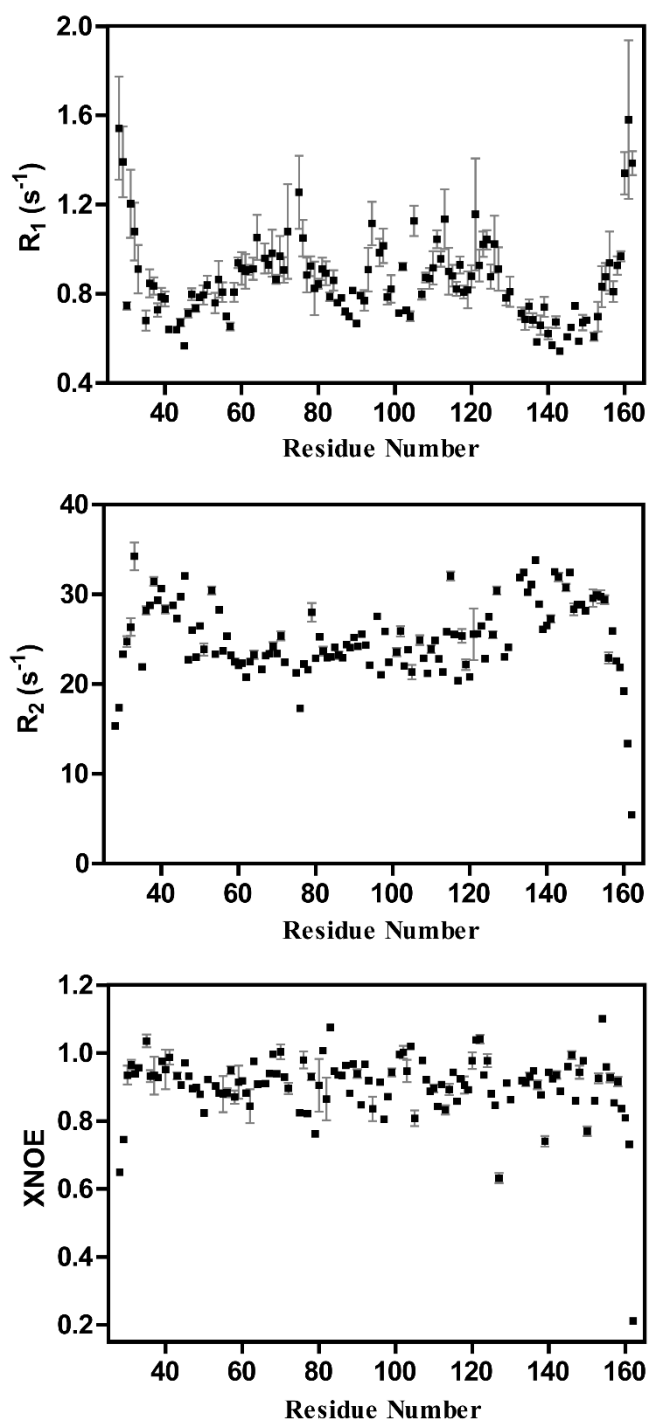


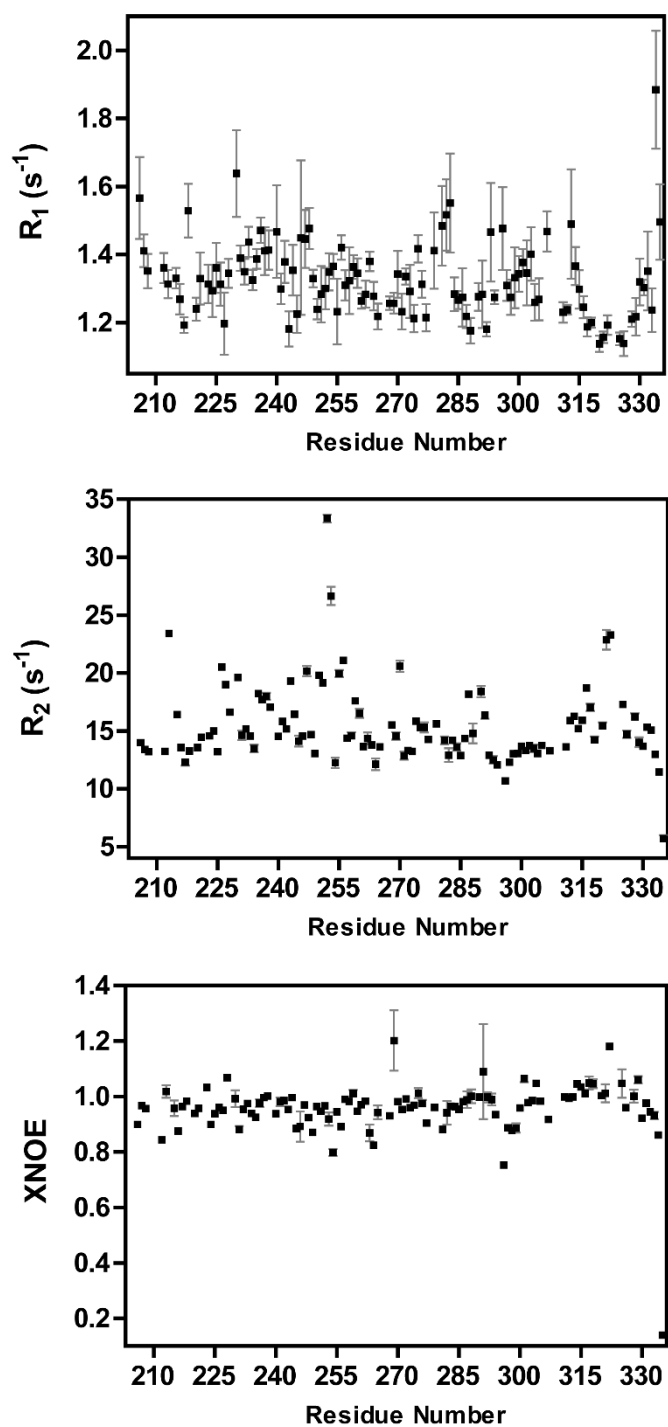
## Supporting information

Figure S1



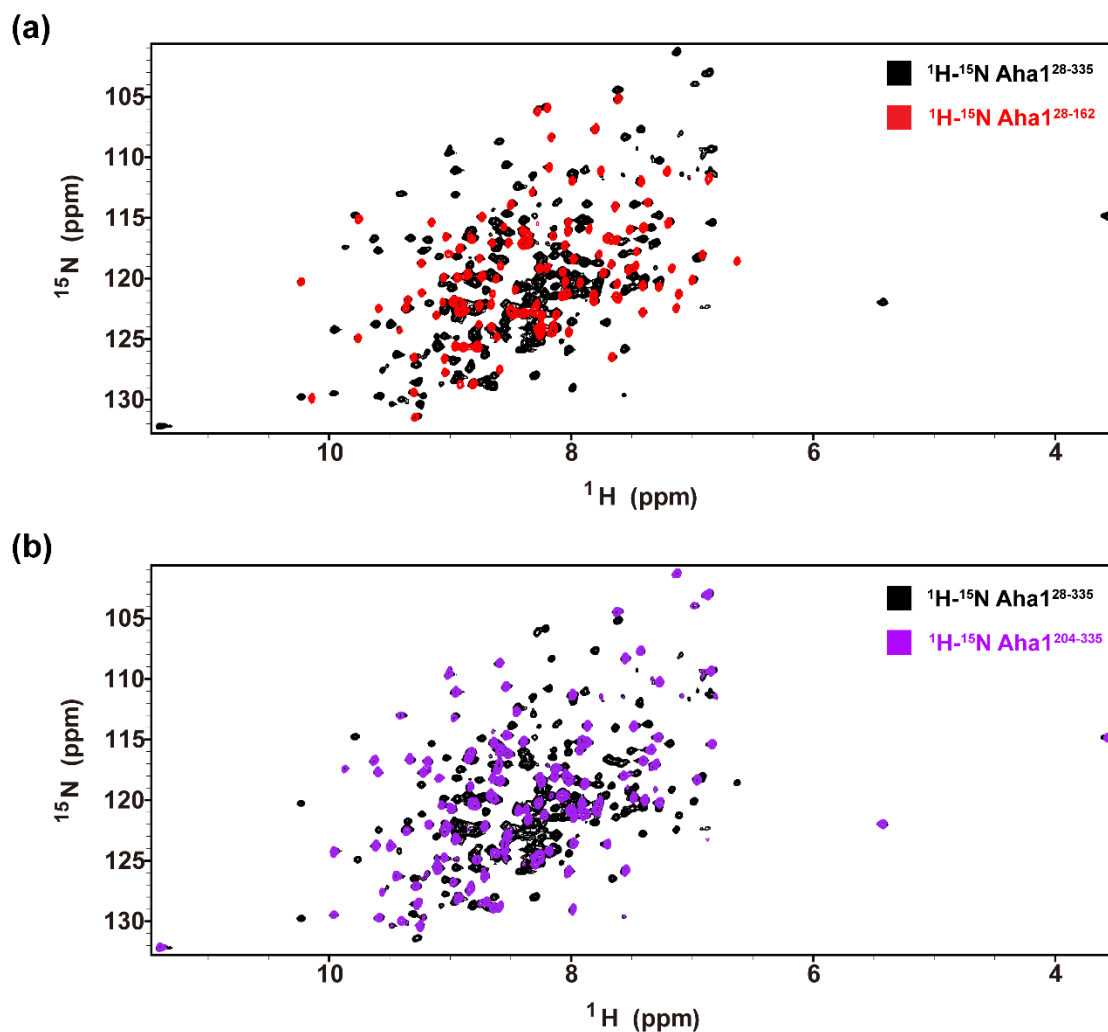
**Figure S1.** <sup>15</sup>N longitudinal relaxation rates  $R_1$ , transverse relaxation rates  $R_2$  and  $\{^1\text{H}\}$ -<sup>15</sup>N heteronuclear steady NOEs of the N-terminal domain of human Aha1 (Aha1<sup>28-162</sup>) in its free state.

Figure S2



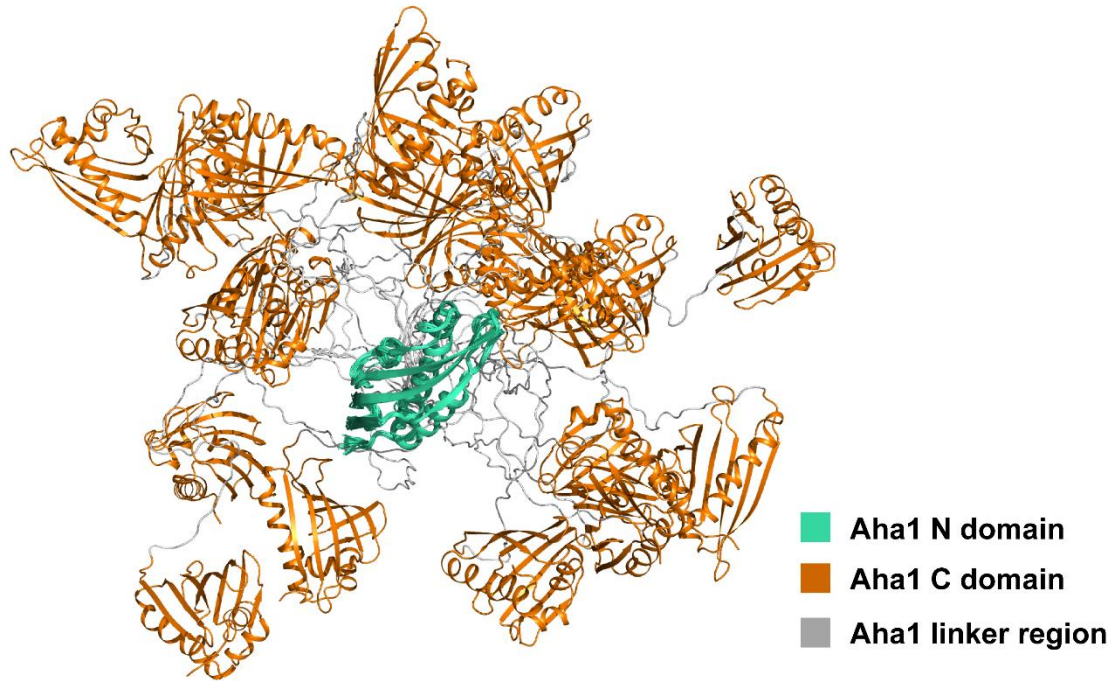
**Figure S2.** <sup>15</sup>N longitudinal relaxation rates  $R_1$ , transverse relaxation rates  $R_2$  and  $\{^1\text{H}\}$ -<sup>15</sup>N heteronuclear steady NOEs of the C-terminal domain of human Aha1 (Aha1<sup>204-335</sup>) in its free state.

Figure S3



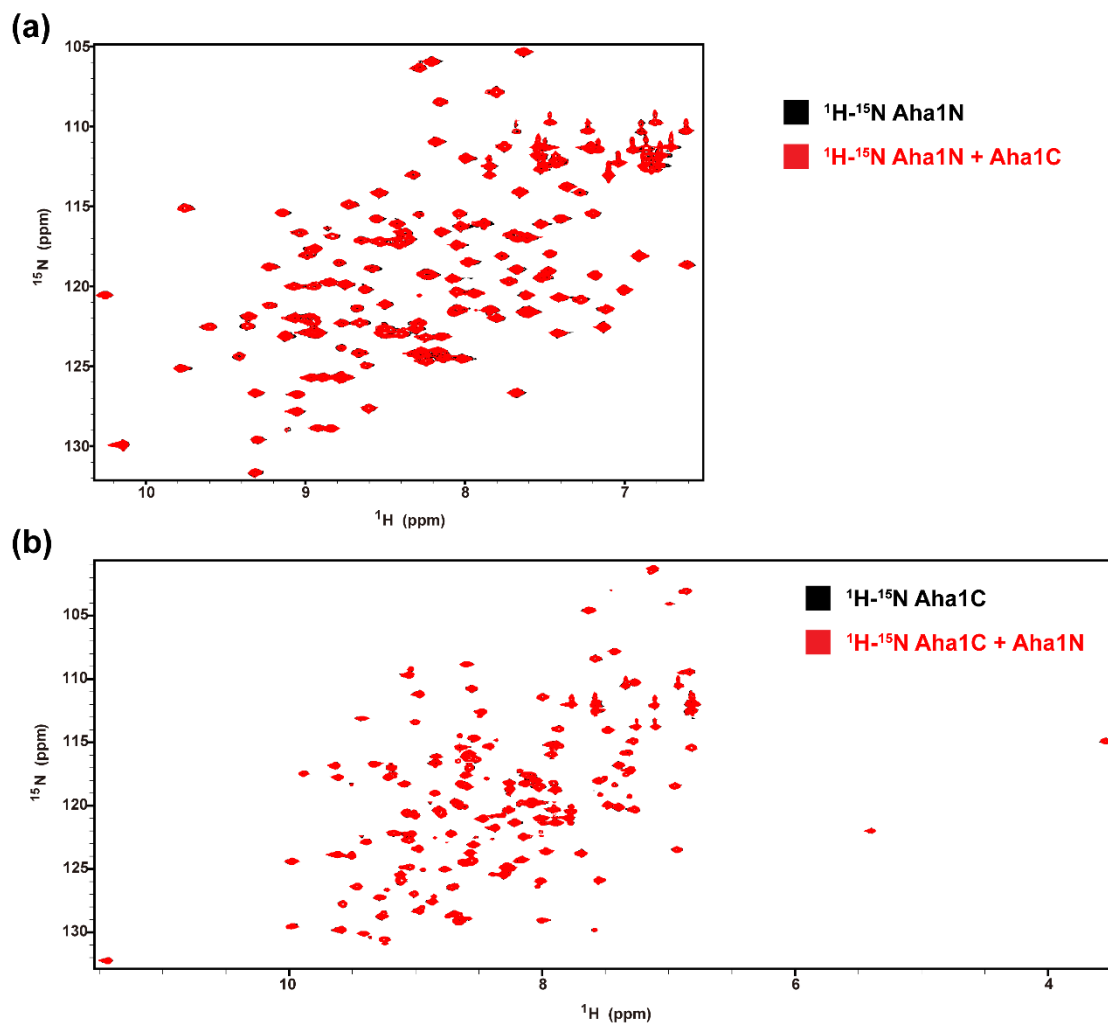
**Figure S3.** (a) Superposition of  $^1\text{H}$ - $^{15}\text{N}$ -HSQC spectra recorded on  $^{15}\text{N}$ , 50%  $^2\text{H}$ -double labeled Aha1<sup>28-335</sup> (colored in black) and  $^{15}\text{N}$ , 50%  $^2\text{H}$ -double labeled Aha1<sup>28-162</sup> (colored in red). (b) Superposition of  $^1\text{H}$ - $^{15}\text{N}$ -HSQC spectra recorded on  $^{15}\text{N}$ , 50%  $^2\text{H}$ -double labeled Aha1<sup>28-335</sup> (colored in black) and  $^{15}\text{N}$ , 50%  $^2\text{H}$ -double labeled Aha1<sup>204-335</sup> (colored in purple).

**Figure S4**



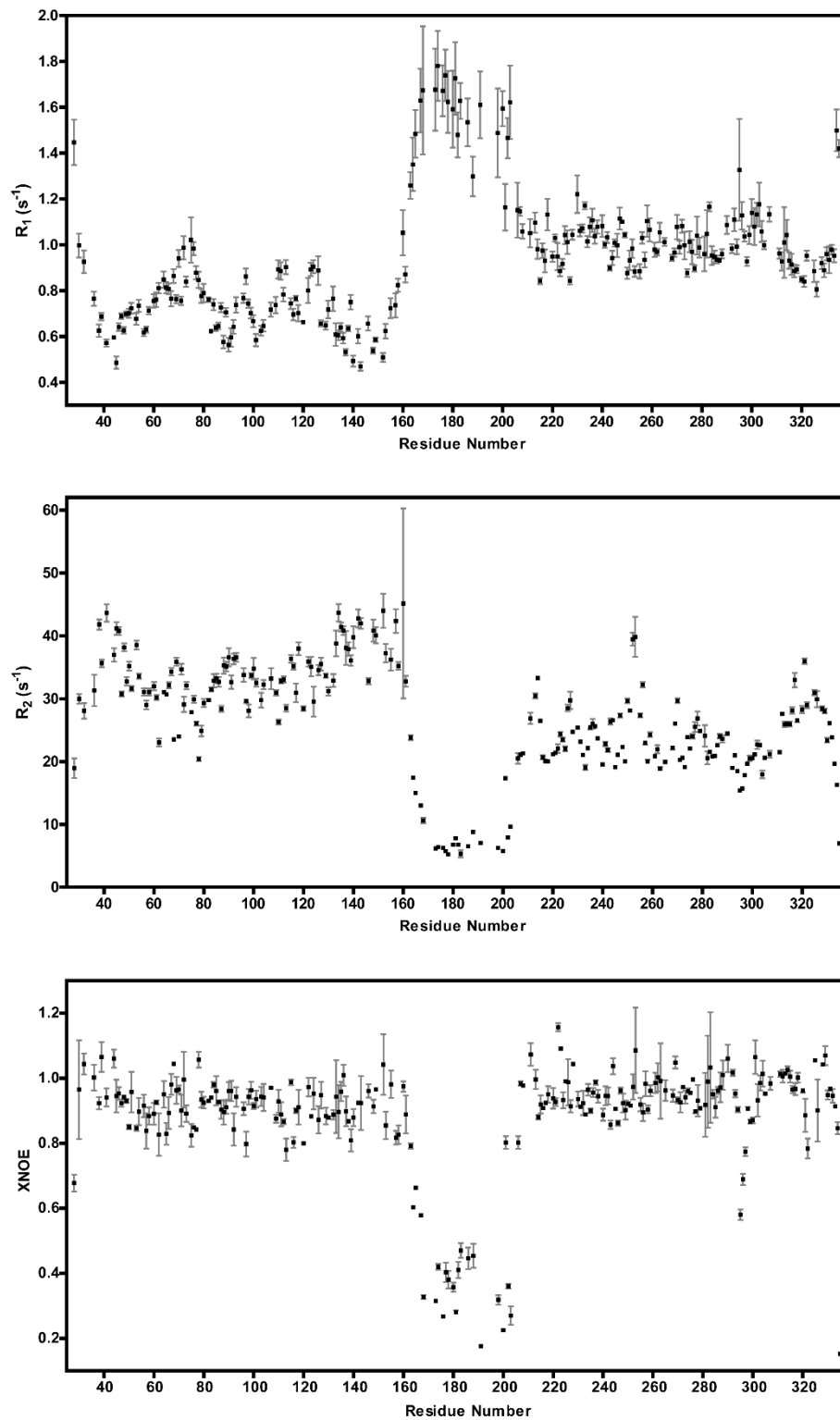
**Figure S4.** Structural ensemble of 20 best structures of human Aha1<sup>28-335</sup> (PDB code: 7DME). A jellyfish-like shape was formed when human Aha1's N-terminal domain served as an anchor in the superposition of Aha1<sup>28-335</sup>'s solution structures.

**Figure S5**



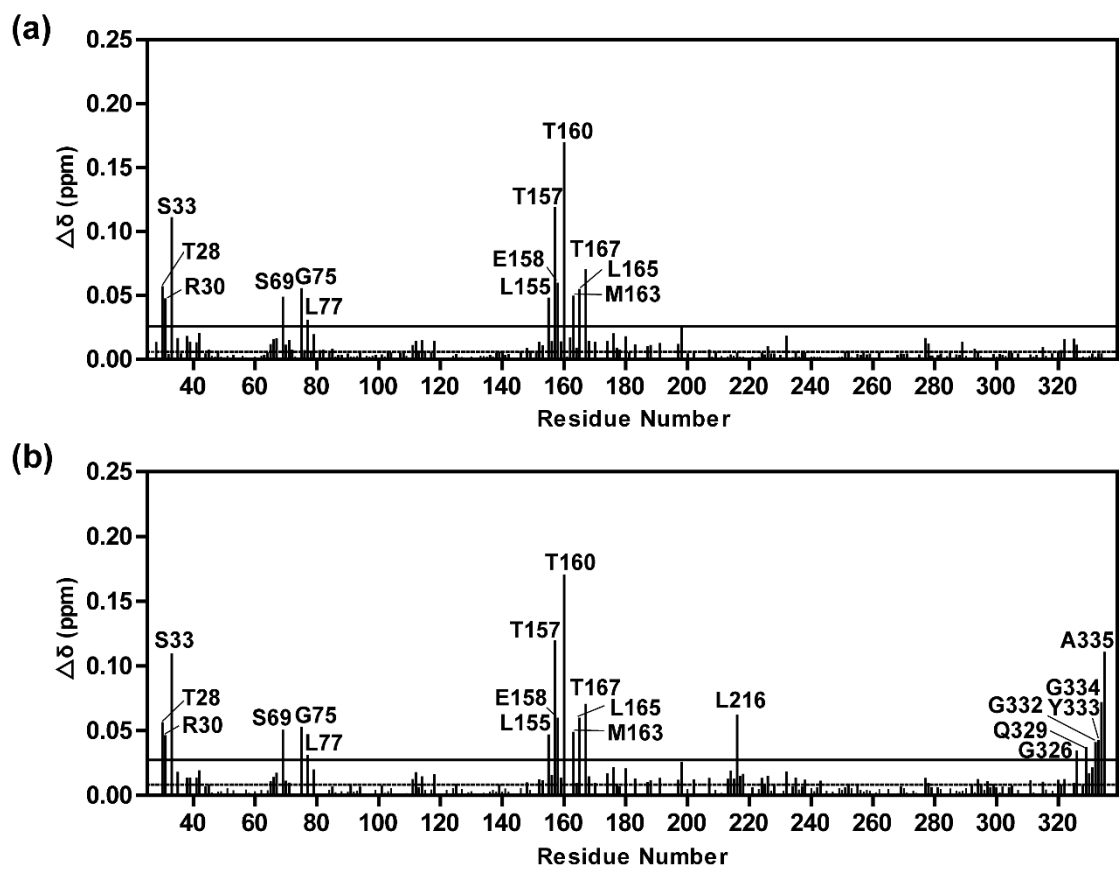
**Figure S5.** The two domains of human Aha1 do not interact with each other in solution. (a) Superposition of  $^1\text{H}$ - $^{15}\text{N}$ -HSQC spectra recorded on  $^{15}\text{N}$ -labeled Aha1<sup>28-162</sup> (Aha1N) without (colored in black) or with (colored in red) the addition of an equal amount of unlabeled Aha1<sup>204-335</sup> (Aha1C). (b) Superposition of  $^1\text{H}$ - $^{15}\text{N}$ -HSQC spectra recorded on  $^{15}\text{N}$ -labeled Aha1<sup>204-335</sup> (Aha1C) without (colored in black) or with (colored in red) the addition of an equal amount of unlabeled Aha1<sup>28-162</sup> (Aha1N).

**Figure S6**



**Figure S6.** <sup>15</sup>N longitudinal relaxation rates  $R_1$ , transverse relaxation rates  $R_2$  and  $\{^1\text{H}\}$ -<sup>15</sup>N heteronuclear steady NOEs of Aha1<sup>28-335</sup> in its free state.

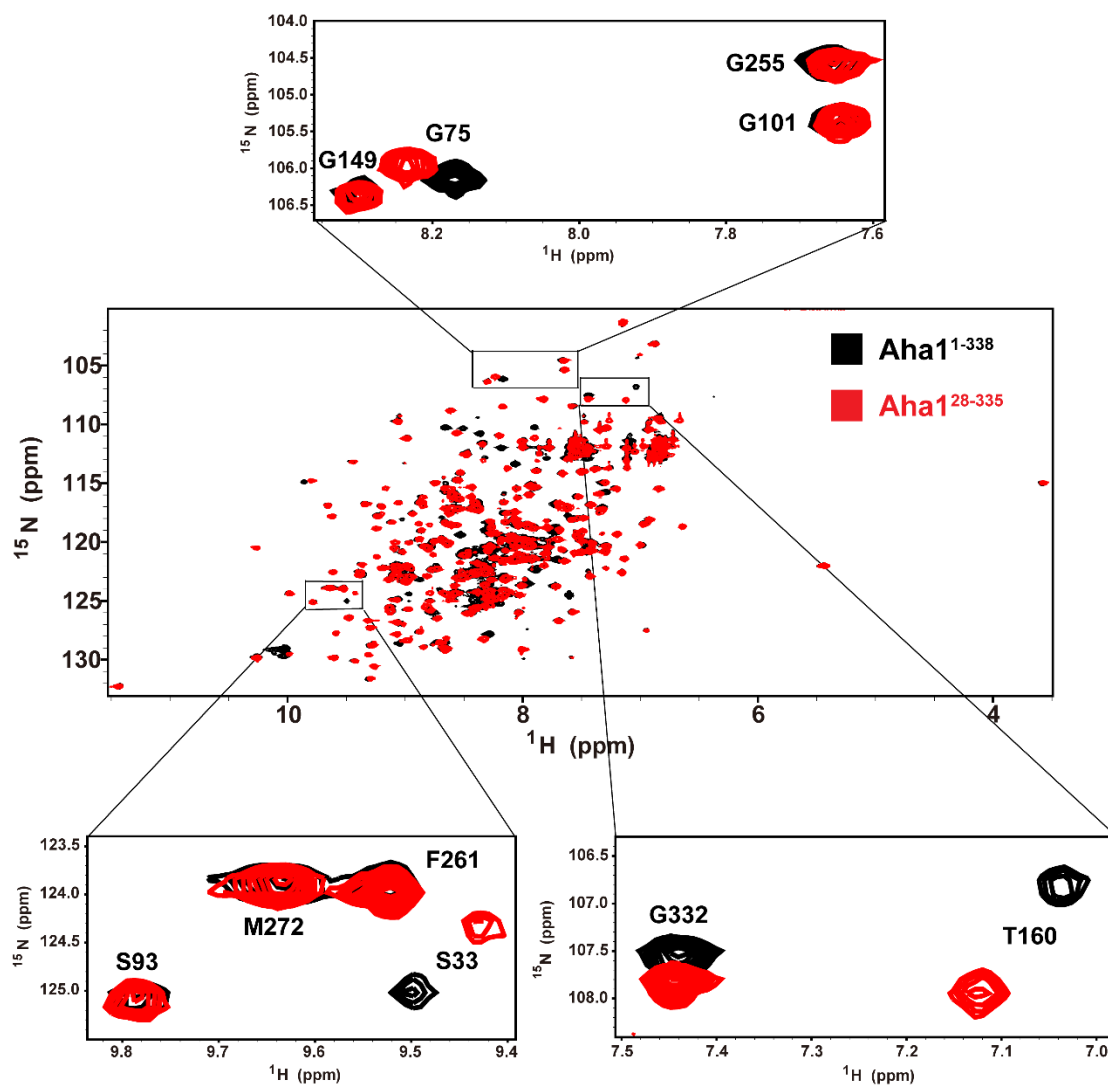
Figure S7



**Figure S7.** Chemical shift changes for (a) Aha1<sup>1-338</sup> vs Aha1<sup>28-338</sup> and (b) Aha1<sup>1-338</sup> vs Aha1<sup>28-335</sup> were calculated by using equation (1) listed below.

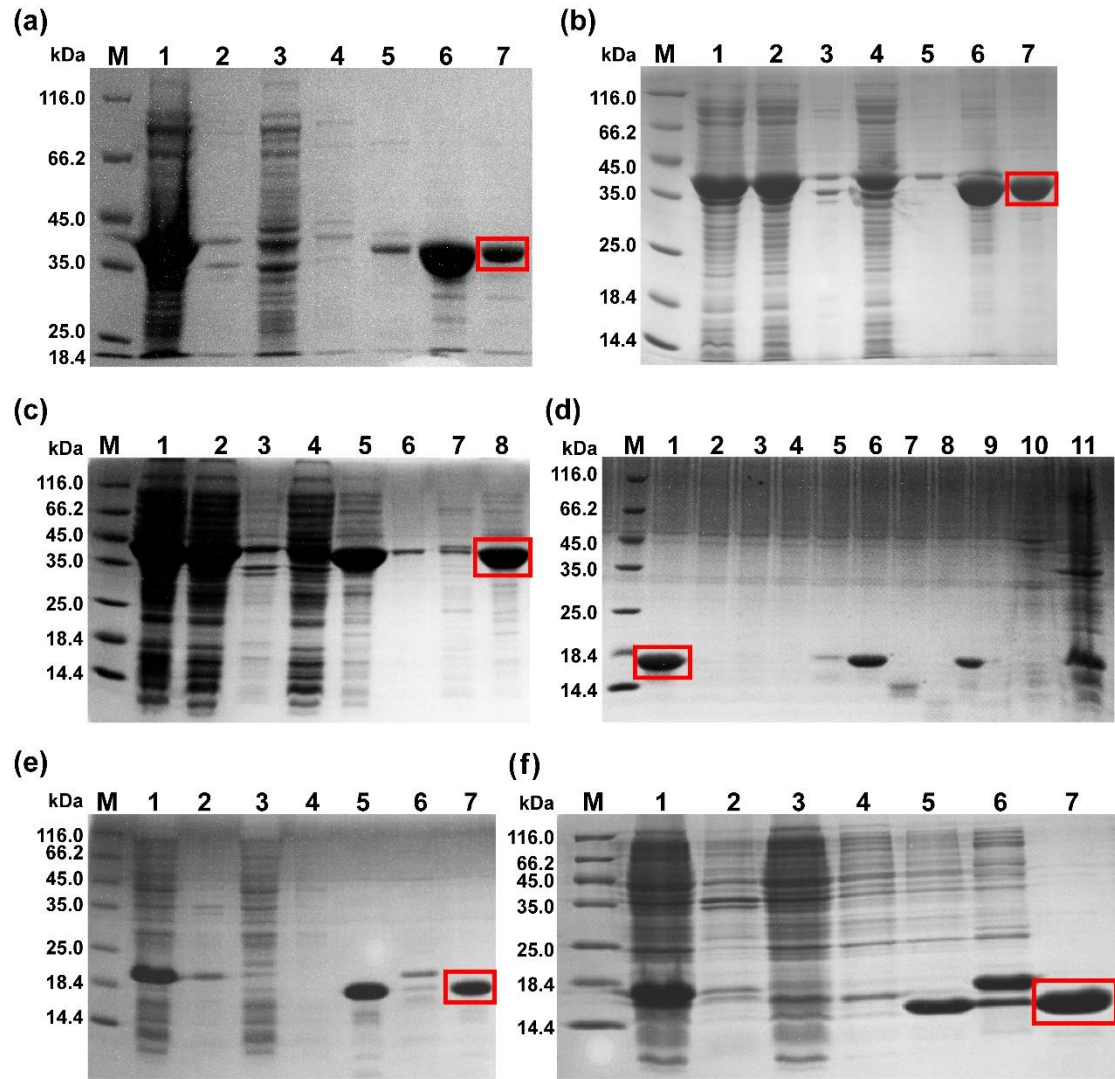
$$\Delta\delta = \sqrt{((\Delta\delta_N/5)^2 + \Delta\delta_H^2)/2} \quad (1)$$

**Figure S8**



**Figure S8.** Superposition of  $^1\text{H}$ - $^{15}\text{N}$ -HSQC spectra recorded on  $^{15}\text{N}$ -labeled Aha1<sup>1-338</sup> (colored in black) and  $^{15}\text{N}$ -labeled Aha1<sup>28-335</sup> (colored in red). Selected  $^1\text{H}$ - $^{15}\text{N}$ -HSQC spectra regions are expanded to view representative residues which undergo resonance shifting upon the absence of Aha1's N-terminal fragment spanning M1-W27.

**Figure S9**



**Figure S9.** Representative SDS-PAGE gels for the preparations of protein samples used in the study. SDS-PAGE (15% gel) results for the purifications of (a) Aha1<sup>28-338</sup>, (b) Aha1<sup>28-335</sup>, (c) Aha1<sup>1-338</sup>, (d)  $\alpha$ -synuclein, (e) Aha1<sup>28-162</sup> and (f) Aha1<sup>204-335</sup>. The protein molecular weight marker lane (26610, Thermo Scientific™) is labeled with M, and the gel staining results for the purified protein samples used in the experiments are highlighted with red box.