Supplemental Information

Table S1 – List of 15N-1H amino acid selective labelling schemes in association with wild-type or ataxin-3(Q13) mutants. All the resonances of residues of wild-type ataxin-3(Q13) (indicated as Wt) that could be detected and assigned are listed. The residues assigned via a combination of selective amino acid labelling and inspection of the HNCACB spectrum are highlighted in bold. The resonances of only the residues of mutants whose assignment was achieved by analysis of chemical shift perturbation with respect to the spectrum of the wild-type protein are shown.

^{15}N-aa	***Mutant***	***Detected and assigned***	***Comments***
^{15}N-Arg	Wt	**R203**, R211, **R231**, **R237**, **R251**, **R262**, **R282**, **R284**, **R285**, **R318**, R352	R188 could not be assigned unequivocally. There is potential candidate resonance
	R282H	R282, R284, R285	
	R284H	R282, R284, R285	
^{15}N-Leu	Wt	**L191**, **L196**, L199, L209, L213, L222, L229, L233, L235, L249, **L255**, L276, **L281**, L308, L326, L330, L340, L348, L355	
	L191I	L191, L196, L199	
	S256A	L255	
	R282H	L281	
^{15}N-Ile	Wt	I240, I253, I264	The NH resonances of I192 not detected in the ^{15}N-Ile HSQC spectrum
^{15}N-Val	Wt	V183, V204, V212, V344, V351	
^{15}N-Glu	Wt	**E194**, **E195**, **E210**, E214, E224, E226, E227, E239, E243, E245, E246, **E279**, **E280**, **E286**, E317, E336, E337, E349, E358	NH resonances of E201and E290 not identified
^{15}N-Gln	Wt	Q198, **Q202**, Q230, **Q238**, **Q254**, **Q258**, Q266, Q270, Q298, Q299, Q300, Q301, Q302, Q303, Q304, Q305, Q311, Q341	NH resonances of Q184 and Q185 not identified
	T207A	Q202	
	S256A	Q254, S258	
	R284H		The NH resonances of Q292, Q293, Q294, Q296, Q297 were identified but sequential assignment was impeded by overlap in the CACB plane of the HNCACB experiment
^{15}N-Ala	Wt	**A197**, A215, **A232**, A234, A247, **A252**, **A287**, A320, A325, A333, A342, A343	
	L191I	A197	

	D228E	A232	The NH resonances of A232 and A252 were close but distinguishable in the HSQC spectrum. Their $C\alpha_i/C\alpha_{i-1}$ and $C\beta_i/C\beta_{i-1}$ resonances overlap completely
	S256A	A252	
	R282H	A287	
^{15}N-Met	Wt	M221, M242, **M257**, M268, M334, M339, M346	M186 could not be detected
	L191I		No chemical shift perturbations observed as referred to the spectrum of the wt protein
	S260A	M257	
^{15}N-His	Wt	**H187**, H314	The HN resonance of H205 not detected
	T207A		No chemical shift perturbation observed with respect to the spectrum of the wt protein
^{15}N-Lys	Wt	K200, **K206**, **K283**, K356, K360, K361	NH resonance of K190 not detected K291, K295: NH resonances not assigned because of the low resolution in the C plane of the HNCACB.
	R282H	K283	
^{15}N-Tyr	Wt	**Y288**	
^{15}N-Phe	Wt	**F289**	

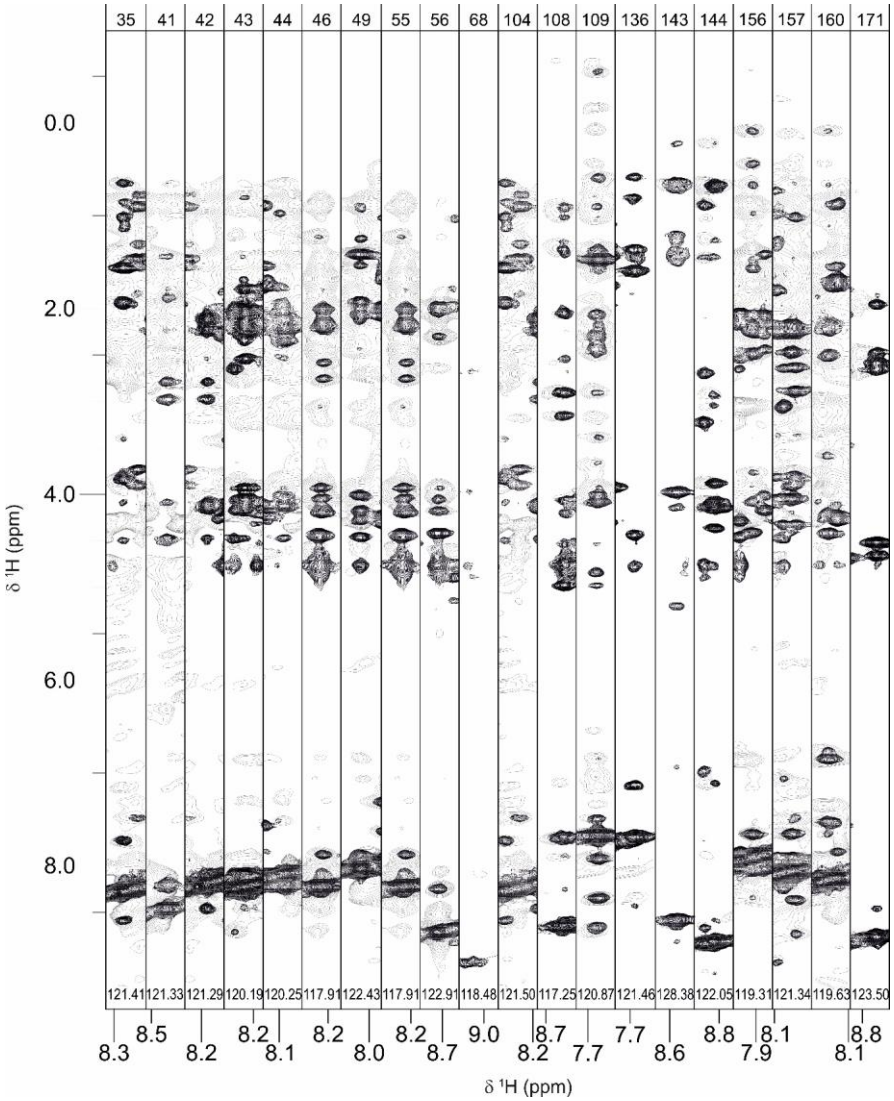

Figure S1 - NOESY-HSQC strips of josephin and ataxin-3(Q13). The strips show the H-H correlations to the HSQC resonances of josephin residues (top) that were assigned exclusively through the comparison of the spectrum of the isolated josephin domain (black) and of ataxin-3(Q13) (grey).

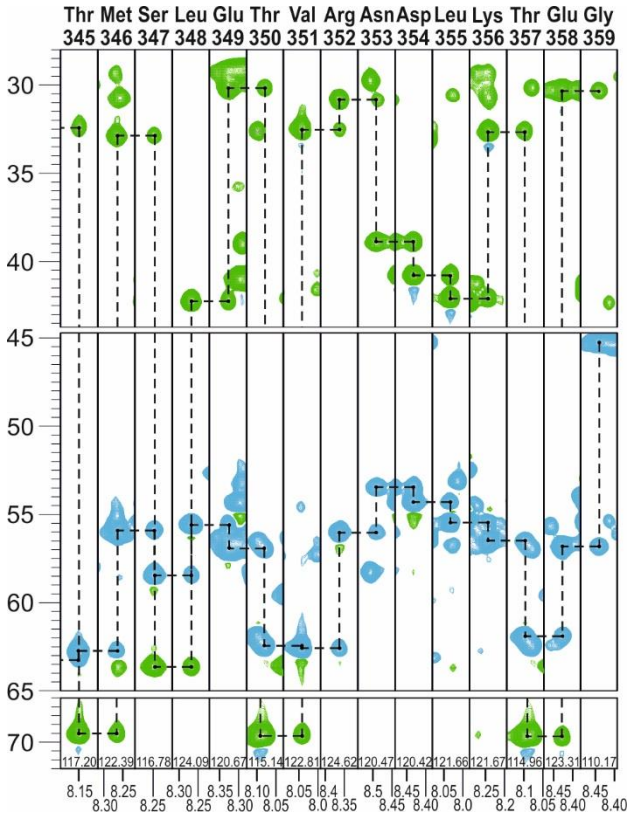

Figure S2 - Representative ^{13}C-^{1}H strips of a HNCACB experiment of ataxin-3(Q13) (residues 343-359). The β-carbons are coloured in green, the α-carbons in light blue. The correlated resonances used for the sequential assignment are connected by dashed lines.

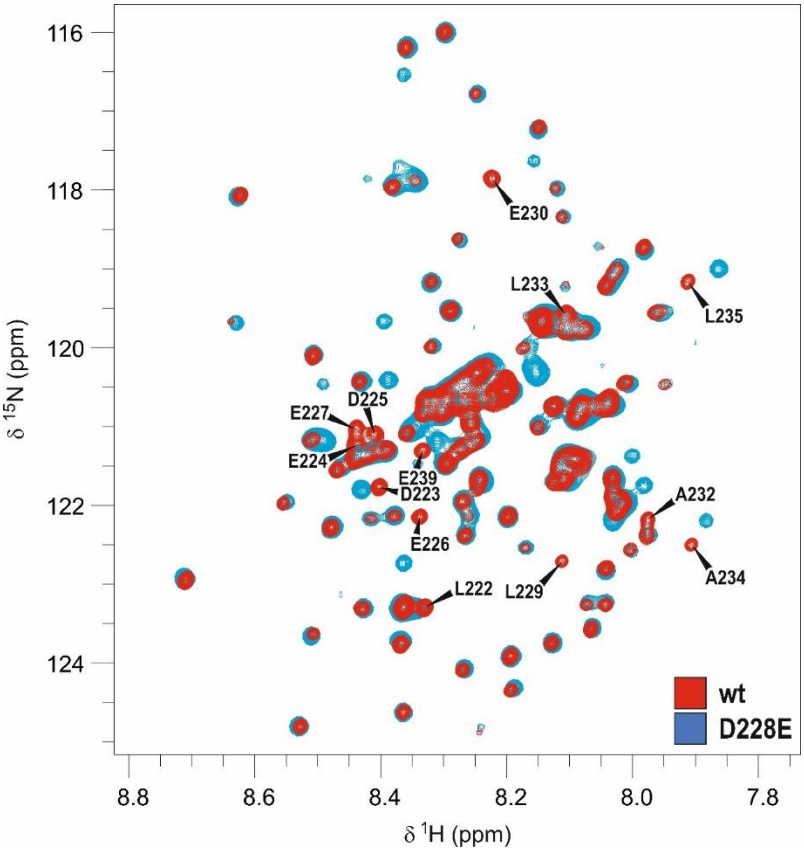

Figure S3 – Overlay of the central region of the ^{15}N-^{1}H HSQC spectra of uniformly ^{15}N labelled wild-type ataxin-3(Q13) (red) and mutant D228E (blue). The counter level was adjusted to simplify the spectral complexity and highlight most of the resonances perturbed by the mutation.

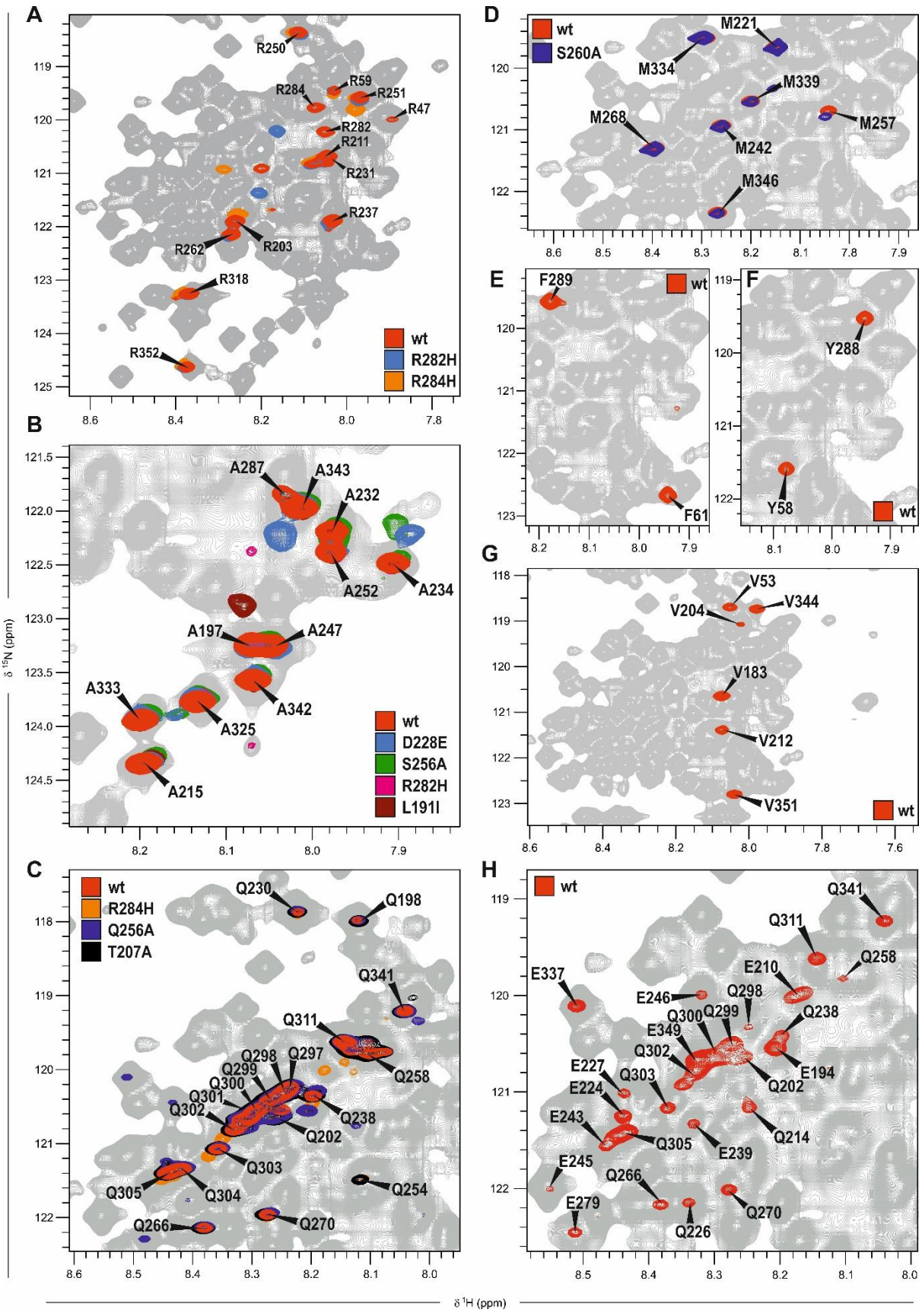

Figure S4 – Examples of ^{15}N selective amino acid labelling of wild-type and mutated ataxin-3(Q13). A: ^{15}N-arginine, B: ^{15}N-alanine, C: ^{15}N-glutamine, D: ^{15}N-methionine, E: ^{15}N-phenylalanine, F: ^{15}N-tyrosine, G: ^{15}N-valine, H: ^{15}N-glutamate.

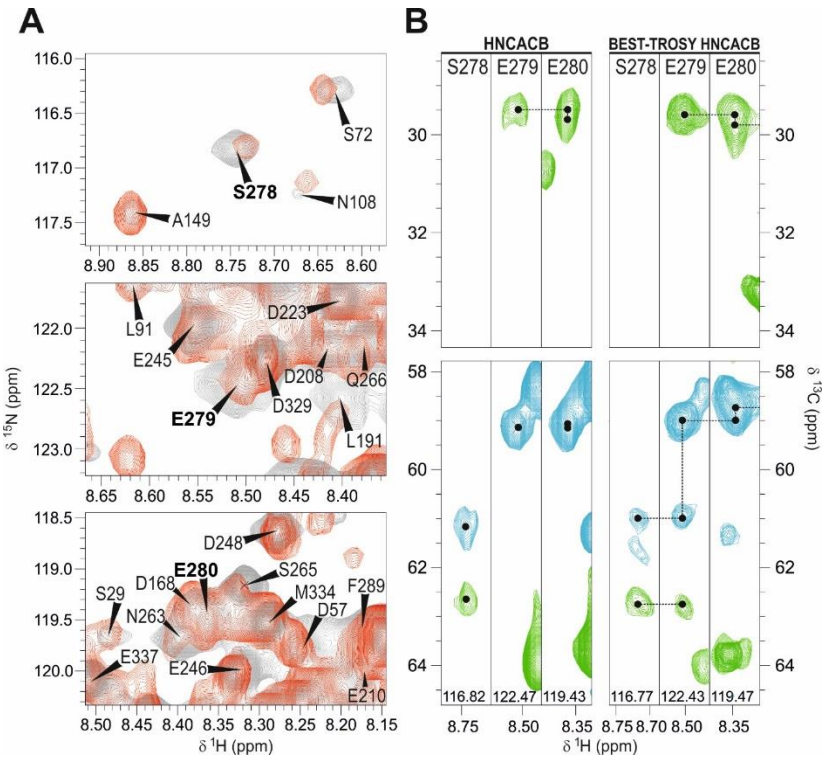

Figure S5 – Sequential assignment of residues of residues 278-280 using BEST-TROSY HNCACB. A: Areas of the ^{1}H-^{15}N BEST-TROSY HSQC (red) and conventional ^{1}H-^{15}N HSQC (grey) of ataxin-3(Q13) containing residues S278, E279, E280 (bold). B: CH strips of the HNCACB and BEST-TROSY HNCACB spectra associated with S278, E279 and E280 (Cα: light blue, Cβ: green).

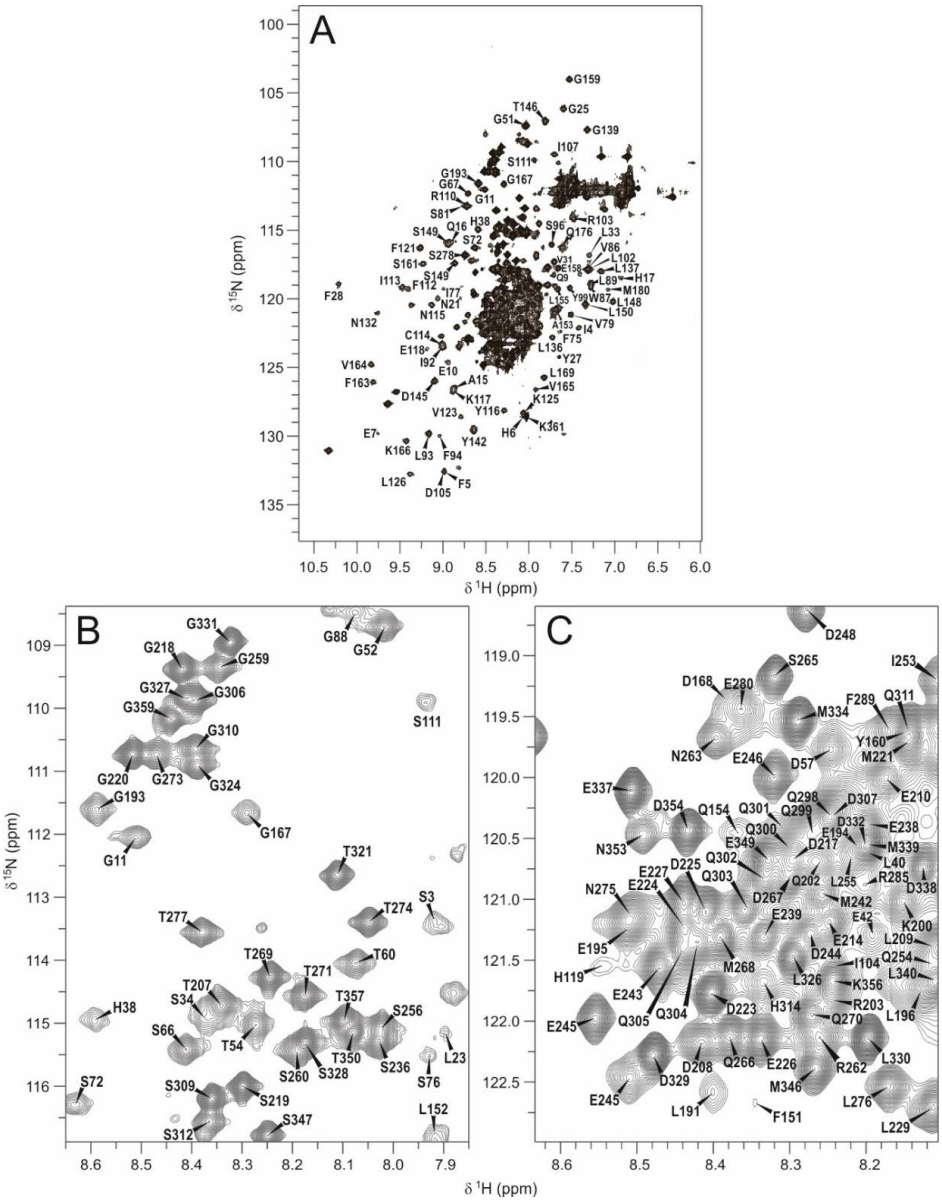

Figure S6 – ^{1}H-^{15}N HSQC spectrum of ataxin-3 and assignment of the NH resonances. A) Spectrum of ataxin-3(Q13) with the assignment of the well resolved resonances. B) Close-up of the poorly dispersed area containing glycines, threonines and serines. C) Close-up of the area containing glutamines within the polyQ tract that could be assigned, alongside with some poorly dispersed UIM residues.