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Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1

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Supplementary Figure Legends

Figure S1. Validation of RBM17 antibody.
(a) Western blot of HEK293T cell extracts transiently transfected with the siRNA targeting human RBM17 or Lamin A/C (negative control). The RBM17 anti-serum recognized two bands (marked by arrows), both of which were strongly reduced by the RBM17 siRNA, but not by the Lamin A/C siRNA. Two independent experiments were performed and GAPDH was used as a loading control. Preimmune serum did not recognize RBM17 protein.
(b) RBM17 protein is phosphorylated. Upper band of RBM17 protein was abolished when HEK293T cell extracts were treated with Lambda phosphatase.

Figure S2. Enhanced interaction of RBM17 and ATXN1 depends on S776-phosphorylation.
(a) Representative westerns of enhanced interaction of RBM17 and ATXN1 depending on S776-phosphorylation. Note that wild-type ATXN1 and ATXN1-S776D strongly, but not ATXN1-S776A, interacted with RBM17 (red arrow). Also note that ATXN1-S776D showed much stronger interaction with RBM17 than wild-type ATXN1. In addition, RBM17 expression in total cell lysates was increased when cotransfected with ATXN1 (blue arrow) likely via a physical interaction. Co-affinity purification (co-AP) assays in all figures were performed in HEK293T cells unless otherwise mentioned.
(b) Normalized levels of co-affinity purified myc-RBM17. Mean relative levels (GST-ATXN1[30Q]-S776=100%) and standard error are shown (n=5, *P=0.033, **P=0.025).
(c) Normalized levels of myc-RBM17 expression in HEK293T cell extracts. Mean relative levels (GST-empty vector=100%) and standard error are shown (n=5, *P<0.01, **P=0.025, ***P<0.001).

Figure S3. Mapping of the ATXN1-RBM17 interaction domains.
(a,b) The C-terminal region (amino acids 695-816, marked by red line) of ATXN1 including the phosphorylated S776 residue is required for RBM17 binding. Schematic representation of mapping the RBM17-interacting region by serial deletions of the ATXN1 constructs (a).
(c,d) The C-terminal region (amino acids 300-401, marked by red line) of RBM17 including the RRM motif is required for ATXN1 interaction. Schematic representation of mapping the ATXN1-interacting sequence by serial deletions of the RBM17 constructs (c).

Figure S4. Enhanced interaction of RBM17 and ATXN1 depends on polyglutamine expansion.

(a) Representative westerns of enhanced interaction of RBM17 and ATXN1 depending on glutamine-tract expansion. Note that RBM17 expression in total cell extracts was also increased depending on ATXN1's glutamine tract expansion.
(b) Normalized levels of co-affinity purified myc-RBM17. Mean relative levels (GST-ATXN1 FL[2Q]=100%) and standard error are shown (n=4, *P<0.02, **P<0.0014).
(c) Normalized levels of myc-RBM17 expression in HEK293T cell extracts. Mean relative levels (GST-empty vector=100%) and standard error are shown (n=4, *$P<1.6x10^{-6}$).
(d) Normalized levels of myc-RBM17 expression in HEK293T cell extracts. Mean relative levels (GST-ATXN1 FL[2Q]=100%) and standard error are shown (n=4, *P<0.002).

Figure S5. Polyglutamine-expansion in ATXN1 does not affect its phosphorylation at S776.
(a) Representative westerns of ATXN1-S776 phosphorylation level depending on polyglutamine expansion using HEK293T cells. Flag-ATXN1[82Q]-S776A was used as a negative control for S776-phosphorylation. Anti-PN1168 antibody was used for detecting specific phosphorylation at S776 of ATXN1.
(b) Normalized levels of ATXN1 phosphorylation at residue S776. Mean relative levels (Flag-ATXN1 FL[2Q]=100%) and standard error are shown (n=4, n.s., non-significant).

Figure S6. Expression of ATXN1 native complexes in cerebellar extracts of SCA1 transgenic mice.
Expression of CIC, ATXN1, and RBM17 was remained high in cerebellar extracts of 18-week-old wild-type (FVB) and SCA1 transgenic (ATXN1[82Q], B05 line) homozygote mice. GAPDH was used as a loading control.

Figure S7. RBM17 contributes to polyglutamine-expanded ATXN1 toxicity in the *Drosophila* eye.
Scanning electron microscopy (SEM) of adult *Drosophila* eyes. A heterozygous loss of one allele of *dRBM17* suppressed ATXN1[82Q]-mediated ommatidial disorganization (a), while overexpression of dRBM17 worsened the ommatidial abnormalities induced by ATXN1[82Q] but not by ATXN1[30Q] (b, c). Flies were raised at 30°C (a) or 25°C (b-e), and genotypes are the following: (a) *GMR-Gal4>UAS-ATXN1[82Q]; Df(2Lh)D1/+*, (b) *GMR-Gal4>UAS-ATXN1[82Q]; UAS-dRBM17*, (c) *GMR-Gal4>UAS-ATXN1[30Q]; UAS-dRBM17*, (d) *GMR-Gal4>UAS-hRBM17; UAS-GFP*, and (e) *GMR-Gal4>UAS-dRBM17; UAS-GFP*. SEM images in the left side were magnified into the right side of each panel. Scale bars in low and high magnification images in (e) are 100μm or 10μm, respectively. More than 50 adult flies examined at day 2 after eclosion showed similar phenotype. Additional data with controls are available in Fig. 3.

Figure S8. RBM17 and ATXN1 interact in the large protein complexes.
(a) Elution profile of endogenous CIC, ATXN1, and RBM17 proteins in HEK293T cells using gel-filtration chromatography. Representative westerns of 1.0ml gel-filtration fractions of human HEK293T cells analyzed for CIC, ATXN1, and RBM17. RBM17 elutes in a broad range with two peak fractions of 8-10 (red boxes, large protein complexes) and 12-13 (blue boxes, small protein complexes). Elution profile of RBM17 covers those of ATXN1 and CIC. CIC-L and CIC-S are the long or short isoforms of Capicua (CIC), respectively. The column void volume (V_o), gel-filtration standards thyroglobulin (669kDa) and ADH (150kDa), and elution volume

(ml) of each collected fraction are indicated. All figures of gel-filtration chromatography have similar configurations unless otherwise indicated. Ex, extract. n.s., non-specific. Bottom panel shows the elution profile for RBM17 protein plotted as the average percent protein (± standard error) in each fraction from six independent extracts.

(b) RBM17 and ATXN1 interacted in the large protein complexes. Representative westerns of gel-filtration fractions of HEK293T cells transfected with Flag-ATXN1[82Q] analyzed for Flag-ATXN1[82Q] and RBM17. Red and blue boxes represent large or small native protein complexes containing a peak fraction of RBM17 protein, respectively. Monoclonal anti-HA agarose conjugated beads and anti-FLAG M2 affinity gel were used as a negative or positive control for immunoprecipitation (IP), respectively. Ex, extract. Note that RBM17 co-immunoprecipitated with ATXN1 only in fractions containing large complexes (red box at the bottom row). Bottom panel illustrates the co-immunoprecipitated RBM17 plotted as the average percent protein (± standard error) in each fraction from five independent experiments. Co-immunoprecipitated RBM17 signal in each fraction is divided by the total signal in all the fractions to determine the percentages.

Figure S9. ATXN1 is not differentially phosphorylated at residue S776 between the large and small protein complexes.
Similar elution profiles of S776-phosphorylated ATXN1[82Q] and total ATXN1[82Q] proteins. Representative westerns of 1.0ml gel-filtration fractions of HEK293T cells transfected with Flag-ATXN1[82Q] analyzed for ATXN1. PN1168 antibody is specific to phosphorylated S776 of ATXN1. Ex, extract.

Figure S10. Atxn1 is not essential for RBM17 incorporation into the large protein complexes.

Representative westerns of 1.0ml gel-filtration fractions of *Atxn1* knock-out mouse cerebellum analyzed for CIC, Atxn1, and RBM17. RBM17 elution profile in *Atxn1* knock-out mice was not much different from wild-type control. Bottom panel illustrates the elution profiles for RBM17 protein plotted as the average percent protein (± standard error) in each fraction from four independent cerebellar extracts.

Figure S11. RBM17 and CIC compete with each other.
(a) CIC competed with RBM17 for interaction with ATXN1. The interaction of myc-tagged ATXN1[82Q] with GST-tagged RBM17 was strongly decreased in the presence of HA-tagged mouse CIC-S (arrow, compare lane 3 with lane 4 at the second row). Note that RBM17 co-affinity purified ATXN1 (arrow), but not HA-tagged CIC-S (bracket), suggesting that RBM17 and CIC are present in different complexes.
(b) RBM17 competed with CIC for the ATXN1 interaction. The interaction of myc-ATXN1[82Q] with HA-CIC-S was strongly decreased in the presence of GST-RBM17 (arrow, compare lane 3 with lane 4 at the second row). Note that CIC co-immunoprecipitated ATXN1 (arrow), but not GST-tagged RBM17 (bracket), suggesting that CIC and RBM17 are present in different complexes.

Figure S12. Polyglutamine-expanded ATXN1 nuclear inclusion formation is enhanced by CIC, but not by RBM17.

(a) RBM17 is expressed in nuclear speckles. HeLa cells transfected with myc-RBM17 were used for confocal microscopy analysis using anti-myc antibody (red) and Toto3 (blue, nuclear marker). RBM17 is known to co-localize with SR proteins in nuclear speckles (marked by arrows) (Sampath et al., 2003).
(b) HeLa cells were transfected with GFP-ATXN1[82Q] and HA-CIC-S. Confocal microscopy analysis was performed 48 hours after transfection. In cells transfected with GFP-ATXN1[82Q] alone (GFP, green arrows), ATXN1[82Q] formed small nuclear inclusions. However, CIC strongly enhanced formation of ATXN1[82Q] nuclear inclusions and co-localized with ATXN1[82Q] in the nuclear inclusions of double transfected cells (yellow arrows). In contrast, CIC was distributed to the nucleoplasm in cells transfected with CIC alone (anti-HA, red arrows).
(c) HeLa cells were transfected with GFP-ATXN1[82Q] and myc-RBM17. In cells transfected with RBM17 alone (anti-myc, red arrows), RBM17 was distributed to the nucleoplasm as well as cytoplasm when extensively overexpressed. RBM17 may slightly decrease ATXN1[82Q] nuclear inclusion formation when it was highly co-expressed with ATXN1[82Q] (yellow arrows). Note that RBM17 did not co-localize with ATXN1[82Q] in the nuclear inclusions, suggesting that the soluble RBM17/ATXN1[82Q] might mediate toxic interaction. Toto3 (blue) was used as a nuclear marker.

Sampath, J. et al. Human SPF45, a splicing factor, has limited expression in normal tissues, is overexpressed in many tumors, and can confer a multidrug-resistant phenotype to cells. *Am J Pathol* **163**, 1781-1790 (2003).

Figure S13. Polyglutamine expansion causes the decreased expression of CIC and wild-type Atxn1 in SCA1 knock-in mice.
Expression of CIC and wild-type Atxn1 was reduced in SCA1 knock-in (*Atxn1 154Q/+*) mice compared to wild-type (*Atxn1 +/+*) control. Right panels show the normalized levels of CIC-S, wild-type Atxn1 (Atxn1[2Q]), and RBM17. Mean relative levels (wt=100%) and standard error are shown (n=6, t test *$P<5x10^{-4}$, **$P<4.4x10^{-5}$, n.s., non-significant). GAPDH was used as a loading control.

Figure S14. Formation or maintenance of large ATXN1/RBM17 complexes is dependent on RNA.
Elution profiles of CIC, ATXN1[82Q], and RBM17 proteins in the absence (a) or the presence (b) of RNase in Neuro-2a cells stably transfected with GFP-ATXN1[82Q]. RBM17 and ATXN1[82Q] proteins eluted from fraction 8 (red box) were strongly decreased in the presence of RNase (compare red boxes in a and b). RBM17 elution at fractions 15-17 strongly increased in the presence of RNase (compare blue boxes in a and b).

Figure S15. Polyglutamine expansion in ATXN1 does not affect its self-association with wild-type ATXN1.
(a) Representative westerns of ATXN1's self-association depending on glutamine-tract expansion.
(b) Normalized levels of co-affinity purified Flag-ATXN1[30Q]. Mean relative levels (GST-ATXN1 FL[2Q]=100%) and standard error are shown (n=4, n.s., non-significant).

(c) Normalized levels of Flag-ATXN1[30Q] expression in HEK293T cell extracts. Mean relative levels (GST-empty vector=100%) and standard error are shown (n=4, *P<0.002). Expression level of wild-type ATXN1 in total cell lysates was increased likely via a self-association (blue arrow in a).

Figure S16. Wild-type Atxn1 inhibits incorporation of polyglutamine-expanded Atxn1 into the large protein complexes.

(a) Expression of CIC, Atxn1[154Q], and RBM17 was analyzed using cerebellar extracts of *Atxn1 154Q/+* and *Atxn1 154Q/-* mice. Right panel shows the normalized levels of CIC-S, Atxn1[154Q], and RBM17 expression. Mean relative levels (*Atxn1 154Q/+* mice=100%) and standard error are shown (n=6, n.s., non-significant). GAPDH was used as a loading control.
(b) Atxn1 expression in the nuclei of Purkinje cells of 9- or 21-week-old mice from either *Atxn1 154Q/+* or *Atxn1 154Q/-*.
(c) Elution profiles of CIC, Atxn1[154Q], and RBM17 in *Atxn1 154Q/-* mouse cerebellum. Westerns of 1.0ml gel-filtration fractions of *Atxn1 154Q/-* mouse cerebellum analyzed for CIC, Atxn1[154Q], and RBM17. RBM17 elutes in a broad range with at least two peak fractions of 8-10 (red box, large protein complexes) and 12-13 (blue box, small protein complexes). Bottom panel shows the elution profiles for RBM17 protein plotted as the average percent protein (± standard error) in each fraction from three independent cerebellar extracts. Ex, extract.
(d) Elution profiles of Atxn1[154Q] protein in *Atxn1 154Q/-* mouse cerebellum compared to *Atxn1 154Q/+* mice. Representative westerns of 1.0ml gel-filtration fractions analyzed for Atxn1[154Q]. Graph in the middle panel shows the elution profiles for Atxn1[154Q] protein plotted as the average percent protein (± standard error) in each fraction (n=5 for *Atxn1 154Q/+* mice, n=3 for *Atxn1 154Q/-* mice). Bottom panel shows the relative ratio of Atxn1[154Q] incorporation into fractions 10-11 to fraction 13, showing dramatically increased Atxn1[154Q] incorporation into fractions 10-11 in *Atxn1 154Q/-* mice.