Supplementary Information to

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

Andrew C. Elden\textsuperscript{1,8}, Hyung-Jun Kim\textsuperscript{2,8}, Michael P. Hart\textsuperscript{1,8}, Alice S. Chen-Plotkin\textsuperscript{3,4,8}, Brian S. Johnson\textsuperscript{1}, Xiaodong Fang\textsuperscript{1}, Maria Armakola\textsuperscript{1}, Felix Geser\textsuperscript{3}, Robert Greene\textsuperscript{3}, Min Min Lu\textsuperscript{1}, Arun Padmanabhan\textsuperscript{1}, Dana Clay\textsuperscript{3}, Leo McCluskey\textsuperscript{4}, Lauren Elman\textsuperscript{4}, Denise Juhr\textsuperscript{5}, Peter J. Gruber\textsuperscript{5}, Udo Rüb\textsuperscript{6}, Georg Auburger\textsuperscript{7}, John Q. Trojanowski\textsuperscript{3}, Virginia M.-Y. Lee\textsuperscript{3}, Vivianna M. Van Deerlin\textsuperscript{3}, Nancy M. Bonini\textsuperscript{2,9}, Aaron D. Gitler\textsuperscript{1,9}

1. Department of Cell and Developmental Biology and
2. Department of Biology, the Howard Hughes Medical Institute and
3. Center for Neurodegenerative Disease Research and
4. Department of Neurology, the University of Pennsylvania, Philadelphia PA, 19104
5. The Children’s Hospital of Philadelphia, Philadelphia, PA 19104
6. Institute of Clinical Neuroanatomy, Dr. Senckenberg Anatomy, Goethe University, Frankfurt am Main, Germany
7. Molecular Neurogenetics, Department of Neurology, Goethe University, Frankfurt am Main, Germany
8. These authors contributed equally.
9. Correspondence should be addressed to: A.D.G. or N.M.B.

Aaron D. Gitler
1109 BRB II/III
421 Curie Blvd.
Philadelphia, PA 19104
215-573-8251 (phone)
215-898-9871 (fax)
gitler@mail.med.upenn.edu

Nancy M. Bonini
306 Leidy Labs
Philadelphia, PA 19104
215-573-9267 (phone)
215-573-5754 (fax)
bonini@sas.upenn.edu
Supplementary Data

Ataxin-2 localization in ALS and FTLD-TDP and TDP-43 localization in SCA2

Five out of six of the ALS cases analyzed in Fig. 4 were negative for SOD1 mutations and these all displayed TDP-43-positive pathology. Interestingly, one case harbored an SOD1 mutation (D124V) and also displayed TDP-43 pathology in the spinal cord, albeit a lower level than the SOD1-negative ALS cases (see Fig. 4 legend). There are conflicting reports in the literature about the presence of TDP-43 pathology in SOD1-positive ALS; it is possible that Ataxin-2 could influence TDP-43 pathology even in SOD1-ALS. Thus, Ataxin-2 mislocalization may be a common feature of both sporadic and familial forms of ALS.

We also examined Ataxin-2 in another TDP-43 proteinopathy, FTLD-TDP, which shares clinicopathological overlap with ALS. Both FTLD-TDP cases showed distinct Ataxin-2 cytoplasmic accumulations (Fig. S10h,i), similar to ALS. We also observed several instances where TDP-43 and Ataxin-2 co-localized in the same cytoplasmic inclusion (Fig. S10j-l). Interestingly, co-localization was only observed in round inclusions, but not in skein-like aggregates (Fig. S10j-l); we did not observe co-localization in the ALS patient spinal cords examined (data not shown). This might reflect a difference in the TDP-43/Ataxin-2 interaction in brain vs. spinal cord. Notably, pathogenic TDP-43 C-terminal fragments are preferentially enriched in the brain but not spinal cord in FTLD-TDP and ALS, underscoring the possibility for regionally distinct pathogenic mechanisms as underlying the etiology of TDP-43 proteinopathies. It will be important to define how Ataxin-2 may contribute to these regional differences.

To further address the significance of interactions between TDP-43 and Ataxin-2, we examined the localization of TDP-43 in SCA2 patient tissue. Although a rare disease, we obtained tissue from two SCA2 patients and examined the cerebellum and brain stem nuclei for TDP-43 pathology. Normally, TDP-43 was restricted to the nucleus of cerebellar Purkinje neurons (Fig. S10a); however, in SCA2 tissue, surviving Purkinje cell neurons displayed abundant TDP-43 immunoreactivity in the cytoplasm with typical corkscrew-shape neurodegenerative signs (Fig. S10b). TDP-43 immunoreactive inclusions were also present in motor neurons of the abducens and the hypoglossus nucleus, and in noradrenergic afferent neurons of the locus coeruleus within the brainstem (Fig. S10c-e). These data indicate that TDP-43 proteinopathy occurs in SCA2, and that TDP-43 and Ataxin-2 interactions could play important roles in the pathogenesis of both ALS and SCA2. This finding also provides a molecular explanation for the observed clinicopathological similarities between the two diseases.

Clinical Anecdote

In the process of reviewing ALS cases with the treating neurologist for this project, we were surprised to find that one of the probands in this study had an intriguing clinical phenotype. Specifically, at age 26, this female patient first experienced bilateral leg weakness that progressed over five years and led to evaluation by a neurologist. At neurologic presentation, she had bilateral upper and lower motor neuron signs in all four extremities with preserved sensation. An EMG demonstrated acute and ongoing chronic denervation in the cervical, thoracic, and lumbosacral segments, and a diagnosis of ALS was made. Weakness of the extremities progressed, and she subsequently developed dysarthria and dysphagia. After 12 years of a disease course that was relatively typical for ALS with the exception of the long duration and early onset, she developed cerebellar signs and symptoms. Specifically, ataxia, cerebellar speech, ocular dysmetria, and nystagmus were noted – unusual for ALS but reminiscent of spinocerebellar ataxia. After a disease duration of 15+ years, the patient died of neuromuscular respiratory failure. Autopsy examination revealed typical TDP-43-positive ALS and our genetic analysis revealed an Ataxin-2 expansion of 27 repeats.

Family history was notable for a brother who also had ALS with early onset in his late 20s and an
unusually long disease duration of 10 years. Unfortunately, he died suddenly of unrelated causes before a DNA sample could be obtained. While anecdotal, this clinical information is in agreement with our hypothesis that Ataxin-2 repeat expansions can present with a spectrum of phenotypes, with the length of the repeat expansion determining whether motor neuron or cerebellar features predominate. These findings underscore the idea that Ataxin-2 may integrate the etiology of multiple neurodegenerative diseases raised in the Discussion.

Supplementary Discussion

The physical and genetic interactions between TDP-43 and Ataxin-2 suggest a model whereby Ataxin-2 serves as a bridge, either directly or via RNA, to bring TDP-43 to sites of a toxic function. Consistent with this, deleting Pbp1 in yeast or Ataxin-2 in the fly mitigated TDP-43 toxicity. Mutating the RRM2s of TDP-43, in addition to blocking the interaction with Ataxin-2, eliminated TDP-43 toxicity. These data suggest that Ataxin-2 is an essential mediator, likely via protein-protein or protein-RNA interactions, of TDP-43 toxicity in the cytoplasm. We did not observe a native interaction between endogenous TDP-43 and Ataxin-2 (M.P.H and A.D.G. unpublished observations), supporting the notion that the TDP-43/Ataxin-2 interaction is associated with pathogenesis, rather than reflecting the normal physiological state. Notably, Ataxin-2 localization was also perturbed in motor neurons of ALS patients harboring normal Ataxin-2 polyQ repeat lengths, indicating that Ataxin-2/TDP-43 interactions are likely a component of ALS generally. Because of this and the findings that reduction of Ataxin-2 strikingly suppresses TDP-43 toxicity in yeast and flies, the Ataxin-2/TDP-43/RNA complex may define a critical new target for therapeutic intervention in disease.

PolyQ repeat lengths in Ataxin-2 exceeding 34 cause SCA2. Perplexingly, if the expanded trinucleotide repeats that encode the polyQ tract are not comprised of pure CAG, but rather interrupted with CAA (also encoding glutamine), evidence suggests patients are more likely to present with levo-dopa responsive parkinsonism than classic spinocerebellar ataxia. Consistent with this connection to PD, we have observed Ataxin-2 mislocalization in some PD cases (M.P.H and A.D.G. unpublished observations). Our studies now indicate that intermediate-length Ataxin-2 polyQ repeat expansions confer genetic risk for ALS. These findings are consistent with a model in which the Ataxin-2 repeat expansion is dominant, as has been observed in all the SCAs and most polyQ diseases. How then do different alterations in a single gene, ATXN2, contribute to at least three distinct clinical presentations (SCA2, parkinsonism, and ALS)? Long polyQ repeats in Ataxin-2 have been shown to increase aggregation whereas smaller repeat expansions do not. Aggregated Ataxin-2 could have toxic gain-of-function properties that cerebellar Purkinje neurons are particularly sensitive to, resulting in SCA2. Intermediate polyQ expansions are not predicted to be aggregation-prone and therefore could function to bring TDP-43 to its toxic location in the cytoplasm, where it is perhaps more deleterious to motor neurons, resulting in ALS. Our studies show that stress, which may occur normally with age, promotes the interaction between TDP-43 and Ataxin-2, with the interaction more severe with intermediate length polyQ repeat expansions (see Fig. 6). Additionally, polyQ expansions of different lengths could alter in different ways the protein-protein and/or protein-RNA complexes with which Ataxin-2 normally associates. For example, polyQ expansions in another Ataxin protein, Ataxin-1, which underlies spinocerebellar ataxia 1 (SCA1), shift the balance of Ataxin-1 from one complex containing Capicua to another complex containing RBM17, resulting in both gain- and loss-of-function interactions mediated by the same mutation.

More globally, intermediate-length Ataxin-2 polyQ expansions could also strain the cellular proteostasis machinery akin to other disease situations in a way that favors cytoplasmic accumulation and aggregation of TDP-43. Intuitively, this predicts a situation that might not be simply limited to Ataxin-2; that is, expansions in other polyQ repeat containing proteins may also contribute to TDP-43
pathogenesis. Consistent with this idea, recent reports suggest TDP-43 pathology in additional polyQ diseases, including SCA3 and Huntington’s disease\(^{15,16}\). Moreover, in *Drosophila*, Ataxin-2 can interact genetically with Ataxin-1\(^{13}\) and Ataxin-3\(^{17}\) to modify degenerative effects. Given our findings of a critical role in ALS, this might be just the tip of an iceberg for Ataxin-2, which could contribute to the pathogenesis of many other diseases involving TDP-43. As the list of TDP-43 proteinopathies expands (for example, up to ~50% of Alzheimer’s disease cases are characterized by altered TDP-43\(^{18}\)), it will be imperative to define the potential role of Ataxin-2. Determining which subsets of ALS cases, as well as other TDP-43 proteinopathies (e.g. FTLD-TDP, see Fig. S10) involve Ataxin-2 may facilitate further stratifying disease cases, which will ultimately aid the development of effective therapeutic approaches. On the other hand, if this interaction contributes broadly, its importance as a therapeutic target becomes even more urgent.

**Supplementary Notes**

36. Mackenzie, I.R. et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann Neurol* **61**, 427-34 (2007).
37. Tan, C.F. et al. TDP-43 immunoreactivity in neuronal inclusions in familial amyotrophic lateral sclerosis with or without SOD1 gene mutation. *Acta Neuropathol* **113**, 535-42 (2007).
38. Neumann, M. et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* **314**, 130-3 (2006).
39. Igaz, L.M. et al. Enrichment of C-terminal fragments in TAR DNA-binding protein-43 cytoplasmic inclusions in brain but not in spinal cord of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Am J Pathol* **173**, 182-94 (2008).
40. Infante, J. et al. Spinocerebellar ataxia type 2 with Levodopa-responsive parkinsonism culminating in motor neuron disease. *Mov Disord* **19**, 848-52 (2004).
41. Nanetti, L. et al. Rare association of motor neuron disease and spinocerebellar ataxia type 2 (SCA2): a new case and review of the literature. *J Neurol* (2009).
42. Imbert, G. et al. Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nat Genet* **14**, 285-91 (1996).
43. Lorenzetti, D., Bohlega, S. & Zoghbi, H.Y. The expansion of the CAG repeat in ataxin-2 is a frequent cause of autosomal dominant spinocerebellar ataxia. *Neurology* **49**, 1009-13 (1997).
44. Pulst, S.M. et al. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. *Nat Genet* **14**, 269-76 (1996).
45. Sanpei, K. et al. Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nat Genet* **14**, 277-84 (1996).
46. Payami, H. et al. SCA2 may present as levodopa-responsive parkinsonism. *Mov Disord* **18**, 425-9 (2003).
47. Huynh, D.P., Yang, H.T., Vakharia, H., Nguyen, D. & Pulst, S.M. Expansion of the polyQ repeat in ataxin-2 alters its Golgi localization, disrupts the Golgi complex and causes cell death. *Hum Mol Genet* **12**, 1485-96 (2003).
48. Al-Ramahi, I. et al. dAtaxin-2 mediates expanded Ataxin-1-induced neurodegeneration in a Drosophila model of SCA1. *PLoS Genet* **3**, e234 (2007).
49. Gidalevitz, T., Ben-Zvi, A., Ho, K.H., Brignull, H.R. & Morimoto, R.I. Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* **311**, 1471-4 (2006).
50. Schwab, C., Arai, T., Hasegawa, M., Yu, S. & McGeer, P.L. Colocalization of transactivation-responsive DNA-binding protein 43 and huntingtin in inclusions of Huntington disease. *J Neuropathol Exp Neurol* **67**, 1159-65 (2008).
51. Tan, C.F. et al. Selective occurrence of TDP-43-immunoreactive inclusions in the lower motor neurons in Machado-Joseph disease. *Acta Neuropathol* **118**, 553-60 (2009).

52. Lessing, D. & Bonini, N.M. Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in Drosophila. *PLoS Biol* **6**, e29 (2008).

53. Arai, T. et al. Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. *Acta Neuropathol* **117**, 125-36 (2009).
Supplementary Figure S1. Intermediate-length Ataxin-2 polyglutamine expansions increase risk for ALS. Schematic of Ataxin-2 protein containing a polyQ tract (yellow). Normally, this polyQ tract is 22Qs (although can be variable (see Fig. 5). Long polyQ expansions (>34Q) cause SCA2 (ref. 44). We found that intermediate-length polyQ expansions in Ataxin-2 (27-33Q) are associated with increased risk for ALS (see Fig. 5 and Table 1).
Supplementary Figure S2. TDP-43 expression levels in transgenic *Drosophila* lines. **a)** Immunoblot showing the level of expression of TDP-43 for flies shown in Fig 2a. * is a non-specific band detected by rabbit anti-TDP-43 polyclonal antibody. Quantitation was performed on 4 independent experiments, normalizing to tubulin. Genotypes: *gmr-GAL4(YH3)* in trans to *UAS-YFP* (control), *UAS-TDP-43(M)* or *UAS-TDP-43-YFP(S)*. (M) and (S) refer to moderate and strong TDP-43 expression levels, respectively. **b)** Immunoblot of transgenic lines used in Fig. 2b showing that the TDP-43 WT and Q331K proteins are expressed at similar levels. Quantitation was performed on 4 independent experiments, normalizing to tubulin. TDP-43.Q331K causes a more severe loss of motility than the WT protein at the same level of expression (see Fig. 2b). Genotypes: *D42-GAL4* in trans to +, *UAS-TDP-43* or *UAS-TDP-43.Q331K*. 
Supplementary Figure S3. The effect of dAtx2 on the level of TDP-43 protein cannot account for the enhanced degeneration. a) Upregulation of dAtx2 with the dAtx2<sup>EP3145</sup> (dAtx2<sup>EP</sup>) allele together with TDP-43 causes dramatically enhanced external eye degeneration with internal collapse of the retina. This effect is more severe than upon expression of TDP-43 on its own at a level of protein expression in the range as that due to the effect of dAtx2<sup>EP</sup>. To achieve an effect of TDP-43 like that of the situation with dAtx2<sup>EP</sup>, we generated transgenic lines that expressed two copies of the TDP-43 transgene (2xTDP-43). We confirmed a similar finding with other transgenic lines of TDP-43 that express at basal levels that are higher (TDP-43-YFP, not shown). Top, external eyes; bottom, retinal sections of (left) TDP-43 with dAtx2<sup>EP</sup>, and (right) 2xTDP-43. b) Immunoblot of the levels of TDP-43 protein in the two situations. c) Quantitation was performed on four independent crosses and immunoblots, normalizing to tubulin. Genotypes, TDP-43/dAtx<sup>EP</sup> is UAS-TDP-43/+;gmr-GAL4(YH3)/dAtx2<sup>EP3145</sup>. 2xTDP-43 flies are genotype UAS-TDP-43/UAS-TDP-43; gmr-GAL4(YH3)/+. 
**Supplementary Figure S4.** dAtaxin-2 does not modulate the GAL4-UAS expression system, and TDP-43 toxicity is not modulated by Hsp70 or SCA3-Q27 in *Drosophila*. 

**a)** Western immunoblot to detect β-galactosidase protein levels in flies with down-regulated or up-regulated dAtx-2 levels (25°C). Genotypes of lacZ lanes are (dAtx2<sup>X1</sup>) gmr-GAL4 UAS-lacZ in trans to dAtx2<sup>X1</sup>, (+) gmr-GAL4 UAS-lacZ in trans to +, and (dAtx2<sup>EP3145</sup>) gmr-GAL4 UAS-lacZ in trans to dAtx2<sup>EP3145</sup>, the upregulation allele of dAtx2. + only lane is gmr-GAL4/+.

**b)** Hsp70 and SCA3-Q27 do not modulate TDP-43 toxicity, shown with a strong (S) TDP-43 phenotype. External eyes and internal retinal sections of flies expressing (left) TDP-43-YFP alone, (middle) TDP-43-YFP together with Hsp70, and (right) TDP-43-YFP together with SCA3-Q27. Genotypes: (left) UAS-TDP-43-YFP(S)/+; gmr-GAL4(YH3)/+, (middle) UAS-TDP-43-YFP(S)/UAS-Hsp70; gmr-GAL4(YH3)/+, and (left) UAS-TDP-43-YFP(S)/UAS-SCA3-Q27; gmr-GAL4(YH3)/+. 

**c)** Hsp70 and SCA3-Q27 do not modulate TDP-43 toxicity, shown with a moderate (M) TDP-43 phenotype. External eyes and internal retinal sections of flies expressing (left) TDP-43 alone, (middle) TDP-43 together with Hsp70, and (right) TDP-43 together with SCA3-Q27. Genotypes: (left) UAS-TDP-43(M)/+; gmr-GAL4(YH3)/+, (middle) UAS-TDP-43(M)/UAS-Hsp70; gmr-GAL4(YH3)/+, and (left) UAS-TDP-43(M)/UAS-SCA3-Q27; gmr-GAL4(YH3)/+.
Supplementary Figure S5. TDP-43 and Pbp1 physically associate in yeast cells. **a)** Yeast cells co-expressing CFP-tagged Pbp1 and YFP-tagged TDP-43. CFP-Pbp1 and TDP-43-YFP show accumulations in yeast that frequently co-localize (arrows) in the cytoplasm. Scale bar is 1 µm. **b)** Co-immunoprecipitation assays in yeast. Yeast cells co-transformed with untagged TDP-43 and either CFP-Pbp1 or CFP alone were lysed and subjected to immunoprecipitation with anti-GFP antibody (also detects CFP), then subjected to immunoblotting with anti-TDP43. CFP-Pbp1 immunoprecipitated with TDP-43, but CFP did not.
Supplementary Figure S6. Specificity of TDP-43/Ataxin-2 interaction and dependence on RNA. a) HEK293T cells were transfected with expression constructs encoding YFP, TDP-43-YFP, or TDP-43ΔNLS-YFP (NLS mutant that localizes the protein to the cytoplasm). Protein was immunoprecipitated with anti-GFP antibody (detects YFP), and then subjected to immunoblotting with anti-Ataxin-3 to detect endogenous Ataxin-3. Whereas TDP-43 interacts with Ataxin-2 (b,c and Fig. 3), it does not associate with Ataxin-3. (b,c) To rule out potential effects of five RRM mutations on protein-protein interactions or TDP-43 stability, we performed several additional control experiments. We tested single, double, and triple mutants within the RRMs of TDP-43 and found that even single mutations markedly diminished the interaction with Ataxin-2 (b), with double and triple mutations abolishing the interaction completely (c). We also found that an ALS-linked TDP-43 mutant, Q331K, interacted with Ataxin-2, by co-immunoprecipitation (c).
Supplementary Figure S7. Ataxin-3 localization in ALS. The localization of another polyQ protein, Ataxin-3, in ALS spinal cord neurons was unaffected (a,b). Ataxin-3 localized properly to the nucleus (arrows). Interestingly, it was not expressed, or expressed at very low levels, in motor neurons of control (not shown) and ALS spinal cords (a, arrowheads).
Supplementary Figure S8. PolyQ repeat expansions increase Ataxin-2 stability and enhance TDP-43 mislocalization. 

a) To determine the effect of intermediate-length polyQ expansions on Ataxin-2, Ataxin-2 protein stability and steady state levels were determined from control (n=4, all with Ataxin-2 polyQ lengths of 22) and ALS patient-derived cells with intermediate-length polyQ expansions (n=4, Ataxin-2 polyQ lengths 24, 27, 29, and 31) lymphoblastoid cell lines. Although steady-state levels of Ataxin-2 were comparable between control and intermediate-length polyQ repeat cells, cycloheximide treatment revealed an increase in stability of Ataxin-2 with intermediate-length repeat expansions compared to Ataxin-2 with normal polyQ length 

b) Quantitation of Ataxin-2 stability. 

c) PolyQ expansions in Ataxin-2 enhanced its interaction with TDP-43. HEK293T cells were co-transfected with YFP alone or TDP-43-YFP and Ataxin-2 constructs harboring polyQ lengths of 22, 31, or 39. TDP-43-YFP immunoprecipitated endogenous Ataxin-2 (long exposure). Top row of immunoblot shows TDP-43-YFP immunoprecipitating more Ataxin-2 with longer polyQ than Ataxin-2 22Q, despite Ataxin-2 39Q being expressed at slightly lower levels (row 3, Ataxin-2 Input). 

d-e) Functional interaction between TDP-43 and polyQ-expanded Ataxin-2 in patient-derived lymphoblastoid cells. 

d) Under normal conditions, TDP-43 was localized to the nucleus and Ataxin-2 in the cytoplasm in both control cells and cells harboring Ataxin-2 polyQ repeat expansions. Following 1-hour heat shock at 44°C, Ataxin-2 coalesced into multiple discreet cytoplasmic foci. By blinded analysis, we observed a significant increase in the number of cells with TDP-43 mislocalized to the cytoplasm in polyQ-expanded Ataxin-2 cases vs. controls (arrows). 

e) Quantitation of TDP-43 mislocalization.
Supplementary Figure S9. Transfected Ataxin-2 co-immunoprecipitates with transfected TDP-43-YFP. HEK293T cells were transfected with an expression construct encoding TDP-43-YFP and expression constructs for Ataxin-2 with 22Q, 31Q, or 39Q. Protein was immunoprecipitated with anti-Ataxin-2 antibody, and then subjected to immunoblotting with anti-GFP (detects YFP).
Supplementary Figure S10. TDP-43 localization in SCA2 and Ataxin-2 localization in FTLD-TDP. a) Control cerebellum showing normal nuclear TDP-43 staining in Purkinje cells (arrows). b) SCA2 cerebellar Purkinje neurons. TDP-43 immunoreactivity is concentrated in the nucleus, but also extrudes into the dendritic arbor, with some dendritic branches displaying the corkscrew shape typical of neurodegenerative processes (arrowheads). c) SCA2 brainstem motor neuron in the hypoglossus nucleus. TDP-43 aggregates protrude from the nucleus into the cytoplasm (arrow). d) SCA2 brainstem abducens nucleus. A TDP-43 aggregate (arrow) remains in the neuropil between motor neurons. e) SCA2 brainstem locus coeruleus neuron. Thread-like inclusion along the nuclear membrane and throughout a neurite (arrows). f-i), Ataxin-2 immunostaining of FTLD-TDP temporal lobe neurons showing examples of both diffuse (f,g) as well as distinct Ataxin-2 cytoplasmic accumulations (h,i), similar to ALS. j-l) Double immunofluorescence revealed several instances where round TDP-43 (j) and Ataxin-2 (k) co-localized in the same cytoplasmic inclusion (l). Note that the two proteins co-localize in round (arrow) but not skein-like (arrowhead) inclusions. * indicates TDP-43 is localized to the nucleus. Scale bar is 10 µm for k-o; 2.5 µm for p-v.
Supplementary Figure S11. Localization of Ataxin-2 in spinal cord neurons of a control. Immunohistochemistry to detect Ataxin-2 shows cytoplasmic localization. Scale bar is 5 µm.