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# **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.

<sup>15</sup> N-aa	Mutant	Detected and assigned	Comments
<sup>15</sup> N-Arg	Wt	<b>R203</b> , R211, <b>R231</b> , <b>R237</b> ,	R188 could not be assigned
		R251, R262, R282, R284,	unequivocally. There is potential
		<b>R285, R318,</b> R352	candidate resonance
	R282H	R282, R284, R285	
	R284H	R282, R284, R285	
<sup>15</sup> N-Leu		L191, L196, L199, L209,	
	Wt	L213, L222, L229, L233,	
		L235, L249, <b>L255</b> , L276,	
		<b>L281</b> , L308, L326, L330,	
		L340, L348, L355	
	L191I	L191, L196, L199	
	S256A	L255	
	R282H	L281	
15NJ 11-	Wt	1240, 1253, 1264	The NH resonances of I192 not detected
<sup>15</sup> N-Ile			in the <sup>15</sup> N-Ile HSQC spectrum
15N Vol	<b>W</b> 74	V183, V204, V212, V344,	
IN-Val	wt	V351	
	Wt	<b>E194, E195, E210</b> , E214,	
<sup>15</sup> N-Glu		E224, E226, E227, E239,	NH resonances of E201and E290 not identified
		E243, E245, E246, <b>E279</b> ,	
		E280, E286, E317, E336,	Identified
		E337, E349, E358	
	Wt	Q198, <b>Q202</b> , Q230, <b>Q238</b> ,	
		<b>Q254</b> , <b>Q258</b> , Q266, Q270,	NH resonances of Q184 and Q185 not identified
		Q298, Q299, Q300, Q301,	
		Q302, Q303, Q304, Q305,	Identified
		Q311, Q341	
<sup>15</sup> N-Gln	T207A	Q202	
N-OIII	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
	Wt	A197, A215, A232, A234,	
<sup>15</sup> N A lo		A247, <b>A252</b> , <b>A287</b> , A320,	
IN-Ala		A325, A333, A342, A343	
	L191I	A197	

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



**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 <sup>13</sup>C-<sup>1</sup>H strips of a HNCACB experiment of ataxin-3(Q13) (residues 343-359). The  $\beta$ -carbons are coloured in green, the  $\alpha$ -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 <sup>15</sup>N selective amino acid labelling of wild-type and mutated ataxin-3(Q13). A: <sup>15</sup>N-arginine, B: <sup>15</sup>N-alanine, C: <sup>15</sup>N-glutamine, D: <sup>15</sup>N-methionine, E: <sup>15</sup>Nphenylalanine, F: <sup>15</sup>N-tyrosine, G: <sup>15</sup>N-valine, H: <sup>15</sup>N-glutamate.



**Figure S5** – Sequential assignment of residues of residues 278-280 using BEST-TROSY HNCACB. A: Areas of the <sup>1</sup>H-<sup>15</sup>N BEST-TROSY HSQC (red) and conventional <sup>1</sup>H-<sup>15</sup>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 (Ca: 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.