Supplementary figure legends

Supplementary result 1. *Boat* transcripts are abundantly expressed in different human tissues and cell lines. Dot blot membrane consisting of normalized loadings of poly A+ RNA from 72 different indicated human tissues and different control RNAs and DNAs, was hybridized with ^{32}P-dCTP labeled probes corresponding to human *Boat* and *Smrt* cDNA respectively.

Supplementary result 2. Boat and ataxin-1 interact with each other in Drosophila salivary glands. (A) *Hsp70-Gal4>UAS-GFP* larvae showing the specific expression of GFP in salivary glands (SG), but not in fat bodies (FB). (B-F) Nomarski images showing the morphology of salivary glands expressing *GFP* (B), *ataxin-1(82Q)* (C), *FLAG-ataxin-1(0Q)* (D), *FLAG-Boat (*E), and *HA-Boat(NBA) (*F) larvae. For all experiments, *Hsp70-Gal4* driver was used. (G-K) Immunostaining images showing protein localization of ataxin-1 and Boat variants in salivary glands. The genotype of each tested larvae is indicated. The pressed salivary glands were immunostained with ataxin-1, Boat, or HA antibody (Texas-Red) and with SMRTER antibody (FITC, green). All tissues were counterstained with 4‘, 6-diamidino-2-phenylinodole (DAPI). The enlarged images correspond to the cells marked with arrows. FL: FLAG. The scale bar: 50μM. (L-P) Immunostaining images showing the co-localization of ataxin-1 and Boat, Boat(NBA), or ataxin-1(0Q). The pressed salivary glands were co-immunostained with ataxin-1 and Boat, HA, or FLAG antibodies. For (L, M), ataxin-1(4-197) antibody (rabbit) was used; for (N-P) ataxin-1(477-575) antibody (guinea pig) was used. RedStinger is a modified red fluorescent protein, which was used as a control.

Description and interpretation for the experiments and results

We used salivary glands for our cell biology studies because their unusually large cells allow us to visualize the localization of each protein and their interactions more easily. Accordingly, a salivary gland-specific Gal4 driver line, *HSP70-Gal4,* was used to express GFP (a control protein), ataxin-1(82Q), FLAG-ataxin-1(0Q), FLAG-Boat, and HA-Boat(NBA) respectively. Consistently with our previous observations (Tsai et al., 2004), GFP is specifically expressed in salivary glands without heat shock treatment (Supplementary result 2A). As expected, GFP-expressing salivary glands display a *wild-type* phenotype characterized by well organized tissue structure and wedge shaped cell contours (Supplementary result 2B). In contrast, ataxin-1(82Q) causes severe defects, including a significant reduction in overall tissue volume, disarray in tissue organization, and loss of visible cell-cell boundaries (Supplementary result 2C).

Somewhat unexpectedly, ataxin-1(0Q) also causes deformity to salivary glands (Supplementary result 2D), although the resulting phenotype is markedly less severe than that caused by ataxin-1(82Q). This observation indicates that ataxin-1 possesses intrinsic toxicity in fly independent of its glutamine-repeat. In contrast to the effects of both examined ataxin-1 variants, Boat- and Boat(NBA)-expressing salivary glands show little or no defective morphology (Supplementary result 2E, F), indicating that these two Boat variants are not toxic when expressed in fly.

Immunostaining of ataxin-1 or Boat-expressing salivary glands further revealed the two proteins' differences at the cellular level. Ataxin-1(82Q) forms large protein aggregates that sequester endogenous SMRTER (compare the results in Supplementary result 2G, H). This result agrees with our previous report (Tsai et al., 2004). In the case of ataxin-1(0Q), aggregates do not form. Instead, ataxin-1(0Q) displays a dispersed pattern within the nucleus (Supplementary result 2I). However, in some cells, ataxin-1(0Q) forms nuclear foci (see enlarged image). The formation of ataxin-1(0Q) nuclear foci in Drosophila salivary glands is reminiscent to its nuclear pattern found in mammalian cells (Tsai et al., 2004) (see also Figure 3B). Therefore, the formation of nuclear foci is an intrinsic property of ataxin-1 that is independent of its glutamine-repeat. Consistently, the nuclear pattern of endogenous SMRTER also overlaps with the nuclear focal pattern formed by ataxin-1(0Q), confirming our hypothesis that ataxin-1's interaction with SMRTER is independent of the glutamine-repeat in ataxin-1. The interaction between ataxin-1 and SMRTER is specific, moreover, based on our findings that Ultraspiracle, another nuclear protein, is not sequestered into ataxin-1-mediated aggregates and that SMRTER is not sequestered into nuclear aggregates formed by long glutamine-repeat alone (Supplementary result 3).

Unlike its ataxin-1 counterparts, Boat in most cases localizes to both the nucleus and the cytoplasm. However, in a few cells, Boat does form nuclear speckles, where concentrated SMRTER was also detected (Supplementary result 2J, see enlarged image). The co-localization of Boat speckles and SMRTER suggests a direct protein-protein interaction between these two proteins. In the case of the nuclear signal-tagged Boat(NBA), its dispersed nuclear pattern does not alter the distribution of SMRTER (Supplementary result 2K). The divergence between the cellular patterns of Boat and that of either form of ataxin-1 may partly explain why Boat and ataxin-1s behave differently in Drosophila salivary glands.

The protein-protein interactions that take place between Boat and ataxin-1 in mammalian cells, can be visualized in Drosophila cells as well when fly lines expressing different combinations of

ataxin-1(82Q), FLAG-ataxin-1(0Q), FLAG-Boat, or HA-Boat(NBA). In the presence of either ataxin-1(82Q) or ataxin-1(0Q), Boat translocates from the cytoplasm to the nucleus and becomes concentrated in the regions where ataxin-1(0Q)-mediated nuclear foci or ataxin-1(82Q)-mediated aggregates reside (Supplementary result 2L, M). Such dramatic changes in the cellular patterns of Boat reflect direct protein-protein interactions between Boat and the ataxin-1s. Interestingly, the opposite effect (translocation from the nucleus to the cytoplasm) was not observed for either form of ataxin-1, indicating that the ataxin-1-Boat complexes predominantly reside within the nucleus.

Boat(NBA) and ataxin-1(0Q) also localize to ataxin-1(82Q) aggregates (Supplementary 2N, O). These *in vivo* phenomena validate our findings in yeast that Boat(NBA) binds ataxin-1 (Figure 4A, E) and confirm a previous report on the self-association of ataxin-1 (Burright et al., 1997). To determine the specificity of the ataxin-1-Boat interactions, we additionally tested a fly line that expresses a modified red fluorescent protein, RedStinger (Barolo et al., 2004). Because the nuclear pattern of RedStinger does not overlap with that of ataxin-1(82Q) (Supplementary result 2P), we conclude that Boat is a specific *in vivo* binding partner of ataxin-1 and that their interaction is, in part, mediated through the NBA domain of Boat.

Supplementary result 3. Control experiments to demonstrate a specific interaction between ataxin-1 and SMRTER. Salivary gland cells isolated from *Hsp70-Gal4>UAS-ataxin-1(82Q)* larvae (A) or from *Hsp70-Gal4>UAS-HA-Q127* larvae (B) were co-immunostained with ataxin-1 (Texas-Red, red) and with Ultraspiracle (USP) (FITC, green) antibodies (A) or with HA (Texas-Red) and SMRTER (FITC) antibodies (B). The enlarged images correspond to the cells marked with arrows. *UAS-HA-Q127* is a fly line expressing a long glutamine-repeat tract (Kazemi-Esfarjani and Benzer, 2000); USP is a nuclear hormone receptor (Oro et al., 1990).

Supplementary result 4. Boat expression is specifically reduced in the Purkinje cells of *SCA1* mice. (A-E) Frozen brain sections prepared from 8-week-old *wild-type* (*FVB/N*) and $SCA1^{tg/tg}$ mice were immunostained with ataxin-1(477-575), Boat(284-412), or SMRT(972-1151) antibody (FITC, green) and NeuN antibody (Texas-Red, red). The enlarged images show the Purkinje cells of focus. The staining in the dendritic regions of normal Purkinje cells and the affected SCA1 Purkinje cells is marked with orange arrows and open arrows respectively. (F. G) Frozen brain sections prepared from 3-week-old *wild-type* and $SCA1^{tg/+}$ mice were immunostained with Boat (284-412) antibody (FITC, green) and Calbindin antibody (Texas-Red,

red). The unchanged expression of Boat in the cerebellar cells is indicated with an asterisk. The molecular cell layer, Purkinje cell layer, and granular cell layers are labeled MCL, PCL, and GCL respectively. The scale bars: 1000μM.

Supplementary result 5. Testing the cross-reactivity of Boat and ataxin-1 antibodies.

Salivary glands isolated from *Hsp7-Gal4>UAS-FLAG-ataxin-1(0Q)* larvae (A) and from *Hsp70-Gal4>UAS-FLAG-Boat* (B) larvae were co-immunostained with FLAG and guinea pig Boat (284-412) antibodies (A) or with FLAG antibody and guinea pig ataxin-1(477-575) antibodies (B) respectively. In both cases, no cross-reactivity was detected.

References

Barolo, S., Castro, B. and Posakony, J.W. (2004) New Drosophila transgenic reporters: insulated P-element vectors expressing fast-maturing RFP. *Biotechniques*, **36**, 436-440, 442.

Burright, E.N., Davidson, J.D., Duvick, L.A., Koshy, B., Zoghbi, H.Y. and Orr, H.T. (1997) Identification of a self-association region within the SCA1 gene product, ataxin-1. *Hum Mol Genet*, **6**, 513-518.

Kazemi-Esfarjani, P. and Benzer, S. (2000) Genetic suppression of polyglutamine toxicity in Drosophila. *Science*, **287**, 1837-1840.

Oro, A.E., McKeown, M. and Evans, R.M. (1990) Relationship between the product of the Drosophila ultraspiracle locus and the vertebrate retinoid X receptor. *Nature*, **347**, 298-301.

Tsai, C.C., Kao, H.Y., Mitzutani, A., Banayo, E., Rajan, H., McKeown, M. and Evans, R.M. (2004) Ataxin 1, a SCA1 neurodegenerative disorder protein, is functionally linked to the silencing mediator of retinoid and thyroid hormone receptors. *Proc Natl Acad Sci U S A*, **101**, 4047-4052.