Perturbation in long-range contacts modulates kinetics of amyloid formation in α-Synuclein familial mutants

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Supporting Information

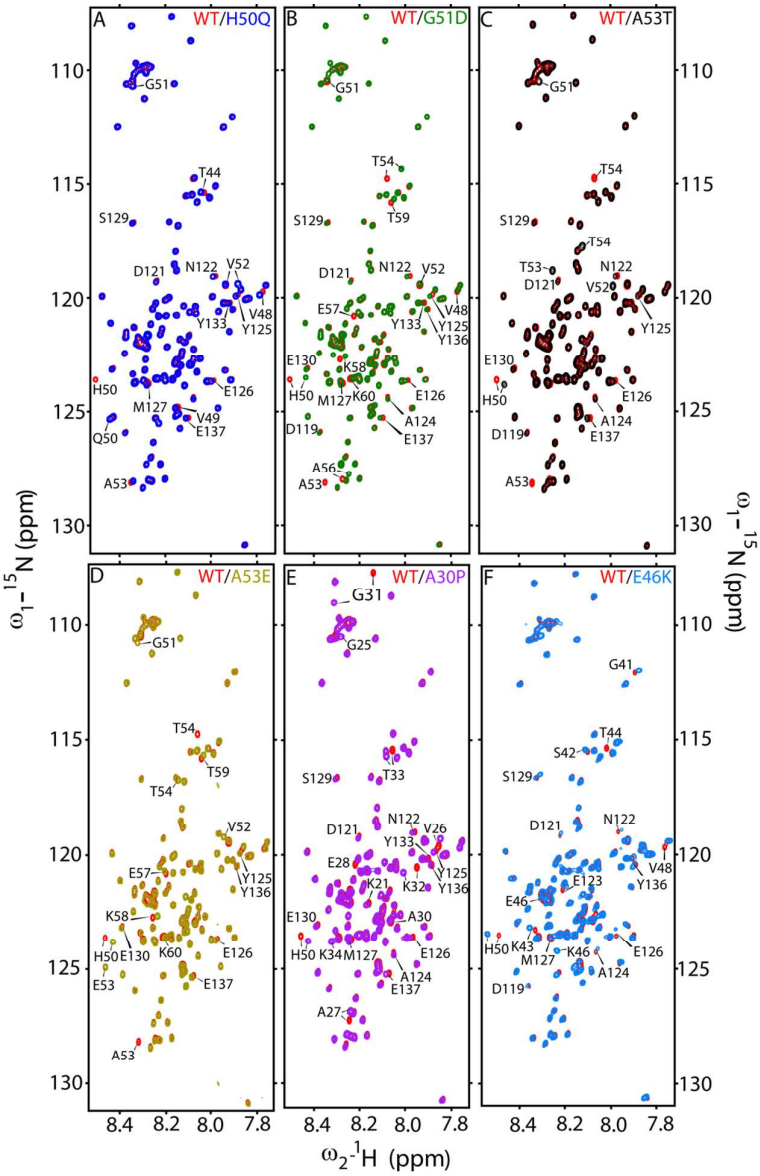

Figure S1. Overlaid ^{1}H-^{15}N HSQC spectra of PD associated familial mutants and α-Syn wild type. Demarcated residues are those showing significant chemical shift perturbations in case of H50Q (A), G51D (B), A53T (C), A53E (D), A30P (E), and E46K (F).^{1}H-^{15}N HSQC spectra of α-Syn WT has been shown in red.

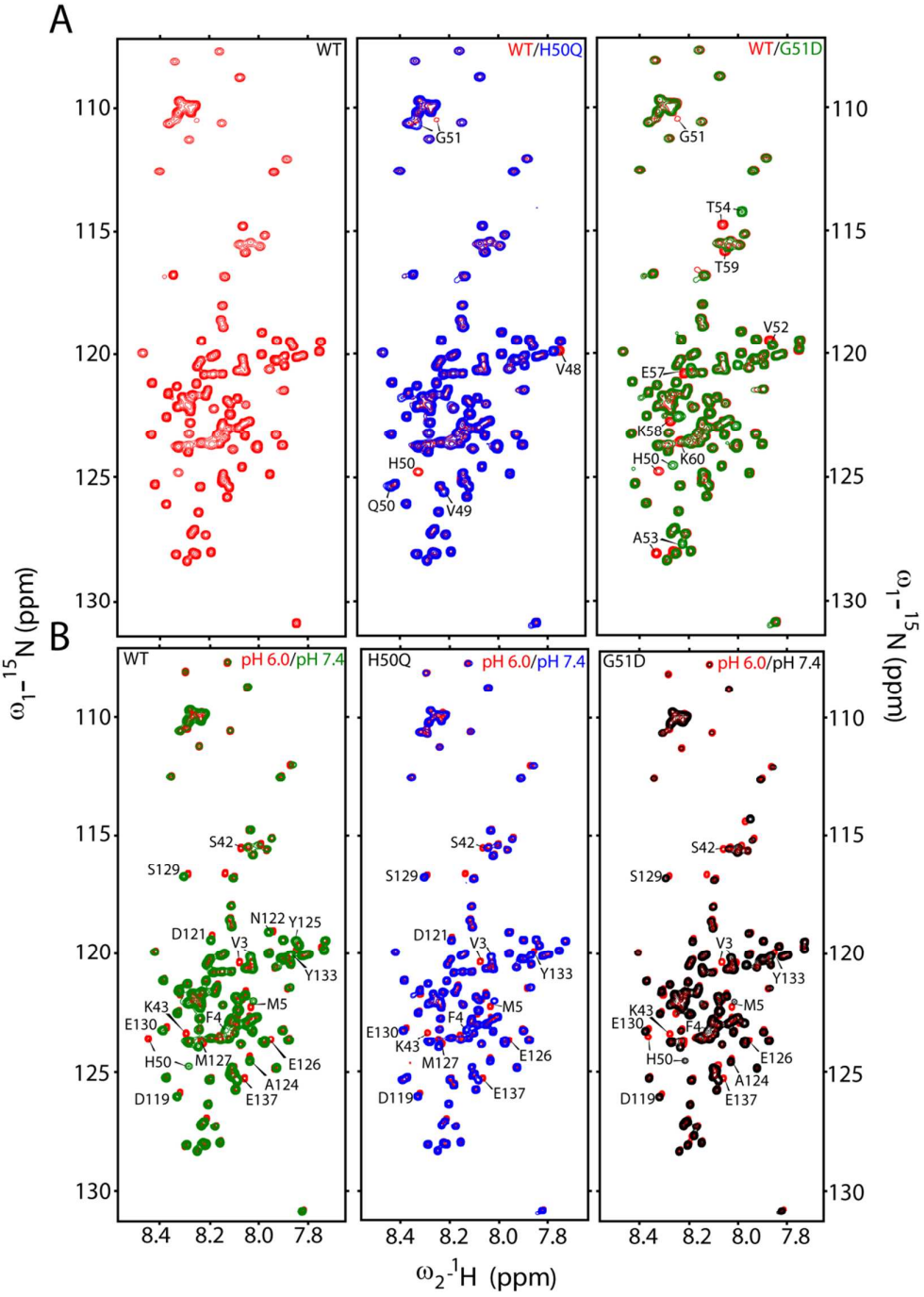

Figure S2. Chemical shift perturbation at the mutation site only in case of the familial mutants at physiological pH. (A) ^{1}H-^{15}N HSQC spectra recorded for WT (left panel), H50Q (middle panel), and G51D (right panel). Residues showing significant perturbations have been indicated in case of H50Q and G51D. (B) Overlaid ^{1}H-^{15}N HSQC spectra recorded at two different pH for α-Syn WT (left panel), H50Q (middle panel) and G51D (right panel).

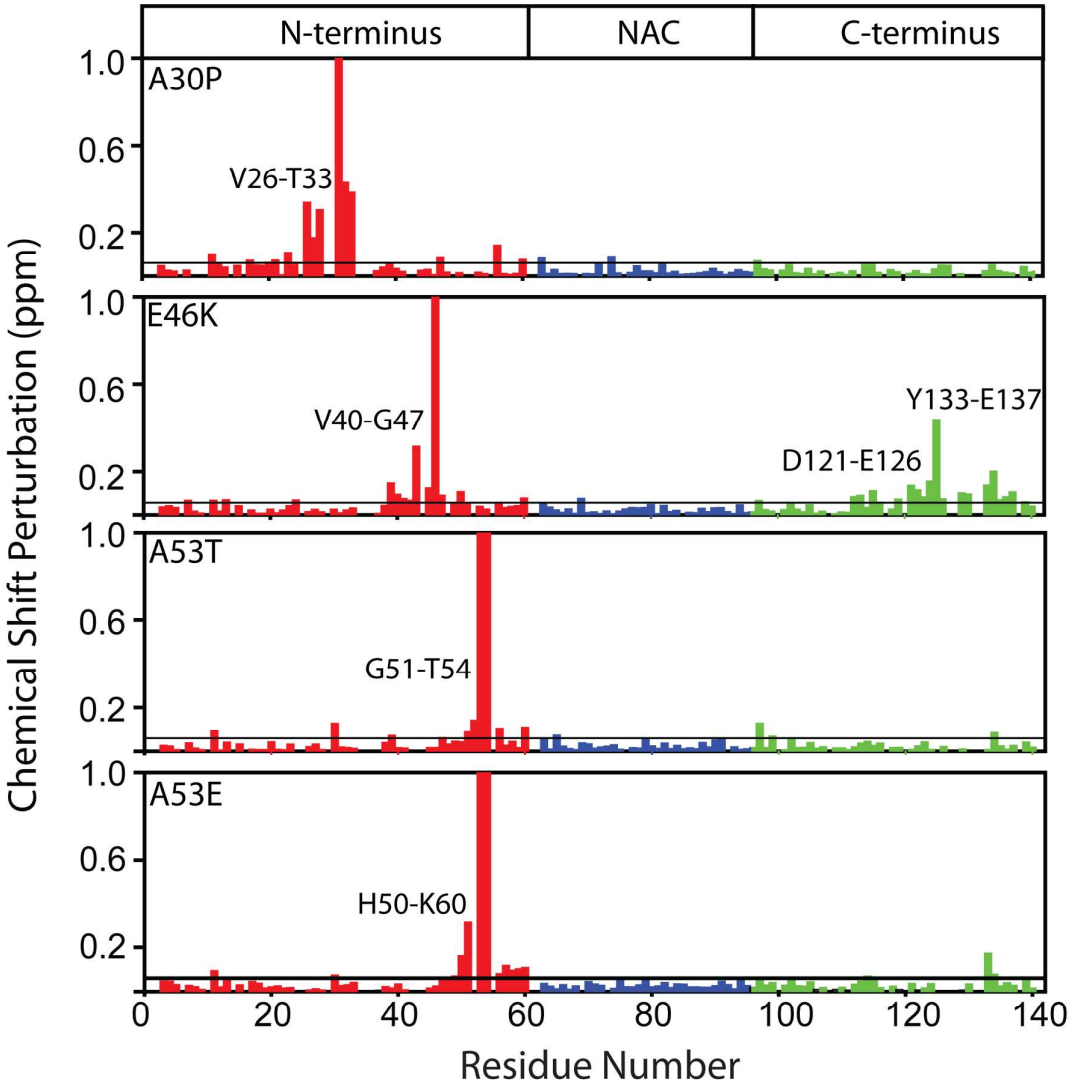

Figure S3. Residue-specific chemical shift perturbations for the familial mutants at physiological pH. Significant CSPs were seen only at the mutation site in A30P, A53T and A53E. However, E46K showed significant CSPs both at the mutation and C-terminus site. Red, blue and green colour denotes the basic N-terminal domain, the amyloidogenic NAC domain, and the acidic C-terminal domain respectively.

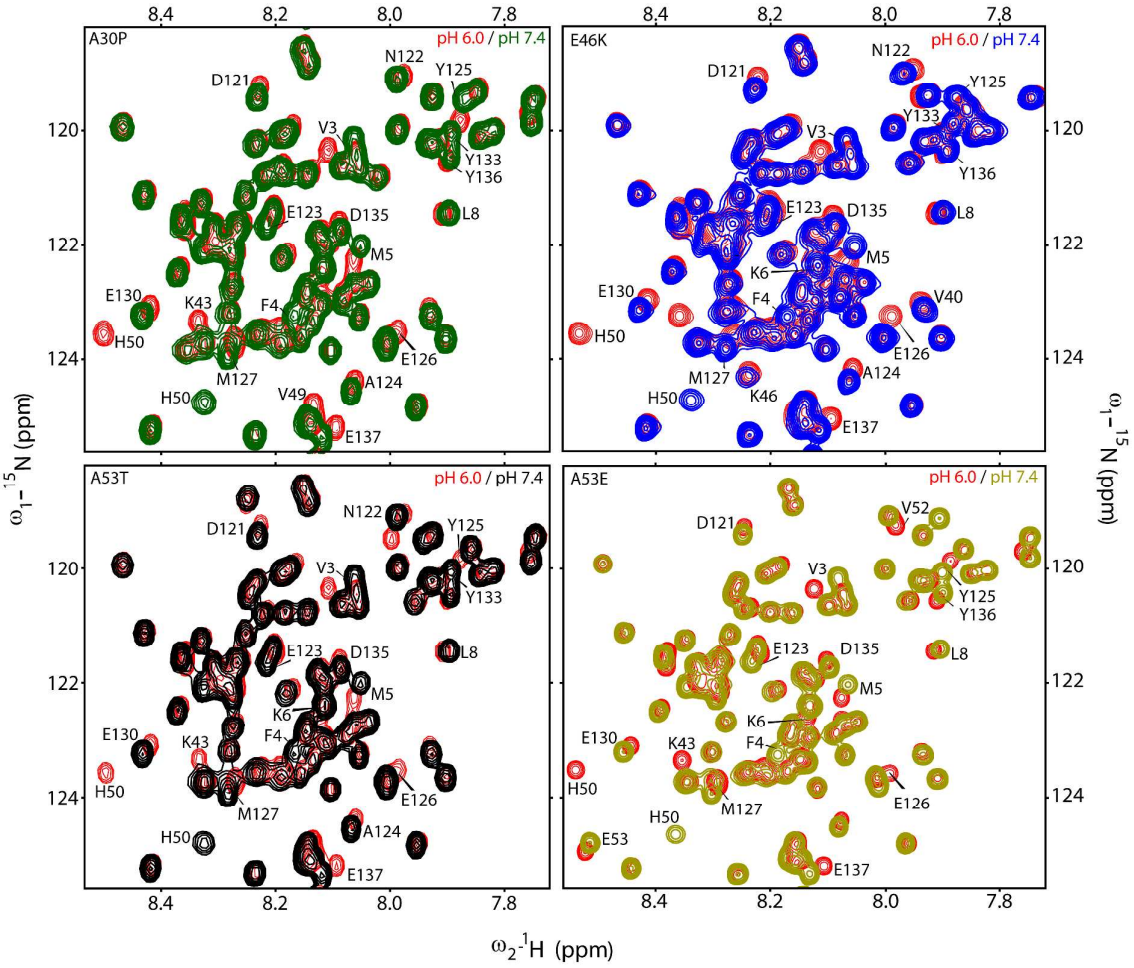

Figure S4. Residues showing significant perturbations at different pH in the overlaid ^{1}H-^{15}N HSQC spectra. Expansion of the overlaid ^{1}H-^{15}N HSQC spectra recorded at two different pHs (pH 6.0 and pH 7.4) for A30P, E46K, A53T, and A53E. In all the synucleins, the spectra recorded at pH 6.0 has been shown in red while the spectra recorded at pH 7.4 has been shown in green, blue, black, and yellow for A30P, E46K, A53T, and A53E.

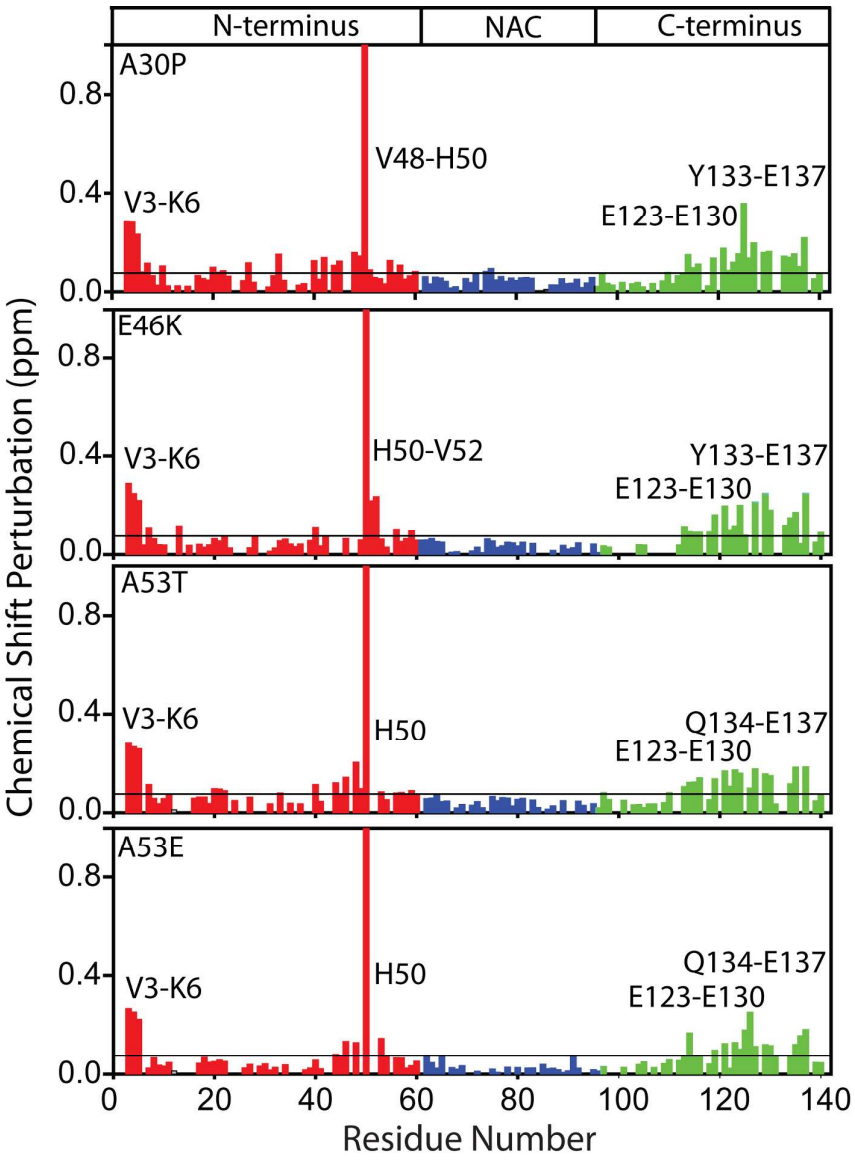

Figure S5. Redistribution of long-range contacts in α-Syn familial mutants. Residue-specific CSP due to change in pH was calculated for A30P, E46K, A53T, and A53E. Red, blue and green colour denotes the basic N-terminal domain, the amyloidogenic NAC domain, and the acidic C-terminal domain respectively.

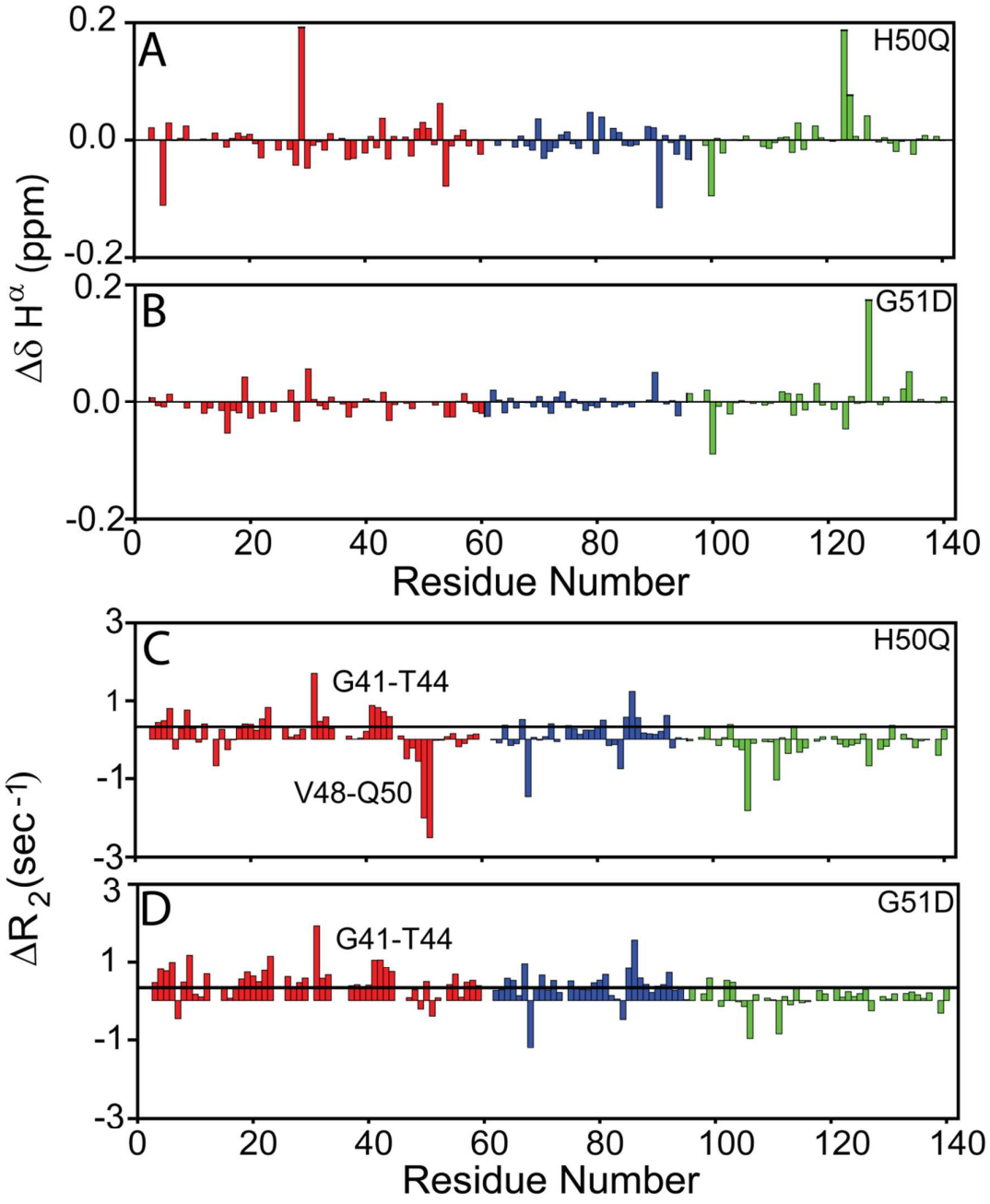

Figure S6. Difference in the secondary structure and ^{15}N transverse relaxation rate (R_2) of mutants compared to the WT. No such significant change in the secondary structure was seen for the familial mutants H50Q (A) and G51D (B). Difference in R_2 values were calculated for H50Q (C) and G51D (D). Monomeric α-Syn G51D was rigid in nature compared to the WT.

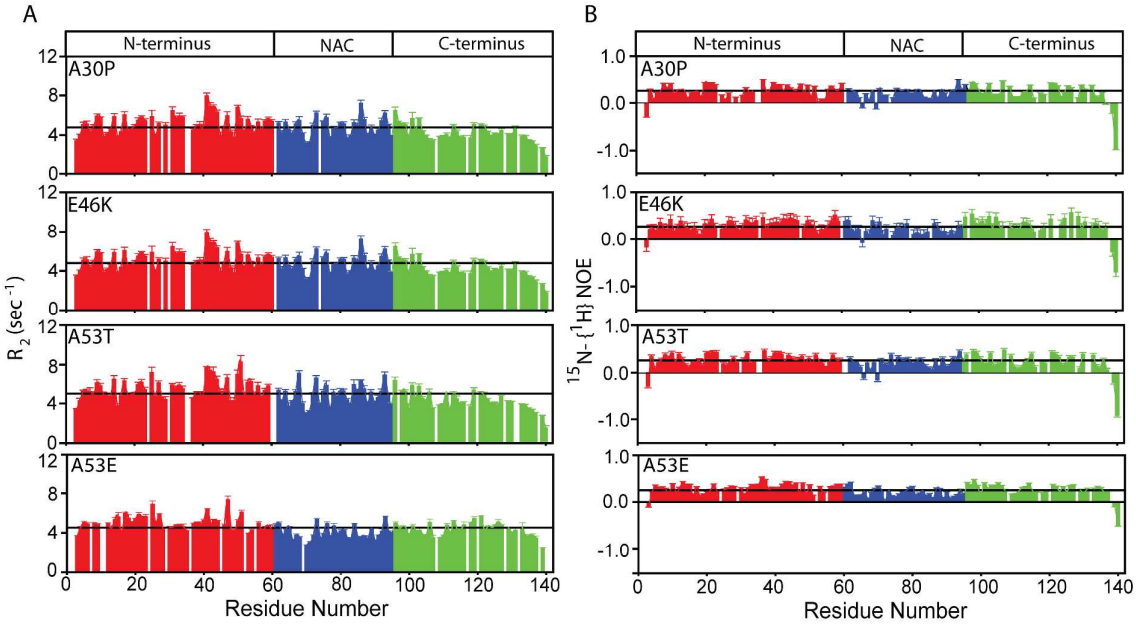

Figure S7. Residue-specific backbone dynamics calculated for α-Syn familial mutants. (A) ^{15}N transverse relaxation rates (R_2) for A30P, E46K, A53T, and A53E. (B) ^{15}N-{1H} heteronuclear NOE calculated for A30P, E46K, A53T and A53E. Red, blue and green colour denotes the basic N-terminal domain, the amyloidogenic NAC domain, and the acidic C-terminal domain respectively.

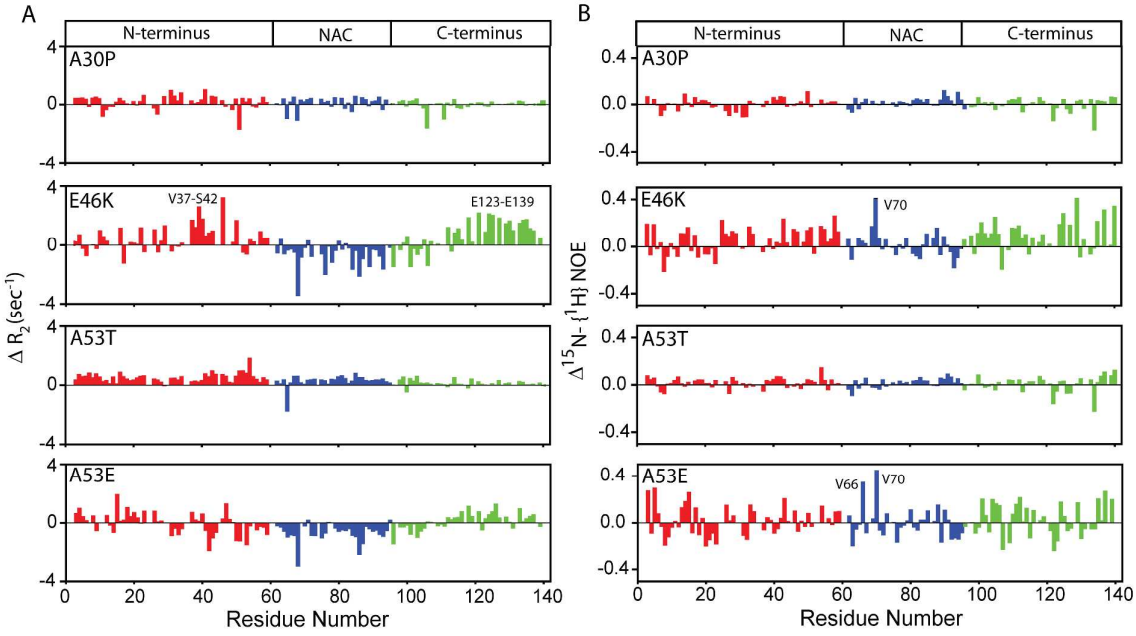

Figure S8. Difference in the ^{15}N transverse relaxation rate (R_2) and ^{1}H-^{15}N heteronuclear NOE of mutants compared to the WT. Difference in R_2 (A) and ^{15}N-{1H} heteronuclear NOE (B) values were calculated for A30P, E46K, A53T, and A53E. Monomeric E46K showed increased rigidity at the N- and C-terminus compared to the other mutants.

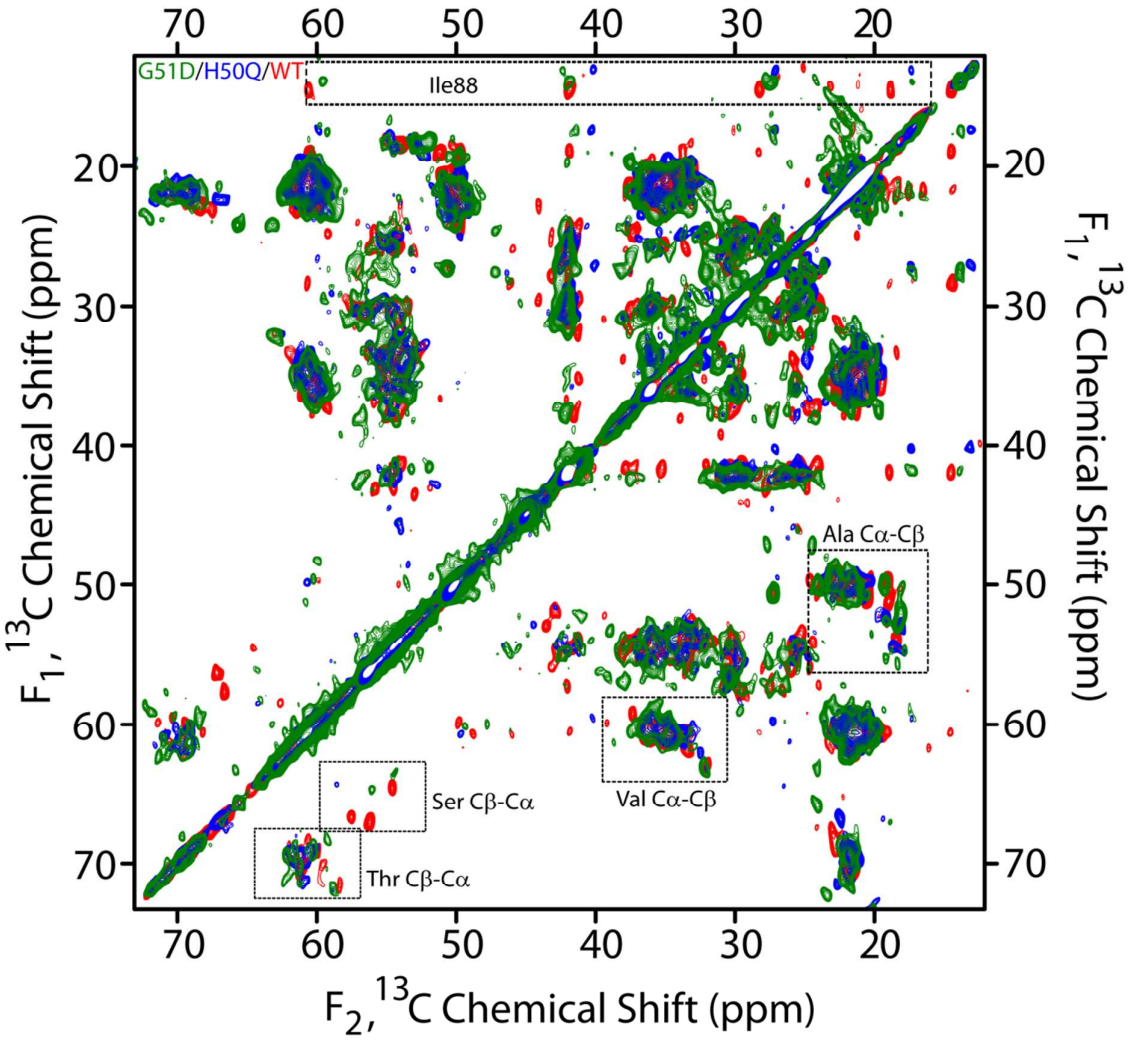

Figure S9. Perturbation of the characteristic amino acids region in the mutants H50Q and G51D. Overlaid ^{13}C-^{13}C 2D PDSD spectra of WT (red), H50Q (blue) and G51D (green).

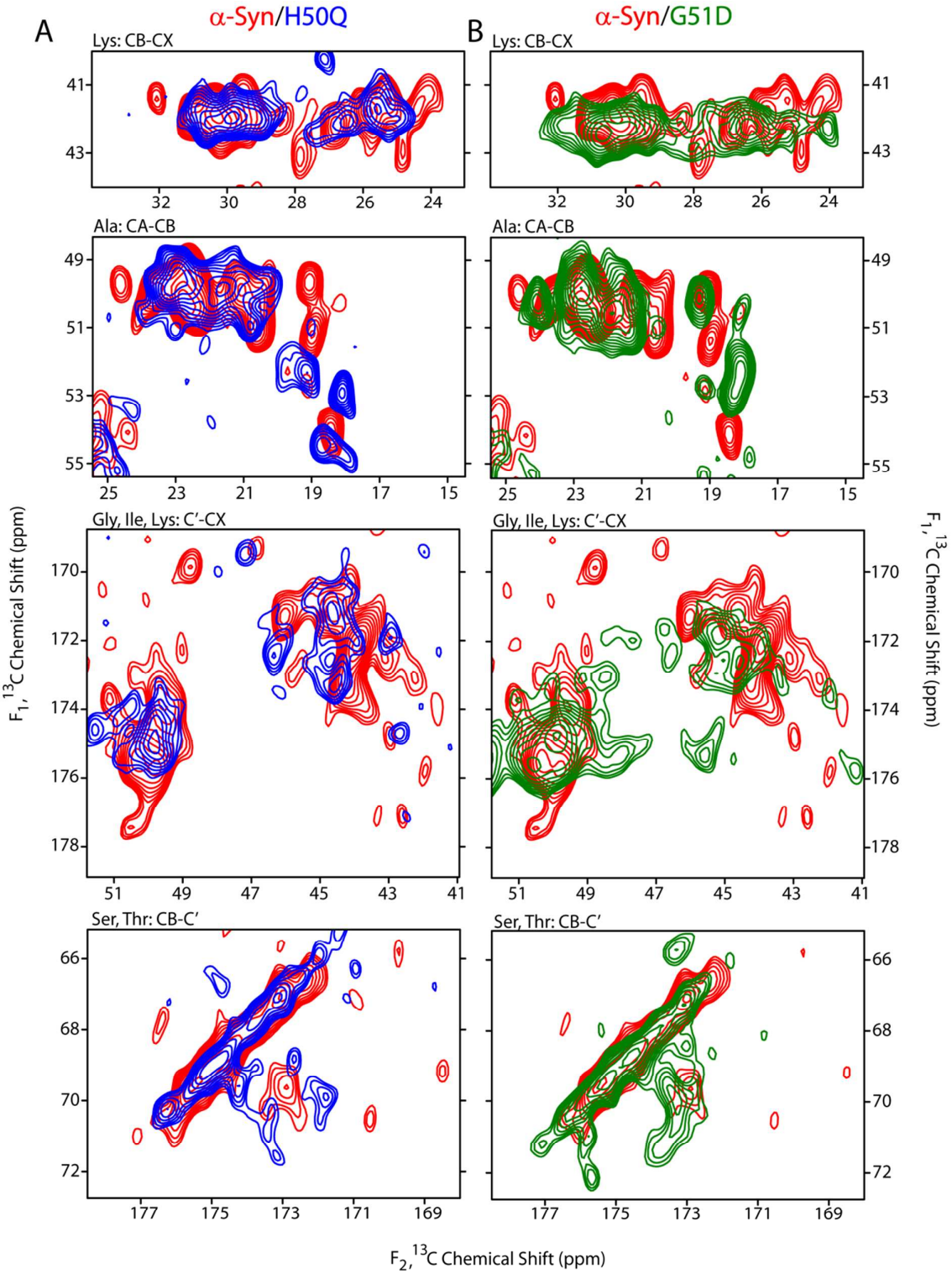

Figure S10. Residue-specific perturbations in the fibrillar structure by the familial mutants. Expansions of ^{13}C-^{13}C 2D PDSD spectral overlays (50 ms) of WT (red) and H50Q (blue) fibril samples (A) and WT (red) and G51D (green) fibril samples.

Table S1. Average R_2 value calculated for the individual domain in α-Syn WT, A30P, E46K, A53T, and A53E.

Synuclein and its familial mutants	R_2 (sec^{-1})	NOE
WT (1-60)	4.99 ± 0.18	0.26 ± 0.04
WT (61-95)	4.64 ± 0.17	0.19 ± 0.04
WT (96-140)	4.16 ± 0.11	0.21 ± 0.04
A30P (1-60)	5.17 ± 0.21	0.26 ± 0.002
A30P (61-95)	4.81 ± 0.19	0.20 ± 0.002
A30P (96-140)	4.19 ± 0.14	0.21 ± 0.002
E46K (1-60)	5.45 ± 0.37	0.30 ± 0.09
E46K (61-95)	3.95 ± 0.23	0.21 ± 0.09
E46K (96-140)	4.87 ± 0.32	0.29 ± 0.09
A53T (1-60)	5.53 ± 0.23	0.27 ± 0.04
A53T (61-95)	4.91 ± 0.20	0.20 ± 0.04
A53T (96-140)	4.28 ± 0.15	0.21 ± 0.04
A53E (1-60)	5.03 ± 0.14	0.29 ± 0.02
A53E (61-95)	4.01 ± 0.10	0.19 ± 0.02
A53E (96-140)	4.35 ± 0.12	0.25 ± 0.02