Supplementary information

Figure S1: Anti Atx1 phospho S776 antibody staining of COS cells transfected with S-Atx1 YFP, A-Atx1-YFP and D-Atx1-YFP. COS cells were transfected with YFP tagged indicated plasmid constructs and were stained with phopshoS776 specific Atx1 antibodies (ab63376, Abcam) followed by anti rabbit IGG conjugated to Alexa 633. Left panels show YFP fluorescence, middle panels show Alexa 633 staining and right panels show merged images.

Figure S2: Expression patterns of untagged Atx1, U2AF65 and 14-3-3 proteins. Ataxin1 and its mutants, U2AF65, and 14-3-3 were expressed in COS cells after deleting fluorescent protein tags and were stained with specific antibodies (Atx1, mouse monoclonal, UC/Davis/NINDS/NIMH NeuroMab Facility, U2AF65, rabbit polyclonal: Santacruz, 14-3-3, rabbit polyclonal: Abcam) followed by FITC conjugated secondary antibodies.

Figure S3: Untagged Ataxin-1 addition decreases FRET in a dose-dependent manner. COS cells were transfected with S-Atx1-YFP and CFP-U2AF65 in the absence (control) and in the presence of increasing amounts of untagged Atx1. DNA concentration was kept constant at 400 ng by adding empty vector DNA where this was required. Corrected FRET is shown for control (200 ng S-Atx1-YFP and 200 ng CFP-U2AF65 per transfection); low (150 ng S-Atx1-YFP,200 ng CFP-U2AF65 plus 50 ng untagged S-Atx-1); med (100 ng S-Atx1-YFP , 200 ng CFP-U2AF65 plus 100 ng untagged S-Atx-1); and high (50 ng S-Atx1-YFP, 200 ng CFP-U2AF65 plus 150 ng untagged S-Atx-1) as box and whisker plots.

Figure S4: Co-localization of Atx1 mutants and CFP-14-3-3-NLS in COS cells. A) Confocal microscopic images of cells co-expressing CFP-14-3-3-NLS (left panel)and A-Atx1-YFP (middle panel) .Merged image is shown in the right panel**.** B) Confocal microscopic images of cells co-expressing CFP-14-3-3-NLS (left panel) and D-Atx1-YFP (middle panel). Merged image is shown in the right panel.

Figure S5: Correlation coefficients between fluorescent protein fusions and immunofluorescence counterstaining. A) Pearson's Correlation Coefficients for the channels used to calculate corrected FRET in Figure 5. Mean correlation coefficient (r) +/- standard error are shown below each box plot. Sample size (N, images used) from left to right are: N=4, N=4, N=4, N=4, N=10, N=5, N= 4. B) COS Cells were trasfected with YFP-Atx1 or CFP tagged 14-3-3 and U2AF65 constructs. Pearson's Correlation Coefficients was calculated for fluorescently labelled protein vs antibody- detected counterpart. C) First Mander's overlap coefficient (M1) showing the fraction of fluorescently- tagged protein that is overlapped by antibody-detected protein from the same images used in (B). N for both (B) & (C) are (left to right): N=8, N=4, N=4, N=4.

Figure S6: Mean radii distances between FRET pairs. Barcharts showing radial distance between fluorophores (R_0, nm). A) CFP-U2AF65 Vs YFP-Atx1 (wild-type, Ala and Asp substitution mutants). B) CFP- Wt-14-3-3 (orange) and CFP-14-3-3- NLS (blue) Vs YFP-

Atx1 (wild-type, Ala and Asp substitution mutants). Statistical significance bars are shown and represent results of unpaired t-tests of mean difference = 0 (* = P<0.05,** = P<0.01, *** = P<0.001). N numbers (left to right) are: (A) N=16, N=10, N=24, N=22, N=21, N=21; (B) N=15, N=12, N=22, N=26, N=22, N=14, N=19,N=15; and represent number of individual bleach events pooled from at least 4 individual cells.

Figure S7: Representative cells showing FRET and ROI intensity plots for U2AF65-CFP Vs YFP-Atx1 and mutants. 'Rainbow' look-up table (LUT)-encoded pseudocolour pre- and postbleach images of CFP-U2AF65 and YFP-Atx1 from the same representative cells in Figure 3. Line profiles across the bleach ROI show intensities of CFP and YFP before and after bleach

Figure S8: Representative cells showing FRET and ROI intensity plots for CFP-14-3-3-NLS Vs YFP-Atx1 and mutants. 'Rainbow' look-up table (LUT)-encoded pseudocolour pre- and postbleach images of CFP-14-3-3-NLS and YFP-Atx1 from the same representative cells in Figure 3. Line profiles across the bleach ROI show intensities of CFP and YFP before and after bleach.

Table S1: Expression pattern of Fluorescent fusion proteins in COS cells. YFP and CFP tagged fusion proteins were individually expressed in COS cells and analysed by confocal microscopy to determine the percentage of cells that show nuclear expression of the proteins. Results represent an average of at least 500 cells counted for each protein type.

Table S2: Co-expression pattern of 14-3-3 and Ataxin1 in COS cells. YFP tagged Atx1 and CFP tagged 14-3-3 fusion proteins were co-expressed in COS cells and analysed by confocal microscopy to determine the percentage of cells that show nuclear expression of the proteins. Results represent an average of at least 500 cells counted for each co-expression pair.

Table S3: Distances between residue 776 of Atx1 and surrounding residues of 14-3-3.