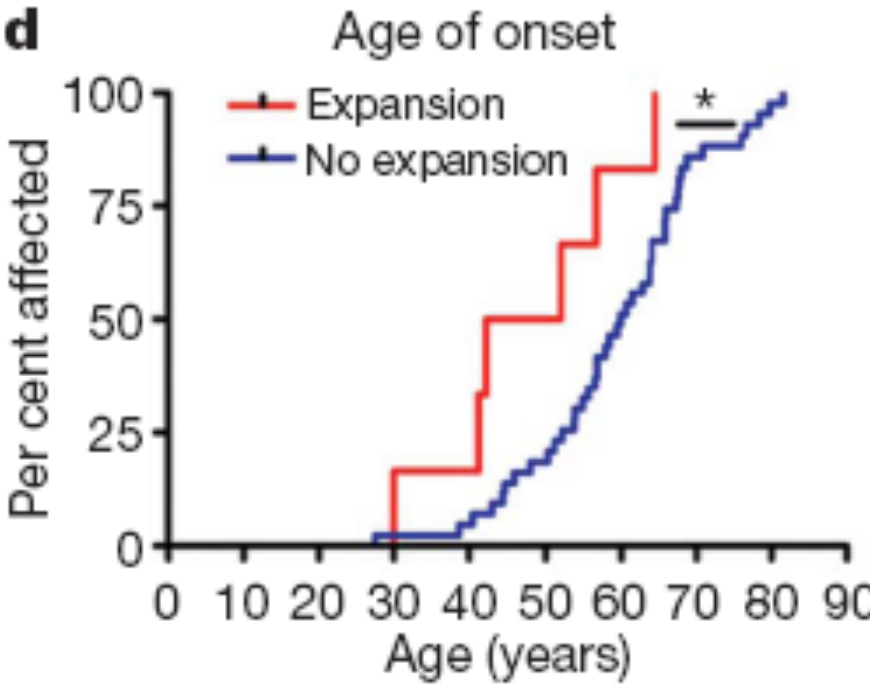
OR, odds ratio; CI, confidence interval

ATXN2 PolyQ in ALS

Smaller subset of ALS patients (n=65)

- Neg for mutations of SOD1, TARDP & FUS
- N=8 (> 24Q)
- N=57(< 24Q) ?

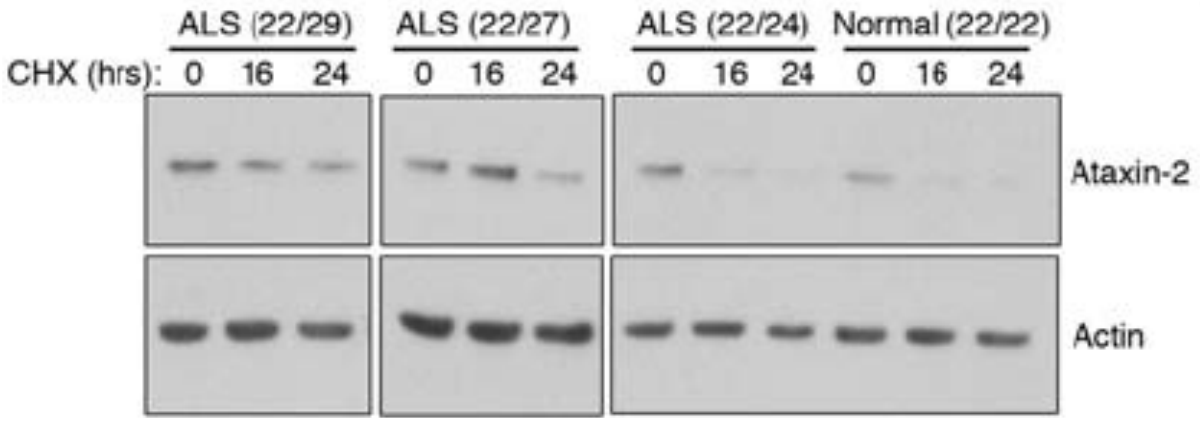
- AoO sig earlier for intermed Q affected individuals
  - mean = 47.8 v 59.4 AoO

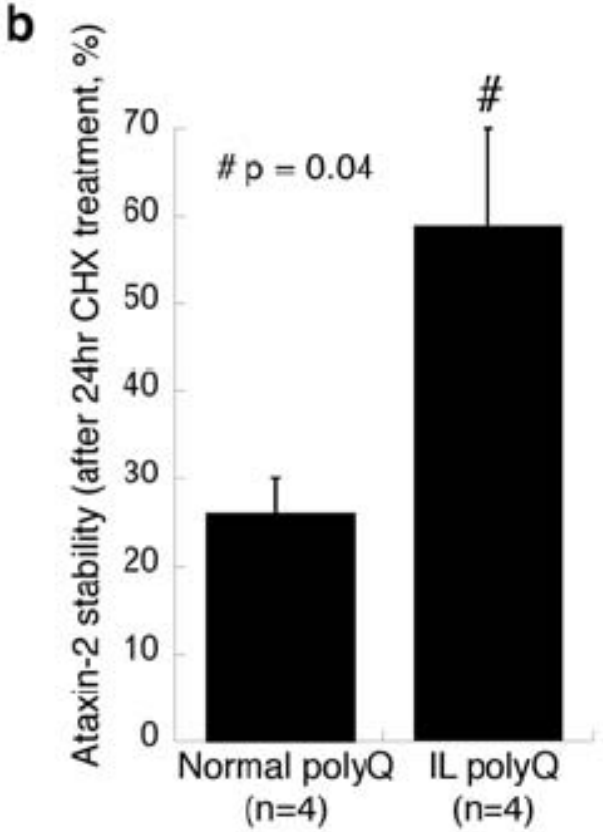


Atxn2 polyQ: stability

Supplemental Fig 8

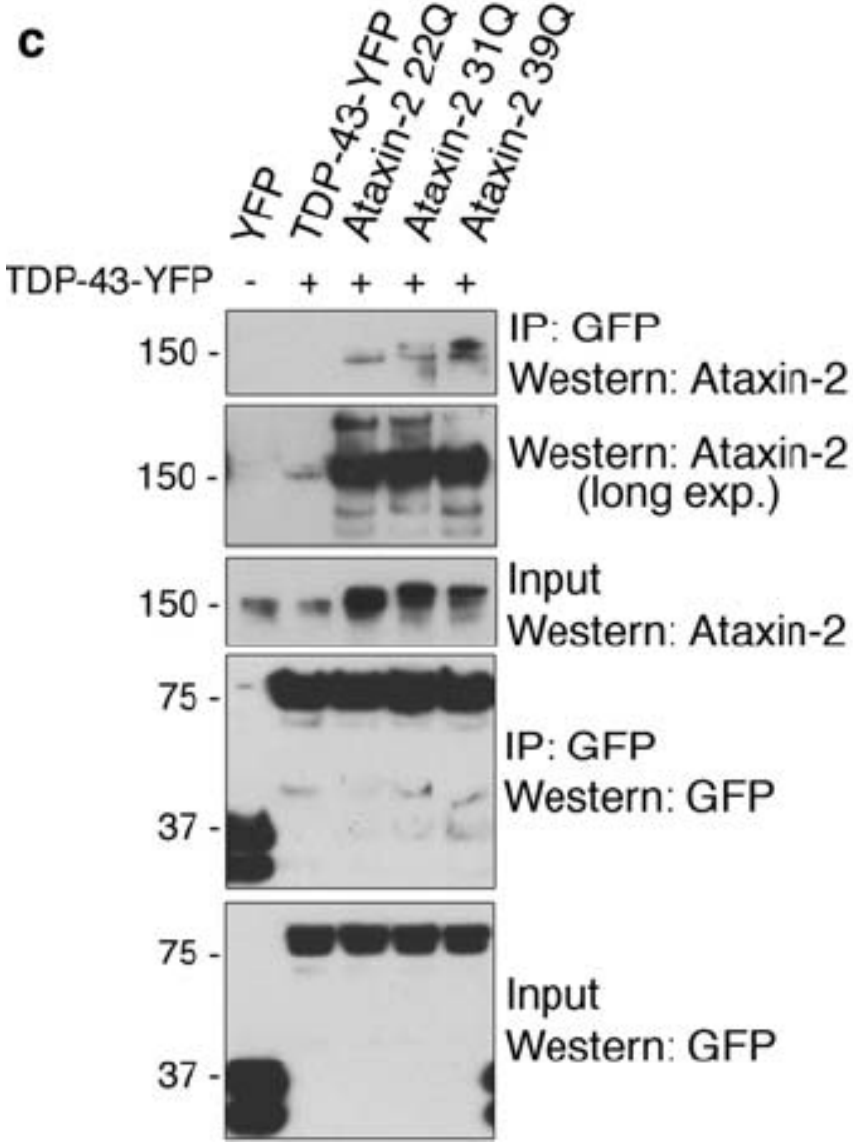
- Lymphoblastoid cells from:
  - ALS patients with intermed Q
  - ALS patients normal Q
  - Healthy controls
  - Treatment with cycloheximide





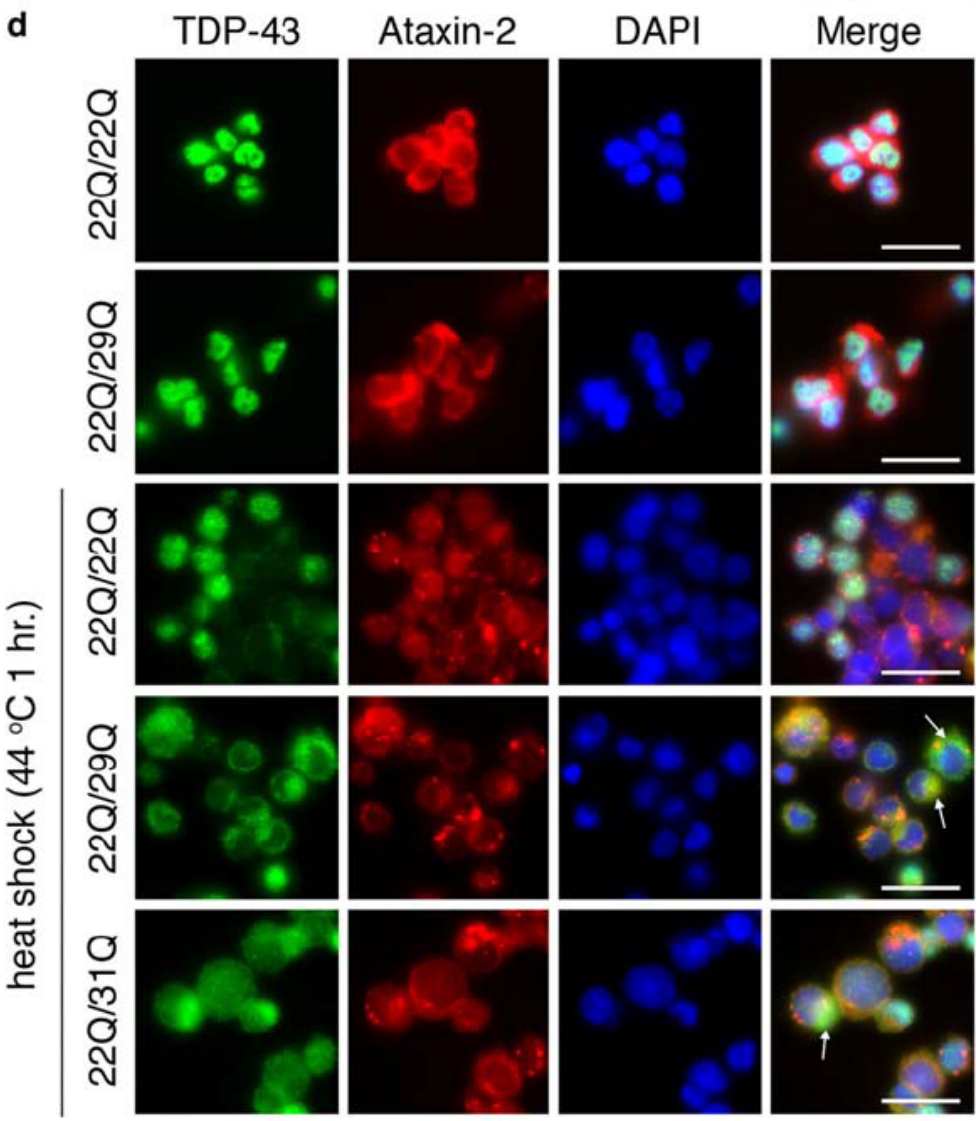
Atxn2 polyQ; TDP-43 Interaction

Supplemental Fig 8



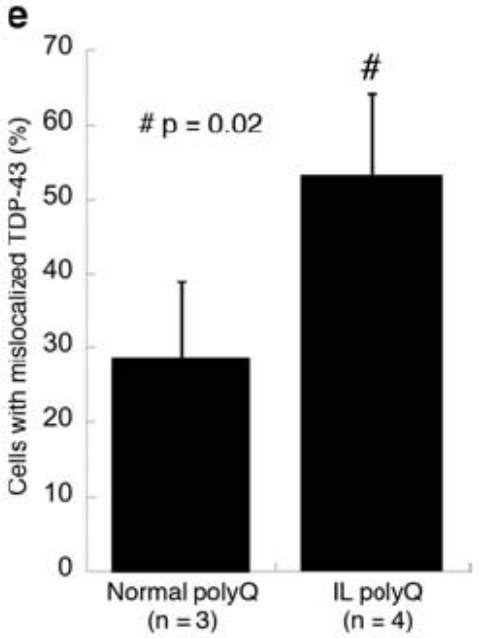
Atxn2 polyQ; TDP-43 Interaction

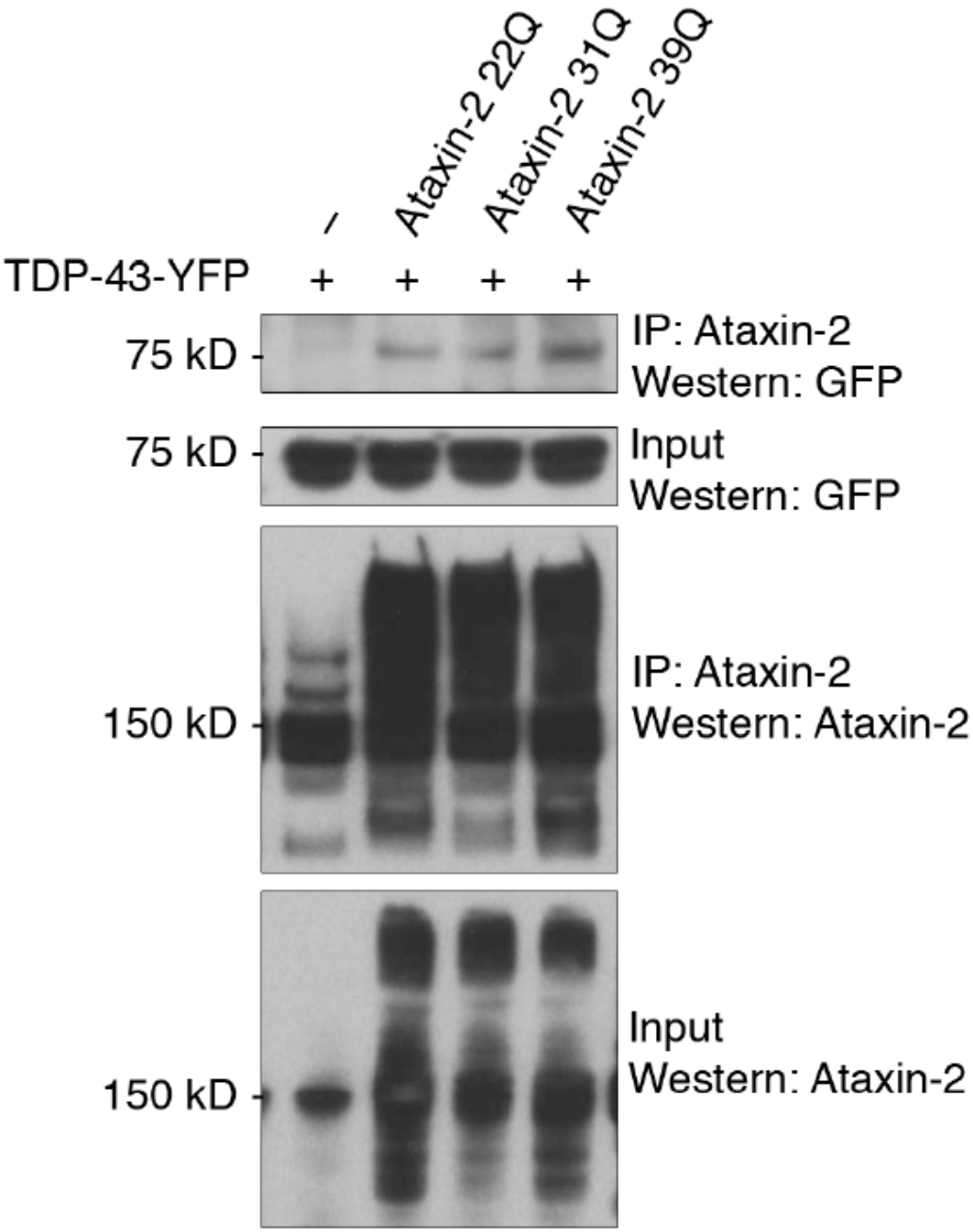
Supplemental Fig 8



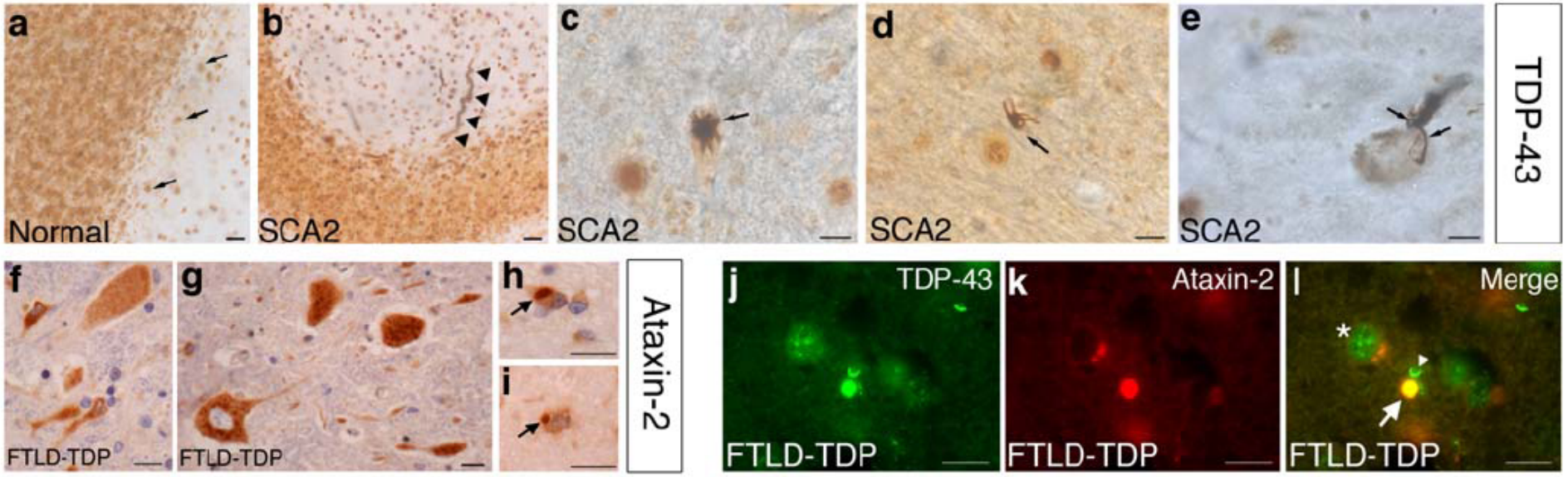
ALS patient derived lymphoblastoid cells

Heat shock 44c resulted in ATXN2 and TDP-43 foci in the cytoplasm



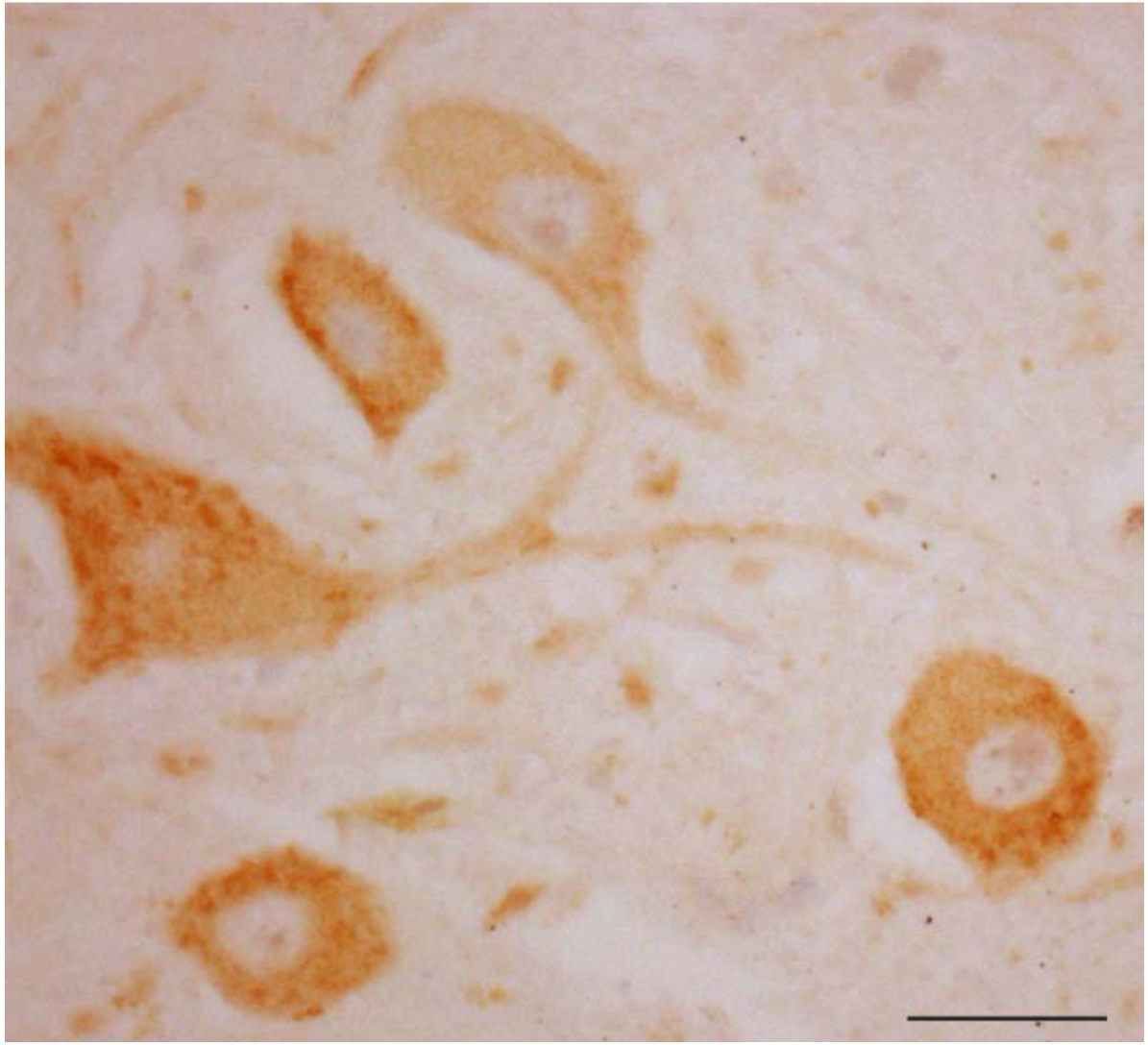


- Supplemental 9
  - Transfected atxn2 co-IP with transfected TDP-43-YFP



- Localization of TDP-43 in SCA2 patients
  - A,B: cerebellum
  - C: hypoglossus nucleus of brainstem
  - D: Abducen nucleus
  - E: Locus Ceruleus
- Localization of Ataxin-2 in FTLD-TDP
  - F,G: temporal lobe (diffuse)
  - H,I: similar to ALS
  - J,L: double stain reveal colocalization

Supplemental fig 10



Cytoplasmic localization of Atxn2 in control spinal cord neurons

Supplemental fig 11