Supplementary information

Of

Structural characterization of PHOX2B and its DNA interaction shed lights into the molecular basis of the + 7Ala variant pathogenicity in CCHS

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Table S1. NMR structural statistics of the PHOX2B-HD NMR ensemble

NMR constraints	
Completeness of resonance assignments	
Backbone (%)	87.3
Carbon β (%)	87.3
Structure precision	
RMSD from mean structure (ordered residues) (Å)	
All backbone atoms	0.80 (0.50)
All heavy atoms	1.30 (1.00)
RMSD from mean structure (residues 12-57) (Å)	
All backbone atoms	0.78 (0.50)
All heavy atoms	1.30 (1.02)
Structure quality	
MOLPROBITY	
Clash score	1.02 ± 1.01
Poor rotamers (%)	0.05 ± 0.22
MolProbity score	0.91 ± 0.28
Residues with bad bonds (%)	0 ± 0
Residues with bad angles (%)	0 ± 0
Cβ deviations > 0.25 Å	0 ± 0
PROCHECK	
G-factors phi-psi/all dihedral angles	0.49 /0.54
Ramachandran plot statistics (%)	
Most favored regions	94.3
Additional allowed regions	5.7
Generously allowed regions	0
Disallowed regions	0

The statistical value reported in parenthesis is related to the NMR structural ensemble containing 10 structures with the lowest energy as reported by the software CS-Rosetta

Figure S1. Backbone dynamics of PHOX2B-HB. (A) Mapping of the S^2 order parameter values, derived from the backbone chemical shifts, on the representative PHOX2B-HD NMR structure. (B) Comparison of the transverse relaxation rates (R_2) estimated by analyzing in the $^{1}H,^{15}N$-HSQC spectrum the ^{15}N linewidth ($R_2^{linewidth}$) with the R_2 values predicted, using HYDRONMR software, from the NMR structural ensemble ($R_2^{structure}$) reported as function of the HD domain primary sequence.

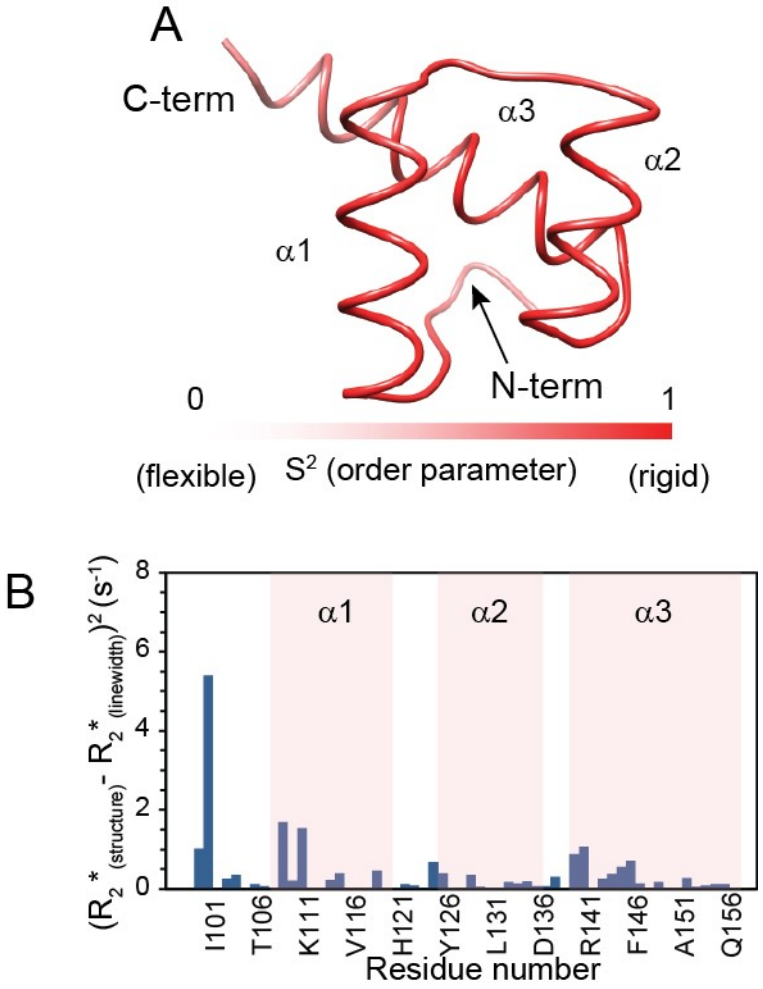

Figure S2. Comparison of the NMR spectra. Superposition of the ^{1}H,^{15}N-HSQC spectra acquired for PHOX2B-HD (red) and PHOX2B-20A (blue) at 298 K using a 600 MHz spectrometer

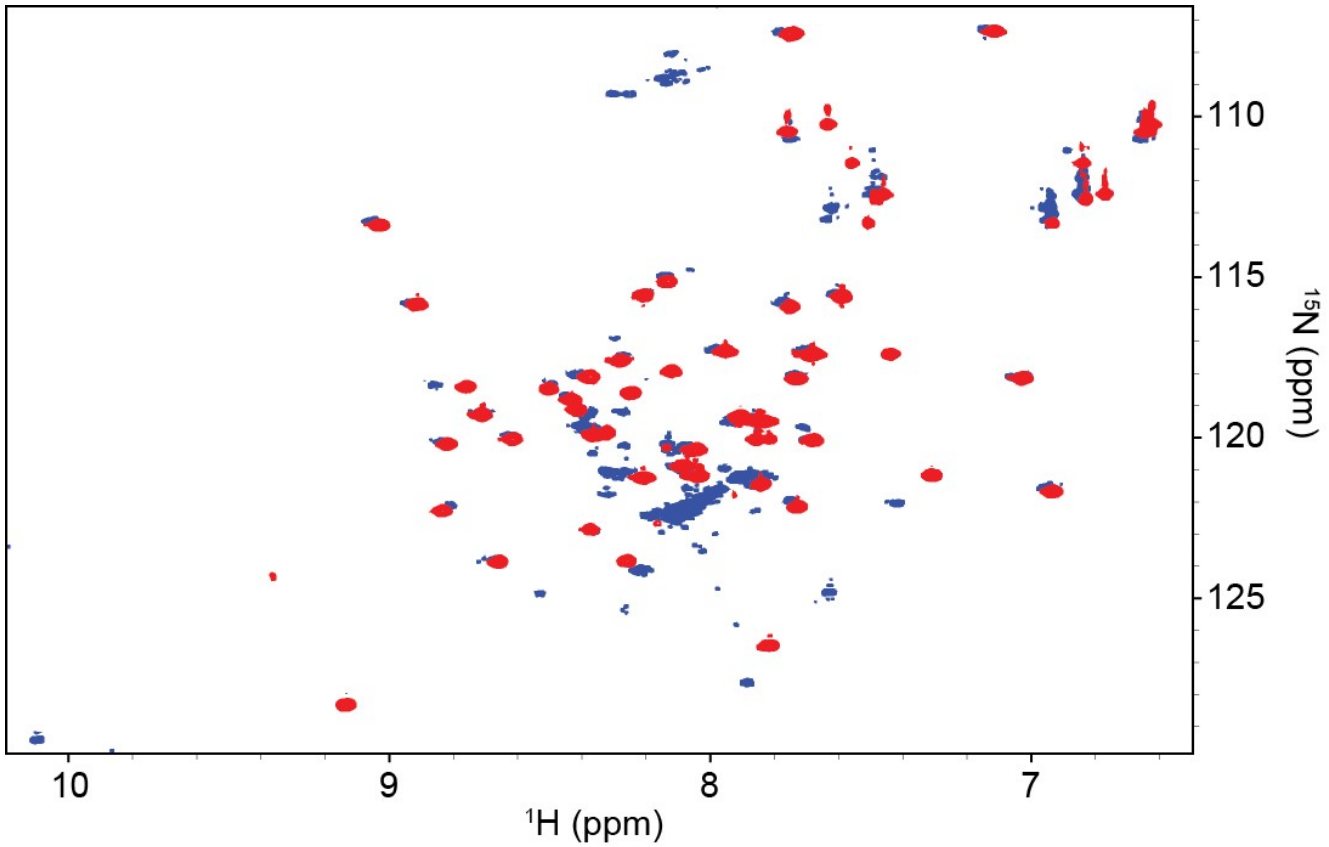

Figure S3. Completeness of PHOX2B-20A chemical shift assignments. Mapping on the primary sequence of PHOX2B-20A of the assigned residues. In blue are reported the residues for which HN, N, Cα, and Cβ chemical shifts were assigned; whereas in light blue are highlighted the residues for which only Cα, and Cβ resonances have been assigned. IDRs are also illustrated as grey-scale.

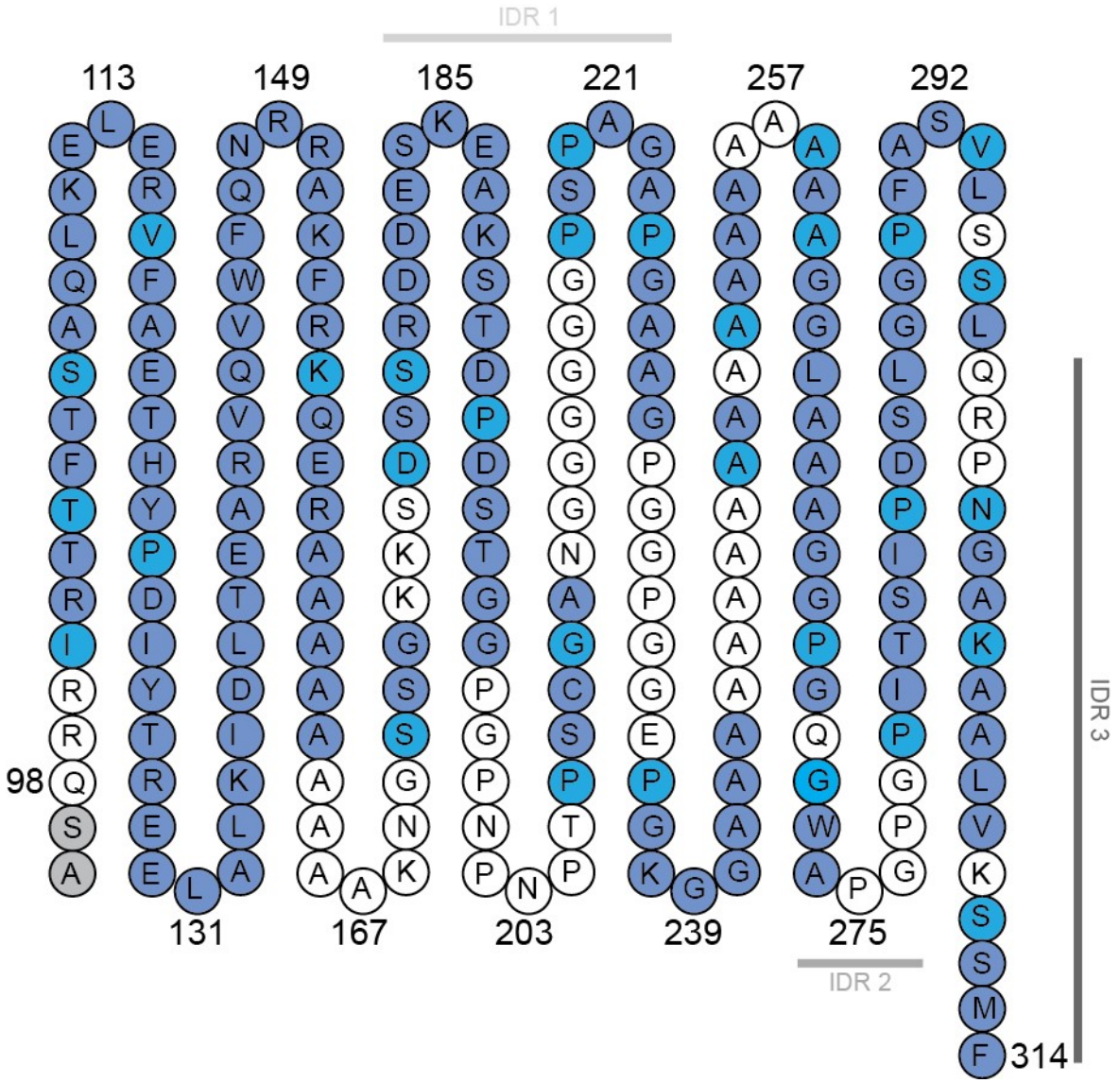

Figure S4. $^{1}H,^{15}N$ T2-filter HSQC of PHOX2B-20A. The HSQC based spectrum was acquired at 600 MHz using a relaxation-compensated Carr-Purcell-Meiboom-Gill sequence (CMPG) period of 100 ms.

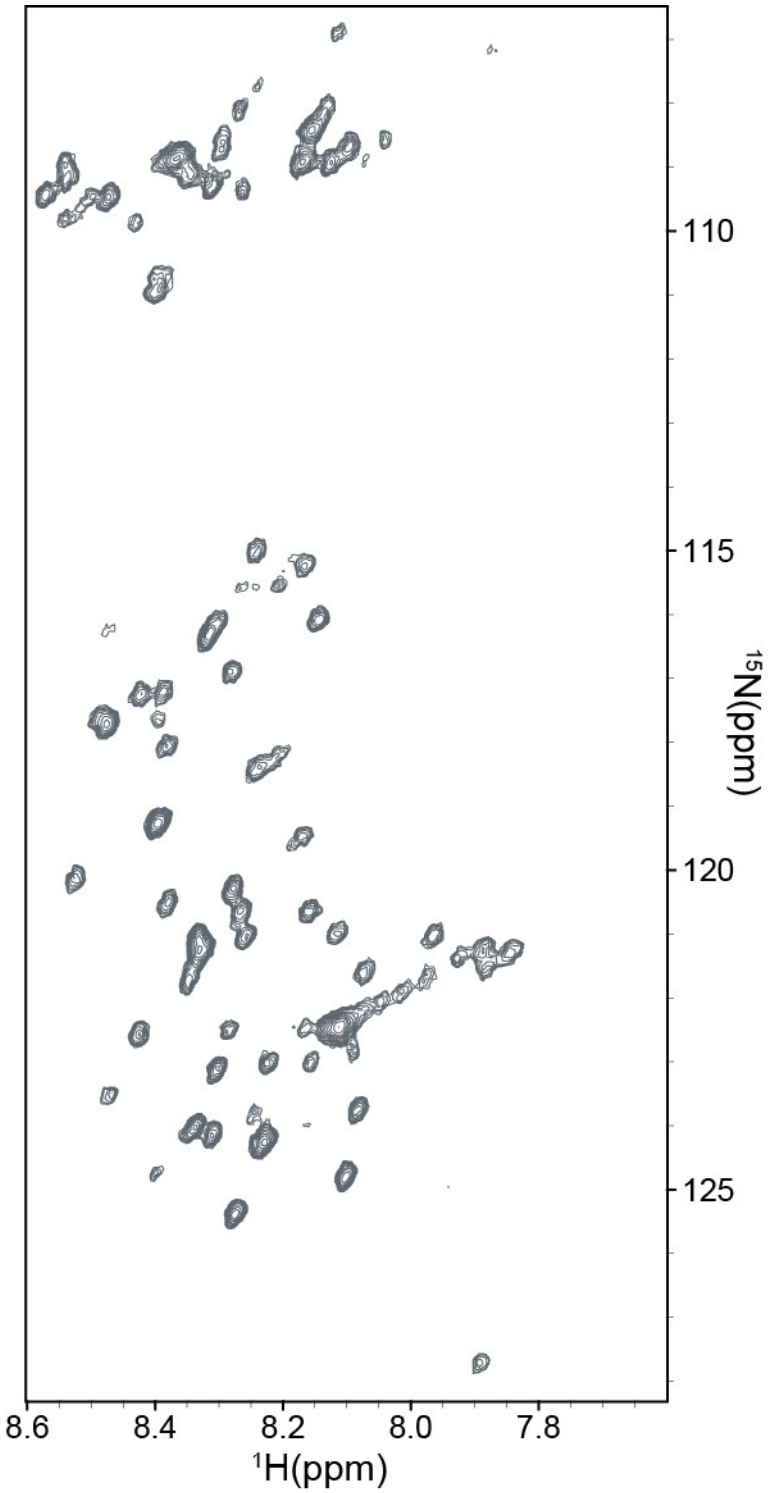

Figure S5. NMR structural investigation of Helix3 and polyAla regions of PHOX2B-20A. Cα secondary chemical shifts for the residues located within the helix 3 (A) and the polyAla stretch (B). Dashed lines in the panels indicate the cut-off values for the identification of secondary structure elements as define by Wishart et al. (Biochemistry. 1992;31(6):1647-1651).

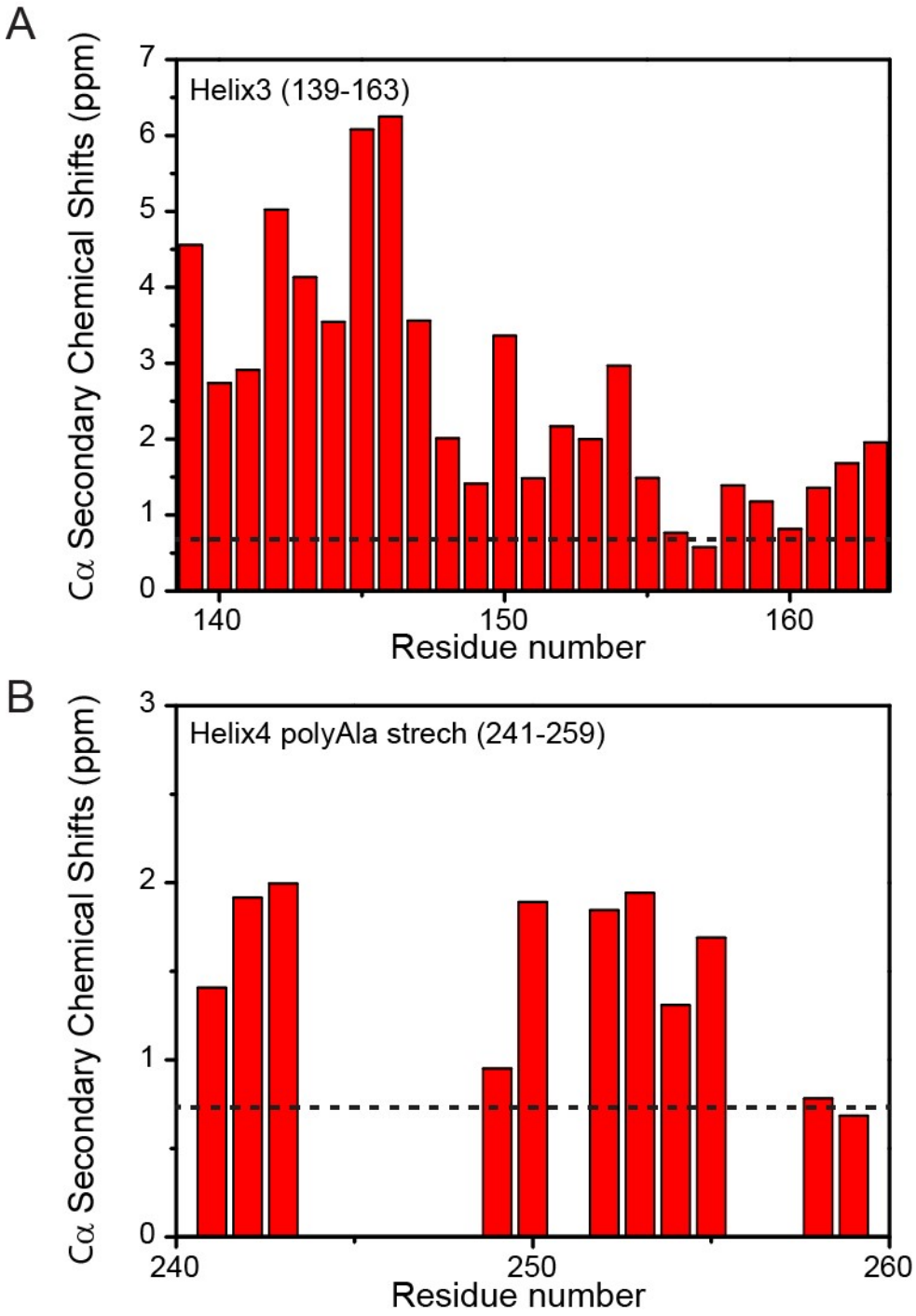

Figure S6. NMR structural investigation of the intrinsically disordered regions of PHOX2B-20A. Cα secondary chemical shifts for the residues located within the three intrinsically disordered regions: IDR1 (Gly^{170}-Gly^{240}) (A), IDR2 (Ala^{260}-Pro^{290}) (B) and IDR3 (Leu^{298}-Phe^{314}) (C). Dashed lines in the panels indicate the cut-off values for the identification of secondary structure elements as define by Wishart et al. (Biochemistry. 1992;31(6):1647-1651).

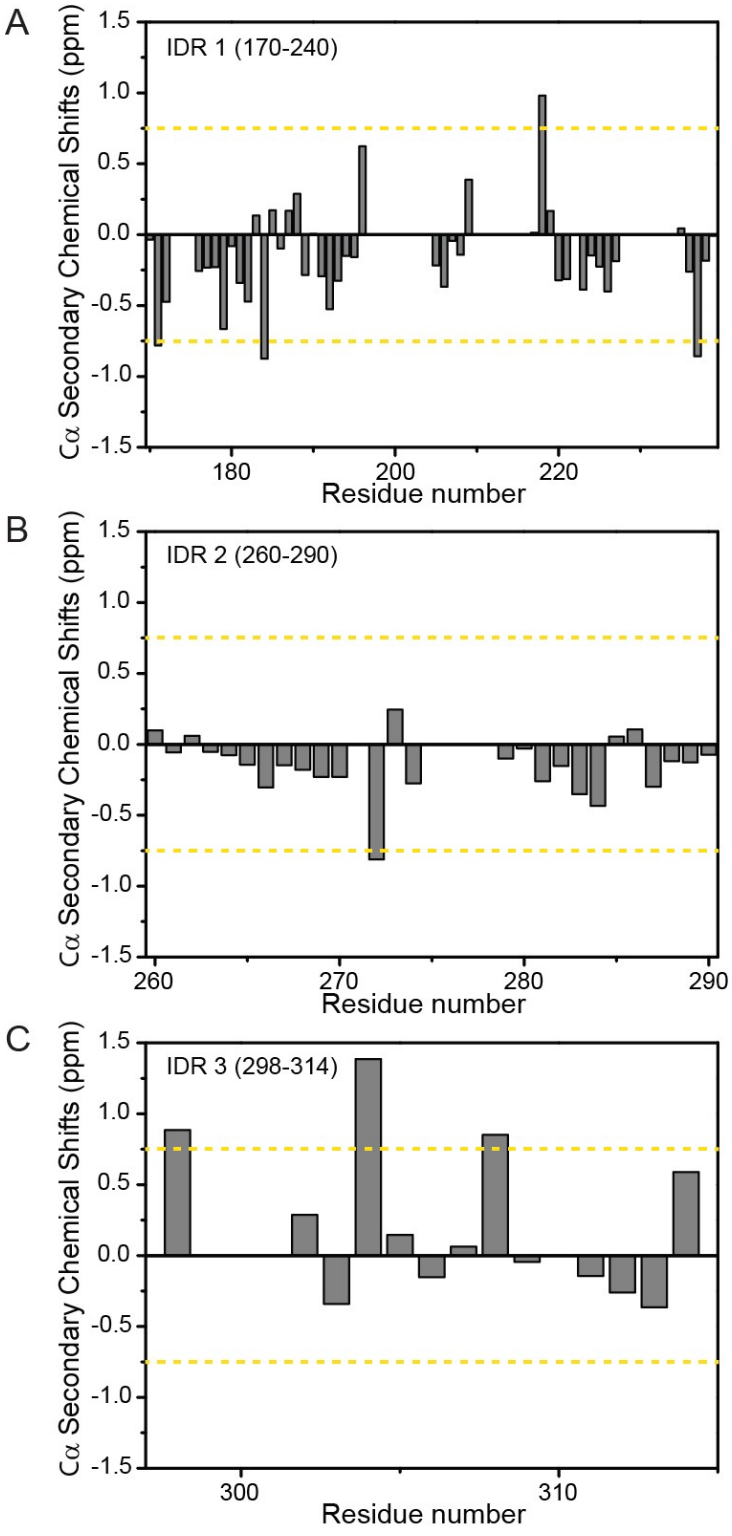

Figure S7. NMR spectra of the HD domain alone and within PHOX2B-20A. Portions of the overlapped ^{1}H,^{15}N-HSQC spectra acquired for the HD domain (red) and PHOX2B-20A protein (blue).

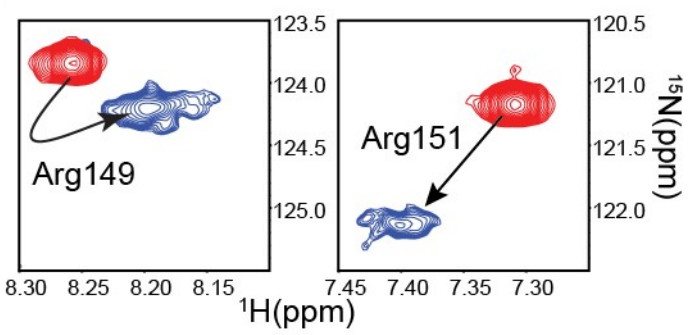

Figure S8. NMR investigation of PHOX2B-27A. ^{1}H-^{15}N HSQC spectrum of PHOX2B-27A acquired using a 600 MHz NMR spectrometer at 298 K pH 7.4.

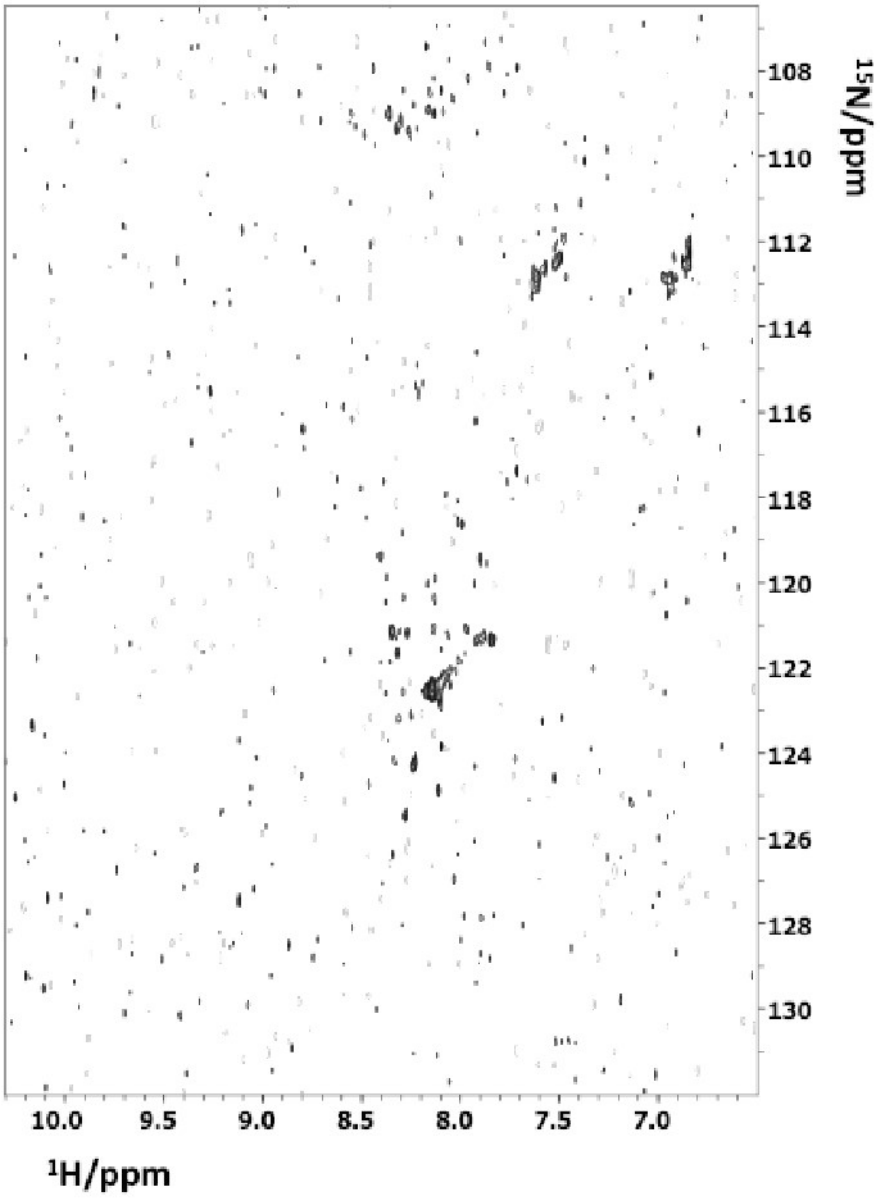

Figure S9. HN/N chemical shifts correlation of HD domain. Correlation plot of the HN (A) and N (B) chemical shifts observed for the homeodomain of PHOX2B-20A with the values obtained for PHOX2B-HD.